IOBC/WPRS

Working Group "Use of Pheromones and Other Semiochemicals in Integrated Control"

OILB/SROP

Groupe de Travail "Utilisation des Phéromones et Autres Médiateurs Chimiques en Lutte Intégrée"

PROCEEDINGS WORKING GROUP MEETING

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Preface

NRI through its predecessor organisations has been associated with the IOBC Working Group on Use of Pheromones and other Semiochemicals in Integrated Control since its inception in 1975?, and it was a great pleasure to host this meeting on Pheromone Technology in Europe and the Developing Countries. This meeting brought together colleagues from both the "developed" and the "developing" countries with the aim of reviewing the current state of pheromone technology in Europe and whether and how these techniques might be transferred to developing countries.

In all, 134 delegates from 30 countries attended the meeting, 20 of the delegates representing 11 developing and 4 East European and CIS countries. We should like to thank the British Council, the UK Overseas Development Administration and the Technical Centre for Rural and Agricultural Cooperation (CTA) who made it possible for many of these delegates to attend the meeting.

This publication illustrates the quality and breadth of the work presented at the meeting.

It also includes, as an Annex, the results of a survey on the role of pheromones in integrated pest management. Most of the delegates to the meeting contributed to this survey and its findings are pertinent to the aims of the meeting and of the Working Group.

The meeting was convened by Heinrich Arn and Albert Minks, and we are grateful to the IOBC, Agrisense-BCS (UK), the Central Science Laboratory (UK), Insects Limited (USA), International Pheromone Systems (UK), Oecos (UK), Russell Fine Chemicals Ltd. (UK) and Trécé (USA) for contributing towards the costs of running the meeting.

Finally, we should like to thank our colleagues at NRI for assisting with the organisation and running of the meeting - John Perfect, David Hall, Dudley Farman, Jane Smith, Rose White, Phil Stevenson and Michele Bryson

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BASIC RESEARCH

IDENTIFICATION AND SYNTHESIS OF NEW PHEROMONES

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Abstract. Lepidoptera can still surprise the chemist with new structures for their sex pheromone components. The female cotton leafperforator, *Bucculatrix thurberiella* (Lepidoptera: Lyonetiidae), uses two nitrate esters, one of them with an odd number of carbon atoms. Female cotton leafworms, *Alabama argillacea* and *Anomis texana* (Lepidoptera: Noctuidae), produce closely-related, chiral monomethyl-substituted hydrocarbons, but the males differ in their enantiomeric fastidiousness. The female groundnut leafminer, *Aproaerema modicella* (Lepidoptera: Gelechiidae), uses a blend of ten-carbon acetates including a terminal conjugated diene and one of the corresponding monoenes of opposite stereochemistry.

Introduction

Although there are many exceptions, the majority of Lepidopterous sex pheromones consist of components derived from fatty acid biosynthesis, having unbranched chains of even numbers of carbon atoms with one, two or three double bonds and a terminal alcohol, acetate or aldehyde functionality. This review of some recent pheromone identifications at NRI shows that Lepidoptera can still surprise the chemist with novel structures.

Cotton Leafperforator, *Bucculatrix thurberiella*

The cotton leafperforator, *Bucculatrix thurberiella* Busck. (Lepidoptera: Lyonetiidae), is one of the main Lepidopterous pests of cotton in Peru, although in the Southern U.S.A. it is now only a problem when pesticides are used excessively. The production of a sex pheromone by the virgin female moth was reported by Rejesus and Reynolds (1970).

Laboratory Work.

Insects collected in Peru and the U.S.A. were reared on cotton plants at NRI. Volatiles were collected from groups of virgin female moths during the dark period on micro charcoal filters and eluted with dichloromethane. The collections were analysed by gas chromatography (GC) linked to

electroantennographic (EAG) recording from a male moth (Cork *et al.*, 1990), and two responses were recorded during analyses on fused silica capillary columns coated with non-polar or polar phases. The second response coincided with a GC peak representing approximately 1 ng per female per night, but no GC peak could be reliably associated with the first response.

The retention times of the two active compounds suggested strongly that they were conventional straight-chain, unsaturated acetates or aldehydes (Equivalent Chain Lengths relative to retention times of *n*-alkyl acetates: 13.04 and 14.05 on non-polar CP Sil 5CB, 14.08 and 15.12 on polar CP Wax 52CB). However, as they were clearly one-carbon homologues, one of the components had to have an odd number of carbon atoms in the alkyl chain.

The EI mass spectrum of the major component showed a fragmentation pattern that supported the above supposition, although an unusual ion at m/z 46 was also present in the spectrum. Single ion monitoring showed the presence of a minor component with a significant ion at m/z 46 in its mass spectrum, and the retention times of this coincided with the first EAG responses on the two GC phases. High resolution mass spectrometry gave an accurate mass of 45.9931 for this ion, the closest fit being 45.9929 for NO_2.

Nitrate esters are known to show a significant ion at m/z 46 in their mass spectra, and some saturated alkyl nitrate esters were synthesised in high yield by reaction of the corresponding alcohol with concentrated nitric acid in acetic anhydride. The GC retention times of these compounds indicated that the pheromone components of *B. thurberiella* were mono-unsaturated 13- and 14-carbon nitrate esters. Ranges of positional and geometric isomers were synthesised, and detailed comparison of GC retention times showed that only the (*Z*)-9-tetradecenyl (Z9-14:NO_2) and (*Z*)-8-tridecenyl nitrates (Z8-13:NO_2) (Fig. 1: I and II) had retention times identical to those of the major and minor natural EAG-active components respectively.

Attempts to synthesise these monounsaturated nitrate esters by reaction of the corresponding alcohol with concentrated nitric acid and acetic anhydride gave very variable yields, and the product showed extensive isomerisation of the double bond. However, nitration of the corresponding acetylenic alcohol proceeded in high yield, and the alkynyl nitrate was smoothly hydrogenated to the *Z* olefin over Lindlar catalyst in hexane or aqueous ethanol at 0°C.

Field Testing

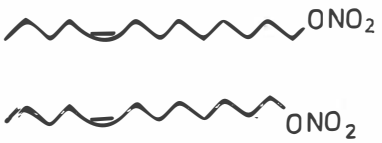
A blend of the two synthetic compounds in their estimated naturally-occurring ratio of 100:2 Z9-14: NO_2 + Z8-13:NO_2 was shown to be highly attractive to male *B. thurberiella* moths in both the U.S.A. and Peru, although the individual components were relatively unnattractive. The synthetic blend was significantly more attractive than a virgin female moth (Table 1).

Discussion

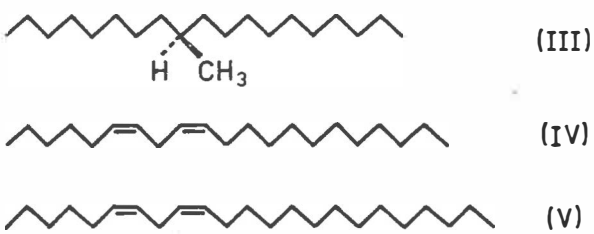
Nitrate esters have rarely been found as natural products, and this is the first occasion on which they have been found as components of a moth sex pheromone (Hall *et al.*, 1992). However, at least one other *Bucculatrix* species has been trapped with these compounds in Europe (Miklos Toth, *pers comm*).

Fig. 1. Structures of the pheromone components

Bucculatrix thurberiella



Alabama argillacea



Anomis texana

Aproaerema modicella

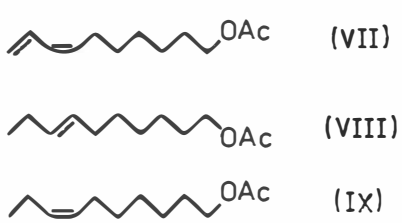


Table 1. Catches of Male *B. thurberiella* Moths in Pheromone Traps

Pheromone Components (µg)			Mean Catch/Trap/Night[1]			
Z9-14:NO_2	Z8-13:NO_2	Z9-13:NO_2	polythene vial		rubber septum	
1. Arizona, USA (4 replicates; 5 periods)						
1000[2]	0	0	25.8	b	26.9	b
1000[2]	20[2]	0	584.8	a	528.0	a
1000[2]	0	20[2]	21.5	b	20.4	b
unbaited trap			5.8	c		
2. Arizona, U.S.A. (6 replicates; 1 night)						
1000[2]	20[2]	0	865.1	a		
virgin female moth			39.1	b		
virgin male moth			2.1	c		
unbaited trap			22.3	b		
3. Piura, Peru (10 replicates, 30 nights)						
1000	20	0	9.1			
1000[2]	20[2]	0	12.6			

[1] mean catches per replicate transformed to log(x+1) for analysis of variance; means followed by different letters in each experiment are significantly different at $P<0.05$ by Duncan's Multiple Range Test.
[2] compounds contain approx 50% of the *E* isomer

Cotton Leafworms, *Alabama argillacea* and *Anomis texana*

The cotton leafworms, *Alabama argillacea* Hb. and *Anomis texana* F. (Lepidoptera: Noctuidae) are also pests of cotton in Peru and other S. American countries. The former used to be an important pest in the U.S.A., but is now rarely a problem with early-planted varieties. The presence of a female sex pheromone in *Alabama argillacea* was reported by Berger (1968), and this was partially characterised as a branched hydrocarbon with 20 carbon atoms. Roelofs and Hammond (unpublished) suggested this was 9-methylnonadecane, but the synthetic, racemic compound did not attract male moths in the field.

Laboratory Work

Insects were obtained from Peru and reared on cotton at NRI. Hexane ovipositor washings were made from virgin female moths and analysed by GC-EAG. Those from *Alabama argillacea* showed three EAG responses coinciding with GC peaks in 10 : 0.5 : 1 ratio. Those from *Anomis*

texana showed two responses associated with GC peaks in 4 : 1 ratio, the second component having identical retention times to the third component from *Alabama argillacea*.

In each species, the first, major response coincided with a GC peak representing over 20 ng per female, and the GC retention times and mass spectra indicated that both these were methyl-substituted hydrocarbons. Racemic, methyl-substituted hydrocarbons were synthesised by Wittig reaction of methyl ketones with the appropriate alkyl phosphonium salt and hydrogenation of the resulting olefin. Comparison of GC retention times and mass spectra of a range of compounds showed that the major components of the pheromones were 9-methylnonadecane (9Me-19:HC) and 7-methylheptadecane (7Me-17:HC) for *Alabama argillacea* and *Anomis texana* respectively (Fig. 1: III and VI).

In each species, the GC retention times of the minor pheromone components indicated they were also hydrocarbons, and the mass spectra showed molecular ions indicating diunsaturated 21- and 23-carbon hydrocarbons, and fragmentation patterns characteristic of 6,9-unsaturation. (*Z,Z*)-6,9-Heneicosadiene (ZZ6,9-21:HC) and (*Z,Z*)-6,9-tricosadiene (ZZ6,9-23:HC) (Fig 1: IV and V) were synthesised from methyl linoleate by reduction to the alcohol, tosylation and chain extension with the appropriate lithium dialkyl cuprate, and these were shown to have retention times and mass spectra identical with those of the minor EAG-active components from *Alabama argillacea*. Only the latter was present in ovipositor washings from *Anomis texana* (Fig. 1).

Both homochiral enantiomers of 9Me-19:HC and of 7Me-17:HC were synthesised from methyl (*S*)-2-methyl-3-hydroxypropionate by enantiomeric chain extensions. The final products had negligible optical rotations and could not be separated by GC on a chiral cyclodextrin phase, but the optical purities of the precursor 2-methylalkan-1-ols were shown to be 96% *ee* by NMR analysis of the esters with (*S*)-2-methoxy-2-trifluorophenylacetic acid and GC analysis of the amides of the derived acids and (*S*)-methylbenzylamine.

Field Testing

Field testing of the synthetic compounds in Peru showed that traps baited with racemic 7Me-17:HC caught large numbers of male *Anomis texana* moths, and addition of the minor component, ZZ6,9-23:HC, had no significant effect on catches. However, racemic 9Me-19:HC was completely unnattractive to male *Alabama argillacea* moths when tested alone or in combination with the minor components ZZ6,9-21:HC and ZZ6,9-23:HC, confirming previous results of Roelofs and Hammond (unpublished).

When the homochiral compounds became available, further field testing in Peru showed that the (*S*)-9Me-19:HC (Fig. 1: III) was highly attractive to *Alabama argillacea* male moths, and addition of the minor components, ZZ6,9-21:HC and ZZ6,9-23:HC, had no significant effect on catches (Table 2). The *R* enantiomer and the racemic material were completely unnattractive. Male *Anomis texana* moths were attracted to (*S*)-7Me-17:HC (Fig.1: VI), but not to the *R* enantiomer (Table 3). The difference in catches with the pure *S* enantiomer and the racemic mixture is consistent with the latter lure containing only 50% of the active enantiomer.

Discussion

Monomethyl-substituted hydrocarbons have not been found as pheromone components of other Noctuid moths, although 7Me-17:HC was recently reported as a possible minor component of the pheromone of a Geometrid, *Lambdina fiscillaria lugubrosa* Hulst., by Gries *et al.* (1993). The males of both *Alabama argillacea* and *Anomis texana* are attracted by the *S* enantiomers of their pheromones and not by the *R* enantiomers, and it is assumed that the female moths produce only the *S* enantiomers. However, whereas *Anomis texana* males are apparently unaffected by the *R*

enantiomer of the pheromone, in *Alabama argillacea* the attractiveness of the *S* enantiomer of the pheromone to males is completely destroyed by the presence of the *R* enantiomer in the racemic mixture.

Methylene-skipped unsaturated hydrocarbons have been found as pheromone components of various Noctuid species, although these are most commonly tri-unsaturated compounds or their mono-epoxide derivatives. ZZ3,6-23:HC has not been reported previously, and ZZ3,6-21:HC only rarely (e.g. Landolt & Heath, 1989). Although no behavioural effects on A*labama argillacea* or *Anomis texana* males have been observed in trapping tests so far, their significance warrants further investigation.

Table 2. Catches of Male *Alabama argillacea* Moths in Funnel Traps in Peru

Pheromone Components (µg)			Mean Catch/Trap/Night[1]	
9Me-19:HC	ZZ6,9-21:HC	ZZ6,9-23:HC		
1000 (*S*)	50	100	8.33	a
1000 (*S*)			14.17	a
1000 (*R*)	50	100	0.02	b
1000 (*R*)			0.04	b
1000 (*RS*)	50	100	0.24	b
1000 (*RS*)			0.24	b

[1] mean catches per replicate transformed to log(x+1) for analysis of variance; means followed by different letters in each experiment are significantly different at P<0.05 by Duncan's Multiple Range Test.

Table 3. Catches of Male *Anomis texana* Moths in Funnel Traps in Peru

Pheromone Component (1 mg)	Mean Catch/Trap/Night[1]	
S 7Me-17:HC	732.5	a
R 7Me-17:HC	0.05	c
RS 7Me-17:HC	601.25	b

[1] mean catches per replicate transformed to log(x+1) for analysis of variance; means followed by different letters in each experiment are significantly different at P<0.05 by Duncan's Multiple Range Test.

Groundnut Leafminer, *Aproaerema modicella*

The groundnut leafminer, *Aproaerema modicella* Deventer (Lepidoptera; Gelechiidae) has been quoted as the most important insect pest of groundnuts in India, and is a serious pest of groundnuts and soya beans throughout South and Southeast Asia. The presence of a sex pheromone was reported by Nandagopal (unpublished) and Lalita Kumari and Reddy (1991).

Laboratory Work

Insects originating from ICRISAT were reared on groundnuts at NRI. Volatiles from virgin female moths were collected on micro-charcoal filters, eluted with dichloromethane and analysed by linked GC-EAG. Two significant EAG responses were observed, the major response eluting second on both polar and non-polar GC phases.

The major EAG response was associated with a GC peak whose retention times indicated that it could be a 10-carbon acetate with two conjugated double bonds, and this was confirmed by GC-MS analyses which showed $(M-60)^+$ at m/z 136. Isomers of 7,9- and 6,8-decadienyl acetates were synthesised, and the former cochromatographed with the natural EAG-active component. The isomers of terminal conjugated dienes are notoriously difficult to separate by GC on "normal" phases (e.g. Lester, 1978), but it was found that the *Z* and *E* isomers of 7,9-decadienyl acetate could be separated on a 50 m column coated with the chiral cycoldextrin-β-2,3,6-M19 phase (Chrompack). Analysis of the volatiles from *A. modicella* on this column indicated that the major EAG-active component was >97% (*Z*)-7,9-decadienyl acetate (Z7,9-10:Ac) (Fig. 1: VII). The latter was synthesised most conveniently by Wittig reaction of the tetrahydropyranyl ether of 7-hydroxyheptyl-(triphenyl)phosphonium bromide with acrolein giving the terminal diene in 95:5 *Z/E* ratio. After deprotection and acetylation, the *E* isomer was removed by reaction with tetracyanoethylene.

GC-MS analysis of the volatiles showed two components at the retention time of the minor EAG response with mass spectra having $(M-60)^+$ indicative of 10-carbon, monounsaturated acetates. These were shown to be the *E* and *Z* isomers of 7-decenyl acetate (E7-10:Ac and Z7-10:Ac) (Fig. 1: VIII and IX) by comparison of their GC retention times with those of all the decenyl acetate isomers on the cycoldextrin phase as well as on normal polar and non-polar phases. The ratio of Z7,9-10:Ac : E7-10:Ac : Z7-10:Ac was 100:20:14.

Field Testing

Field testing was carried out at two locations in India (Table 4). Results from Gujurat indicated that both minor components increased the attractiveness of the major component to male *A. modicella* moths. In experiments at ICRISAT, addition of E7-10:Ac greatly increased the attractiveness of Z7,9-10:Ac, but Z7-10:Ac had no effect when added to either the major component alone or to the blend of Z7,9-10:Ac and E7-10:Ac. At both locations, traps baited with the best synthetic blends caught significantly more moths than traps baited with virgin female moths.

Further tests showed that the naturally-occurring ratio of Z7,9-10:Ac and E7-10:Ac is optimal, and that the 95:5 *Z/E* mixture of isomers of 7,9-10:Ac produced in the Wittig reaction is as effective as the pure *Z* isomer.

Table 4. Catches of Male *A. modicella* Male Moths in Pheromone Traps

Pheromone Components (µg)			Mean Catch/Trap/Night[1]			
Z7,9-10:Ac	E7-10:Ac	Z7-10:Ac	Gujurat[2]		ICRISAT[3]	
1000	-	-	14.9	b	0.9	c
1000	200	-	23.6	a	31.2	a
1000	-	140	23.6	a	1.7	bc
1000	200	140	37.1	a	28.0	a
virgin female moth			12.3	b	3.4	b
unbaited trap			5.2	c	0.2	d

[1] data transformed to log(x+1) for analysis of variance; means followed by different letters significantly different at $P<0.05$ by Duncans Multiple Range Test
[2] sticky plate traps; 16 replicates, 7 nights
[3] delta traps; 16 replicates, 7 nights

Discussion

Conjugated, terminal dienes are unusual pheromone components. The 12-carbon acetates (Nesbitt *et al.*, 1975) and 14-carbon aldehyde (Baker *et al.*, 1991) have been found previously as Lepidopterous sex pheromone components, but this is the first time that the 10-carbon acetate has been reported. It is surprising that, although the major pheromone component of *A modicella* is the *Z* isomer of 7,9-10:Ac, the E7-10:Ac seems to be the key minor component. The reason for the different results at Gujurat and ICRISAT on the effect of the Z7-10:Ac is not known at present, although it may be associated with the different trap designs used. At present the 100:20 blend of Z7,9-10:Ac and E7-10:Ac is recommended for use in monitoring *A. modicella*.

References

BAKER, T.C., FRANCKE, W., MILLAR, J.G., LOFSTEDT, C., HANSSON, B., DU, J.-W., PHELAN, P.L., VETTER, R.S., YOUNGMAN, R. & TODD, J.L., 1991. Identification and bioassay of sex pheromone components of carob moth, *Ectomyelois ceratoniae* (Zeller). *Journal of Chemical Ecology* **17**: 1973-1988.

BERGER, R.S., 1968. Sex pheromone of the cotton leafworm. *Journal of Economic Entomology* **61**: 326-327.

CORK, A., BEEVOR, P.S., GOUGH, A.J.E. & HALL, D.R., 1990. Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. In *Chromatography and Isolation of Insect Hormones and Pheromones*, eds. A.R. McCaffery & I.D. Wilson, Plenum Press, New York, 271-279.

GRIES, G., GRIES, R., KRANNITZ, S.H., LI, J., SKIP KING, G.G., SLESSOR, K.N., BORDEN, J.H., BOWERS, W.W., WEST, R.J. & UNDERHILL, E.W., 1993. Sex pheromone of the western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst) (Lepidoptera: Geometridae). *Journal of Chemical Ecology* **19**: 1009-1019.

HALL, D.R., BEEVOR, P.S., CAMPION, D.G., CHAMBERLAIN, D.J., CORK, A., WHITE, R.D., ALMESTAR, A. & HENNEBERRY, T.J., 1992. Nitrate esters: novel sex pheromone components of the cotton leafperforator, *Bucculatrix thurberiella* Busck. (Lepidoptera: Lyonetiidae). *Tetrahedron Letters* **33**: 4811-4814.

LALITA KUMARI, V.L. & REDDY, D.D.R., 1991. Sex pheromone in the groundnut leaf miner, *Aproaerema modicella*. *International Arachis Newsletter*, **May**: 15-16.

LANDOLT, P.J. & HEATH, R.R., 1989. Lure composition, component ratio, and dose for trapping male *Mocis latipes* (Lepidoptera: Noctuidae) with synthetic sex pheromone. *Journal of Economic Entomology* **82**: 307-309.

LESTER, R., 1978. Smectic liquid crystal for the gas-liquid chromatographic separation of lepidopterous sex pheromones and related isomeric olefins. *Journal of Chromatography* **156**: 55-62.

NESBITT, B.F., BEEVOR, P.S., COLE, R.A., LESTER, R. & POPPI, R.G., 1975. The isolation and identification of the female sex pheromones of the red bollworm moth, *Diparopsis castanea*. *Journal of Insect Physiology* **21**: 1091-1096.

REJESUS, R.S. & REYNOLDS, H.T., 1970. Demonstration of the presence of a female sex pheromone in the cotton leaf perforator. *Journal of Economic Entomology* **63**: 961-964.

CONTRIBUTION OF SINGLE SENSILLUM RECORDINGS TO THE UNDERSTANDING OF PHEROMONE COMMUNICATION IN LEPIDOPTERA

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Abstract The pheromone detection system of several close related species of Noctuidae was studied by single sensillum recordings. Two types of olfactory hairs were characterized by their morphology, their position on the antenna, and their responses to pheromones. In addition to receptor cells selectively tuned to components of the pheromone blend there are receptor cells tuned to compounds not produced by conspecific females. Similiraties between species in receptor cell and sensillum types are discussed in respect to the evolution of pheromone detection systems.

Introduction

The different components of moth pheromone blends are detected by highly specialized receptor neurones whose activity can be recorded from single hairs sampled from the tens of thousands of olfactory hairs present on the male antennae. It is admitted that pheromone detection involves a labelled line system of quality encoding. Under this scheme, each component of the pheromone requires its own type of specialist receptor neurone for its detection. Electrophysiological methods, electroantennography (EAG) and single sensillum recording (SSR), are of great help to pheromone studies. However, there are several examples in the literature of discrepancies between the compounds emitted by conspecific females and the key-compounds of the receptor cell characterized on the male antennae. To have a better understanding of their pheromone detection system, a comparative study was undergone in close related Noctuidae species. The pheromone detection system of 5 species of the Hadeninae genus *Mamestra* was studied by single sensillum recordings. Their pheromone blends have in common the same main component, Z11-16:Ac, but their minor components, though biochemically related, differ. A survey of receptor neurone types was also done in some *Orthosia* and a few other Hadeninae species.

Morphology

The antennae of male *Mamestra brassicae* are filiform, 12 mm long, and comprises up to 72 segments. They bear about 10,000 thrichoid sensilla, pheromone sensitive for the majority. SSR associated with morphological studies revealed different types of pheromone sensitive sensilla, housing receptor cells with different response spectra. On the lateral margins of the ventral side of

the segments are located long olfactory hairs (60-190 µm), characteristically arranged in 4 to 5 parallel rows. These lateral hairs (LHs) are found only in males which possess about 2,200 of them per antenna. Medio-ventrally on the segments are located shorter hairs (35-55 µm), not lying in rows. These medial hairs (MHs) are more numerous than the lateral hairs. The number of olfactory hairs per segment decreases from about 230 at the base of the flagellum to less than 55 in the ten last segments. More than 80 % of the olfactory hairs are located on the segments 1 to 45. Furthermore, the LHs disappear after segment 50.

Characterization and distribution of the receptor cell types

Receptor cell types were characterized from their response spectra by screening pheromone compounds on individual sensilla sampled among the LHs or the MHs, mostly on segments 10 to 40. The same cell types are shared by different species. Very similar patterns in the distribution of the receptor cells were observed in the 5 *Mamestra* species showing that it is a stable character throughout the genus evolution. Even the correspondence between key compound and spike amplitude of the companion cells in a sensillum type was preserved from one species to the other.

A receptor neurone type tuned to Z11-16:Ac was found in the LHs of the 5 species (Lucas & Renou, 1989; Renou, 1991). When clear differences in spike amplitude were seen this Z11-16:Ac cell was always firing with large spikes. A second cell in LH responded either to Z9-14:Ac and Z11-16:OH (*brassicae* and *suasa*) or to Z11-16:OH only (*oleracea*, *thalassina* and *contigua*). Z11-16:OH was found in the gland extracts from the females of these 3 species but not in *brassicae* and *suasa*.

A cell responding to Z11-16:Ald was found in the MHs of our five species. The sensilla responding to Z11-16:Ald also responded to Z9-14:Ac in *brassicae* and *oleracea* and to Z7-12:Ac in *contigua* with the same spike class amplitude. The absence of differences in the shape of spikes and cross adaptation experiments indicated that in *brassicae* Z11-16:Ald and Z9-14:Ac stimulated the same receptor cell (Renou and Lucas, 1993). A second functional type of MHs presented the same response profile as the LHs in *contigua*, *oleracea* and *suasa* (Lucas and Renou, 1991) but not in *thalassina* nor *brassicae*. According to the species, from 10 % up to 30 % of the MHs did not respond to any pheromone compound tested.

Specificity of the receptor cells

Pheromone receptor cells are specialist sensory neurones that usually respond to only a few compounds that resemble their key compound. In contrast to the high specificity of the Z11-16:Ac receptor cell, several neurone types were found to be tuned to more than one compound. For example, in *suasa* and *brassicae* the same cell responded to Z9-14:Ac and Z11-16:OH, which both inhibit the attraction flight of males to females. In return, its homolog receptor cell in *oleracea* responded only to Z11-16:OH. Less specificity is expected from receptor cells tuned to behavioural inhibitors and, when fine discrimination is not critical, it could be selectively advantageous to detect several compounds with a same cell expressing more than one type of pheromone acceptor site.

Interspecific communication

The sensory capacity of males is not limited to the detection of the components of the pheromone blend of conspecific females. For example in the European sunflower moth (*Homeosoma nebulellum* Den. & Schiff.) the trichoid sensilla house a large-spike firing cell tuned to Z9,E12-14:Ald and a small-spike firing cell tuned to Z9,E12-14:Ac (Zagatti et al., 1991). Z9,E12-14:Ac reduces the catches when added to the attractive blend. This acetate is the main component of the sex pheromone of the American sunflower moth, *Homeosoma electellum*.

In *brassicae* and *suasa* the number of cells tuned to Z9-14:Ac, not produced by females, reaches that of cells tuned to Z11-16:Ac, the main pheromone component. Such a functional specialization may indicate that the specificity of communication is ensured not only by the recognition of a specific blend, but also by the detection of compounds emitted by other species. Z9-14:Ac is produced by the females of *M. pisi* (Renou et al., 1981) and *M. biren* (Frérot et al., 1987). Mutations ending the production of Z9-14:Ac by the females may have occured in some *Mamestra* although chemoreceptors detecting this compound were preserved. Alternatively, Z9-14:Ac-receptors could have appeared as a response to selection pressure exerced by competition with other species emitting blends where attractive compounds like Z11-16:Ac were mixed with Z9-14:Ac.

Detection of the minor components

While receptor cells for Z11-16:Ac, the main component of the blend, were easily characterized, more investigations were needed to find receptor cells tuned to specific minor components. In spite of extensive investigation, a specialist receptor cell type could not be associated to each of the components with behavioural activity in *brassicae*.

Receptor neurones tuned to minor components may have been missed because they are present in very low number on the antenna. Only 2 % of the sampled sensilla were found to respond to Z9-14:Ac in *Agrotis segetum* (Löfstedt et al., 1982). On the other hand, the sampling may be altered if the receptor cells are housed in sensilla either localized in specialized areas of the antenna or belonging to a different morphological type.

This small number of receptor cells for minor components implies lowered detection performances in comparison to the sensitivity to the main components. This is confirmed by the generally low EAG responses recorded for minor components in Hadeninae (Renou et al., 1991). The sensory system of these noctuid moths seems adapted to a qualitative detection of the presence of minor components rather than to a precise measurement of their ratio in the blend.

Evolution of pheromone receptor systems

Single sensillum recordings from species belonging to other genera of Hadeninae revealed other receptor neurone types and other patterns. The lateral hairs in *Mythimna impura* house a large-spike receptor cell tuned to Z11-16:Ac and a small-spike cell tuned to Z9-14:Ald. In *Pseudaletia unipuncta*, a first type of sensilla houses a large-spike cell tuned to Z11-16:Ac and a small-spike cell tuned to Z11-16:Ald and Z7-12:Ac. A less common type of hairs responded to Z11-16:Ac, Z9-14:Ac and Z9-12:Ac.

Single sensillum recordings in the genus *Orthosia* revealed a greater variety of receptor cell types than in *Mamestra*. In the 3 investigated species 6 different neurone types were found. Two

types of trichoid sensilla, one responding to Z11-16:Ac and Z11-14:Ac, the other tuned to Z9-14:Ac were found in *Orthosia gothica*. Responses from a large-spike cell tuned to Z9-14:Ald and a small-spike cell tuned to Z11-16:Ald were recorded from the same hairs in *Orthosia incerta*. In *Orthosia munda*, a large-spike cell tuned to Z11-16:OH and a small-spike cell tuned to Z9-14:OH were found together in the same olfactory hairs.

The comparison of the pheromone receptor systems of these 5 *Mamestra* species reveals 3 potential mechanisms by which these specialized olfactory organs could have evolved. First, the percentage of a receptor cell type varies from one species to the other. Less than 30 % of the medial hairs were found to respond to Z11-16:Ald in *brassicae*, versus 60 % in *suasa* (Lucas and Renou, 1991) and above 90 % in *thalassina*. These variations in the number of Z11-16:Ald receptor cell are correlated with variations in its proportions in the female pheromone secretion (0.1 % of the total blend in *brassicae*, 1 to 2 % in *suasa* and 12 % in *thalassina*).

Secondly, mutations may affect a cell type, inducing the expression of an acceptor site with enlarged sensitivity, or more probably, of another site on the same cell. This new sensitivity leads to a decrease in specificity but to an increase in the number of compounds detected with a limited number of cell types. The B cell of the lateral hairs that exhibit various levels of sensitivity to Z9-14:Ac could have evolved through this way.

Finally, both mechanisms, if continued, would lead to sensory systems with different receptor cell types. Alternatively, discrete changes may result in the apparition, at once, of a new receptor cell type, then spreading over the population in response to selection (Mankin, 1991). Such a mechanism might have arisen in the *Orthosia*, a genus where the pheromone receptor systems do not share receptor cell type as in *Mamestra*.

Conclusions

Morphological observations reveal functional specializations on the male antennae due to different specificities between lateral and medial hairs on each segment. This precise setting of the receptor cell types in MHs and LHs suggests the coexistence of two sub-systems of pheromone detection. However, its functional significance is still unknown.

Besides receptor cells selectively tuned to components of the pheromone blend emitted by conspecific females, male antennae contain cells responding to compounds emitted by females of other species. There is a number of similarities between the tuning and the distribution of the pheromone receptor cells of the *Mamestra*, contrasting with the diversity encountered in the other Hadeninae species. This suggest that numerous plesiomorphic characters have been preserved throughout the coevolution of the sender and the receiver of the chemical message.

The differences among *Mamestra* pheromone detection systems lie primarily in the proportions of common receptor cell types. This implies different integrations of the neural responses in the central nervous system to insure communication specificity. In other words, when pheromone receptor cells tuned to the same compound are present on the antennae of different species, the behavioural responses to it may have evolved differently for each species.

From a practical point of view, SSR approaches should take into account these considerations to improve their contribution to chemical communication studies by: (i) including a minimal morphological study and sampling from different morphological types of sensilla, (ii) comparing if possible with close related species (iii) never forgetting that different behavioural effects may follow the detection of a compound by homolog types of receptor cell, (iv) providing quantitative data on the proportions of receptor cell types.

References

FREROT B., GALLOIS M., MALOSSE C. & BUES R., 1987. Identification de la phéromone sexuelle de *Mamestra biren* (Goeze) ex *M. glauca* (Hb.). (Lepidoptera, Hadeninae). C. R. Acad. Sci. Paris, **304**: 139-142.

LÖFSTEDT C., Van der PERS J. N. C., LOFQVIST J., LANNE B. S., APPELGREN M., BERGSTRÖM G. & THELIN B., 1982. Sex pheromone components of the turnip moth, *Agrotis segetum*: chemical identification, electrophysiological evaluation and behavioral activity. J. chem. Ecol., **8**: 1305-1321

LUCAS P. & RENOU M., 1989. Responses to pheromone compounds in *Mamestra suasa* (Lepidoptera: Noctuidae). J. Insect Physiol. **35**:837-845.

LUCAS P. & RENOU M., 1991. Mise en évidence d'un neurone récepteur au (Z)-hexadéc-11-én-al, composé minoritaire de la phéromone, chez *Mamestra suasa* (Lepidoptera, Noctuidae). C. R. Acad. Sci. Paris, **312**: 71-76.

MANKIN R. W., 1991. Evolution of pheromonal specificity in insect chemoreceptors. in Chemical Senses Vol. 3 Genetics of perception and communications. C. J. Wysocki and M. R. Kare Eds. Marcel Dekker, New-York.

RENOU M., 1991. Sex pheromone reception in the moth, *Mamestra thalassina*. Characterization and distribution of two types of olfactory hairs. J.Insect Physiol. **33**: 617-626

RENOU M., LALANNE-CASSOU B., FREROT B., GALLOIS M., & DESCOINS C., 1981. Composition de la sécrétion phéromonale émise par les femelles vierges de *Mamestra (Polia) pisi* (L.) (Lépidoptère, Noctuidae, Hadeninae). C. R. Acad Sc. Paris, **292**: 1117-1119.

RENOU M. & LUCAS P. 1993 Sex pheromone reception in *Mamestra brassicae* L. (Lepidoptera): Responses of olfactory receptor neurones to minor components of the pheromone blend. Submitted to J. Insect Physiol.

RENOU M., LUCAS P., DORE J.-C., LALANNE-CASSOU B., CHAMBON J.-P. & COCQUEMPOT C., 1991. A comparative study of sex pheromone reception in the Hadeninae (Lepidoptera: Noctuidae). Physiol. Entomol. **16**: 87-97.

ZAGATTI P., RENOU M., MALOSSE C., FREROT B., PAVIS C., DESCOINS C;, PERMANA A., PIVOT Y. & LECLANT F, 1991. Sex pheromone of the European sunflower moth, *Homeosoma nebulellum* (Den. & Schiff.) (Lepidoptera: Pyralidae).

RECEPTION OF PRINCIPAL PHEROMONES IN THREE SPECIES OF HELIOTHINE MOTHS

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Abstract The mechanisms for peripheral coding of information from pheromones and interspecific signals are compared in three species of Heliothine moths; the two sympatric, American species *Heliothis virescens* and *Helicoverpa zea*, and *Helicoverpa assulta* living in other continents. Information from the principal pheromones and interspecific signals is received via a pure labelled-line system in *H. virescens* and *H. assulta*, whereas in *H. zea*, a modified labelled-line system seems to be used for receiving the information. All three species possess receptor neurons (RNs) tuned to cis-11-hexadecenal (Z11-16:Al) which is one of their principal pheromones. These RNs seem to be functionally similar, suggesting that their membrane receptors are conserved through evolution. The information from the second principal pheromone components in *H.virescens* and *H. assulta* is likewise received via RNs tuned specifically to these components, cis-9-tetradecenal (Z9-14:Al) and cis-9-hexadecenal (Z9-16:Al), respectively. However, in *H.zea* the information from the second principal pheromone Z9-16:Al seems to be received via RNs tuned to the interspecific signal, Z9-14:Al. The specificity of this type of RNs in *H.assulta*, however, differs from that of the corresponding types of RNs in *H.virescens* and *H.zea* and can can be correlated to the ratio of the pheromone components produced by the females of *H.assulta* as compared to *H.zea*. In *H.assulta*, producing about 200 times more Z9-16:Al than *H.zea*, the RNs tuned to Z9-14:Al do not respond at any concentration to Z9-16:Al, whereas the corresponding RNs in *H.zea* and *H. virescens* respond second best to Z9-16:Al. It means that when the *H.assulta* males are flying in the pheromone plume, two types of RNs are strongly activated, the Z9-16:Al RNs and the Z11-16:Al RNs. If the RNs tuned to the interspecific signal Z9-14:Al had responded to Z9-16:Al (like in *H.zea)*, they might have become too strongly activated, resulting in interruption of the attraction. No RNs tuned to minor components were found in any of the Heliothine species investigated. It is suggested that recruitment of RNs with lower sensitivities tuned to the major component, might be important for close range behaviour rather than recruitment of RNs tuned to minor components.

Introduction

Pheromone reception in several species of Heliothine moths is investigated in our laboratory. Furthermore, in a collaboration with Division of Neurobiology, University of Arizona, Tucson, we

are studying how the pheromone information is processed in the antennal lobe of two Heliothione species (Christensen et al 1991, Christensen et al, unpublished). Beside elucidating the basic mechanisms for pheromone reception and CNS processing, such comparative studies also give information on how the processing of pheromone information has undergone changes through evolution, which may e.g. explain why the same compounds, acting as attractants in one species are blocking the attraction in a closely related species. I shall here focuse upon three species; the two sympatric, American species *Heliothis virescens* and *Helicoverpa zea*, and *Helicoverpa assulta*, living on other continents (Asia, Australia, Africa).

In all Heliothine species studied so far, 4-9 components are identified as pheromone constituents (cf. Arn et al 1992). However, two compounds seem to be nessesary and sufficient for eliciting the full sequence of male sexual behaviour or attraction to the pheromone source (Roelofs et al 1974, Tumlinson et al 1975, Klun et al 1980,1982, Teal et al 1981, Rothschild et al 1982, Vetter and Baker 1983,1984, Teal et al 1986, Dunkelblum and Kehat 1989, Kehat and Dunkelblum 1990, Cork et al 1991). We have named these two components, principal pheromones. The other, minor components, are in some species suggested to have a suttle effect at close range. In all Heliothine species, cis-11-hexadecenal (Z11-16:Al) is one of the principal pheromone components, and in all of them, except *H.assulta*, this compound is produced in largest amount (major pheromone). It gives the impression that Z11-16:Al is the original principal pheromone in the Heliothine moths. In *H.virescens*, cis-9-tetradecenal (Z9-14:Al) is the second principal pheromone, and the natural ratio between the two principal compounds Z11-16:Al : Z9-14:Al is about 16:1 (ng) (one female equivalent), whereas in *H.zea* the ratio between the principal pheromones Z11-16:Al: cis-9-hexadecenal (Z9-16:Al) is about ca 16: 0,1 (ng) (one female equivalent). *H.assulta* uses the same principal pheromones as *H.zea*, however with Z9-16:Al as the major component. The ratio here between the two principal components is about 15:1, i.e. there is about 200 times more Z9-16:Al in a *H.assulta* than in a *H.zea* female gland.

Labelled-lines

Most studies of pheromone receptor neurons (RNs) in insects have given the general impression that pheromone compounds are received by specialist types of RNs, i.e. that each RN is tuned to one compound (key compound) in the pheromone blend (cf Masson and Mustaparta 1990). However, at higher concentrations, the RNs may also respond to another pheromone compound. Therefore, in order to find out whether compounds with lower effect might have a biological function, we have compared the the RN responses to its key compound and to mixtures where the less effective compounds were added (Almaas and Mustaparta 1991). The dose-response curves obtained for the key compound and the mixtures overlapped completely, suggesting that no interaction of the compounds takes place on the RN. This means that, as long as the key compound is present (which is the case when a moth is flying in its pheromone plume), each RN will respond to its key compound as if it were present alone. This is what we mean with a labelled-line system. It is important to express this, since most pheromone receptor neurons respond at high concentrations to chemical analogues (often other pheromone components which are structurally similar).

Heliothis virescens: reception of principal pheromones and possible mechanisms for close range behaviour.

The reception of the principal pheromones (Z11-16:Al and Z9-14:Al) in *H.virescens* seems to follow the classical labelled-line system as defined above. In this species is identified two types of pheromone RNs in the males, both located in the male specific *sensilla trichodea* type 1 and in *s. trichodea type* 2 which is present in both sexes (Almaas and Mustaparta 1990a, 1990b). These two specialist types of neurons, tuned to Z11-16:Al and Z9-14:Al respectively, responded at high concentrations to other pheromone components. The RNs tuned to the major compound Z11-16:Al responded second best to Z9-14:Al and the RN tuned to Z9-14:Al responded second best to Z9-16:Al, both compounds being 100-1000 times less effective than the key compound.

No RN has as yet been found which responds specifically to the minor components in *H.virescens* (Almaas and Mustaparta 1990a,1990b, Berg and Mustaparta, unpublished). Furthermore, the minor components do not seem to influence the effect of the principal pheromones. For instance, in tests with minor components added to the major pheromone Z11-16:Al, the same responses of RNs were elicited as that obtained by the major pheromone alone on RNs tuned to the major phermone (Almaas and Mustaparta 1990). It is, therefore, an open question how the minor components are received in this species. In many other species it has also been hard to identify RNs tuned to minor components. This is in general ascribed to a presumed low number of such neurons, which seems to be the case in some species where less than 1% of identified pheromone RNs were tuned to minor components (Priesner 1979, Akers & O'Connel, 1991). The possibility can not be excluded that also minor components are important in *H.virescens*. However, in the species investigated by us, we have not been able to detect any RNs tuned to minor pheromone components. It may be questioned, therefore, whether RNs tuned to minor components are lost in lab reared insects, whether RNs for minor components are present in other sensilla than those we have recorded from, or whether there are individual differences (heterogenous populations) concerning the importance of minor components. For instance, one might ask whether the suttle effect of the minor components observed in wind tunnel experiments, are seen in all individuals or only in some individuals.

In any case, it may be that the minor components are of little importance for close range orientation which rather is dependent on increased concentration of Z11-16:Al. In *H.virescens* it has been found that the sensitivity particularly of the Z11-16:Al RNs varies, and that it roughly can be correlated with the lenght of the sensilla hairs (Almaas and Mustaparta 1991). At proximal segments and laterally on each segment, where the sensilla hairs are longer, the sensitivity appeared highest. Accordingly, the RNs of *s.trichodea* type 2 with short hairs, exhibited low sensitivity. It appears possible therefore, that when the males, flying in the pheromone plume, come closer to the female and the pheromone concentration gets higher, RNs with lower sensitivity become activated. Such recruitment of new Z11-16:Al RNs could thus be a mechanism for close range behaviour by which the male can determine the the distance to the female. In principal, this is a similar mechanism for modulation of close range behaviour as that suggested regarding the minor components. It seems possible, therefore, that the close range behaviour in the investigated Heliothines rather is modulated via the Z11-16:Al RNs with different sensitivities than by RNs tuned to the minor components.

Helicoverpa zea: the same RNs receive information from a principal pheromone and an interspecific signal

In spite of a different pheromone blend, *H.zea* has the same two groups of RNs as *H.virescens* for receiving information about its principal pheromones (Almaas et al 1991). Furthermore, by comparing the specificities (i.e. the relative effect of analogues), the two types of RNs, tuned to Z11-16:Al and Z9-14:Al respectively, are similar in the two sympatric species, suggesting that these receptors are conserved through evolution. The presence of RNs tuned to Z9-14:Al in *H.zea* is not surprising, since this compound interrupts the attraction of the *H.zea* males. The effect of such interspecific signals, activating separate RNs, is found in many species (Mustaparta et al 1977, Priesner 1979, Renou this vol). What was surprising, was that no RNs were found tuned to the second principal pheromone Z9-16:Al, which is nessesary for upwind flight and male sexual behaviour. However, as indicated below, we assume that the information from this compound is mediated via the RNs tuned to Z9-14:Al which respond second best to Z9-16:Al.

The information from the RNs is conveyed to neurons in the antennal lobe (AL) of the CNS. The AL contains two morphologically different classes of neurons, the local neurons (without axons) and the projection neurons with axons which convey the information from the AL to neurons in the protocerebrum (Christensen et al 1991). We have in particular studied the responses of the projection neurons in the AL of *H.zea* (Christensen et al 1991). Two major groups of neurons could be functionally classified; one responding to antennal stimulation with Z11-16:Al and the other to stimulation with Z9-14:Al. The responses of the neurons in both groups reflected the responses of the two RN groups, tuned to Z11-16:Al and Z9-14:Al, respectively. Furthermore, the Z9-14:Al neurons in the AL also responded to Z9-16:Al at higher concentrations and no projection neurons responded best to Z9-16:Al. The integrated results have formed the basis for the hypothesis that the information from the Z9-16:Al is received via the RNs tuned to Z9-14:Al. It would mean that the same RNs, when weakly activated by Z9-16:Al in the pheromone plume, elicit attraction. However, when strongly activated by Z9-14:Al in the plume of *H.virescens*, the same neurons would mediate interruption of the attraction. Hence, the two compounds may substitute each other depending on their relative consentration in the mixture with the major compound Z11-16:Al. This assumption lead to a wind tunnel study, which confirmed that indeed each of the two compounds could simulate the effect of the other at certain ratios in a mixture with the major component Z11-16:Al (Vickers et al 1991). Thus, it appears that in *H.zea*, the information from the two principal pheromones are received via a modified labelled-line system, where one group of RN respond to Z11-16:Al and the other group respond to Z9-16:Al when the males are flying in the pheromoen plume. However, the latter group of RNs, which is most sensitive to the interspecific signal, would be strongly acvtivated when the male comes into the plume of the sympatric species and would then mediate interruption of the attraction. It means that the labelling of one group of RNs is dependent upon which kind of plume the males are in contact with. This could be characterized as a modified labelled-line system.

In addition to the two groups of AL neurons, three neurons showed a strong synergistic response to the mixture of Z11-16:Al and Z9-14:Al (Christensen et al 1991). No neurons activated by the pheromone were inhibited by the interspecific signal. In this connection it is important to remember that the pheromone plume of the sympatric species is the mixture of the two compounds. It is, therefore, likely that these three neurons, either alone, or in addition to the Z9-14:Al neurons, mediate the important message that interrupts the attraction. The neuronal mechanism for the interruption of the attraction might thus take place at higher orders of neurons in the protocerebrum.

H.assulta: conservation and evolutionary changes of RNs

Since no RNs were found tuned to Z9-16:Al in *H.zea* or in *H.virescens*, it was of interest to study the pheromone reception in *H.assulta*, the only species that uses Z9-16:Al as a major pheromone. It was recently found that *H.assulta* possesses three types of RNs; two tuned to the principal pheromones Z9-16:Al and Z11-16:Al,respectively and the third to Z9-14:Al (Berg and Mustaparta, unpublished). The smallest group were the RNs tuned to Z11-16:Al. By comparing the specificities of these neurons with the corresponding types of neurons in *H.virescens*, it was found that these RNs were functionally similar to the Z11-16:Al neurons in the American species, indicating a conservation of their membrane receptors. The two other types of RNs were always recorded simultanously in the same sensillum and were found in equal numbers. Thus, a large number of RNs were here tuned to Z9-16:Al, showing that the pheromone information in this species is received via the classical "labelled-line" system.

The presence of specific RNs tuned to Z9-14:Al raised the question of whether Z9-14:Al is an interspecific signal in *H.assulta*. The behavioural effect of Z9-14:Al has recently been tested in greenhouse and in pepper field, in collaboration with Dr.K.Boo, National University of Seoul, Korea and Dr. D. Hall, National Resources Institute, Chatham, England. It was found that by adding Z9-14:Al to the four component pheromone blend, the attraction of *H.assulta* males was decreased (unpublished). The results indicate that Z9-14:Al is an interspecific signal which interrupts the pheromone attraction of *H.assulta* males.

The RNs tuned to Z9-14:Al showed different specificities than the corresponding neurons in *H.virescens* and *H.zea*, e.g. by showing no responses to Z9-16:Al at any concentration (Berg and Mustaparta, unpublished). Thus, the Z9-14:Al neurons in *H.assulta* are evolved differently than the corresponding RNs in *H.virescens* and *H.zea*, living in other continents. Of further interest is that the specificity of these neurons in *H.assulta* seem to be an adaptation to the ratio of the principal pheromone components produced. Thus, when *H.zea* males are flying in the conspecific pheromone plume, the Z11-16:Al neurons are strongly activated, whereas the neurons tuned to Z9-14:Al are weakly activated by Z9-16:Al. When the males of *H.assulta* is flying in its pheromone plume, two types of RNs (tuned to Z9-16:Al and Z11-16:Al, respectively) are both strongly activated. If the Z9-14:Al neurons in *H.assulta* had responded to Z9-16:Al, like in *H.zea*, they might have become too strongly activated by the high amount of Z9-16:Al produced by *H.assulta* females, which might have interrupted the attraction of the males. Thus, there seem to be an adjustment between the produced amount of the pheromone components and the specificities of the receptor neurons.

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References

AKERS, R.P., & O'CONNEL, R.J. 1988. response specificity of male olfactory receptor neurons for the major and minor components of a female pheromone blend. Phys. Entomol. **16**: 1-17

ALMAAS, T.J. & MUSTAPARTA, H. 1990. Pheromone reception in tobacco budworm moth, *Heliothis virescens*. J.Chem. Ecol. **4**: 1331-1347

ALMAAS, T.J. & MUSTAPARTA, H. 1990. *Heliothis virescens*: Response characteristics of receptor neurons in *sensilla trichodea* type 1 and type 2. J.Chem.Ecol. **5**:953-972

ALMAAS, T.J., CHRISTENSEN, T.A. & MUSTAPRTA, H. 1991. Chemical communication in heliothine moths. I. Antennal receptor neurons encode several features of intra- and interspecific odorants in the corn earworm moth *Helicoverpa zea*. J.Comp.Physiol. **169**: 249-258

ARN, H., TOTH, M & PRIESNER, E. 1992. List of sex pheromones and related attractants. International Organization for Biological control, West Palearctic Regional Section.

CHRISTENSEN, T.A., MUSTAPARTA, H., & HILDEBRAND, J.G. 1991. Chemical communication in heliothine moths. II Central processing of intra- and interspecific olfactory messages in the male corn earworm moth *Helicoverpa zea*. J. Comp. Physiol. **169**: 259-274

DUNKELBLUM, E & KEHAT, M. 1989. Female sex pheromone components of *Heliothis peltigera* (Lepidoptera: Noctuidae) Chemical identification from gland extracts and male response. J. Chem. Ecol. **15**:2234-2245

KEHAT, M. & DUNKELBLUM, E. 1990. Behavioural responses of male *Heliothis armigera* (Lepidoptera: Noctuidae) moths in a flight tunnel to combinations of components identified from female sex pheormone glands. J. Insect Behaviour **3**:75-83

KLUN, J.A., PLIMMER, J.R., BIERL-LEONARD T,B.A., SPARKS, A.N., PRIMIANI, M., CHAPMAN, O.L., LEE, G.H. & LEPONE, G. 1980a. Sex pheromone chemistry of female corn earworm moth, *Heliothis zea*. J.Chem.Ecol. **6**: 165-175

KLUN, J.A., BIERL-LEONARDT, B.A., PLIMMER, J.R. SPARKS, A.N., PRIMIANI, M., CHAPMAN, O.L., LEPONE, G. & LEE, G.H. 1980b. Sex pheromone chemistry of the female tobacco budworm moth *Heliothis virescnes*. J.Chem. Ecol. **6**: 177-183

MASSON, C. & MUSTAPARTA, H. 1990. Chemical information processing in the olfactory system of insects. Physiol. rev. **70**: 199-245

MUSTAPARTA, H., ANGST, M.E., & LANIER, G.N. 1977. Responses of single receptor cells in the pine engraver beetle, *Ips pini* (Say) (Coleoptera: Scolytidae) to its aggregation pheromone, ipsdienol, and its aggragation inhibitor, ipsenol. J. Comp. Physiol. **121**: 343-347

PRIESNER, E. 1979. Specificity studies on pheromone receptors of noctuid and tortricid lepidoptera. In: Chemical Ecology: Odour communication in animals (ed. Ritter, F.J.) Elsevier. North-Holland. pp. 57-71

ROELOFS, W.L., HILL, A.S., CARDÉ, R.T. & BAKER, T.C. 1974 Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. Life Sci. **14**: 1555-1562

ROTSCHILD, G.H.L., NESBITT, B.F., BEEVOR, P.S., CORK, A., HALL, D.R. & VICKERS R.A. 1982. Studies of the female sex pheormone of the native budworm, *Heliothis punctiger*. Ent. exp. & appl. **31**: 395-401

TUMLINSON, J.H., HENDRICKS, P.E., MITCHELL, E.R., DOOLITTLE, R.E. AND BRENNAN, M.M. 1975. Isolation, identification and synthesis of the sex pheromone of the tobacco budworm. J. Chem. Ecol. **1**: 203-214

TEAL, P.E.A., TUMLINSON, J.H., & HEATH, R.R. 1986. Vhemical and behavioural analyses of volatile sex pheromone components released by calling *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae). J. Chem. Ecol. **12**: 107-125

TEAL, P.E.A., HEATH, R.R., TUMLINSON, J.H. & McLAUGHLIN J.R. 1981. Identification of sex pheromone of *Heliothis subflexa* (GN.) (Lepidopera: Noctuidae) and field trapping studies using different blends of components. J.Chem. Ecol. **7**: 1011-1022

VETTER, R.S & BAKER, T.C. 1983. Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. J.Chem. Ecol. **9**: 747-759

VETTER, R.S. & BAKER, T.C. 1984. Behavioral reponses of male *Heliothis zea* moths in sustained-flight tunnel to combinations of 4 compounds identified from female sex pheormone gland. J. Chem. Ecol. **10**: 193-202

ANALYSIS OF PHEROMONE PLUME STRUCTURE IN THE FIELD USING SINGLE SENSILLUM RECORDING

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Abstract Efforts to improve the effectiveness of insect pest control techniques based on mating disruption require detailed knowledge of pheromone concentrations and dispersion under actual field conditions. Unfortunately, the concentrations of pheromones as emitted by the insects or given off by pheromone dispensers are too low to be measured by common physico-chemical detectors. The only way this could be achieved is to use the olfactory system of the insect itself as a detector offering both high sensitivity and selectivity of the specific pheromone components. Recently several devices based on the technique of electroantennography (EAG) have been developed and successfully applied in the field. These EAG devices allow measurement of ambient pheromone concentrations under various fired conditions. However, due to their limited time resolution they lack the possibility to record fast fluctuations in pheromone concentration.

In order to enable registration of the dynamic properties of pheromone plumes in the field a portable recording module was used consisting of a compact assembly 0f manipulators, electrode holders and signal conditioning electronic circuits. Continuous recording of single pheromone receptors is possible via glass micropipettes or tungsten electrodes from a wide variety of insect species. The momentary air velocity over the antenna is simultaneously recorded by means of a fast responding miniature thermistor sensor. During field operation the signals can be monitored by a loudspeaker or headphones and are stored on a portable cassette tape recorder for analysis in the laboratory. The recording module, measuring 25 x 20 25 cm, and the cassette recorder are powered by batteries; the whole system is truly portable.

Explorative recordings were made in apple orchards treated with pheromone dispensers for mating disruption using male antennae of *Aegeria* (*Synanthedon*) *myopaeformis*, *Adoxophyes orana* and *Pandemis heparana*. The recordings indicated that the firing frequency of the pheromone receptor cell was strongly correlated with the momentary air velocity indicating a homogeneous distribution of pheromone molecules in this situation.

Another series of experiments was carried out in an open field in which a small number of artificial pheromone sources were placed at various distances from the recording site. Recordings from these experiments show that the pheromone cell responses are not associated with the momentary air speed fluctuations indicating that the variations in pheromone flux over the antennae are due to distinct filaments of the pheromone plume.

The responses obtained from the field recordings from antennal sensilla of *A. orana* and *P. heparana* were subsequently compared to recordings from coupled gas chromatography and single sensillum recording in the laboratory. The latter technique enabled estimation of the average pheromone concentration in the field. Experiments to gain information about the ability of the antennal olfactory system to discriminate pheromone plumes from a homogeneous background concentration are currently in progress.

EVALUATION OF COMMUNICATION DISRUPTION IN THE PINK BOLLWORM IN FIELD WIND TUNNELS

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Abstract. Marked male pink bollworm moths (*Pectinophora gossypiella*) were released into the downwind end of walk-in field wind tunnels placed over two rows of cotton. The diel timing and percentage of capture of males in pheromone-baited traps were used as indicators of how Shin-Etsu rope and other formulations altered male attraction. An electroantennogram device was used to measure relative airborne concentrations of disruptant. Together these techniques provided insight into the mechanisms that effect disruption. Observations supported disruption of attraction being induced by several mechanisms: a camouflage of natural plumes; following of plumes from formulated pheromone; and an advancement of the diel rhythm of attraction.

Introduction

The use of formulated synthetic pheromone to interfere with mate finding and therefore effect population control has been realized for a number of moth pests, but the underlying mechanisms responsible for mating disruption remain speculative. Probable modes of action when the disruptant formulation releases a copy of the natural attractant include:

1. Sensory adaptation of peripheral receptors or habituation at a central neuronal level. These mechanisms could raise the threshold of responsiveness or eliminate responsiveness altogether.

2. Competition between calling (pheromone-releasing females) and point sources of synthetic pheromone. Males orienting to pheromone plumes emanating from point source formulations diminish the time available for locating a female. This has also been termed "**false trail following**."

3. Camouflage of the plumes from calling females. A background of pheromone generated by the formulation renders the plume from the calling female imperceptible at some distance downwind.

These mechanisms have been reviewed by Bartell (1982) and Cardé (1990). If the disruptant is an off ratio of pheromone components, an analogue, or the pheromone is combined with an insecticide, then other mechanisms may contribute to a reduction in mating (Cardé 1990). Here we consider the case of a pheromone used as a disruptant.

The actions of Mechanisms 1-3 may be synergistic. For example, a close encounter (Mechanism 2) with a high dose pheromone source may elevate a male's threshold (Mechanism 1), thereby causing a female's plume to be less discernible (Mechanism 3). Mechanisms may thus act in concert, with their proportionate contribution to disruption being contingent on the nature of the formulation, the behaviour of the particular pest species and its population density.

The nature of the formulation is a primary factor in the mechanism of disruption. A relatively even permeation of the atmosphere may be achieved by application of a sprayable, microdispersible formulation. But such matrices may not have point sources of pheromone of sufficient concentration to lure males and alter subsequent responsiveness. High-dose point sources, on the other hand, could administer substantial doses of pheromone, but the plumes of pheromone they generate may not extend equally throughout the plant canopy. Within these voids matings may occur. Such examples offer explanations for variability in the field of differing formulations applied at the same rate of active ingredient. Additional problems arise from various formulation types possessing differing characteristics in decay of release rates over time.

Other stipulations to understanding variability in field performance of any formulation are the pest's initial population level and its dispersal characteristics. Even given the overly simplistic presumption that the efficacy of mating disruption will not diminish as population density increases, it is obvious that populations exceeding a set starting level cannot be regulated by mating disruption, unless virtually all mating is prevented. As well, if the area to be managed is not sufficiently isolated from an influx of gravid females from the periphery of the treated crop, mating disruption will be ineffectual. These caveats suggest that establishing the mechanisms that set the efficacy of mating disruption in the field will be exceedingly complex, because they involve the interactions of the pest's population dynamics with the manipulation of normal patterns of mate finding by disruptant. Thus there are two avenues of understanding to explore:

1. What degree of disruption efficacy is required to manage a given population? This question leads to the necessity with most species of establishing a sophisticated monitoring program in which a threshold catch of males in pheromone-baited traps triggers the application of disruptants or possibly other control measures.

2. What are the mechanisms responsible for mating disruption? Answering this question could lead to improvements in strategies of formulation and application.

Establishing the principles of mating disruption under field conditions has not had a high priority. There are considerable difficulties in observing moth reactions in the field, especially given that the sexual behaviour of most moth species is nocturnal. Documenting the mate-finding patterns of moths responding to isolated, point sources of pheromone in the field nonetheless is feasible (e.g., Murlis et al., 1982, David et al., 1983, Charlton and Cardé, 1990, Willis et al., 1991). Parallel observations in disruptant-treated plots, however, are rendered almost intractable by the infrequency of encountering free-flying males and knowing the pattern of airborne disruptant that they are encountering. Indeed until recently (Sauer et al., 1992), the distribution and concentrations of disruptant in the field have been measured only for sample intervals of hours or longer (e.g., Flint et al., 1993).

Use of field wind tunnels

One approach to understanding the process of disruption is to use large wind tunnels in the field. This system was employed in Arizona in 1991 and 1992 with the pink bollworm moth (*Pectinophora gossypiella*) to discover some of the mechanisms involved is successful mating disruption. The female-emitted pheromone is an approximately 1:1 blend of (*Z,Z*)- and (*Z,E*)-7,11-hexadecadienyl acetates. (Hummel et al., 1973). The use of several formulations of pheromone for direct population control of this pest in the southwestern United States was reviewed by Baker et al. (1990) and there are similar reports from other cotton growing areas worldwide (e.g., McVeigh et al., 1990). However, despite some 15 years of commercial use of disruptant for control of this species, little is known of how the omnipresence of synthetic pheromone interferes with mate finding.

The use of field wind tunnels offered the potential of exploring how various formulations and deployment strategies manipulate male behaviour. The tunnels are 6.2 m long, 2.5 m wide and 1.85 m high. The frame of the tunnel is constructed of plastic tubing and its working section is covered with a translucent polyethylene plastic sheet. The ground on which the tunnel is set serves as the tunnel's floor. The tunnel is positioned over two rows of cotton. Air is pushed into the tunnel and a sheet of open-ended hexagonal cells at the upwind end of the tunnel ensures a laminar air flow. Air velocity is set at 0.7 to 0.8 m sec^{-1}. Various formulations are applied by hand onto the cotton in the tunnel or, in some experiments, also onto the cotton surrounding the tunnel. In the latter situation, air drawn through the tunnel would have the same concentration of disruptant as the **average** concentration of disruptant in the ambient air outside the tunnel. The tunnels can be readily moved on site or disassembled and set in new locations.

One method of using such tunnels is to release marked males (usually 150) at a tunnel's downwind end and monitor subsequent capture in two pheromone-baited traps positioned near the top of the cotton canopy at the end of each row of plants 5.5 m upwind. The levels of disruption are assessed by comparing the catch of males in pheromone-baited traps in an untreated tunnel vs. a tunnel containing a disruptant treatment. Marked males are released at dusk and their capture tallied hourly until dawn. For a given comparison of treatments a minimum of 3 nights of replication is performed.

The recapture of males in the check tunnel on the first night following release typically fluctuates between 60-75%. When high release rate rope formulation (Shin-Etsu) was applied in standard fashion to the base of three plants on one row of cotton, trap catch on the rope-side of the tunnel was suppressed 98%, and on the non-rope side 35% (Fig. 1B). This finding indicates that "local" suppression of trap catch is pronounced. The mechanisms responsible for this include false trail following, because video records of the activity of males on and near the ropes show that numerous males arrive within cm of the ropes and often contact the ropes for as long as several seconds. How such exposure alters the males' threshold and orientation is not yet known, but attraction of males to high dose lures was unexpected, given that they emit pheromone (Flint et al., 1985) at a level more than 1000-fold higher than females (Haynes et al., 1984).

Such quenching of attraction along a short row of cotton does not provide insight into the effects produced by application of rope formulation to large areas, which would not only produce locally high concentrations of pheromone near individual ropes, but also a background of disruptant generated by all ropes in the area. This effect was monitored in a third tunnel with an identical placement of three ropes imbedded within a 50 x 50 grid of ropes, also with the same inter rope spacing. Trap catch patterns showed that the background of pheromone from outside the grid was sufficient to disrupt attraction along both rows (Fig. 1D). Thus plumes from both traps would appear to be camouflaged by the overall background of disruptant in the plot.

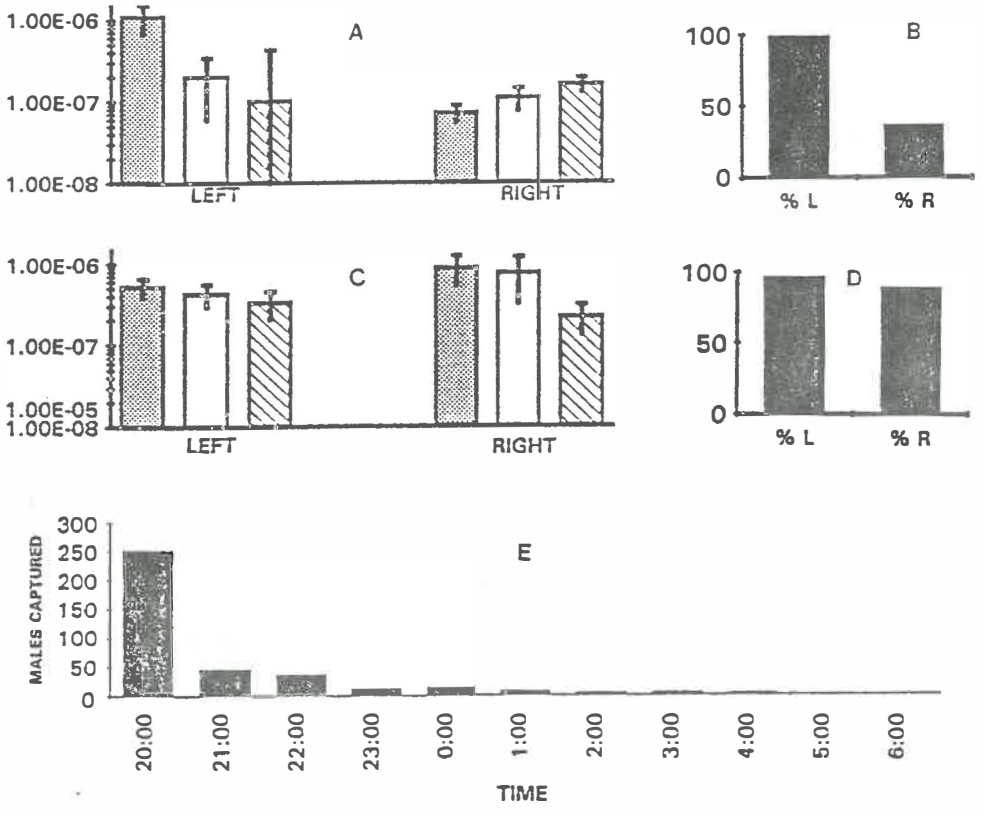

Fig. 1A-D. 1A and 1B provide values for a tunnel with 3 ropes on the left side and Fig. 1C and 1D show values for a tunnel also with 3 ropes on the left, but with this tunnel situated in the center of a 50 x 50 m grid of 360 ropes. 1A and 1C depict relative levels of airborne concentrations of pheromone (±SD) at heights of 32 (stippled), 100 and 172 cm (hatched) above ground level at the downwind end of the two rows of cotton on the right and left sides of a tunnel. Concentrations are given in relative units. The actual field concentration of (*Z,Z*)- and (*Z,E*)-7,11-hexadecadienyl acetates represented by 10^{-7} is on the order of 100 pg per m^3. 1B and 1D give the percentages of disruption of catch in left (L) and right (R) traps relative to the catch in the check wind tunnel. This experiment was replicated 3 times.

Fig. 1E. Hourly trap catch of laboratory-reared males released at 20:00 at the downwind of a field wind tunnel without disruptant formulation. Tests conducted on 4 nights in June 1992. A total of 600 males were released; of these 379 were captured in two pheromone traps at the upwind end within 8 hours. Sunset occurred close to 17:30.

An unexpected finding in the check tunnels and in the disruptant-treated tunnels was a pronounced shift in the periodicity of male attraction. Experiments in Arizona have shown that attraction to traps occurs within a relatively short time window (Lingren et al., 1989), mostly from about 3 or more hours after sunset. In our wind tunnel experiments, males were attracted to ropes within 10-15 seconds after their release 10-30 minutes after sunset (Fig. 1E). Such an advance in their diel rhythm of responsiveness could contribute to disruption by insuring that males experience any modification of their threshold before the normal time of female attraction. The extent to which a shift in rhythmicity occurs in native males in field plots remains to be verified, but this phenomenon appears to be a new mechanism contributing to mating disruption.

Use of field electroantennograms to estimate pheromone concentration

The camouflage mechanism is supported by measurements of the relative concentration of pheromone using a portable electroantennogram system, similar to the one described by Sauer et al. (1992). Comparisons of known standards with ambient pheromone at three heights inside both tunnels (Fig. 1A & 1C) show vertical stratification of pheromone on the rope-treated side of the tunnel, which is to be expected given that the ropes are placed near ground level. In contrast, another tunnel also with 3 ropes placed within a 2500 m^2 rope-treated plot shows little vertical stratification of pheromone.

Conclusions

Field wind tunnels provide new approaches for understanding mating disruption. Orientation of males can be observed under reasonably realistic conditions of formulation placement within the plant canopy. Measurements of ambient pheromone concentration in the field and within the wind tunnel by the electroantennogram indicate the patterns of formulated disruptant encountered by the male, and suggest the probable apparency of natural plumes. Combined these techniques offer the prospect of defining the principles of mating disruption and identifying effective formulation strategies.

References

BAKER, T.C., STATEN, R.T. & FLINT, H.M. 1990. Use of the pink bollworm pheromone in the southwestern United States. pp. 417-436 *In*, Behavior-modifying chemicals for insect management (R.L RIDGWAY, R.M. SILVERSTEIN, R.M. & M. INSCOE, eds.). Marcel Dekker, New York. 761 p.

BARTELL, R.J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. Physiol. Entomol. **7**: 353-364.

CARDÉ, R.T. 1990. Principles of mating disruption. pp. 45-71 *In*, Behavior-modifying chemicals for insect management (R.L. RIDGWAY, R.M. SILVERSTEIN, & M. INSCOE, eds.). Marcel Dekker, New York. 761 p.

CHARLTON, R.E. & CARDÉ, R.T. 1990. Orientation of male gypsy moths, *Lymantria dispar* (L.), to pheromone sources: the role of olfactory and visual cues. J. Insect Behav. **3**: 443-469.

DAVID, C.T., KENNEDY, J.S. & LUDLOW, A.R. 1983. Finding a pheromone source by gypsy moths released in the field. Nature **303**: 804-806.

FLINT, H.M., MERKLE, J.R. AND YAMAMOTO, J. 1985. Pink bollworm (Lepidoptera: Gelechiidae): field testing a new polyethylene tube dispenser for gossyplure. J. Econ. Entomol. **78**: 1431-1436.

FLINT, H.M., YAMAMOTO, A.K., PARKS, N.J. & NYOMURA, K. 1993. Aerial concentrations of gossyplure, the sex pheromone of the pink bollworm (Lepidoptera: Gelechiidae), within and above cotton fields treated with long-lasting dispensers. J. Econ. Entomol. **22**: 43-48.

HAYNES, K.F., GASTON, L.K., POPE, M.M. & BAKER, T.C. 1984. Potential for evolution of resistance to pheromone: interindividual and interpopulational variation in chemical communication system of the pink bollworm moth. J. Chem. Ecol. **10**: 1551-1565.

HUMMEL, H.E., GASTON, L.K., SHOREY, H.H., KAAE, R.S., BYRNE, K.J. & SILVERSTEIN, R.M. 1973. Clarification of the chemical status of the pink bollworm sex pheromone. Science. **181**: 873-875.

LINGREN, P.D., HENNEBERY, T.J. & POPHAM, T.W. 1989. Pink bollworm (Lepidoptera: Gelechiidae): nightly and seasonal activity patterns of male moths as measured in gossyplure-baited traps. J. Econ. Entomol. **82**:781-787.

McVEIGH, L.J., CAMPION, D.G. & CRITCHLEY, B.R. 1990. The use of pheromones for control of cotton bollworms and *Spodoptera* spp. in Africa and Asia. pp. 407-415 *In*, Behavior-modifying chemicals for insect management (R.L. RIDGWAY, R.L., R.M. SILVERSTEIN & M. INSCOE, eds.). Marcel Dekker, New York. 761 p.

MURLIS, J., BETTANY, B.W, KELLY, J. & MARTIN. L. 1982. The analysis of flight paths of male Egyptian cotton leafworm moths, *Spodoptera littoralis*, to a sex pheromone source in the field. Physiol. Entomol. 6: 71-86.

SAUER, A., KARG, G., KOCH, U.T., De KRAMER, J.J. & MILLI, R. 1992. A portable EAG system for measurement of pheromone concentrations in the field. Chem. Senses 17: 543-553.

WILLIS, M.A., MURLIS, J. & CARDÉ, R.T. 1991. Pheromone-mediated upwind flight of male gypsy moths, *Lymantria dispar*, in a forest. Physiol. Entomol. 16: 507-521.

SYSTEM FOR LABORATORY INVESTIGATION OF INSECT BEHAVIOUR

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Abstract A system has been set up to investigate the behaviour of small non-flying insects due to attractants and repellents. The package includes a tracking system, data acquisition and control software and a comprehensive data analysis software. The system has been extensively used for the study of *Oryzaephilus surinamensis*.

Introduction

It is now common practice to use pheromones and other chemicals to attract insects to a certain location. Insect behaviour is primarily dependent on the attractant chemical used, however the mode of delivery, delivery rate, condition of insect and type of environment will also play a major role in controlling insect behaviour. The paper describes a system recently developed at the University of Glamorgan in collaboration with Central Science Laboratory at Slough, to provide a means of undertaking detailed studies of insect behaviour with or without the influence chemical attractants. The system may also be used to study the effects of repellents.

A number of workers have reported the use of a video camera to track insects, for example McGeachie (1988), Witzgall (1990), Hoy (1984) and Keyserlingk (1982). The authors are however not aware of any previous automated tracking and delivery system.

The system currently developed can be used for the study of individual non-flying insects in the approximate size range 0.5 mm to 10 mm (long) in the presence of pheromone or an attractant delivered under computer control. Work is in progress to enable the study of (a) flying insects and (b) two or three insects concurrently.

The system is shown in Figs. 1 and 2, and is essentially an arena in which the insects may move, illuminated by low intensity red light and viewed by a video camera. The arena base is normally a white filter paper and the arena boundary is a painted metal ring approximately 10 mm high coated with a material which will not allow the insects to climb over the ring. A computerised micro-syringe delivery system enables precise amounts of chemicals in the liquid state to be delivered to the underside of the filter paper from where the material may vaporise. The currently used delivery rates are within the range 1 nls^{-1} to 800 nls^{-1} (3.6 μlh^{-1} to 2880 μlh^{-1}).

Design of Arena and Optical System

The arenas used to date have all been white filter papers supported by a stainless steel plate with arena boundary being a matt white painted metal ring. Fluon is painted on the inside of the ring to ensure that the insects remain within the arena. Three arena diameters are currently available, namely 100 mm, 160 mm and 215 mm. Attractant material is delivered at the arena centre, details of the system used will be discussed later.

The design of the optical system is central to the success of the whole system and uses a standard PC together with an image processing card supplied by Brian Reece Scientific Limited. The image processing card essentially allows a rectangle to be defined in the field of view and then determines the co-ordinates of the highest point, within that rectangle, of intensity greater than 50% of maximum intensity. Each rectangle may be defined and scanned in a time of 40 ms. Five rectangles are used to cover an arena, therefore minimum acquisition time is 200 ms.

The basic system is therefore ideal for the study of movement of bright objects on a dark background provided the illumination levels are correct. In order to allow tracking of dark insects on a white filter paper a video signal processor was designed and built to invert the video signal and to allow easy adjustment of the actual intensity at which the tracked object can be detected.

Correct arena illumination is essential to efficient operation of the system. The type of light source is very important since good resolution between the observed insect and the filter paper is essential. High sensitivity video cameras tend to be sensitive within the approximate wavelength range 450 nm to 900 nm, namely the visible and near infra-red. Careful observation with the naked eye will give an approximate guide to resolution in the range 500 nm to 650 nm, however a detailed study of insect resolution was undertaken for the whole wavelength range.

The parameter use to evaluate the resolution of the insect with respect of filter paper is the ratio $\beta = (r_i/r_f)_\lambda$ where r_i is insect reflectance, r_f is filter paper reflectance and λ denotes the wavelength.

Fig. 3 shows the variation of β with λ for *O. surinamensis*. Similar results were observed for a number of other beetles. The plot shows that wavelengths greater then 800 nm should be avoided. Filament lamps emit in the near infra-red as well as the visible, hence they are not used. The first system developed used a circular fluorescent tube however in order to facilitate overall design and to use red light (650 nm to 700 nm), which is preferred for studies of insect behaviour, an array of "ultra bright" red LED's is now used, see Fig. 1.

It is essential that the light reflected by the arena (including the metal ring) towards the camera is as uniform as possible in order that the difference between insect and background remains approximately constant across the field of view of the camera. This is easily achieved with the LED array and diffuser, except in the partial shadow of the boundary ring.

The light intensity leaving the shadow region is approximately 60% of the intensity leaving the central regions of the arena. This means that insects may be theoretically tracked if β is less than 0.6. Results of tracking experiments suggest that β should be greater than 0.4 for guaranteed trouble free tracking.

Insect size also plays a major role in successful tracking. especially in the case of a large arena. Investigations show that the system should be capable of tracking insects which are reasonably "black" in the wavelength region 650 nm to 700 nm, provided arena area divided by the horizontal area projected by the insect is not greater than approximately 7,000.

Delivery Unit

Delivery of precise amounts of liquid attractants is arranged by means of a computer controlled motorised micro-syringe, as shown in Fig. 1. A number of different methods were tested in order to vaporise the carob extract attractant which was used with *O. surinamensis*. The carob extract contains a large number of different chemicals of different volatilities hence systems were tested where the carob extract delivered by a micro-syringe was gently heated to assist vaporisation and then allowed to diffuse into the arena through a hole of diameter 10 mm in the filter paper. Such methods proved ineffective since, (a) it was effectively impossible to clean the delivery unit between individual runs, and (b) large amounts of vapour was absorbed onto the surfaces of the tube leading from the heated area to the actual arena.

The system currently used ensures that neither liquid or vapour from the unit escapes to the arena during any settling or pre-treatment periods, by ensuring that the tip of the micro-syringe is embedded in a septum until actual delivery is required. When delivery is required the needle is pushed through the septum until its (blunt) tip pushed gently against the underside of the filter paper. During actual delivery the material diffuses into the filter paper and then evaporates.

The delivery procedure used is as follows:

(i) With both linear drive motors in the down positions a recently filled gas tight micro-syringe is fixed to the support frame and a new septum located in the needle guide.

(ii) Motor B automatically lifts the micro-syringe so that the tip of the needle is inside the septum.

(iii) Insect is placed in area, allowed to settle for an agreed time and then tracking is started.

(iv) At the beginning of the delivery period the computer suspends tracking for a few seconds to allow motor B to lift the micro-syringe until the needle tip gently pushes against the underside of the filter paper.

(v) Delivery is undertaken by motor A.

It is appreciated that this system does not overcome the difficulty due to different volatilities of the carob extract components.

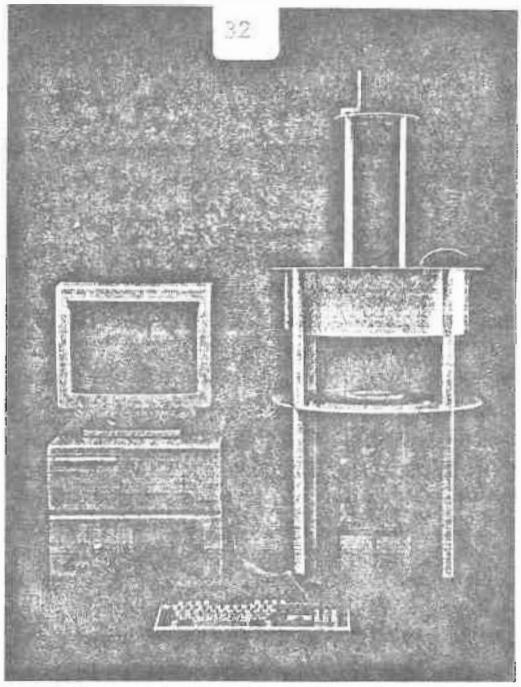

Fig. 1 Actual tracking system

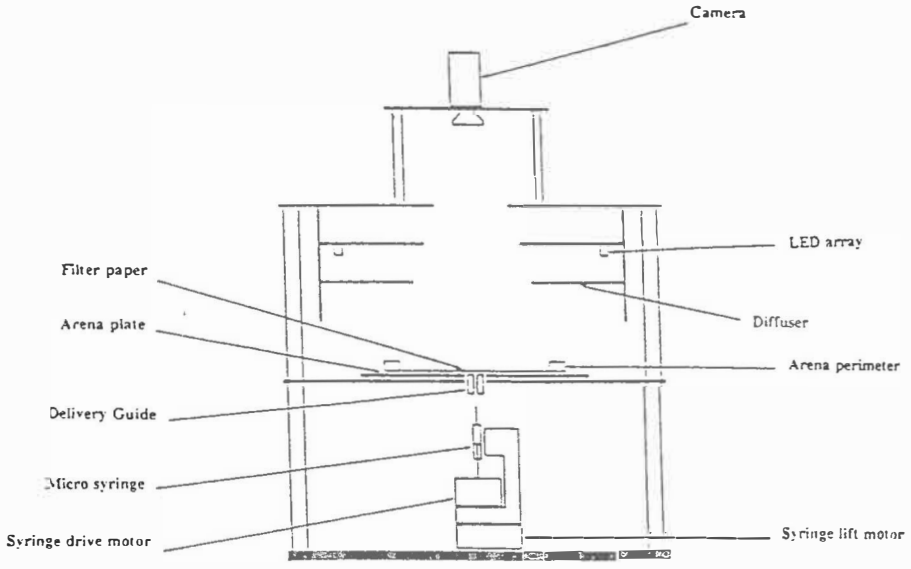

Fig. 2 Complete unit

Data Acquisition and Control Unit

This unit tracks the movement of the insects, controls delivery and provides the operator with user friendly management of tracking parameters and data files.

The position of the insect is determined, displayed and stored every 0.5 s, 1.0 s or 2.0 s as directed by operator. A 1.0 s sampling period was used in the case of the work on *O. surinamensis*. Some of the work undertaken on *O. surinamensis* involved the operator watching the insects and recording specific behaviour such as antennae response. Such behaviour can be stored as an event and is recorded by pressing the keyboard spacebar.

The delivery mode and rate(s) are set by the operator at the beginning of each run. Three modes are possible, namely (a) constant delivery, (b) manually operated delivery and (c) delivery equivalent to a previous run. In each mode delivery rates are possible within the range 1 nls^{-1} to 800 nls^{-1}. In the case of mode (b) the delivery rate may be changed at the keyboard every 10 s interval as the actual run progresses. The delivery details for the duration of the run are stored and then may be re-used for subsequent runs as mode (c). This means that the same complicated delivery routine, such as a number of pulses, may be easily applied to a large series of runs.

Each actual run can be precisely defined by such parameters as run duration, pre-treatment duration, sampling period, arena diameter, and whether or not delivery is required or the pre-treatment is to be stored. These parameters can be set for the first run of a series and then quickly accepted for subsequent runs. The following is an example of a possible mode of operation.

Time (sec)	Operation
0	Insect introduced and system initiated
0 → 600	Pre-treatment with track stored
600 → 1200	Post-treatment with track stored
600 → 610	Delivery of 250 nl of chemical
900 → 910	Delivery of 500 nl of chemical
1200	Data stored and run completed

Each run is identified by a six character series title followed by two digits identifying the run number. The run number is automatically updated on completion of each run. Additional information is stored for each such as a one line description of run or series, and date and time of start of series and last completed run. During tracking operation insect position will be shown on the computer monitor.

Data Analysis

A comprehensive software package has been developed to analyse the recorded data. This provides three main display modes which are as follows:

(a) Screen or printed plots of whole or part of insect track including the recorded events.

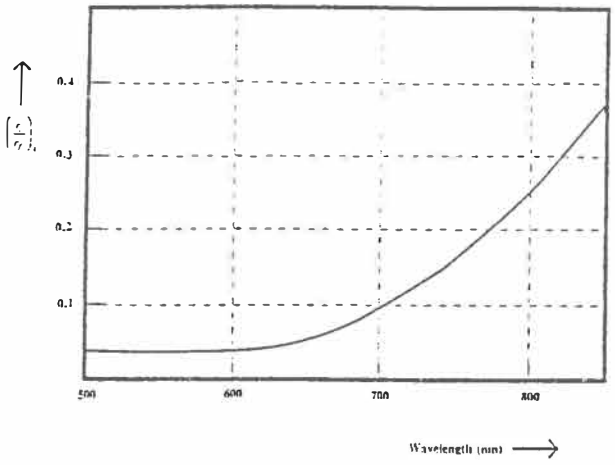

Fig. 3 Variation of insect filter paper resolution parameter $\left(\frac{r_i}{r_f}\right)_\lambda$ with wavelength for *O. Surinamensis*

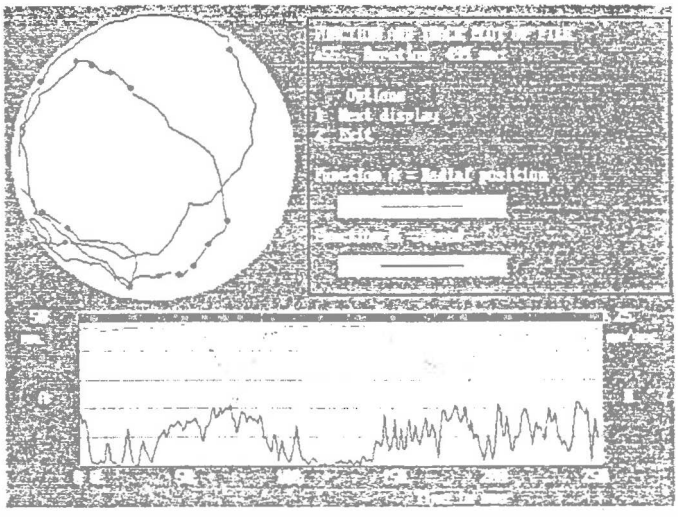

Fig. 4 Typical screen of analysis software

(b) Simulated real-time insect tracking at x2, x4 or x6 speed of stored track. In this case the insect is represented by a small white cursor which moves along its track followed by a tail which displays the insect track during the previous 10 s, 20 s, 40 s, or 60 s as defined by the operator. A recorded event is displayed as a red circle with the diameter of the circle being a function of duration of event.

(c) Screen and printed plots of any two of the following parameters (plus events) with respect to time:

(i) Radial position, defined as distance of insect from the centre.
(ii) Angular position, defined as the angle between the radial on which the insect is located and a reference radial.
(iii) Insect speed in mms^{-1}.
(iv) Direction of movement, defined as the angle between insect velocity direction and radial on which insect is located.
(v) Delivery rate.

Fig. 4 shows such screens and includes:

(i) Plot of actual track of insect.
(ii) Location of events on track.
(iii) Plot of radial positions and speed with time.
(iv) Timing of events (on the event bar).

References

MCGEACHIE, W.J. 1988. A remote sensing method for the estimation of light-trap efficiency. Bull Entomol Res **78** (3). 379-386.

WITZGALL, P. and ARN, H. 1990. Direct measurement of the flight behaviour of male moths to calling females and synthetic sex pheromones. Zeitschrift fur Naturforschung. Section C, Biosciences, **45** (9-10). 1067-1069.

HOY, J.B. and DAHLSTEN, D.L. 1984. Effects of malathion and Staley's bait on the behaviour and survival of parasitic Hymenoptera. ENVIRON. ENTOMOL.; **13**(6), pp. 1483-1486.

KEYSERLINGK, H. von. 1982. The response of Scolytus scolytus to volatile host plant stimuli. Proceedings of the 5th International Symposium on Insect-Plant Relationships, Wageningen, the Netherlands, [edited by Viser, J.H. and Minks, A.K.].

INHIBITION OF THE SEX PHEROMONE PERCEPTION IN THE PROCESSIONARY MOTH *Thaumetopoea pityocampa*

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ABSTRACT

Several aliphatic and aromatic trifluoromethyl ketones **I-X** have been synthesized and evaluated in the laboratory and in the field as inhibitors of the pheromone response of the processionary moth *Thaumetopoea pityocampa* males. Among them **IX** and **X** are closely-related analogs of the natural pheromone (*Z*)-13-hexadecen-11-ynyl acetate (**XII**). In the laboratory compounds **I, II, V** and **XI** displayed notable blockage of the pheromone perception on EAG. The compounds, however, exhibited only a modest or null EAG intrinsic activity. In the field, trifluoromethyl ketones **III, IV, TFAF, IX, X** showed a remarkable disruptant effect when mixed with the pheromone in 1:0.1, 1:1 and 1:10 ratios. Compound **XI** has been found to be a modest agonist of the natural pheromone, being the attractant activity threefold lower than the parent molecule **XII**.

INTRODUCTION

Fluorinated ketones have received in recent years an special interest because of their utilization as starting materials in the preparation of more complex biologically-active fluorinated molecules as well as enzyme inhibitors (Begué and Bonnet-Delpon, 1991). Their particular features arise from the unique physical and biological properties induced by fluorine, which closely mimics the steric requirement of hydrogen at the enzyme receptor sites. In this context, trifluoromethyl ketones (TFMKs) have been tested as inhibitors of the antennal esterases in insects. Thus, Prestwich and Streinz (1988) evaluated the activity of 1,1,1-trifluoro-(*Z*)-14-nonadecen-2-one, a putative mimic of (*Z*)-11-hexadecenyl acetate, one major component of the sex pheromone of *Plutella xylostella*. The compound showed only a weak EAG activity and a modest esterase inhibition activity in contrast to the potent inhibitory effect displayed by 1,1,1-trifluorotetradecan-2-one on the sensillar esterase of *Antheraea polyphemus* (Vogt et al., 1985).

Continuing our efforts on the development of inhibitors of the pheromone perception process of the processionary moth *Thaumetopea pityocampa* (Camps et al., 1990), we have prepared and tested a variety of several TFMKs **I-X**, two of them (**IX, X**) being closely related analogs of (*Z*)-13-hexadecen-11-ynyl acetate (**XII**), the only pheromonal component found so far in the sex pheromone gland (Guerrero et al., 1981). In both cases, the enyne function and stereochemistry have been preserved, whereas the acetate group has been replaced by the isosteric trifluoropanone moiety, thus mimicking the putative enzyme-bound intermediate in the pheromone catabolic process. In our study we have also included methyl ketone **XI**, which formally results from the isosteric replacement of the oxygen atom of the pheromone acetate function by a methylene group.

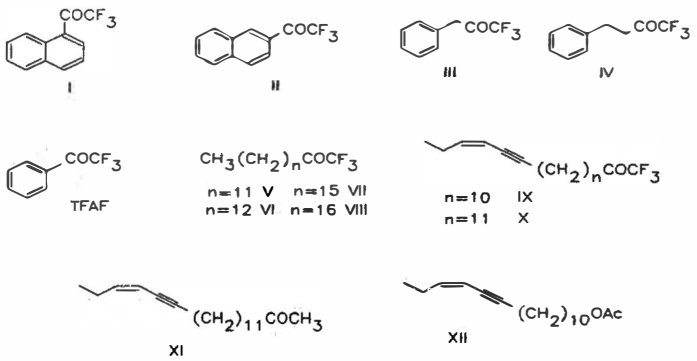

Figure 1

MATERIALS AND METHODS

Laboratory bioassays.

Wild pupae were collected in the field, sexed and sent to our laboratory, where they were placed in plastic boxes of 30x27x10 cm. The insects were covered with a 3-5 cm layer of sawdust and kept at 20-22°C and 70±10% humidity in a 16:8 light:dark regime until emergence. The inhibition assays were carried out by placing 1-day old individual insects in 12 cm diameter Petri dishes, which contained a small squared-shape piece of filter paper (2x2 cm) on which several amounts of the test compounds had been absorbed (10, 100, 500 and 1000 μg). The moths were exposed to vapors of the compounds for 4 h in the dark, taken out and their antennae excised. Between 8 and 12 moths were tested for each treatment. The electroantennogram responses were recorded at 40 seconds intervals and the values normalized to compensate the time-dependent decay of antennal responses. For every concentration of inhibitor between 6 and 12 insects were used, and the inhibition values were calculated as percent of the relative decrease of the EAG average response of treated moths in relation to the mean value displayed by control insects. The results were analysed statistically for significance according to the LSD test.

Field tests.

The trials were held in Mora de Rubielos (Teruel) from 1989 to 1992. The baits were prepared by dissolving the specified amounts of the test compounds and the pheromone in nanograde hexane (1 ml), mixed with paraffin wax (2.5 mg) and transferred to closed polyethylene vials (3x1.1 cm ID). The vials were used as dispensers and the traps contained no glue or insecticide. They were hung on pine branches 1.7-2.0 m high and separated a minimum of 50 m apart. Between parcels the minimum distance was 150 m. The traps were set out in statistically randomized blocks and revised and rotated every day. The inhibition activity of the compounds was measured by the relative trap catch decrease of the specified blend in comparison with the natural pheromone **XII**. Five traps were utilized for each formulation and the data subjected to analysis of variance by LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

In our effort to develop inhibitors of the pheromone perception process, we have prepared and tested a number of structurally-related analogs of the sex pheromone of the processionary moth, through modifications of the three putative key active sites of the parent molecule **XII**, with remarkable inhibition activity (Camps et al., 1988). We now report new inhibition studies with a variety of aliphatic and aromatic TFMKs structurally related or not with the structure of the natural pheromone **XII**. TFMKs have been proven to inhibit the action of a variety of serine esterases, juvenile hormone esterase or mammalian carboxyl esterases. In our case, the inhibition tests were carried out by evaluation of the relative average pheromone response of moths, which have been kept in contact with several doses of the chemicals, in comparison with that of untreated males. The doses used were generally in the 10 to 1000 μg range, although for the most active compounds **V** and **XI** lower doses (0.1 and 1 μg) were also tested. While naphthyl TFMKs **I**, **II** behaved similarly at 10, 100 and 500 μg doses, the latter exhibited a much better inhibitory effect at 1000 μg (>95%) than the former (60%). This result parallels the good affinity for sensillar esterase shown by 2-naphthyl acetate compared with the more sterically hindered 1-naphthyl derivative (Prestwich, 1987). Ketones **III** and **IV** showed a much lower inhibition effect than the naphthalene-derived compounds **I** and **II**. High concentrations were needed to obtain significantly different results from control ($P<0.05$, LSD test), being specially remarkable the inhibition obtained with 1000 μg of **IV** (>97%). When the aliphatic TFMKs **V-XI** and **TFAF** were tested (compounds **VI** and **VII** not assayed), only compounds **V** and **XI** showed a notable blockage of the pheromone perception at relatively low concentrations. Thus, whereas **V** displayed significantly inhibitory action at 100 μg (60% inhibition), **XI** showed a low (20%) but statistically differentiated effect at only 1 μg. As expected, both compounds exhibited increasing inhibitory potency at higher concentrations (Figure 2). Enyne **XI**, which formally results from isosteric replacement of the alcohol oxygen of the natural structure **XII** by a methylene group, has been the best inhibitor found in the laboratory bioassays. The close structural and stereoelectronic similarity with the pheromone may account for this result. When we tested the EAG intrinsic activity of the chemicals, only compounds **IX**, **X**, and **XI**, i.e. those having the most closely-related structures to the natural pheromone, displayed some activity (ca. 20%) in comparison with **XII**. This reflects once more that strict molecular and stereoelectronic requirements are needed for a successful recognition of the incoming signal by the antennal receptors.

Field tests.

As noted above, formulations based on blends of the synthetic chemicals with **XII** in different ratios (1:0, 1:0.1, 1:1, 1:10 and 0:1) were tested. The inhibition effect was assessed by the relative decrease in catches shown by an specific formulation in comparison with the pheromone alone. Among the aromatic ketones assayed, **TFAF** and compound **III** displayed potent inhibitory effects when mixed with the pheromone in 1:1 and 10:1 ratios (63-96% inhibition, Figure 3). By contrast, the less volatile TFMKs **I**, **II**, and **IV** showed only a moderate disruptant effect (35-42%) when mixed with the pheromone in equivalent amounts. Higher or lower proportions of the chemicals with respect to **XII** did not imply any significant inhibitory action. Although the order of activity displayed **TFAF** > **III** > **IV** correlates well with the relative volatilities of the compounds involved, other factors (bulkiness, stereoelectronic, etc.) related to the association-dissociation process with the receptors may also be involved. The less volatile compounds **I** and **II** were practically inactive, except compound **II** in a 1:1 mixture with the pheromone, which displayed a moderate effect (47%). While aliphatic non-functionalized TFMKs **V-VII** did not exhibit any significant disruptant activity, those containing the enyne group (compounds **X** and **XI**) presented

a good inhibitory action, particularly **X**, the most closely structural analog of the pheromone. The inhibition displayed by the latter increased with the concentration in the baits but the compound possessed no attractant activity, as the other TFMKs tested, when used alone. It is interesting to note that compound **IX**, the homolog with one-carbon less in the aliphatic chain, showed a remarkable synergistic effect, increasing the number of catches by 56% when mixed with the pheromone in a 0.1:1 ratio. Although it is still unknown whether the inhibitors and synergists interact with the same receptor sites than the pheromone specific cells, our results confirm in any case that the presence of the enyne function in the molecular structure of the analog is required for achieving biological activity (Camps et al., 1988).

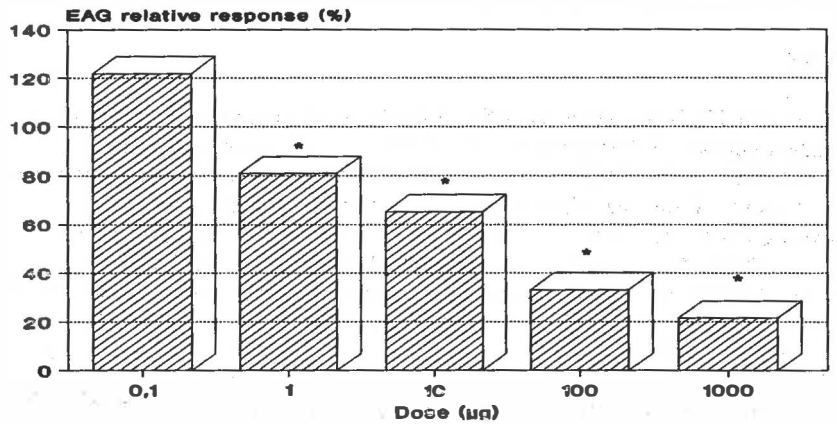

Figure 2. EAG relative response induced by different doses of compound **XI**. Bars represent the mean values of 6-12 experiments. Responses with an asterisk (*) are significantly different from control at $P < 0.05$ (LSD test).

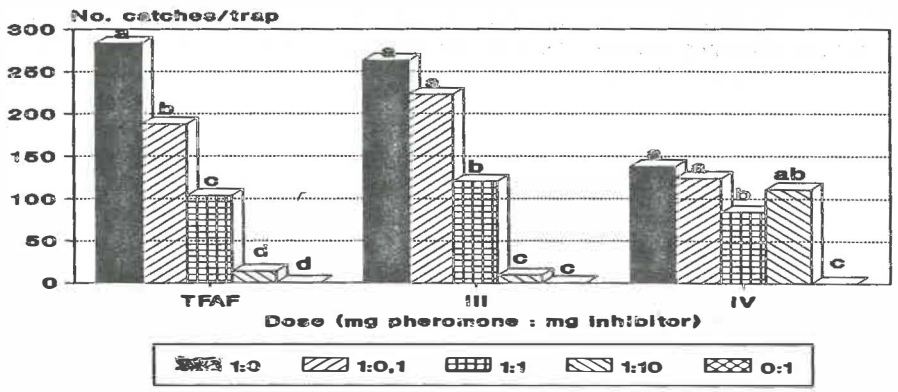

Figure 3. Catches per trap of *Thaumetopea pityocampa* males obtained with blends of **TFAF** and compounds **III** and **IV** in several ratios with the natural pheromone. Five replicates per trap were used. Statistically significant values of inhibition are displayed by different letters ($P < 0.05$, LSD test).

When methyl ketone XI was tested, the compound behaved as a modest agonist of the pheromone action, since traps baited with the compound alone caught 30.8 males per trap, i.e. 29% of the attractant activity of the natural pheromone. The compound was not a synergist, however, when mixed with **XII** in the usual ratios.

ACKNOWLEDGEMENTS

We gratefully acknowledge CICYT (PB90-0089 and AGF 92-0178) for financial support and MEC for a predoctoral fellowship to A.P. We are also indebted to R. Hernández (ICONA) for providing the insects and for conducting the field trials.

REFERENCES

BEGUÉ, J.P. & BONNET-DELPON, D. 1991. Preparation of trifluoromethyl ketones and related fluorinated ketones. Tetrahedron **47**:3207-3258, and references cited therein.

CAMPS, F., GASOL, V. & GUERRERO, A. 1990. Inhibitory pheromonal activity promoted by sulfur analogs of the sex pheromone of the female processionary moth *Thaumetopoea pityocampa* (Denis & Schiff.). J. Chem. Ecol. **16**:1155-1172, and treferences cited herein.

CAMPS, F., FABRIÀS, G., GASOL, V., GUERRERO, A., HERNÁNDEZ, R. & MONTOYA, R. 1988. Analogs of sex pheromone of processionary moth *Thaumetopoea pityocampa*: Synthesis and biological activity. J. Chem. Ecol. **14**:1331-1346.

GUERRERO, A., CAMPS, F., COLL, J., RIBA, M., EINHORN, J., DESCOINS, CH. & LALLEMAND, J.Y. 1981. Identification of a potential sex pheromone of the processionary moth *Thaumetopoea pityocampa*. Tetrahedron Lett. **22**:2013-2016.

PRESTWICH, G.D. 1987. Chemical studies of pheromone reception and catabolism, pp. 473-527 *in* G.D. Prestwich and G.J. Blomquist (eds.). Pheromone Biochemistry. Academic Press, New York.

PRESTWICH, G.D. & STREINZ, L. 1988. Haloacetate analogs of pheromones. Effects on Catabolism and Electrophysiology in *Plutella xylostella*. J. Chem. Ecol. **14**:1003-1021.

VOGT, R.G., RIDDIFORD, L.M. & PRESTWICH, G.D. 1985. Kinetic properties of a pheromone degrading enzyme: The sensillar esterase of *Antheraea polyphemus*. Proc. Natl. Acad. Sci. USA **82**:8827-8831.

MONITORING APPLICATIONS

DEVELOPMENT OF AN EXTENSIVE PHEROMONE TRAP MONITORING SYSTEM FOR FOREST PESTS

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Abstract An extensive sex pheromone trap monitoring program for the spruce budworm (*Choristoneura fumiferana*) has been in operation in eastern North America since 1986. The objective is to provide early warning of outbreaks so that harvesting plans can be modified to remove threatened stands. Interpretation of the data has been complicated by the capture of non-target species, by contamination of the traps with pheromone from previous use, and by variation in potency of lures from different years. Only the last of these, lure-potency, is a serious problem. It has been resolved by the annual calibration of trap catches against larval populations in selected areas, by calibrating successive batches of lures against the previous batch, and by securing a supply of high quality pheromone sufficient to last for many years. After calibration, the data are being analysed using geostatistical analytical techniques. Contour maps are being developed using variograms to weight the data. GIS-based overlays of successive spatial patterns will detect trends over time, and overlays on forest cover-type maps will optimise trap deployment.

Introduction

In contrast to agricultural pests, which are often chronic, persistent problems, forest pests tend to be cyclical. For many years they are absent, which usually means they are present but at low densities. Periodically they 'erupt' to cause widespread damage, before collapsing back to low 'endemic' levels. Traditionally the reaction of forest managers has been to wait until trees are about to die and then to spray the forests with insecticide to kill enough insects to keep the trees alive. More recently, such 'crisis management' is being replaced by a more far-reaching policy of 'pest management' in which the problem is dealt with before it reaches crisis proportions. For this to work, however, prediction of when and where outbreaks will occur is essential, and for this purpose, sex pheromone trapping programs show considerable potential. In western Canada an operational program has been initiated for monitoring the onset of outbreaks of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Shepherd et al. 1985). In eastern Canada efforts have focussed on the spruce budworm *Choristoneura fumiferana* (Clem.), and it is hoped that once an operational program exists for this species the program can be extended to other important pest species. The development of this sex pheromone trapping program was reviewed in 1987 (Sanders 1990). This report is an update on the program since then, detailing some of the problems encountered on the way, which may help others to avoid the same pitfalls.

The Problem

The spruce budworm, a tortricid moth with a wingspan of about 2 cm, is the major economic pest of the boreal, pulpwood forest of eastern North America. Outbreaks last 5 to 10 yr and occur somewhat irregularly, but there is evidence of a 35 to 40 yr cycle (Royama 1984). The control strategy over the past 40 yr has been to apply chemical or biological (Bt) insecticides to kill the feeding larvae and to protect the foliage. Such treatments are never extensive enough to influence the course of an outbreak, and treatments may have to be repeated for several years to keep the trees alive while the outbreak runs its course.

Over the past decade there has been a move away from the strategy of using chemicals to keep trees alive to one of 'living with the budworm' and avoiding serious losses by scheduling harvesting to remove threatened trees before the budworm kills them. Harvesting in the boreal forest requires long-term planning, and involves major investments of money in building roads and bridges. A key to successful budworm management is therefore advanced warning of when and where the next outbreak will occur. Conventional sampling systems are not sensitive enough to detect the first signs of an impending outbreak, but pheromone traps are. Because spruce budworm outbeaks occur on such long cycles it is difficult to fully assess the potential of the traps, but the available data from one location where traps have been deployed annually for 25 years is very encouraging (Fig. 1).

In 1986 a standardised trapping program was implemented across the range of the spruce budworm in North America to monitor long-term population trends. This involves 9 of the 10 Canadian Provinces and 6 of the States of the USA. Multi-pher traps are deployed by federal, state, provincial and industrial employees following standardised procedures. During the first 2 years of the program a number of locations recorded no moths at all. This was attributed to the low potency of the polyvinyl chloride baits used at that time. Zero counts are of course meaningless for trend analyses, so the potency of the lures was increased and at the same time the program switched to the use of commercial Biolures produced by Consep Membranes Inc.

Since that time the program has run smoothly and we are now starting analyses to interpret and present the results. However, before discussing this, we will review some of the problems, both potential and real that have occurred, and that might occur with the implementation of other trapping programs.

Contamination

When adsorbed onto other materials, pheromones can be remarkably persistent. We were therefore concerned that re-usable plastic traps might become sufficiently contaminated with pheromone to affect catches in subsequent years. We found that unbaited Multi-pher traps which had contained pheromone baits the previous year did catch significant numbers of moths. Moreover, this attraction persisted even after the traps were washed with detergent, rinsed in chlorine solution or exposed to sunlight for a week (Fig. 2). The good news is that contamination did not result in increased catches in baited traps. Therefore, in the case of the spruce budworm, trap contamination is not an operational problem. However, it might be with other species, and clearly this is a potential problem that users should be aware of. Also it is possible that contamination with the pheromone of one species might affect catches of another species if the traps are subsequently used for different species. It is therefore a good policy to assign individual traps to only one species.

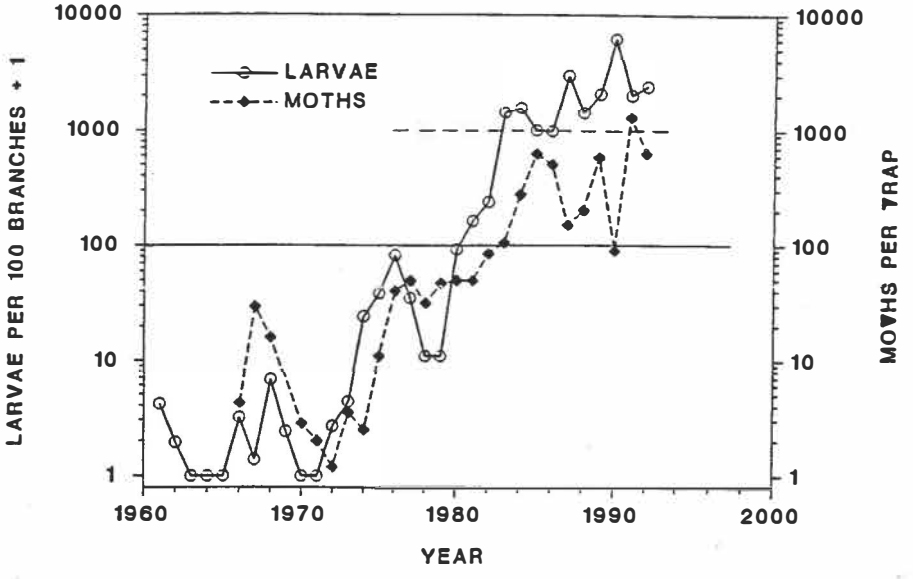

Figure 1. Relationship between larval populations of the spruce budworm and moth catch in pheromone traps, northwestern Ontario, Canada.

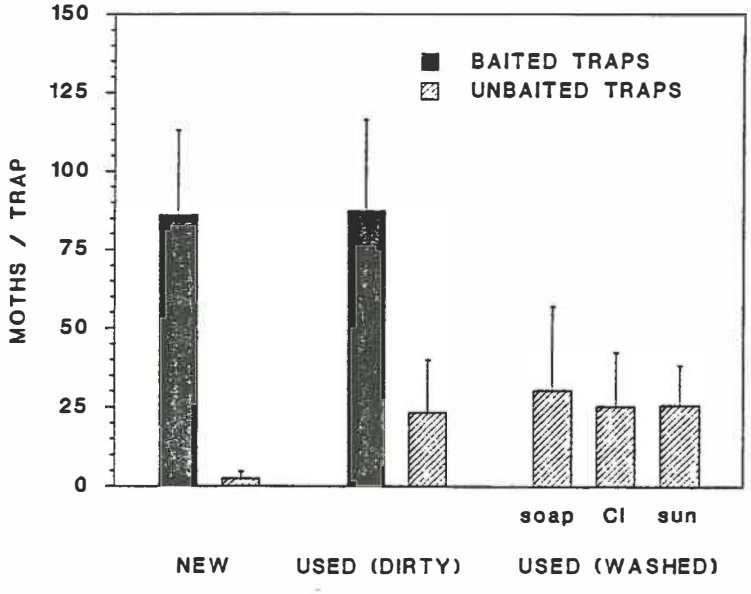

Figure 2. Spruce budworm moth catches in previously baited Multi-pher traps compared to catches in new, unused traps and traps that had been washed with detergent, rinsed with chlorine bleach (Cl), or left out in sunlight for 1 week (sun), showing the effect of contamination by residual pheromone.

Identification of non-target species

In one location in 1988 and 1989 Multi-pher traps baited with spruce budworm lures captured very large numbers of the large aspen tortrix, *Choristoneura conflictana* Walker, a close relative of the spruce budworm, which can easily be confused with the spruce budworm. Further field tests showed that these moths were not attracted by the pheromone, but were attracted to the traps themselves, or that they blundered into the traps. In fact they were actually 'repelled' to a certain extent by the spruce budworm pheromone. With practice the two species can be distinguished, but the episode did raise the question of whether there are other species which could cause confusion. Therefore, in 1990, traps were deployed across the whole of Ontario throughout the entire field season. More than 90 species of Lepidoptera were captured, but only 9 occurred in sufficient numbers to suggest thast they were attracted by the pheromone. Of these only 2 species of *Acleris* showed any similarity to spruce budworm, and they occurred some 4 to 5 weeks before the budworm flight period. Apart from the large aspen tortrix, there are therefore no other species which might occur in sufficient numbers in the traps to bias the counts.

Lure potency

The most serious problem to plague the implementation of the spruce budworm trapping program has been inconsistencies in the potency of different batches of lures produced each year. This is evident from both calibration against other measures of population density and from comparisons among catches with different batches at the same time and place. Each year estimates of larval populations have been carried out in some of the trapping locations. Correlations between trap catch and larval densities have varied widely with the different batches of lures. In the first 2 years that the Biolures were used, 1989 and 1990, regressions between trap catch and larval densities were very similar, suggesting that in the 2 years at least, the lures were similar in potency. This was confirmed by contemporaneous trapping with the 2 batches in 1990 (Fig. 3). However, in subsequent years the relationships varied widely (Fig. 4). The cause of the problem has not been fully resolved. The pheromone used in 1990 and 1991 came from different suppliers and both lots had considerable impurities. In addition release rates varied significantly among the different batches, although the manufacturer claims the specifications were identical. Possibly, the impure batches had undergone significant trimerisation of the aldehyde. The problem of how to interpret the catches was solved by using the contemporaneous catches to calibrate the catches each year, using the 1992 catches as the standard. When this was done, annual differences between the regressions of trap catches and larval counts became insignificant (Fig. 5). Two lessons have been learned from this. First, the quality of the pheromone must be consistent from year to year. To solve this we have made a bulk purchase of pheromone, sufficient to last 10 or more years. Second, calibration between batches of lures must be carried out each year. The problems we have encountered with the spruce budworm may be worse than will occur with other species (aldehyde pheromones are notoriously unstable), but our experience should act as a warning for others to take appropriate precautions to ensure that results are comparable from year to year.

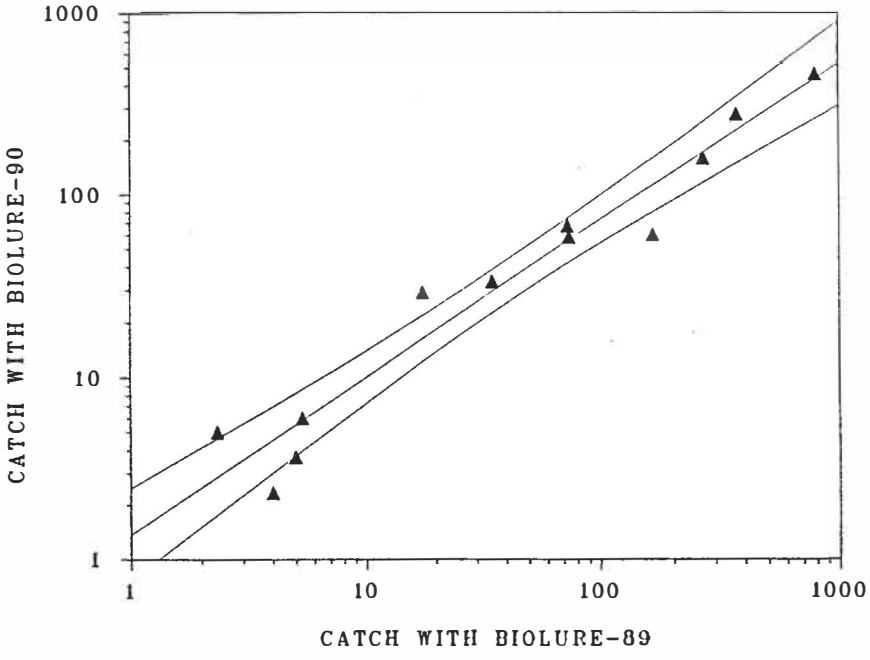

Figure 3. Relationship between catches of spruce budworm moths in traps baited with different batches of Biolures, manufactured in 1989 and 1990 respectively.

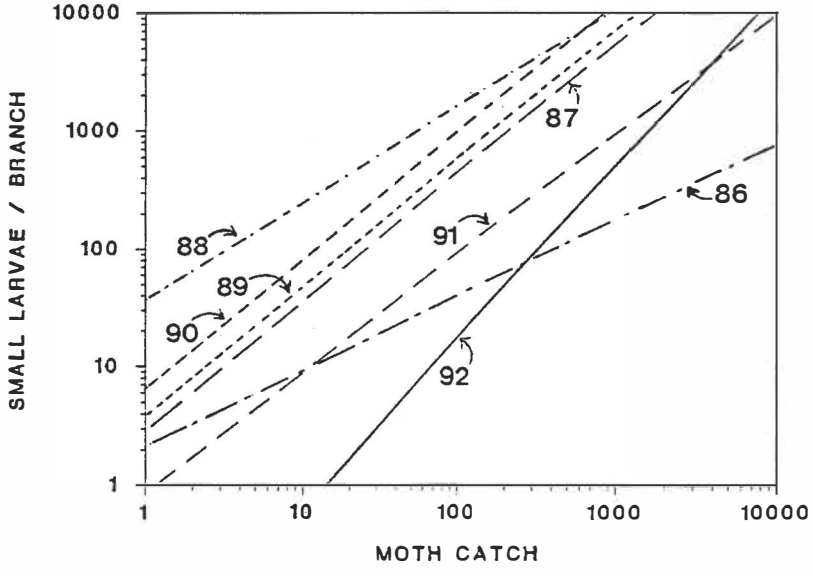

Figure 4. Relationships between larval population densities and catches of moths in pheromone traps over a 7 year period, 1986 to 1992.

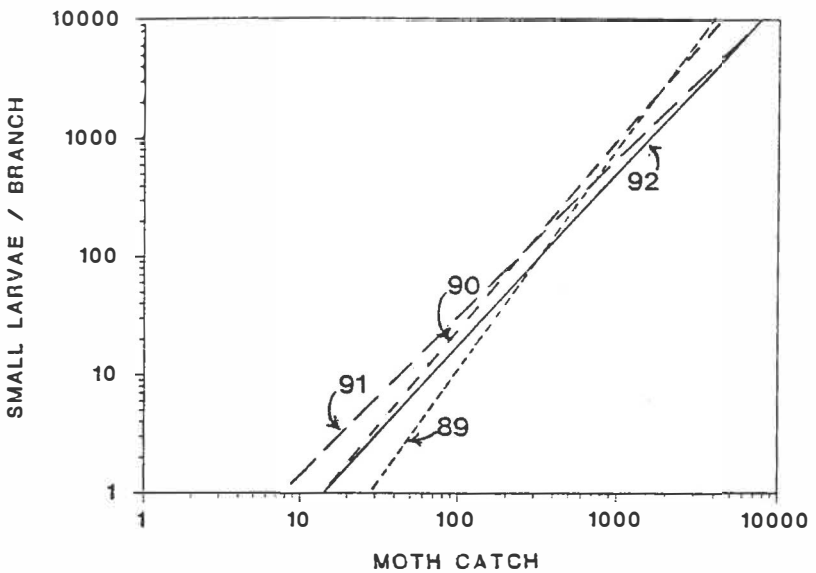

Figure 5. Relationships between larval populations and catches in pheromone traps corrected for annual differences in lure potency.

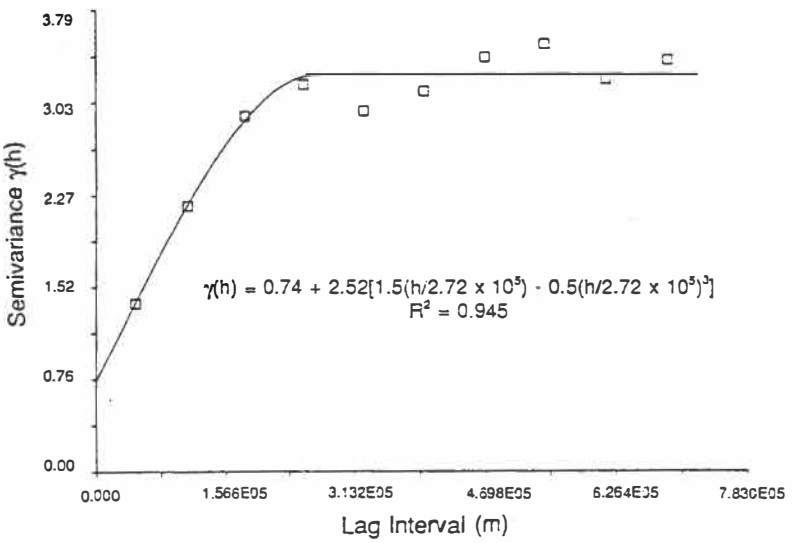

Figure 6. Variogram showing relationship of variance between adjacent traps (Y-axis) to distance between adjacent traps (lag interval, X-axis).

Analysis and interpretation of results

Now that we have been able to calibrate catches from previous years to make them comparable, the analysis of the results has begun. A major goal is to provide annual maps of budworm density, both for their visual impact, but more importantly for spatial analyses to determine where changes in density are occurring (Lyons and Sanders 1993). The potential application of geostatistics and geographical information systems (GIS) to the spatial analysis of insect population dynamics has recently been reviewed by Liebhold et al. (1993).

Analysis by GIS requires contour maps which smooth out variations in trap catch and fill in the blank areas between the sample points. Appropriate geostatistical software is now available to do this. These techniques estimate densities between the trapping locations based on the actual catches at the sample points and on how far they are away from the point where the densities are being estimated; the closer they are the more influence they have. The first step in this procedure is to plot the variance between adjacent sample points as a function of the distance between them. The result is a 'variogram' as shown in Fig. 6. The example in Fig. 6 is derived from an area measuring about 1350 x 540 km covering southern Québec, including the Gaspé peninsular. The point where the curve flattens (i.e. where the variance stabilises) is at a distance of about 270 km. This implies that at distances of more than 270km the sample points, and hence the populations, are independent. Trap-catch is then estimated throughout the area by using the variogram to weight the catches of adjacent sampling points.

GIS software will be used to overlay time series of moth densities to detect population trends. In addition, overlays of the contour maps on forest inventory and forest cover-type maps will be carried out to optimise the deployment of the traps and to relate changes in population density to forest management plans.

References

LIEBHOLD, A.M., ROSSI, R.E. AND KEMP, W.P. 1993. Geostatistics and geographic information systems in applied insect ecology. Ann. Review Entomol. **38** : 303-327.

LYONS, D.B. AND SANDERS, C.J. 1993. The North American spruce budworm pheromone trapping network. Pp. 37-48, in LIEBHOLD, A.M. AND BARRETT, H.R. (eds.). Proceedings: Spatial Analysis and Forest Pest Management. USDA Forest Service, Gen. Tech. Rep. NE-175.

ROYAMA, T. 1984. Population dynamics of the spruce budworm *Choristoneura fumiferana*. Ecological Monographs **54**: 429-462.

SANDERS, C.J. 1990. Practical use of insect pheromones to manage coniferous tree pests in eastern Canada. Pp 345-361, in RIDGWAY, R.L., SILVERSTEIN, R.M. AND INSCOE, M.N. (eds.). Behavior-modifying chemicals for insect management: applications of pheromones and other attractants. Marcel Dekker Inc., NY, pp 761.

SHEPHERD, R.F., GRAY, T.G., CHORNEY, R.J. and DATERMAN, G.E. 1985. Pest management of Douglas-fir tussock moth, Orgyia pseudotsugata (Lepidoptera: Lymantriidae): monitoring endemic populations with pheromone traps to predict incipient outbreaks. Can. Entomol. **117**: 839-848.

PROBLEMS RELATED TO UTILIZATION OF SEX PHEROMONE LURES FOR MAIZE LEPIDOPTEROUS PESTS IN THE SENEGAL RIVER AREA.

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Abstract Preliminary pheromone tests were conducted in Senegal river valley in order to evaluate effectiveness and target specificity of synthetic pheromone (from INRA) of the pink stalk borer, *Sesamia calamistis*. During these tests carried out on irrigated maize at two cultivation periods, no male moth catches of this pest were made, probably for some formulation problems. However, this pheromone lure has shown a good attractiveness to *Mythimna loreyi*, another noctuid pest which causes significant damages before maize flowering. Consequently, this study was focused on this insect. In addition to the trap tests, direct sampling of the infested plants were made in order to complete the information on its biology

Introduction

Like many crops grown in Africa, maize is often attacked by a range of pests, the most important of which are lepidopterous stem-borers (Harris, 1962; Girling, 1980; Moyal, 1988).

The three species which cause the most important damages during the rainy season on the Senegal river area are the following : *Sesamia calamistis* (Noctuidae), *Eldana saccharina* (Pyralidae) which are both stem-borers and *Mythimna loreyi* (Noctuidae), the african leafworm, which has been recorded feeding extensively in the whole plant (Goebel, 1991).

Yield losses can reach 50% during the period of heavy damages but during the mild season (which is another possible period for maize growing) the losses don't exceed 10% (Goebel, 1992).

There are three well-known methods which allow to control the extension of lepidopterous insects and to estimate the population dynamics of stem-borers. Those methods are direct sampling of the plants attacked, the light and pheromone traps.

Light trapping is easy to carry out if the necessary infrastructure such as electricity and cages are available. But the non-specific catches required a great amount of work as far as the sorting and the identification (study of genetilia for the different species of lepidopterous insects) are concerned.

Consequently, particular attention has been given to the pheromone trap methods. The main insect target has been *Sesamia calamistis* whose sex pheromone is known (Zagatti et al, 1988) and preliminary tested in Mali in 1987 and 1988. The results of those tests are satisfactory because the sex attractant is specific at least between the different species of *Sesamia*. The synthetic compound has also been attractive as far as *Mythimna loreyi* (another noctuid) is concerned. Besides, components of sex pheromone of this pest was also identified (Takahashi et al., 1980) and the similarity between pheromones of the two species is known.

In the present study, our field tests in the two stations of Institut Senegalais de Recherches Agricoles (ISRA) were made not only to evaluate the effectiveness and target specificity of the *S.calamistis* sex attractant, but to establish a relation between adult flight, larval infestation periods and the growth stages of the host plant as well. Pheromone traps could be used to monitor the field population of maize lepidopterous in a region where agricultural systems are relatively precarious.

Materials And Methods

Pheromone tests were conducted during the two planting seasons of maize:
in mild season (dry season), from November to March, and in rainy season (the rainfall does not exceed 200 mm per year), from July to October.

In the mild season 1991/92, Pheromone traps were tested only at Fanaye station, because the second station, which is at Ndiol, was not functional at this period (problems with the irrigation system).

In the rainy season 1992, these traps were tested in the two stations.

Lure type

The synthetic pheromone of *Sesamia calamistis* used was manufactured by Institut National de la Recherche Agronomique (INRA). Two concentrations of major acetates per mixture were prepared for the tests :

SC 800 : 600 µg of (Z)-11-hexadecenyl acetate (Z11-16:Ac), 200 µg of (Z)-9-tetradecenyl acetate (Z9-14:Ac) and SC 2000 : 1500 µg of Z11-16:Ac, 500 µg of Z9-14:Ac. The dispenser of pheromone is a rubber septa from INRA, with a diffusion period of aproximately four to six weeks.

Trap design.

The trap type used in the fields was manufactured by Biological Control Systems and was a rigid plastic funnel trap (Dimensions : 230 mm high x 170 mm diameter).

Field tests.

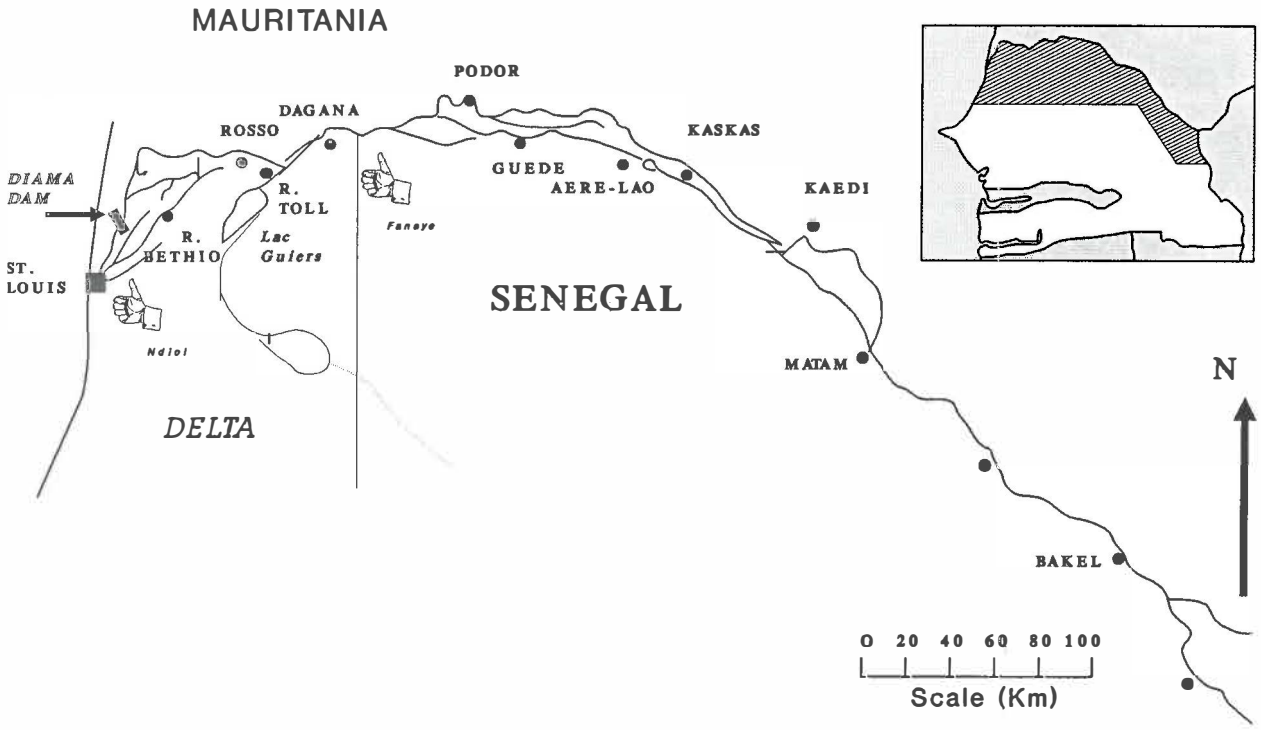
Four pheromone traps were placed in maize fields in each station : two traps containing rubber cap of SC 2000 formulation (maximum load) and two with SC 800. These devices were set 100 m apart, in the middle of maize plot (separated blocks of 200 to 400 m2), in order to cath only the indigenous insects. Traps were hung approximately 1.5 m above the ground.

The trapping were carried out from the seedling emergence to the maturation of maize. A small piece of plastic strip impregnated with dichlorvos was placed in the bottom of each trap, to kill captured insects. The dispensers were replaced at the following intervals based on manufacturer recommandations (every two weeks).

Observations in the field.

Captured moths were collected every day and identified in our laboratory. In order to complete the information about the biology of insect captured in the field, infested plant and larval countings were done on already sampled rows (4 X 10m represented a total of 2O0 plants) of maize plots.

Fig.1 : The Senegal river area and experimental stations



Results And Discussion

Sesamia calamistis.

During our experiments, no male moth of *S.calamistis* was captured in spite of the presence of larvae into maize young plants, stems and ears (particularly in rainy season).

Laboratory tests at INRA (France), revealed some formulation problems with synthetic pheromone of *Sesamia calamistis*, which could explain the lack of selectivity for this species.

The pheromone synthesized in these laboratories was, in fact, the optimal coumpound for *Mythimna loreyi*, another noctuid. This is mainly due to the great similarity of the chemical compounds of sex pheromones for these two species. Nevertheless, in these compounds, the percentage of major acetates was different (Zagatti et al, 1988; Takahashi et al, 1980):

Major acetates (%)

S.calamistis : Z11-16:Ac (**60%**), Z9-14:Ac (**14%**)
M.loreyi : Z11-16:Ac (**19%**), Z9-14:Ac (**74%**)

Generally, selectivity of sex pheromones lures in the noctuid group is not easy to obtain because of a similar composition of major acetates (Arn et al., 1986). About *Ostrinia nubilalis* (Lepidoptera, pyralidae), the compound of Z11-hexadecenyl acetate (Z11-16:Ac) has given a positive response to the noctuid *Mythimna unipuncta* (Pena et al., 1988). Adams et al (1989) reported some problems about the capture of nontarget species of lepidopterous.

In our first tests, the selectivity and performance of the *S.calamistis* pheromone lure for *M.loreyi* was unexpected but not uninteresting. In fact, this result has encouraged us to focus our researches on the leafworm.

Mythimna loreyi.

Total captures of adults for all traps throughout the study periods (regrouped every fortnight during the maize cultivation) are shown in table 1,2 and 3 (and graphically represented on fig. 2, 3 and 4).

A good performance on *M.loreyi* moths was noticed, particularly with SC 2000 formulation (maximum load). In the two stations, most important catches were made before maize flowering which in accordance with the larval density at this period.

Traps catches of M.loreyi in relation to larval infestation on maize.

In the mild season, two peaks of moth activity were observed, the most important occured at the early growth stages of maize plants (during the fortnight 1). First larval infestations were observed two weeks after seedling emergence. At the tassel emergence stage (during the fortnight 3), percentage of infested plants due to the leafworm reached the maximum with a mean of 25% (with 2,8 larvae/plant), at Fanaye station. A wider peak also noted during and after flowering (fortnight 4 and 5). Besides some damages were observed on the silks and husks of maize ear (but rarely on the seeds).

In the rainy season, the number of catches was much more important between seedling and tasseling stages (fortnight 2 and 3). In maize plots of the two stations, the first infestation was noticed two weeks after planting. Maximum infestation at the tassel emergence stage were observed with a mean of 29% (with 1,7 larvae/plant) at Fanaye station and 17% (with 2,1 larvae/plant) at

Fig.2 : Trap catches of *M.loreyi*
Fanaye station. mild season 91/92

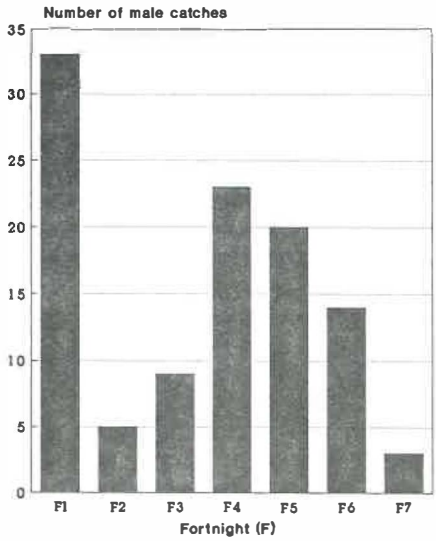


Fig.3 : Trap catches of *M.loreyi*
Ndiol station. rainy season 92

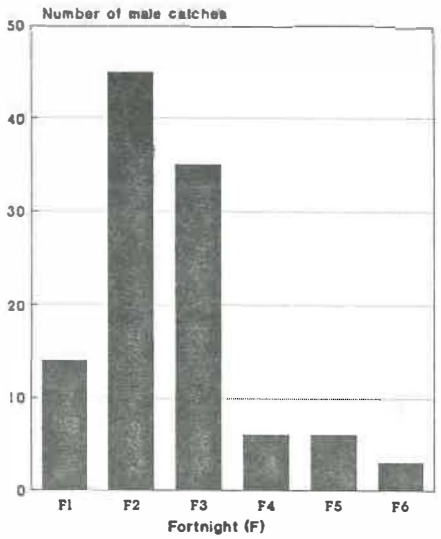
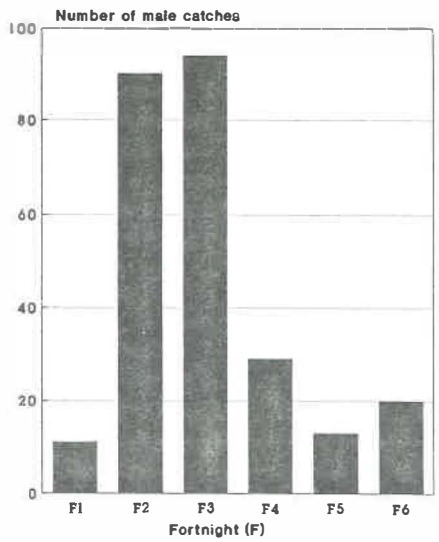


Fig 4 : Trap catches of *M.loreyi*
Fanaye station. rainy season 92



Trap catches of *Mythimna loreyi* moths during the cultivation period of maize, in relation to infestation.

Table 1: FANAYE STATION / MILD SEASON 91/92

Trapping period per fortnight (F)	Pheromone SC 800 (2 Traps)	Pheromone SC 2000 (2 Traps)	Total Captures	infestation (*) %
F1 : O6/O1-20/01	23	33	56	5,5
F2 : 21/01-03/O2	3	5	8	14,5
F3 : 04/O2-17/02	5	9	14	25,0
F4 : 18/02-02/03	16	23	39	25,0
F5 : 03/03-16/03	10	20	30	7,5
F6 : 17/03-30/03	10	14	24	12,0
F7 : 31/03-13/04	2	4	6	12,0
TOTAL	69	108	177	-

(*) : % infestation estimated at the end of each fortnight
F1, F2, F3, F4 : % plants with leaf and tassel damaged
F5, F6, F7 : % plants with ear damaged (silk and husk)

Table 2: NDIOL STATION / RAINY SEASON 92

Trapping period per fortnight (F)	Pheromone SC 800 (2 traps)	Pheromone SC 2000 (2 traps)	Total Captures	infestation(%)
F1 : 01/O8-14/08	4	10	14	8,0
F2 : 15/08-31/08	3	42	45	17,0
F3 : O1/09-14/O9	8	27	35	17,0
F4 : 15/O9-28/O9	1	5	6	17,0
F5 : 29/O9-13/10	2	4	6	3,5
F6 : 14/09-28/10	1	2	3	3,5
TOTAL	19	90	109	-

Table 3: FANAYE STATION / RAINY SEASON 92 (Sowing : 27/O7/92; Harvest : 3O/11/92)

Trapping period per fortnight (F)	Pheromone SC 800 (2 traps)	Pheromone SC 2000 (2 traps)	Total Captures	infestation (%)
F1 : 31/O8-14/09	6	5	11	9,5
F2 : 15/O9-29/09	8	82	90	18,5
F3 : 30/09-14/10	8	86	94	29,0
F4 : 15/10-29/10	3	26	29	29,0
F5 : 30/10-13/11	2	11	13	5,5
F6 : 14/11-28/11	5	15	20	5,5
TOTAL	32	225	257	-

Ndiol station. At the maize flowering, several pupae where found on the plant. After this growth period, the incidence of *M.loreyi* was very low (table 2 and 3).

In the Senegal river valley, the stem-borers *S.calamistis* and *E.saccharina* usually attack during the rainy season, however *M.loreyi* seems indifferent to the growing season. This pest attacked the maize with much the same intensity and results did not differ in the two stations (during the mild season). Nevertheless, different modes of irrigation in the two stations, Ndiol with irrigation by sprinklers (on sandy soil) and Fanaye with surface irrigation (on heavier soil), led to differing levels of infestation. This was shown for stem-borers where infestation is usually higher in maize under irrigation by sprinklers (Goebel, 1991).

During the trapping periods, Several nontarget insects were also captured with the sex pheromone lure of *Mythimna loreyi*, particularly stinging Hymenoptera such as bees, bumble bees (Apidae) and yellowjackets (Vespidae). ADAMS et al. (1989) obtained the same effects with sex pheromone lure of the Fall Armyworm, *Spodoptera frugiperda*, especially with yellow and white traps. Finally, the funnel trap used has shown a good resistance in sahelian conditions (high temperature, sand and dust winds.).

Conclusion

Although we did not catch any *Sesamia calamistis* moth for some problems of synthetic pheromone, we have managed to focus on *Mythimna loreyi*, the only pest attracted by this lure type.

The adult and larvae population surveys in maize fields has shown a good relation between growth stages of crop and infestation periods.

During the two cultivation periods, maximum infestation due to *M.loreyi* occurs at the tassel emergence stage.

Compared to *Sesamia calamistis* and *Eldana saccharina*, *M.loreyi* is the rather uncommon pest, and the data collected on its biology are very useful. This pest does not seem to be sensitive to the maize cultivation periods. This characteristic confers about *M.loreyi*, a real threat for production all the year round in irrigated fields of Senegal river valley.

With respect to the first findings, it would be interesting to pursue the experiments on *M.loreyi* in station with different lure and trap tests.

Concerning *S.calamistis*, new tests with specific formulations should be made.

Finally, these studies could be conducted in farmer's fields. The detection of high infestation periods due to the trapping systems on irrigated crops in Africa would be very useful for integrated pest management.

References

ADAMS, R.G., MURRAY, K.D., and LOS, L.M., 1989. Effectiveness and selectivity of sex pheromone lures and traps for monitoring Fall Armyworm (Lepidoptera : Noctuidae) adults in connecticut sweet corn. J. Econ. Entomol. **82**: 285-290.

ARN, H., TOTH, M. and PRIESNER, E., 1986. List of sex pheromones of Lepidoptera and related attractants. OILB-SROP, PARIS.

GIRLING, D.J., 1980. *Eldana saccharina* as a crop pest in Ghana. Tropical Pest Management. **26** (2):152-156.

GOEBEL, R., 1991. Problèmes phytosanitaires en maïsiculture sur le fleuve Sénégal. Inventaire et suivi des ravageurs. Rapport d'activité 90 de l'opération entomologie. CIRAD-CA/ISRA, 1991/12. 25 p. Doc.interne.

GOEBEL, R., 1992. Synthèse des activités 91/92 en Entomologie du maïs irrigué. Base centre maïs irriguée. Rapport d'activité ISRA/St-Louis (Senegal).

HARRIS, K.M., 1962. Lepidopterous stem borers of cereals in Nigeria. Bull. Ent.Res. **53**: 139-171.

MOYAL, P., 1988. Les foreurs du maïs en zone des savanes en Côte d'Ivoire : Données morphologiques et écologiques. Essais de lutte et relation plante-insecte. Thèse de doctorat es-science. Collection Etudes et Thèses, éd. ORSTOM, Paris.

PENA, A., HARN, H., BUSER, H.R., RAUSHER, S., BIGLER, F., BRUNETTI, R.,
MAINI, S., TOTH, M., 1988. Sex pheromone of European Corn Borer, *Ostrinia nubilalis*. Polymorphism in various laboratory and field strains. J. Chem. Ecol. **14**: 1359-1366.

TAKAHASHI, S., KAWARADANI, M., SATO, Y. and SAKAI, M., 1980. The components of Female Sex Pheromone of the Lorey leafworm, *Leucania loreyi* Duponchel.
Appl. Ent. Zool., **15**: 499-501.

ZAGATTI, P. et al., 1988. Phéromone sexuelle de *Sesamia calamistis* Hmps (Lepidoptera : Noctuidae). C.R. Acad. Sci. Paris, **307**: 837-840.

TRAP DESIGN STUDIES WITH THE PHEROMONE OF *Coniesta ignefusalis* (Hampson) (LEPIDOPTERA: PYRALIDAE) IN SUB-SAHARAN AFRICA

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Abstract Studies were conducted to evaluate a pheromone-baited trap design to monitor *C. ignefusalis* (Hampson) populations, subsequent to the identification and synthesis of its female sex pheromone. A water-oil based trap developed from local material proved to be three-fold as efficient as one commercial trap. Trap efficiency was enhanced by the use of mineral oil as a surfactant. Implications on trap designs and optimization studies for monitoring *C. ignefusalis* are discussed.

Introduction

C. ignefusalis (Hampson) (Lepidoptera: Pyralidae) is an important stem borer pest of pearl millet, *Pennisetum glaucum* (L.) R. Br. a major staple crop, in the West Africa Sahelian and Soudano-sahelian zones (Harris 1962, N'doye and Gahukar 1987, Youm 1990). The type and symptoms of borer damage depend on the crop developmental stage and stem borer generation. Adult moth oviposition occurs primarily in leaf sheath (Youm 1990) and attack from first-generation developing larvae on small plants cause premature death with stand-loss resulting from dead hearts. Second and third generation attacks result in disruption of nutrients flow and increases likely chance of secondary diseases transmission and incidence. The disruption of plant vascular system causes empty heads resulting from poor to no grain formation due to severe stem tunnelling. Yield losses resulting from *C. ignefusalis* damage range from 15% to total crop failure (Harris 1962, Ajayi 1990). During studies on the control of *C. ignefusalis*, the presence of a sex pheromone was demonstrated (Bako 1977, ICRISAT 1989). Subsequently, the sex pheromone was identified and synthesized, and field evaluation showed that a blend of synthesized pheromone attracted more male moths than did virgin females (Beevor et al., In review). The studies reported here were conducted to design and evaluate a water based pheromone-baited trap for monitoring *C. ignefusalis* populations.

Materials and Methods

Field trials

Trials were conducted in village millet farms near ICRISAT, about 45 km from Niamey.

Niger. Experiments were conducted to determine the effect of various trapping surfaces on catches of male *C. ignefusalis* moths in water based traps baited with synthetic pheromone. Traps were positioned approximately 25m apart in a circular arrangement within a replicate. Moth catches were recorded each day when traps with dispensers were moved clockwise one position within a replicate. Three replicates separated by at least 100m were carried out for each experiment, and the experiment was run until each treatment had occupied each position up to two times (4 - 8 nights).

Trap design.

Traps consisted of a tray, 34 cm diameter, and 5.5 cm deep with a 20 cm-wide plastic shade supported 10 cm above the trapping surface to protect pheromone dispensers from direct sunlight. A 40 x 40 cm rectangular wooden plate mounted on a central pillar supported the traps at 60 cm above ground level.

Pheromone dispensers.

All traps in this study were baited with pheromone dispensers consisting of polythene vials (PV) (20 x 10 x 2mm thick) loaded with the three component pheromone blend (Z)-7-dodecen-1-ol (500 ug), (Z)-5-decen-1-ol (3.95 ug) and (Z)-7-dodecen-1-al (2.5ug) and an equivalent amount of BHT (Butyrated Hydroxy Toluene) antioxidant.

Efficiency of traps containing water, water + mineral oil (W/O) and oil alone for catching male C. ignefusalis moths.

Three treatments consisting of trap trays containing water 3cm deep, water 3cm deep containing mineral oil (motor-oil S-40), 20ml (W/O trap) and enough mineral oil to cover the bottom surface of the tray. The experiment was replicated three times and repeated for four nights.

Efficiency of traps containing water + mineral oil (pheromone-baited and unbaited), and non drying insect trap sticker for catching male C. ignefusalis moths.

Three treatments consisting of a W/O trap, an unbaited water trap as control and a trap tray in which water was replaced by a thick layer of non drying insect glue (trap sticker) (Agrisense-BCS Ltd, UK) on the inside surfaces up to 3cm high on the edge. The experiment was replicated three times and repeated for six nights.

Relative efficiency of pheromone baited W/O and sticky board traps for catching of male C. ignefusalis moths.

A W/O trap and a sticky board trap consisting of a 50 cm x 50 cm green plastic board covered with a non drying insect trap sticker spread over the upper surface were used. The sticky board trap had a plastic shade, 20 cm x 20 cm, supported 10cm above the sticker surface, the trap in turn being supported 60 cm above ground level as for W/O traps. The experiment was replicated three times and repeated for eight nights.

Data analysis.

Data from each experiment were transformed using the natural logarithm as: YT=Log(Y+1), where Y = number of daily moth captures per trap. An analysis of variance (ANOVA) was used to compare trap efficiency and differences in trap catches were determined using the least significant difference (LSD) tests (SAS Institute 1987).

Results

Male *C. ignefusalis* catches per trap night were significantly different among the trap types tested ($F = 87.5$, $df = 2,4$, $P< 0.001$) (Table 1). W/O traps caught significantly more moths than traps containing water with no surfactant. There was no significant difference in trap catch between W/O traps and traps containing mineral oil alone. This experiment shows that addition of a surfactant such as mineral oil to water in the trap trays is essential for high trap efficiency.

W/O traps caught significantly more male moths than traps with non-drying insect trap sticker when baited with the same pheromone blend ($F = 1820.6$, $df=1,2$, $P< 0.001$). Unbaited W/O traps caught no male moths (Table 2). Catches in W/O traps were more consistent over time than when non-drying trap sticker was used as the trapping surface.

The W/O traps caught significantly more male moths than the sticky board traps ($F = 156.5$, $df = 1,2$, $P< 0.01$) (Table 3). The W/O trap was also more consistent over time in terms of daily captures of male moths, whereas catches recorded from sticky board trap were much more variable (Figure 1).

Discussion

The addition of a surfactant to the attractive pheromone blend significantly increased the efficiency of the water trap baited with the pheromone blend. Increased efficiency was probably due to the reduced surface tension. The fact that no significant difference in catch of male *C. ignefusalis* was detected between water mixed with mineral oil and mineral oil alone suggests that mineral oil is very efficient as a surfactant. Night observations of a trap containing water without surfactant showed that male moths were able to take off very often after landing on the water surface. In addition, some males would land on the edge of the tray, move on the internal surface above water level then try to reach and mate with the pheromone dispenser. A pheromone-baited trap with oil alone was also efficient, although, it would be more costly to use. The W/O trap was also more efficient than the trap with non-drying insect trap sticker in the tray or the sticky board trap. The reduced efficiency of the sticky board trap over time (Figure 1) could be due to dust, small pieces of plant material, remains of scales from previously caught moths etc. which can reduce effective sticky surface area. Furthermore, sticky traps are more tedious and expensive to maintain. Thus, the water trap with a surfactant offers easily maintained and less expensive trap design that can be used at the subsistence farmer level in the sahelian region of Africa.

Although this trap is efficient, some parameters still need investigation. What is the optimum trap height, tray diameter, shade size and height above the trapping surface? Optimization of the W/O trap will require knowledge of these parameters and their effect on male moth catches in pheromone-baited traps.

In summary, a water trap with oil as surfactant was shown to be more efficient than a sticky board trap. The use of a surfactant was also essential for trap efficiency as it reduces surface tension.

Table 1. Efficiency of traps containing water, water + mineral oil and oil alone for catching male *C. ignefusalis* moths when baited with polythene vial (PV) pheromone dispenser.

Trapping surface in trap tray	Total catch [a/]	Mean ± SE moths/ trap/night [b/]
Water + Mineral oil (W/O)	840 (11)	76.4 ± 11.7 a
Mineral oil	643 (12)	53.6 ± 6.4 a
Water	20 (12)	1.7 ± 1.0 b

a/ Number of trap nights in parentheses
b/ Means followed by the same letter are not significant (P>0.05, LSD tests, SAS Institute, 1987).

Table 2. Efficiency of traps containing water + mineral oil (pheromone baited and unbaited), and non drying insect trap sticker for catching male *C. ignefusalis* moths when baited with a PV pheromone dispenser

Trapping surface in trap tray	Total catch [a/]	Mean ± SE moths/ trap/night [b/]
Water + mineral oil (W/O)	2,770 (18)	153.9 ± 17.5 a
Non-drying glue	208 (18)	11.6 ± 3.5 b
Water + mineral oil, unbaited	0 (18)	- -

a/ Number of trap nights in parentheses
b/ Means followed by the same letter are not significant (P>0.05, LSD tests, SAS Institute, 1987).

Table 3. Relative efficiency of a pheromone baited W/O and sticky board traps for catching of male *C. ignefusalis* moths when baited with a PV pheromone dispenser.

Trap design	Total catch [a/]	Mean ± SE moths/ 2 trap/night [b/]
Water + mineral oil (W/O)	1,036 (23)	45.0 ± 7.6 a
Sticky board	16 (24)	0.7 ± 0.2 b

a/ Number of trap nights in parentheses
b/ Means followed by the same letter are not significant (P>0.05, ANOVA, SAS Institute, 1987).

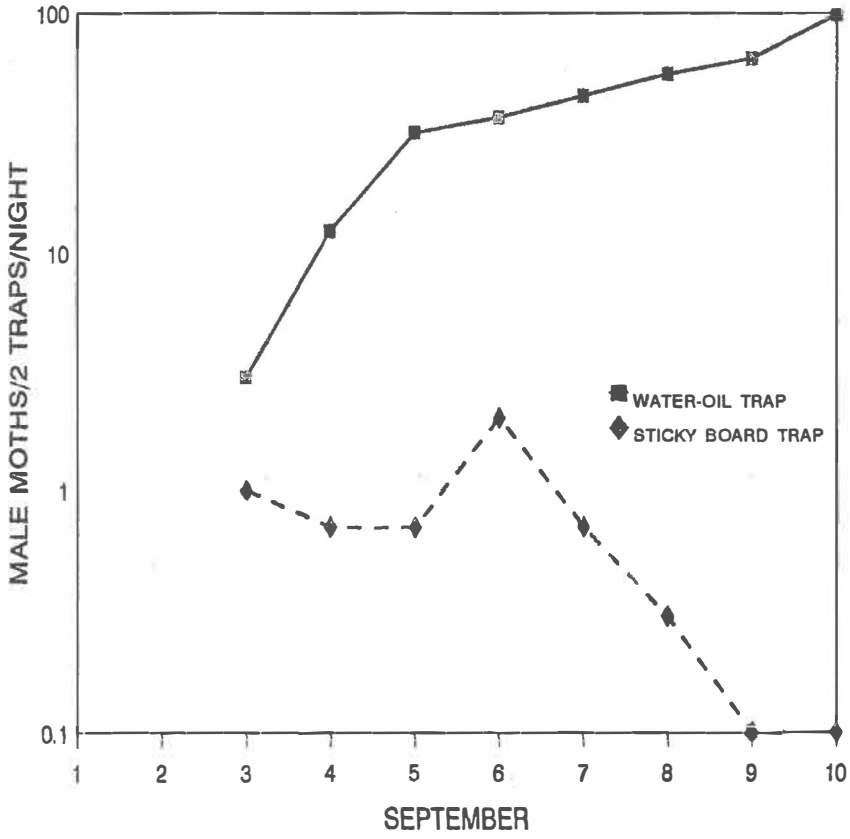

Figure 1. Relative efficiency of pheromone-baited W/O and sticky board traps for catches of male C. ignefusalis

The W/O trap offers an alternative to light traps for population monitoring of *C. ignefusalis*. Further trap optimization studies are suggested to determine optimum parameters such trap diameter and height, shade size and height above the trapping surface and types of surfactants, among others, to improve the trapping efficiency of *C. ignefusalis* pheromone-baited traps.

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References

AJAYI, O. 1990. Possibilities for the integrated control of the millet stem borer, *Acigona ignefusalis* Hampson (Lepidoptera: Pyralidae) in Nigeria. Insect Sci. Applic. **11**: 109-117.

BAKO, O. 1977. Etude biologique de *Haimbachia ignefusalis* (Hamps.) (Lepidoptera: Pyralidae, Crambinae) en vue d'une lutte biologique. Thèse de Maitrise. Université Laval, Quebec. Canada.

BEEVOR, P. S., O. YOUM, D. R. HALL, & A. CORK. 1993. Identification and field evaluation of components of the female sex pheromone of the millet stem borer, *Coniesta ignefusalis* (Hampson) (Lepidoptera: Pyralidae). *In review*

HARRIS, K. M. 1962. Lepidopterous stem borers of cereals in Nigeria. Bull. of Entomol. Res. **53**: 139-171.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) - Sahelian Center. 1989. ICRISAT West African Programs Annual Report 1988. ICRISAT Sahelian Center, BP 12404, Niamey, Niger

N'DOYE, M., & R. T. GAHUKAR. 1987. Insect pests of pearl millet in West Africa and their control. pp. 195-205. In ICRISAT (International Crops Research Institute for the Semi-Arid Tropics): Proceedings of the International Pearl Millet Workshop, 7-11 April 1986. ICRISAT Center, Patancheru, A. P. 502 324, India.

SAS Institute, 1987. SAS/STAT Guide for Personal Computers. Version 6 ed. SAS Institute, Cary, N.C.

YOUM, O. 1990. Evaluation of natural enemies associated with the millet stalk borer, *Haimbachia ignefusalis* (Hampson) (Lepidoptera: Pyralidae) in Niger. Ph.D dissertation. Texas A & M University, College Station, Texas USA. 145 pp.

DEVELOPMENT OF PHEROMONE-BAITED TRAPS AND THEIR USES FOR THE LARGER GRAIN BORER

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Abstract *Prostephanus truncatus,* the larger grain borer (LGB), was a relatively unknown pest of stored maize in meso-America until it was accidently introduced into Tanzania in the late 1970s and Togo in the early 1980s from where it has spread to neighbouring countries. This paper reviews the development of a flight trap, baited with an aggregation pheromone that is produced by adult males, which has been used: to monitor the spread of this pest in Africa; to develop a predictive distribution model in Mexico; and to assess the spread of a *P. truncatus* biological control agent, the histerid beetle *Teretriosoma nigrescens*. Problems of using pheromone-baited traps in stores due to poor response rates and the risk of encouraging infestation are discussed.

Introduction

The bostrichid beetle *Prostephanus truncatus* (Horn) is an important pest of farm-stored maize and cassava which was accidently introduced into Africa in the late 1970s (Golob and Hodges, 1982).

P. truncatus initially became established in western Tanzania from where it has spread to neighbouring countries. A second outbreak occurred in Togo in the early 1980s (Krall, 1984) and there is now an increasing population in west Africa which appears to be spreading at a greater rate than that in the east.

After the Tanzanian outbreak it was clear that *P. truncatus* was a mobile pest with a destructive potential and an effective monitoring method was required. Subsequently, a pheromone-baited trap was developed, which has been used to give an early warning of infestation, and to assess the rate and direction of spread of *P. truncatus* and its natural predator *Teretriosoma nigrescens*. It also has potential to be used for assessing the success of *P. truncatus* control measures and to provide more information on the biology and ecology of *P. truncatus*.

Development of a *P. truncatus* lure and trap system

The pheromone lure

The first response to the need for a monitoring device was the development of a trap for use in stores (Hodges, 1983). This was a cardboard crevice trap, lined with a contact insecticide, which was baited with a component of the male-produced aggregation pheromone of another bostrchid *Rhyzopertha dominica,* namely (±)-1-methylbutyl-(*E*)-2,4-dimethyl-2-pentanoate, known as Dominicalure 2. The pheromone was released from a rubber septum and the trap was successful in catching *P. truncatus.*

Subsequently, a component of the male-produced aggregation pheromone of *P. truncatus* was isolated and identified as 1-methylethyl (*E*)-2-methyl-2-pentenoate and given the trivial name Trunc-call (Hodges *et al.*, 1984; Cork *et al.*, 1991). Trunc-call has a very similar structure to that of the *R. dominica* pheromone. In trials, Trunc-call was twice as efficient at detecting *P. truncatus* when compared to Dominicalure 2 (Hodges *et al.*, 1984). Subsequently a second component of the *P. truncatus* male aggregation pheromone was identified and synthesized (Trunc-call 2: 1-methylethyl *(E)*-2, *(E)*-4-2, 4-dimethyl-2-heptadienoate) (A Cork, D R Hall, J L Smith, J Dendy and R J Hodges, *unpubl.*). The previously identified component was renamed Trunc-call 1. Traps baited with both *P. truncatus* pheromone components were tested in maize stores in Tanzania. The pheromone was loaded into polythene vials as these provided a slower, more uniform release than the rubber septa. The trials in infested village stores showed that mixtures of Trunc-call 1 and Trunc-call 2 caught significantly more *P. truncatus* than Trunc-call 1 alone (Dendy *et al.*, 1991).

In the laboratory the adult male insects release the two pheromone components in a ratio of 10:1. There are differences in volatility between the two components: Trunc-call 1 is released faster than Trunc-call 2, and thus, the ratio alters with time. A mixture of 1:4 in a polythene vial appears to simulate the natural ratio (A Cork *pers. comm.*) but ratios of 1:4 and 1:1 resulted in similar trap catches in both store and field trials (Dendy *et al.*, 1989b). Currently, the baits are manufactured commercially using the 1:1 ratio.

The commercial bait was tested in maize fields using a funnel trap. An anti-oxidant was added to the lure to prolong its life. Ten times as many insects were caught using the pheromone mixture than by Trunc-call 1 alone.

Trap design

Subsequent studies by Dendy *et al.*, (1989a) investigated the trapping efficacy of three field trap designs. Funnels similar to the Graham flight trap (Graham, 1970) and delta traps (pink bollworm traps) proved more efficient than pitfall traps (a pot with a lid that has a peripheral rim and a small central opening). Others have shown that the greater the surface area of the trap the more effective it appears to be (J Key *pers. comm.*). Attempts were also made to improve the design of the store traps. The cardboard insecticide-treated crevice trap was replaced by a corrugated plastic sandwich trap which used a non-drying glue to retain the insects.

Problems associated with the use of traps in stores

Markham *et al.,* (1991) highlighted the need for more research into the efficacy of the crevice traps as little was known about the relationship between store infestations and pheromone-baited trap catches. Pheromone-baited store traps are intended to detect low-density infestations with minimal input. However, recent work has shown that *P. truncatus* resident in maize stores exhibit a limited response to the synthetic pheromone lure over a range of 40-100cm.

Markham *et al.*, (1991) considered that pheromone traps placed within stores could be trapping dispersing insects from outside of the store. Trials have demonstrated that pheromone lures do attract *P. truncatus* into previously uninfested maize cob stores. As less than half of the insects attracted to the store reach the trap (when the trap is within 10cm of the cob stack surface) the presence of the trap is encouraging infestation (V Pike, *unpubl.*). Burying the traps at a depth of 40cm within maize cob stacks does not remove their attractiveness to dispersing beetles (V Pike, *unpubl.*).

Since traps placed in stores catch few of the resident population and attract insects to the stores, their use can no longer be recommended. Instead, monitoring exercises should use flight traps. Delta traps are often used due to their relative inexpensiveness, ease of use, robustness and effectiveness. The traps should be placed at a distance from stored or drying susceptible crops.

Pheromone longevity

As the components of the pheromone are highly volatile their concentration in a plastic vial will deplete rapidly once they are exposed to the environment. It appears that, using 2mg of Trunc-call 1 and Trunc-call 2 in a delta trap, the peak *P. truncatus* capture rate is achieved within the first week (Tigar *et al.*, 1993; Dendy *et al.*, 1989a; D Rees, *pers. comm.*). A recent study has shown that the decrease in trap catch with time can be due to a reduction in attractiveness of the pheromone lure and not to 'trapping out' of a population (Tigar *et al.*, 1993). Some studies have shown that the lures can retain their attractiveness for up to 11 weeks at lower temperatures (Novillo *et al.*, 1991). It is recommended that the lures are used for a maximum of 4 weeks and preferably for 2 weeks to ensure efficient trapping. The actual exposure duration is usually dependent on the trapping programme logistics.

Attractive range of the pheromone

Release and recapture studies broadly agree on the attractive range of the pheromone lure. Farrell (1990) reported a recapture distance of up to 340m. The majority of the insects were recaptured within 24 hours of release, none were recovered after more than 72 hours. The beetles do fly upwind in response to the pheromone but the range depends upon the strength of the wind. Trials in a wind tunnel indicate that *P. truncatus* is capable of matching an 0.9m/s wind speed (M Downham, *unpubl.*). Farrell (1990) recorded upwind flight towards a baited flight trap over a distance of 50m. Novillo *et al.*, (1991) recorded a maximum distance of 300m and Rees *et al.* (1990) a distance of 250m. Laboratory testing of *P. truncatus* flight on flight mills suggests that the beetle is capable of covering up to 25km in 45 hours (V Pike, *unpubl.*). However, it is highly unlikely that the insects would fly so far in the field. Being suspended in mid-air, attached to a wire with the flight mill supporting their weight, insects could feasibly fly greater distances than they would under normal conditions. However, this result suggests that a trap left in place for up to four weeks is likely to trap not only insects that are resident in the immediate vicinity but also individuals that may be dispersing. Much work remains to be done to identify flight causal factors and assess the flight activity of *P. truncatus* populations in the field. Pheromone baited traps have a role to play in this work.

Uses For Pheromone-baited Traps

Monitoring P. truncatus distribution

The pheromone-baited traps have been used successfully to monitor the spread of *P. truncatus* populations in east and west Africa. Trapping data have shown that it is distributed in a wide range of environments including those outside of maize and cassava farming systems (Richter & Biliwa, 1990, 1991; M Wright, *unpub.*). There is mounting circumstantial evidence that *P. truncatus* is not an obligate stored product pest and may have alternative natural host plants. Pheromone trapping results have added weight to this. Trapping surveys in Mexico have recorded high numbers of *P. truncatus* far from regions of maize cultivation or storage (Herrera *et al.*, 1989 & 1991; B Tigar and J Key *unpubl.*) and in Kenya positive trap catches have been recorded in the Tsavo National Park (P H Giles, *unpubl.*).

A national monitoring network in Kenya is giving the government early warning of the pest's spread, thus allowing time for a control campaign to be mobilised. Malawi and Mali are monitoring for the pest (the latter country has yet to be invaded).

Monitoring predator populations

A natural predator of *P. truncatus*, the histerid beetle *Teretriosoma nigrescens* (Lewis), was introduced into Togo by the German Aid Agency GTZ and into Kenya by the UK/Kenya LGB Project as a biological control agent. The pheromone-baited traps have also proven a useful tool for monitoring the distribution of this insect.

Although *T. nigrescens* is not prey-specific (adults are facultative feeders and the larvae obligate predators) it is highly sensitive to the aggregation pheromone, exhibiting a kairomonal response. A release and recapture experiment was undertaken to assess the attractive range of the pheromone to the predator (Helbig *et al.*, 1992). It appears that *P. truncatus* and *T. nigrescens* are attracted to the lure over roughly equal distances. The greatest successful trapping distance for the predator was 450m. However, meteorological data suggests that the beetles were carried downwind towards these traps. The upwind trapping range was around 100m.

Since the release of *T. nigrescens*, pheromone-baited delta traps have been used to monitor the spread of the predator from its release points. In Togo, trap catches showed that *T. nigrescens* had moved a distance of 1km from the release point in three months and 5km in seven months. The latest data from Kenya suggest that four months after the release the first insects were trapped at a distance of 1km from a seeded store and after nine months *T. nigrescens* were trapped 2km from the nearest release site.

The effects of environmental factors on trap catch

Pheromone-baited delta traps were used in a nationwide population monitoring survey in Mexico. The results suggests that time of year and location significantly affected trap catch, with interaction between the two. Catches of the natural predator *T. nigrescens* were similarly affected, but to a lesser extent. For *P. truncatus,* a relationship existed between high trap catches and peaks in rainfall in arid regions. A similar relationship was also found in Honduras (Novillo *et al.*, 1991) and Togo even in areas with comparatively high humidity (M Wright, pers. comm.). However, Farrell (1990) reported a decline in trap catch in the presence of rain. This study involved looking at traps on a daily basis over an eight week period. It could be surmised that overall conditions during rainy seasons may result in increased *P. truncatus* abundance, but, if rain falls during a short trapping period, catches are likely to be reduced. Mexican distribution densities also related to minimum temperature. A regression equation based on temperature, humidity and rainfall explains 83% of the

trap catch. Habitat was a poor indicator of abundance. Trap catches were not related to the presence of maize.

The information from the Mexican trapping survey is currently being used to develop a distribution model and should assist in predicting where else *P. truncatus* could become a major pest in Africa. One of the most interesting findings of this study was that *P. truncatus* abundance predicted by the insects' reproductive behaviour in the laboratory (Haubruge and Gaspar, 1990), differed from that recorded by the field trap catches and that predicted by the model developed from the field data. Laboratory data suggest *P. truncatus* is most productive at 30°C and at humidities over 60%. Trapping stations that attain these conditions in the field appear to have low catches. The field data suggest, and the model agrees, that abundance is greater in the relatively cool, higher altitude regions where the average temperature is in the mid-twenties. This apparent contradiction may be partially or completely explained by the fact that competition with other storage pests increases at higher temperature and the prevalence of predators, parasites and diseases also increases.

Summary and further work

Research has resulted in the development of a highly sucessful flight trap for detecting the presence of *P. truncatus* and its predator *T. nigrescens*. The potential hazard of using pheromone-baited traps in stores has been identified. Extensive pheromone trapping has shown the relationship between trap catch and field abundance to be highly complicated. Although the significance of climatic and environmental factors is now becoming clearer we have still to establish what proportion of the

P. truncatus population responds to the pheromone. For these reasons flight traps have not found a role in quantitative assessment of abundance. Until these relationships are established, exercises such as monitoring the success of control campaigns will depend upon visual inspection.

References

CORK, A., HALL, D.R., HODGES, R.J. & PICKETT J.A., 1991. Identification of major component of male-produced aggregation pheromone on larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). J. Chem. Ecol. **17**(4): 789-803.

DENDY, J., DOBIE, P., SAIDI, J.A. & SHERMAN, C., 1989a. The design of traps for monitoring the presence of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in maize fields. J. Stored Prod. Res. **25**(4): 187-191

DENDY, J., DOBIE, P., SAIDI, J.A., SMITH, J.L. & URONU B., 1989b. Trapping the larger grain borer *Prostephanus truncatus* in maize fields using synthetic pheromones. Entomol. Exp. et Applic. **50**(3): 241-244.

DENDY, J., DOBIE, P., SAIDI, J.A., SMITH, J.L. & URONU B., 1991. Trials to assess the effectiveness of new synthetic pheromone mixtures for trapping *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in maize stores. J.Stored Prod. Res. **27**(1): 69-74.

FARRELL, G., 1990. An investigation of the flight behaviour of the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in response to synthetic pheromone. MSc thesis, University of Birmingham, U.K. iv + 50pp.

GOLOB, P. & HODGES R.J., 1982. Study of an outbreak of *Prostephanus truncatus* (Horn) in Tanzania. Report of the Tropical Products Institute, G164. 23pp.

GRAHAM, W.M., 1970. Warehouse ecology studies of bagged maize in Kenya - I. The distribution of adult *Ephestia (Cadra) cautella* (Walker) (Lepidoptera: Phycitidae). J. Stored Prod. Res. **6:** 147-155.

HAUBRUGE, E. & GASPAR, C. 1990. Determination en laboratoire des zones favorables au developpment des populations du grand capuchin du grain, *Prostephanus truncatus* (Horn), en Afrique. L'agronomie Tropicale **45**: 251-258.

HELBIG, von. J., LABORIUS, G.A. & SCHULZ, F.A., 1992. Untersuchungen zur distanz der anlockwirkung des synthetischen pheromons des grossen kornbohrers *Prostephanus truncatus* (Horn) (Col., Bostrichidae) "Trunc-call (1+2)" aus dessen predator *Teretriosoma nigrescens* Lewis (Col., Histeridae).

HERRERA RODRIGUEZ, F.J., RODRIGUEZ RIVERA, R. & REES, D., 1989. Distribucion del 'Barranador del Grano' *Prostephanus truncatus* y el depredador *Teretrisoma nigrescens* en el estado de Yucatan, pp. 43-55. *In:* Primer Simposio Problemas Entomologico de Granos Almacenados, XXIV Congreso Nacional de Entomologia, Oaxtepac, Morelos, 24 mayo 1989.

HERRERA RODRIGUEZ, F.J., RODRIGUEZ RIVERA, R. & REES, D., 1991. Efecto de la vegetacion natural y el cultivo de maiz en las poblaciones de *Prostephanus truncatus* H. (Coleoptera: Bostrichidae). pp544. *In:* XXVI Congreso Nacional de Entomologia 19-22 de mayo de 1991, Veracruz, Mexico. Sociadad Mexicana de Entomologia.

HODGES, R.J., HALL, D.R., GOLOB, P. & MEIK, J., 1983. Responses of *Prostephanus truncatus* to components of the aggregation pheromone of *Rhyzopertha dominica* in the laboratory and field. Ent. Exp. & Appl. **34:** 266-272.

HODGES, R.J., CORK, A. & HALL, D.R., 1984. Aggregation pheromones for monitoring the greater grain borer *Prostephanus truncatus*. British Crop Protection Conference - Pests and Diseases 4A-3: 255-259.

KRALL, S., 1984. A new threat to farm-level maize storage in west Africa: *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Trop. Stored Prod. Inf. **50**: 26-31.

MARKHAM, R.H., WRIGHT, V.F. & RIOS IBARRA, R.M., 1991. A selective review of research on *Prostephanus truncatus* (Col., Bostrichidae) with an annotated and updated bibliography. CEIBA, **32** (1) pp.90.

NOVILLO, P., WRIGHT, V. & MARKHAM, R., 1991. Population dynamics of *Prostephanus truncatus* in the Zamorano Valley, Honduras, p.42. *In:* Resumenes de trabajos, XXXVII Reunion Anual, Programa Cooperativo Centroamericano para el Mejoramiente de Cultivos y Animales. Instituto de Investigaciones Agropecuaria de Panama. xxxvii + 327pp.

REES, D.P., RODRIGUEZ RIVERA, R. & HERRERA RODRIGUEZ, F.J., 1990. Observations of the ecology of *Teretriosama nigrescens* Lewis (Col: Histeridae) and its prey *Prostephaunus truncatus* (Horn) (Col: Bostrichidae) in the Yucatan Peninsula, Mexico. Trop. Sci, **30** (2) 153-165.

RICHTER, J. & BILIWA, A., 1990. Repartition du *Prostephanus truncatus* au Togo. Sahel PV Info **29**: 20-23.

RICHTER, J. & BILIWA, A., 1991. Landesweite Erhebung mittels pheromonfallen zur verbretung von *Prostephanus truncatus*(Horn) (Coleoptera: Bostrichidae) in Togo. Anzeiger fur Schadlingskunde Pflanzenschutz Umweltschutz **64**(59): 89-92.

TIGAR, B.J., KEY, G.E., FLORES-S., M.E. & VASQUEZ-A., M., 1993. Flight periodicity of *Prostephanus truncatus* and longevity of attraction to synthetic pheromone. Ent. Exp. Appl. **66**: 91-97.

MONITORING THE AFRICAN ARMYWORM, *Spodoptera exempta* in EASTERN AFRICA

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Abstract A network of traps, predominantly now pheromone traps operates, in eastern Africa to monitor armyworm populations and is used for forecasting purposes, through a Regional Forecasting Centre at the Desert Locust Control Organization for Eastern Africa, in Nairobi, Kenya. Forecasting procedures and data base used in conjunction with satellite imagery are important tools. Perfection of the pheromone has eliminated the catching of other species, and long-term lures are being developed. Procedures used for data collection are discussed; the use of pheromones in monitoring low density populations is mentioned. Future developments and the concerns caused by universal lack of foreign exchange and commitment are highlighted.

CHEMICAL ECOLOGY OF *PHORACANTHA SEMIPUNCTATA* (COL., CERAMBYCIDADE) : POTENTIAL ROLE IN EUCALYPTUS PEST MANAGEMENT

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Abstract *Phoracantha semipunctata* originates from Australasia, having spread to Africa, Europe and America where it attains pest status on eucalyptus crops. Field and laboratory experiments were conducted to decode the mechanisms of primary attraction for this eucalyptus borer. The selection process involves olfactory recognition of the volatiles of the host bouquet emitted signalizing susceptibility, as well as other cues. Field experiments showed that logs originating from trees already attacked by the borer captured significantly more adults, and more eggs of *P. semipunctata* , than the ones cut from vigorous trees. A similar tendency was observed for standing trees. Significant differerences were detected between the composition of the bouquets emitted by attacked, and attack free *E. globulus* when analysed by GLC / MS . A model is hypothesized to describe the olfactory behaviour of *P. semipunctata* . Dispersion is probably guided by long range attracting terpenes while host selection and oviposition are mediated by substances like a-felandrene which emanate mainly from the bark and indicate the presence of lesions in the host tissues.

Introduction: A Borer's Eye View

Phoracantha semipunctata Fab. originates from Australasia where it reproduces on Myrtacea of the genus *Angophora* and *Eucalyptus* . Although for this part of the world occasional references of damage to both plantations and individual trees can be found (e.g. Neumann & Marks 1976), attacks to standing trees are not common through most of Australia (Moore, 1963; pers. observ.). The borer was introduced to South Africa (e.g. Took 1949) where economic losses have since been caused to eucalyptus plantations. Its subsequent spread to central and northern Africa has also been associated with records of serious damage (e.g. Powell 1978, Chararas 1969a). In Europe, *P. semipunctata* entered the Iberian Peninsula in 1980, rapidly becoming a menace to this eucalyptus growing region. Economic damage in America started with its introduction to California (Scriven 1986), where *P. semipunctata* now attains the status of a major pest.

Control methods available are still unsophisticated, employing trap trees made from freshly cut eucalyptus logs. Such devices empiricaly rely on the principle of olfactory communication, and remain effective for a period of 1 to 2 weeks. A marked peak of attractivity occurs during the first 1-3 days.

It is generaly accepted that, in an eucalyptus stand not all trees prove equaly attractive to *P. semipunctata*. In the literature "stress" factors are believed to render trees prone to the borer's attack

(e.g. Chararas 1969b, González Tirado 1984) which is sometimes considered as a secondary insect (e.g. Moore 1963). Previous experiments (Farrall et al. unpublished) pointed to differences in the attractivity to ovipositing females, of eucalyptus already attacked by the borer, in comparision with unattacked trees.

Host Attraction : Selection And Colonisation

Methodology

Experiments were conducted to compare the relative efficiency of traps made with *E. globulus* logs originating from trees previously attacked by *P. semipunctata* (AT),but not severely damaged, with others obtained from vigorous trees (NAT).

In a 7 years old *E. globulus* stand located in Concavada, Abrantes, a total of 6 traps was set up for each treatment. A trap consisted of 4 logs disposed horizontaly on a support, placed 20 cm above the soil, and from which crawling predators were excluded. In another experiment, 24 pairs of traps were set up with the aim of collecting adults. The logs were thus covered with a net, mesh 1x1 cm, impregnated with Stikem_, to which the aproaching insects remained glued. Experiments elapsed between July 1991 and September 1992.

In another set of experiments, six aproximately 13 m high standing trees, four of which had been attacked by *P. semipunctata* and 2 vigorous ones, were individualy enclosed in a net of the type described. Due to practical problems, the experiment took place during the last weeks of September. The adults captured were counted daily and the heigth at which they were traped recorded. Further details will be given in Farrall et al. (in prep.).

GC/MS techniques were used to compare the composition of the bouquet emitted by *E. globulus*, between trees attacked by *P. semipunctata* , and trees which were attack free.

The trees used for the experiment were felled at Pedra do Rato, Portalegre, and cut into 1 m long logs. They were transfered to the laboratory, where the analysis commenced within the first 24 hours after cutting, and were completed over a period of 5 days. Samples were taken from the 3 bottom logs of each tree, and analysed by gas chromatography/ mass spectrometrie using a Carlo-Erba Vega 6000 - Finnigan MAT, ITD. Squares of 1 cm2 of bark were cut, placed in a micro-vial, and heated to 70º C for 2 minutes. The gaseous fraction was then collected with a 500 µl seringe, and injected into a gas chromatograph [FID; splitless; capillary columns 25. m x 0.32 mm; Su.ox (polar phase) made at the chemistry department at UNL/FCT; injector 225º C; detector 250º C; oven 60º to 200º C with 4º C /minute rate]. Further details will be given in Mateus et al. (in prep).

Results

Table 1 shows that significantly more eggs and egg batches /dm2 of log surface, and adults / trap, were collected from AT, than from NAT. For standing eucalyptus a similar tendency was observed, although significant differences could not be detected due to the small size of the sample. It was also concluded that 92% of all insects were captured around the bottom 3 metres of each tree trunk.

Figure 1 and Table 2 list the main 9 terpenes identified from the bouquet of E. *globulus*. Quantitative variations were noticed in the relative percentage of volatiles emitted according to the physiological condition of the eucalyptus. Trees attacked by *P. semipunctata* emitted significantly

more a-terpinene, a-felandrene and p-cymene than vigourous ones. On the contrary, significantly more a-pinene was detected in the bouquet of vigorous trees.

TABLE 1: Log surface of *E. globulus* available for oviposition to *P. semipunctata*, mean and standard error for the number of eggs and egg batches collected / dm2 from traps originating from attacked (AT) and attack free trees (NAT). Wilcoxon paired test. LOWER BOX: Mean and standard error for the number of adults trapped on: logs from AT and NAT trees - Wilcoxon paired test; standing attacked and vigourous trees - Man-Whitney U-test (Sokal & Rohlf, 1981). Abrantes, Portugal, July 1991 - September 1992.

Parameters	Trees Attacked (AT)		Trees Not Attacked (NAT)		Level of Significance
	N	X ± s.e	N	X ± s.e	
EGGS					
Log Surface (dm^2)	6	27.7 ± 2.3	6	24.7 ± 1.2	n.s.
Eggs/dm^2	6	11.3 ± 4.6	6	1.2 ± 0.5	*
Egg batches/dm^2	6	0.56 ± 0.16	6	0.06 ± 0.03	*
Adults Trap logs	2.4	12.8 ± 2.7	2.4	6.9 ± 1.7	*
Adults Trap trees	4	18.3 ± 4.9	2	3.0 ± 1.0	•

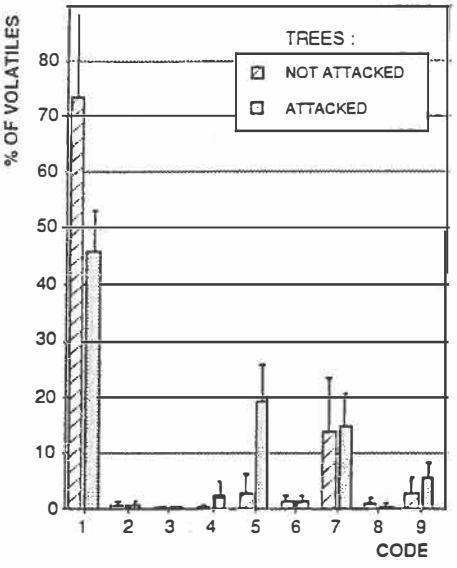
* - significant at 5% level ($p<0.05$)
n.s. - not significant at 5% level ($p>0.05$)
• - not significant due to the small size of the sample

Discussion: Modelling *P. semipunctata* - *eucalyptus* Interactions

Differences in the attractivity and susceptibility of eucalyptus species to *P. semipunctata* have been documented, indicating a non-random host selection process (Powell 1978, Farrall et al. 1987). Furthermore, for this insect a considerable rate of mobility and dispersion has been observed, e.g. in Portugal (Araujo & Paiva 1987) and California (Scriven et al. 1986).

For the eucalyptus borer some electrophysiological and olfactometric studies have been conducted, although results remain largely unpublished. Electroantenogramms showed strong responses of both males and females, to the bouquet emitted by leaves and bark of *E. globulus* , as well as to some synthetic terpenes, mainly a-pinene and cineol (Barata et al.1991; Barata et al. 1992).

Field experiments conducted in Australia, showed that females of *P. semipunctata* oviposited on logs of species unsuitable for larval development, when they were set up adjacent to logs of prefered species (Paiva et al. in prep.). Olfactory stimuli thus apear to override tactile and visual ones at short range, particularly during the search for oviposition sites. Results from Hanks et al. (1993) who worked in California with other eucalyptus species, indicate that *P. semipunctata* adults



CODE	TERPENE	LEVEL OF SIGNIFICANCE
1	a-pinene	**
2	ß-pinene	n.s.
3	mircene	n.s.
4	a-terpinene	*
5	a-felandrene	**
6	∂-limonene	n.s.
7	cineol	n.s.
8	g-terpinene	n.s.
9	o-cymene	**

** - significant at 1% level ($p<0.01$)
* - significant at 2% level ($p<0.02$)
n.s. - not significant ($p>0.05$)

FIGURE 1 and TABLE 2 - Mean values and standard errors for the relative percentage of the main volatile components of the bouquet of *Eucalyptus globulus* (bark) attacked by *Phoracantha semipunctata* and attack-free. List of terpenes and levels of significance for the differences detected between the two tree conditions (Kruskall-Wallis test - Sokal & Rohlf 1981). Trees from Portalegre, Portugal, November 1991- September 1992.

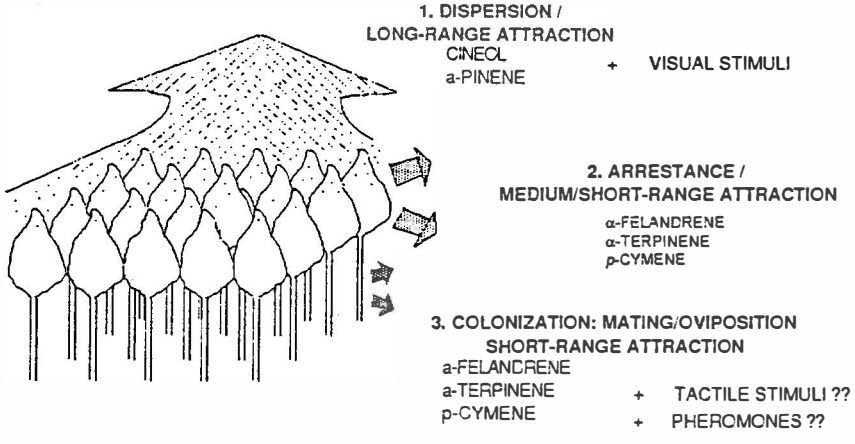


FIGURE 2 - Mechanisms of host selection and colonization by *P. semipunctata* - hypothetical scheme.

distinguished between the attractivity of logs placed at 1 m intervals, although no information is given about oviposition rates.

The mechanisms leading to dispersion and host colonization by *P. semipunctata* are hypothesized on Figure 2.

It is plausible that volatiles of the eucalyptus bouquet, emanated mainly by the leaves, will act as long range stimuli guiding the dispersing adults to host stands, or isolated groups of trees. A key role should be played by a-pinene and cineol, which clearly make up the major fraction of the leaves_emissions (Barata et al. 1991, Mateus et al. 1993). Visual cues involved in the recognition of the shape of the forest mass, will probably act in synergism with the olfactory ones at this stage. Arrestance should be provided by volatiles indicating host susceptibility, namely a-felandrene. This terpene which is normaly not emitted by leaves of healthy trees, has also been detected in eucalyptus under physiological unbalance, due to other causes apart from *P. semipunctata* attack (Mateus et al., unpubl.). Furthermore, substances like a-terpinene and p-cymene, which are mainly emitted by eucalyptus bark, might reinforce the susceptibility signal. According to wind tunnel observations, accoustical stimuli and aphrodisiac pheromones will probably influence the process of mate finding and acceptance.

Work is in progress to further substantiate the hypothesis presented.

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References

BARATA, E. , BONIFACIO, L., FARRALL, M.H., LIMA, M., LOPES, O., MATEUS, E., ARAUJO, J. & PAIVA, M.R., 1991. Identificação dos atraentes primários. In: Yearly Report 1991, Res. Contract "Protection of *Eucalyptus* against *P. semipunctata* Fab."University of Evora, pp. 18-21.

BARATA, E., MATEUS,E., ARAUJO, J. & FONSECA, P., 1992. Host-finding by *P. semipunctata* (Col., Cerambycidae). Host volatiles, electroantennograms and baited field traps. Proc. 8th Int. Symposium on Insect-Plant Relationships, Wageningen, in press.

CHARARAS, C., 1969 a. Biologie et écologie de *Phoracantha semipunctata* F. (Coléoptere Cerambycidae xylophage) ravageur des Eucalyptus en Tunisie, et methodes de protection des peuplements. Ann. Inst. National de Recherches Forestières de Tunie **2**, 1-37.

CHARARAS, C., 1969 b. Etude biologique de *Phoracantha semipunctata* F. (Coléoptere Cerambycidae xylophage) spécifique des Eucalyptus en Tunisie, et recherches sur la vitalité et adaptation de ces essences. C.R. Séances Acad. Agric. Fr. **55**, 47-57.

FARRALL, H., LENCART,P., LIMA, M.,LOURENÇO, T., ARAUJO, J., PAIVA, M.R.,1988. Análise dos níveis de ataque por *Phoracantha semipunctata* (Col.,Cerambycidae) para diferentes espécies de eucaliptos. Actas I Enc. Protec. Eucalipto contra *P. semipunctata,* ACEL, Lisbon. pp.95-109.

FARRALL, M.H., MATEUS, E. & PAIVA, M.R., 1993. Population interactions among exotic species in Portugal: eucalyptus plantations, *Phoracantha semipunctata* Fab. (Col., Cerambycidae) and *Avetianella longoi* (Hym., Encyrtidae), in prep.

GONZALEZ TIRADO, L., 1984. Lucha contra *Phoracantha semipunctata* Fab. el en suroeste español. Bol. Serv. Plagas **10**: 185-204.

HANKS, L.M., PAINE,T.D. & MILLAR, J.G.,1993. Host species preference and larval performance in the wood boring beetle *Phoracantha semipunctata* F.. Ann. Ent. Soc. Am., in press.

MATEUS, E., FARRALL, M.H. & PAIVA, M.R., 1993. Characterization of the physiological condition of *Eucalyptus globulus* Labill. by GC/MS analysis of the bouquet of odours, in prep.

MOORE, K.M., 1963. Observations of some australian forest insects. Proc. Linn. Soc. N.S. Wales, **88**(2): 221-229.

NEUMANN, F.G. & MARKS, G.C.,1976. A Synopsis of Important Pests and Diseases in Australian Forests and Forest Nurseries. Aust. For. **39** (2), 83-102.

PAIVA, M.R. & ARAUJO, J., 1987. Impacto de *Phoracantha semipunctata* na cultura do eucalipto no Alentejo. Actas I Cong. s. Alentejo, Evora, III Vol., pp. 1510-1516.

PAIVA, M.R., WANJURA, W. & FARROW, R.,1993. Ecology of Phoracanthini (Col.,Cerambycidae) borers in Australia, in prep.

POWELL, W., 1978. Colonisation of twelve species of Eucalyptus by *Phoracantha semipunctata* (F.) (Coleoptera: Cerambycidae) in Malawi. Bull. ent. Res. **68**: 621-626.

SCRIVEN, G.T, REEVES, E.L., LUCK, R.F.,1986. Beetle from Australia threatens eucalyptus. Calif. Agric. **40**: 4-6.

SOKAL, R. & ROHLF, J., 1981. Biometry. 2nd. Edition. Freeman & Co. New York.

TOOK, R.J., 1949. Beetles injurious to timber in South Africa. Sci. Bull. Dept. Agric. For. Un. S. Afr. **293**: 1-95.

CONTROL APPLICATIONS

PHEROMONES AND OTHER SEMIOCHEMICALS IN THE CONTROL OF APHIDS

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Abstract Recent studies (Pickett *et al.* 1992) have shown that semiochemicals play a more important role in aphid ecology than originally suggested, particularly in relation to longer range interactions. This increased understanding enhances prospects for aphid control by means of semiochemicals as an alternative to compounds employing toxic modes of action. However, for this objective to be realised, a still greater understanding of aphid chemical ecology is essential. Also, the use of semiochemicals must be integrated with beneficial organisms capable of reducing aphid populations. In such integrated regimes, pheromones and host plant kairomonal attractants play an important role in aggregating pests in traps or on trap crops for destruction by the biological control agents. The harvestable crop is protected partly by inhibiting host plant attraction by using non-host plant components. The study of such kairomone inhibitors is benefiting by recent discoveries on the nature and specificity of host and non-host plant component sensory receptors (Nottingham *et al.* 1991). New identification work will be described, together with attempts to use such agents in the field. Devlopments in exploiting host and non-host plant semiochemicals by molecular biology will be reported.

References

J A PICKETT, L J WADHAMS, C M WOODCOCK AND J HARDIE (1992) The chemical ecology of aphids. *Annual Review of Entomology* **37**, 67-90.

S F NOTTINGHAM, J HARDIE, G W DAWSON, A J HICK, J A PICKETT, L J WADHAMS AND C M WOODCOCK (1991) Behavioural and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology* **17**, 1231-1242.

THE COMMERCIAL IMPLEMENTATION OF MATING DISRUPTION FOR THE CONTROL OF THE RICE STEMBORER, *CHILO SUPPRESSALIS* , IN RICE IN SPAIN.

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Abstract: The mating disruption of *Chilo suppressalis* is the only instance of the successful commercial application of the mating disruption technique to a pest of a cereal crop. This presentation aims to relate some of our experiences with the commercial application of the technique in Spain, in particular, where the technique was first developed.

The development of the system was reported by Jones et al at the OILB meeting in Granada in 1990 and covered the development of the slow release formulations and proving the technology in the field starting in 1986.

Sale of the product **Selibate CS** commenced in 1989 under the Spanish equivalent of an EUP. The field optimised treatment was based on the application of 40g of pheromone per hectare in a 5% formulation applied as 100 point sources. The formulation was produced as a flat sheet which was stapled to wood stakes at a height of about 0.5 metres above the ground.

A total of 4,000ha of rice are treated annually with **Selibate CS.** It's application in the field is a major logistical exercise. The presentation will cover some of the problems and how they were resolved involving the large scale co-ordination of materials and people to get the material into the field in the short time window available.

Introduction

The mating disruption of the rice stem borer *Chilo suppressalis* is, so far, the only instance of the successful commercial application of the mating disruption technique to a pest of a cereal crop. The aim of the presentation is to relate some of our experiences with the commercial application of the technique in Spain where the technique was first developed.

The Pest

Chilo suppressalis is one of the most important pests of rice world wide. It is particularly important in the more temperate rice areas ie in Asia it is most important in Japan, Korea and China with it's importance decreasing as you approach the more tropical areas. In these areas it is replaced by other stemborers such as *Scirpophaga incertulas* and *S. innotata* . *Chilo* is also very important in

rice grown in the Middle East eg. in Iran and the southern countries of the former USSR. Fortunately the pest has never been introduced in the rice areas of the USA, Australia or Italy. In Europe it is only a pest in rice in Spain. Here it affects the rice of the Ebro delta and the Albufera near Valencia where a total of approximately 40,000 ha of rice is grown.

As it's common name indicates the larvae attack the plant by burrowing into the stem and eating out the soft growing tissues. This causes that particular stem to die, a condition often called "dead heart" early and "white head" later in the season. "Dead heart" damage, if mild, is not necessarily very dangerous because the plant is actively tillering and killed stems can usually be replaced. Later, as the plant flowers and the heads are forming, tillering is very much reduced and any damage "white head" at this stage is likely to result in economic loss.

Despite the fact that damage is rarely economically important early in the season the pest is actively controlled to prevent the build up of high populations which will be difficult to control later. There are normally three generations of Chilo in Spain and traditionally these have been controlled by the application of various insecticides mostly by regionally organised aerial ULV sprays. These are in general fairly effective but come with the inherent problems of applying broad spectrum insecticides (discussed later).

History of the Mating Disruption Technique

It is not the intention here to cover this in any great detail since the development of the system was reported by Jones *et al* at the OILB meeting in Granada in 1990 and in the attached references. Briefly the pheromone of *Chilo suppressalis* was identified as a blend of Z11-16Al, Z9-16Al and Z13-18Al. By their nature these aldehydes are very unstable so to make effective use of them a polymer slow release formulation was developed which effectively protected and slowly released the pheromone over a period of approximately 100 days. The first trials commenced in 1986. Trials over successive years optimised and verified the method. These are summarised in Tables 1,2 &3.

TABLE 1. Summary of experiments and results from the Ebro, Spain.

Year	Area treated (ha)	Distance between dispensers (disp/ ha)	Pheromone rate per hectare (g/ha)	Mean pheromone trap catch	Trap catch suppression index	Percent. damage by Chilo
1988	0.25	2x2 (2,500)	20	0	100.0	NE
	0.25	3x3 (1,111)	20	0	100.0	NE
	0.25	3x3 (1,111)	30	0	100.0	NE
	0.25	4x4 (625)	30	1	99.6	NE
		INSECTICIDE	0	232		NE
1989	7.9	4x4 (625)	30	0	100.0	0
	7.2	6x6 (278)	30	1	99.2	0
	8.5	8x8 (156)	40	3	99.2	0
	7.5	12X12 (70)	40	3	97.6	0.6
		INSECTICIDE	0	125		<1.0

NE = Not estimated

TABLE 2. Summary of experiments and results from Huesca, Spain.

Year	Area treated (ha)	Dispenser spacing (disp/ ha)	Pheromone rate per hectare (g/ha)	Mean pheromone trap catch	Trap catch suppression index	Percent. damage by Chilo
1987	1.0	2x2 (2,500)	10	8	95.7	NE
	1.0	2x2 (2,500)	20	0	100.0	NE
	1.0	3x3 (1,111)	10	35	81.2	NE
	1.0	4x4 (625)	10	22	88.2	NE
	1.0	5x5 (400)	10	50	73.2	NE
		CONTROL*	0	187		NE
1988	1.0	2x2 (2,500)	20	1	99.5	10
	1.0	3x3 (1,111)	15.5	0	100.0	10
	1.0	3x3 (1,111)	26.6	0	100.00	5
	1.0	4x4 (625)	30	0	100.0	10
		CONTROL*	0	238		80
1989	1.0	4x4 (625)	20	0	100.0	0
	1.0	4x4 (625)	30	0	100.0	0
	1.0	6x6 (278)	20	21	97.3	2
	1.0	6x6 (278)	30	1	99.8	0
	1.0	8x8 (156)	30	0	100.0	0
	1.0	8x8 (156)	40	0	100.0	0
	1.0	12x12 (70)	30	0	100.0	0
	1.0	12x12 (70)	40	0	100.0	0
		CONTROL*	0	796		>70
1990	6.0	12.5x13 (162)	45	2	99.7	0.11
	6.0	24x6.7 (161)	45	3	99.5	0.16
	6.0	36x4.5 (162)	45	3	99.5	0.18
		INSECTICIDE	0	704		0.08
		CONTROL*	0	704		26.76

NE = Not estimated * Controls without insecticide treatments

TABLE 3. Summary of experiments and results from Valencia, Spain.

Year	Area treated (ha)	Distance between dispensers (disp/ ha)	Pheromone rate per hectare (g/ha)	Mean pheromone trap catch	Trap catch suppression index	Percent. damage by Chilo
1988	60	2x2 (2,500)	20	2	99.7	0.195
		INSECTICIDE	0	692		0.04
1989	60	4x4 (625)	30	15	93.1	5.04
		INSECTICIDE	0	218		0.3
1990	30*	10x10 (100)	40	14	95.0	0.07
	30**	10x10 (100)	40	1.4	99.5	0.46
	1,200	8x8 (156)	40	9.3	97.0	0.26
		INSECTICIDE	0	295		--

* Borders of the treated site. ** Interior of the treated site.

Commercial Application

Sale of the product **Selibate CS** commenced in 1989 under the Spanish equivalent of an EUP. The field optimised treatment was based on the application of 40g of pheromone per hectare in a 5% formulation applied as 100 point sources.

Looking out across the rice areas of Valencia and the Ebro, which are covered by rice as far as the eye can see, it is difficult to believe that, as in most parts of the world, rice is produced on very small individual holdings. The average size of each holding is about 2 hectares. Most of the growers are part timers who either farm other crops elsewhere or hold other jobs and work the rice in their spare time. Because mating disruption works best where treatments are carried out on large contiguous and regular blocks the problem was one of ensuring the co-operation of the multitude of rice growers involved.

Fortunately for us the rice growers of the affected regions both form very close knit communities and because of the need for water management for example are used to working co-operatively. The other advantage was, ironically, that insect control was already handled communally with either the co-operative or the local office of the Ministry of Agriculture organising and/or supervising the application of insecticides which in these areas are applied aerially. It is these organisations which decide which treatments are applied and where. The farmers are then levied at a price to cover the cost of the treatments. Thus with the help of the local co-operative and Ministry of Agriculture it has been relatively easy for us to have the pheromone applied within the narrow parameters that it requires.

While it has been easy to select the treatment area for the pheromones, it is entirely another matter to actually carry out the treatment. The over wintering generation of Chilo adults are on the wing by the end of May. To make maximum use of the pheromone formulation which needs to protect the crop until harvest in early October, application should be timed to as near to the moth emergence time as is safely practicable. In Spain the local workers allow a period of about two weeks in which to apply the pheromone.

Rice (which is effectively a grass growing in water) is not an ideal crop into which to apply point source devices. Unlike top fruits or even cotton there is nothing to which a dispensing device

can be attached. As well as applying the dispensing device the user must also provide a support for the dispenser. It was not for nothing that every effort was made to reduce the number of point source per hectare despite the fact that trial results indicated that half the pheromone rate was equally effective if the dispenser rate was above 500 per hectare. 100 points per hectare was the lowest that we could safely go to.

The current formulation is produced as a flat sheet which needs to be stapled to wooden stakes at a height of about 0.5 metres above the ground. Before application can commence sufficient wooden stakes must be sourced. Each stake is about 0.75 metre in length and about 5mm diameter. To treat for example 1000 ha of rice 100,000 of these must be sourced. Before application the dispensers must then be stapled to the stakes and finally these must be trucked out to the rice paddies where gangs of workers walk 10 metres apart through the paddies with mud and water often up to their knees planting a stake every 10 metres. Assuming it takes a minimum of 1 hour for 1 person to treat 1 hectare, 1000 ha requires 1000 man hours to treat ie 125-8 hour days. To treat each multiple of 1000 ha in 2 weeks requires at least 10 labourers.

In 1992 a total of about 4,000 hectares of rice was treated with **Selibate CS** of a total of about 40,000 hectares with a Chilo problem in Spain. With the current state of resources and the technology this is rapidly approaching the limit which they can physically and financially handle. To go further would require substantial external support. While **Selibate CS** is not much more expensive than the currently used insecticides, the extra costs in application increase the total costs significantly above those of the current insecticide regime. Up to now these extra costs have been met by the farmers with subsides from the Spanish government.

Spain is a fast developing EC nation with labour costs now approaching those of any developed country. To, firstly afford the labour costs, then to actually find and organise enough reliable workers for such a short and fixed period is a major task and requires considerable forward planning. These problems should be much less important in developing countries where labour is more readily available and far less expensive.

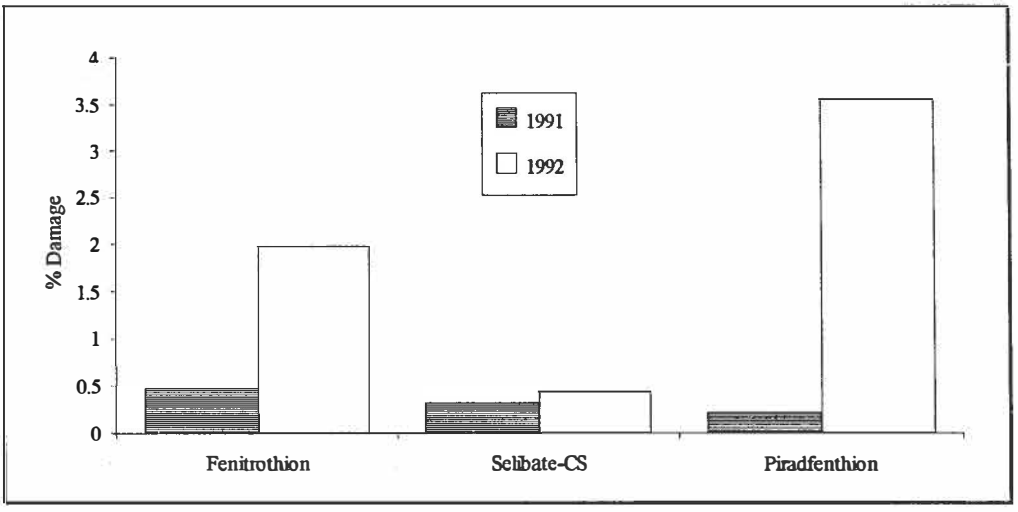
The formulation has been designed to protect the rice for the length of the vulnerable so the dispensers need only applied once. At the end of the season the rice is harvested by combine harvesters. Fortunately the Spaniards have been able to source wooden stakes which are weak and easily degraded. The harvesters have no trouble in cutting through these and discharge both stake and dispenser without contaminating the grain.

With all the difficulties I have outlined one would ask why are they doing it. Firstly, the system works with the pheromone giving as effective protection as the insecticide treatments. The Ministry of Agriculture is particularly supportive of the system since they played a major part in it's development. For the Spaniards the concern is that rice is grown in national parks of great ecological importance. There is a great deal of pressure from various environmentalists movements both local and international to ban the use of insecticides in these areas because of the harm they do to the eco-system. Some factions of these movements have demonstrated violent tendencies and there are many, largely unsubstantiated, stories circulating of sabotage of spray equipment etc. True or not these stories serve to fuel the concern felt by the industry. Environmentalists aside the rice areas are important as natural fish nurseries as well as being the sites of many fish farms. In both cases the application of insecticides, which kill fish, have a serious impact on the fish industry.

The application of pheromones for the large scale control of Chilo has not been without it's problems. The main area of concern has been in the Ebro delta. Here, for reasons unknown, there have been several cases of problems with the pheromone system. This has meant that in some paddies additional insecticide treatments were necessary to control the pest. In fairness the classic insecticide program has also the same problems in the Ebro with many supplementary applications

required in some areas. This is in sharp contrast with the system in the Valencia area where the pheromone has not once experienced any efficacy problems despite some insecticide failures. This is shown by the Graph 1.

GRAPH 1. Comparison of the efficacy of different treatments for the control of Chilo in Valencia in Spain, 1991-92 (region averages).



There has not yet been time to clarify the differences between the Ebro and Valencia. It has been speculated that the different results may be due to factors such as different population pressures, the rice varieties, planting times, proximity to wild hosts and possibly even nitrogen fertiliser levels. Observations in the field last year indicated a strong relationship between variety and damage. Comparison of two neighbouring plots showed an immediate and dramatic change in damage levels from one to the other. One with virtually no damage and the other with damage well above normal thresholds.

Another apparently important factor is planting date. Because of a different system of irrigation Valencia growers are obliged to closely synchronise planting. This is not the case in the Ebro with neighbouring plots often considerably different in age. Again it was observed that this had an effect on damage levels with the early planted rice being much more heavily attacked. Whether this reflects a greater absolute population or merely the concentration of attack is as yet unclear.

To look into these problems this year we plan a major trial program to try to evaluate the role of some of the above factors in determining the effectiveness of the mating disruption system. It is clear already that to assure the success of the method we need a cultural systems approach to assure that mating disruption control of Chilo is given the best conditions in which to operate.

To make the task of applying the pheromones easier we are constantly looking to better methods. Unfortunately with the hand applied formulation there is little we can do about the requirement for the wooden stakes. As mentioned there is little chance of going to an application rate

much below 100 points per hectare. There are opportunities, however, to make the application of the dispensers to the stakes easier. We have recently been working on improved formulations which don't need to be stapled to the stake. These are designed as tubes that will slide down over the top of the stake but remain in position for the season. This should substantially reduce the time taken to apply the dispenser to the stake. In fact this operation can now be done in situ as the worker walks through the paddy.

The Future

The approach which would make everybody's life easier in this area is the development of a sprayable formulation of the pheromone. This solution is fraught with many problems. For us the main problems is to develop a sprayable formulation of the pheromone. This is already very difficult for any kind of volatile compound but is particularly so for the very unstable components of the Chilo blend. At the moment we have a formulation which will last about a week in the field. Unfortunately to be economically viable we need a formulation which will ideally last about three weeks. Our chemists are confident that it can be done using our proprietary technology but it will involve a considerable investment.

The other major problem facing the use of the sprayable pheromone formulation is the nature of the crop. At the time of the first generation there is very little vegetation in the paddy and a vast expanse of water. Of any application of the pheromone at this time at least 90% of the material would be immediately lost into the water. With the high cost of the materials we can not afford to waste this material. Later in the season, as the rice vegetation covers more of the surface the formulation can be applied more efficiently. How we a can overcome the problems of the first generation is yet to be solved. Various options have been proposed eg. application of insecticide first followed by pheromone or application of a small number of stake dispensers around borders followed by sprayed pheromone as the rice crop grows. All these options and others will need to be examined before we can envisage going to a sprayable technology.

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References

ANON. 1988. Ensayo del sistema de confusion sexual en la lucha contra *Chilo suppressalis*, Walker, en el arrozal de Valencia. Phytoma Espana, **3**: 30-37.

BEEVOR, P.S., HALL, D.R., NESBITT, B.F., DYCK, V.A., ARIDA, G., LIPPOLD, P.C. & OLOUMI-SADEGHI, H. 1977. Field trials of the synthetic sex pheromones of the striped rice borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), and of related compounds. Bull. Ent. Res., **67**: 439-447.

KANNO, H., TATSUKI, S., UCHIUMI, K., KURIHARI, M., FUKAMI, J., FUGIMOTO, Y., & TATSUNO, T. 1978. Disruption of sex attraction in the rice stem borer moth, *Chilo suppressalis*, Walker, with components of the sex pheromone and related chemicals. Appl. Ent. Zool., **13**: 321-323.

CORK, A., HALL, D.R., SMITH, J.L. & JONES, O.T. 1989. A new resin formulation for the controlled release of insect pheromones. Controlled Release Society: 16th International Symposium on Controlled Release of Bioactive Materials. Chicago, USA.

KANNO, H., HATTORI, M., SATO, A., TATSUKI, S., UCHIUMI, K., KURIHARI, M., FUKAMI, J., FUGIMOTO, Y., & TATSUNO, T. 1980. Disruption of sex pheromone communication in the rice stem borer moth, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), with sex pheromone components and their analogues. Appl. Ent. Zool., **15**: 465-473.

KANNO, H., HATTORI, M., SATO, A., TATSUKI, S., UCHIUMI, K., KURIHARI, M., OHGUCHI, Y. & M., FUKAMI, J. 1982. Release rate and distance effects of evaporators with Z-11-hexadecanal and Z-5-hexadecene on disruption of male orientation in the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). Appl. Ent. Zool., **17:** 432-438.

LEE, J.O., PARK, J.S., GOH, H.G., KIM, J.H. & JUM, J.H., 1981. Field study on mating confusion of synthetic sex pheromone in striped rice borer. *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). Korean J. Pl. Prot., **20**: 25-30.

MOCHIDA, O., ARIDA, G.S., TATSUKI, S., FUKANI., J. 1984. A field test on a third component of the sex pheromone of the rice stem borer, *Chilo suppressalis*, in the Philippines. Ent. exp. appl., **36**: 295-296.

NESBITT, B.F., BEEVOR, P.S., HALL, D.R., LESTER, R. & DYCK, V.A. 1975. Identification of the sex pheromones of the moth *Chilo suppressalis*. J. Insect Physiol., **21**: 1883-1886.

OHTA, K., TATSUKI, S., UCHIUMI, K., KURIHARA, M. & FUKAMI, J. 1976. Structures of sex pheromones of rice stem borer. Ag Biol. Chem., **40:** 1897-1899.

TANAKA, F., YABUKI, S., TATSUKI, S., TSUMUKI, H., KANNO, H., HATTORI, M., USUI, K., KURIHARI, M., UCHIUMI, K. & FUKAMI, J. 1987. Control effect of communication disruption with synthetic pheromones in paddy fields in the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). Jpn. J. Appl. Ent. Zool., **17:** 432-438.

MATING DISRUPTION OF THE EUROPEAN GRAPEVINE MOTH *LOBESIA BOTRANA* SCHIFF. (LEPIDOPTERA: TORTRICIDAE) INVESTIGATIONS ON THE TEMPORAL AND SPATIAL DISTRIBUTION OF POPULATIONS

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Abstract In order to achieve more information on the role female moths of L. botrana play in mating disruption, investigations were conducted in a pheromone treated system of approximately 40 hectares of vineyards in the vine-growing region of the Palatinate in Germany. Of special interest was the evaluation of the parameters density of moths, fecundity status and sex-ratio and their influence on the infestation.

Results and Discussion

During 1992, our investigations focused on the following issues:

1. Determination of the influence of:
   a. density of moths
   b. fecundity status
   c. sex-ratio

   of *L. botrana* on the infestation within pheromone treated systems.

2. Influence of female moths of *L. botrana* on the temporal and spatial distribution of populations.

3. Influence of problem zones such as
   a. borders of a pheromone treated system
   b. gardens, hedges and shrubs

   on the infestation.

Mating disruption of *L. botrana* was conducted in a coherent area of approximately 40 hectares of vineyards in the vine-growing region of the Palatinate in Germany. The pheromone dispensers (500 dispensers per hectar containing 120 g/ha E7-Z-9 DDA) were supplied by the BASF AG. In a distance of 200-300 m in different directions from the pheromone treated site, 6 control plots each 150 m2, were left insecticide untreated.

The monitoring of the moth flight was accomplished by counting every other day the number of moths caught per trap (pheromone and wine-bait traps). The flight of the first generation of

moths lasted from April 20th until June 1st, the second generation started June 22nd and lasted until August 3rd and a third generation appeared from August 27th until October 5th. A comparison between the pheromone treated site and the control plots showed that the number of female moths caught in the wine-bait traps (food traps) was always significantly lower within the pheromone treated site in comparison to the control plots. It is known that a low population density is a precondition for the effectiveness of the mating disruption technique. Under pheromone conditions male moths as well as female moths show different behaviour patterns compared with the behaviour under pheromone free conditions. These behaviour patterns must result in an altered attractiveness of food traps within a pheromone atmosphere. Female moths do not show a distinct flight activity during their calling and this does not change until mating has occured. Therefore wine-bait traps are not able to catch as many female moths under pheromone conditions as they do under pheromone free conditions. This may lead to the conclusion that the actual density of female moths under pheromone conditions must be higher than indicated by the catches in food traps.

The different behaviour pattern of male moths under pheromone conditions however, leads to an altered sex-ratio, especially during the flight period of the second generation of the pest. For several years it has been observed that the number of male moths caught in wine-bait traps as well as in pheromone traps is much lower during the flight period of the second generation in contrast to the first and third generation. Under pheromone free conditions the sex-ratio is biased in favour of female moths during the second generation. In 1992, the sex-ratio was determined by the use of wine-bait traps and equalled 10% male moths : 90% female moths within the control plots. Within the pheromone treated site however, the sex-ratio was 38% male moths : 62% female moths. It must be assumed that the higher number of male moths caught in the food traps within the pheromone treated site was caused by the higher flight activity of the male moths. The pheromone atmosphere causes confusion of the male moths, which in search for calling female moths, obviously get trapped more frequently in the wine-bait traps. Therefore a higher percentage of male moths caught in food traps within a pheromone treated system could be an indication for the effectiveness of mating disruption.

With respect to the spatial distribution of populations of L. botrana the development of the density of moths (males and females/wine-bait trap) was determined at different spots of the pheromone treated site. The following three situations were observed:

1. Spots or areas with a high density of moths during the first and the second generation (problem zones or "wormholes").

2. Spots or areas in which the density of moths increased from the first to the second generation.

3. Spots or areas in which the density of moths decreased from the first to the second generation.

Regarding the spatial distribution of infestation, it showed that the infestation was high along the borders of the pheromone treated site, especially where houses, gardens, hedges and shrubs were near. The higher "border infestation" could be caused by female moths, which had been mated before entering the pheromone treated system. Whether virgin female moths are able to leave the pheromone treated site in order to get mated before returning for egg-depostion or whether mated female moths enter a pheromone treated system from adjoining pheromone free vinyards is under investigation at present. Although few spots showed higher infestation rates within the center of the

pheromone treated site, the economic threshold of 15-20% during the first and 5-10% during the second generation was not exceeded in general. The infestation rates showed a tendency to decrease from the border zones towards the center of the pheromone treated site.

Within the control plots and the pheromone treated site the following mean infestation rates (larval attacks) were determined (with deviation and number of samples):

	1.Generation	2.Generation
control plots	68.9% (12% - 216.5% ; n = 1075)	235.2% (46% - 632%; n = 650)
pheromone treated site	5.0% (1.77% - 9% ; n = 5000)	6.9% (4% - 13.7% ; n = 3000)

Aiming at a possible prediction of infestation, the density of female moths caught in wine-bait traps was compared with the infestation rates in the close vicinity to the traps. It showed that spots with a high density of female moths generally showed higher infestation rates, while spots with a low density of female moths showed lower infestation rates. The exceptions made it necessary to take a closer look at the fecundity status of the female moths caught in the food traps. Regarding the density of female moths and their fecundity status at spots of determined infestation, it shows that spots with a comparably high density of female moths did not necessarily show a lower percentage of virgin female moths. On the other hand, spots with a comparably low density of female moths could show a high percentage of mated female moths, which lead to higher infestation rates. Spots with low infestation were always characterized by a high density of virgin female moths.

In the future, our investigations are going to be focused on a precise evaluation of population density, fecundity status and sex-ratio at spots of known infestation rates for a possible prediction of infestation.

HOW TO ACHIEVE BETTER RESULTS WITH THE MATING DISRUPTION TECHNIQUE

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In the last few years the mating disruption technique has been significantly improved. In Europe, already in 1986, the first commercial pheromone, RAK 1 or Bocep Viti against Eupoecilia ambiguella Hbn. became available (Neumann et. al., 1986). Consequently other pheromones have been introduced on the market, e. g. RAK 2 (for Lobesia botrana Schiff.), RAK 3 or Bocep Carpo (for Cydia pomonella L.), RAK 5 or Quant Gm (for Grapholitha molesta Busck.). Much of experience was gained in more than ten years of experimental use of pheromones on several thousands of hectares all over Europe. This experience may be used to avoid errors but also for further improvements of the mating disruption technique.

We still do not know exactly how the mating disruption technique works. Is it adaptation, false trail following? And/or when is it or with which pheromone is it adaptation, false trail following, camouflage etc. Therefore true guidelines from basic research as to what to improve in the field are still missing. In order to find out what quantity and release rates of dispensers are required, many experiments in rather large areas were performed. Due to tremendous variations in population densities it was very hard to interprete these experiments. Sometimes we got the impression that some applied formulation worked very well, whereas this effect should in fact be ascribed to a very low population density and vice versa. To avoid such more statistical problems many repetitions on many different sites are required. We at BASF gathered experience in an area of over 5000 ha and with many different application forms.

Before entering the market, we had not only to accumulate much experience about the behaviour of dispensers, farmers, production facilities but also about practical conditions in the field. We were to learn which circumstances fit which dispensers and what application rates were required to be almost certain about success. Something which appeared to be of major importance was to be able to estimate population densities, as beyond a certain limit the efficacy of the method seems to deteriorate, an effect I would like to discuss here in some more detail.

POPULATION DENSITY

The effect of population density are often not fully understood. Even between neighbouring plots population densities may vary enormeously. This can be clearly demonstrated in a trial at Rothenberg (fig. 1).

We would have obtained a completely different view on the effectiveness of our dispensers if we would just have analyzed the local area B. Also between successive years the population density may vary enormeously. Thus satisfactory results may be gained over several years, but due to some regional event damage may suddenly increase (table 1).

Figure 1: Different infestation levels after treatment with pheromones

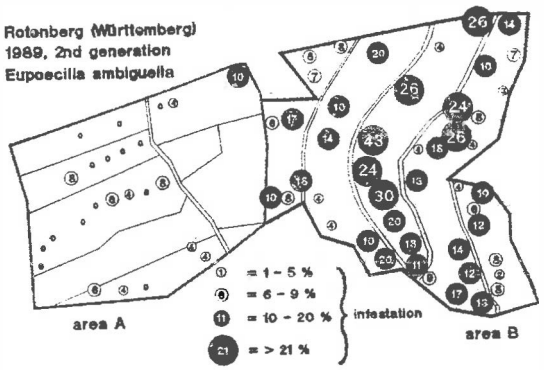


Table 1: Pheromone-trial, viticulture, Switzerland 1988-92
Grape berry moth (Lobesia botrana)
- Venthone -

Chateauneuf - Sion (Wallis)

year	% infestation			
	pheromone		untreated	
	1st gen.	2nd gen	1st gen.	2nd gen.
1988	2.0	4.6	5	16.3
1989	1.16	1.16	15.3	40
1990	0.5	15	8.6	103
1991	0.3	0.5	10	35
1992	0.5	0.25	11	15

It should therefore be emphasized, that the efficacy of a formulation can only be appraised after many experiments of many sites and years.

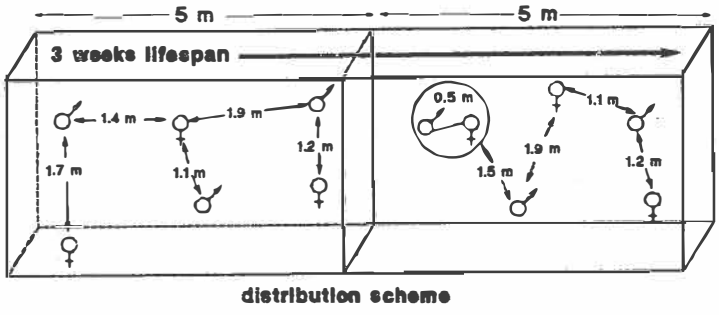
During an IOBC-Meeting in San Michele in 1992 I already discussed that the mating disruption technique may only be applicable at low population densities (Table 2, Neumann 1992). Within a pheromone atmosphere the pheromone plumes emitted by females will always remain attractive locally. Flying males may during their active periods, enter such local attraction zones and mating may occur. Stüber (1988) found that females of the Apple clearwing moth often change calling positions - before the desired mating -, thereby increasing their attractive space.

Table 2: T h r e s h o l d s

Crop	Pest	Dose g/ha/Mon.	previous generation ? which ?	Threshold initial population %
Grapes	Eupoecilia ambiguella Hbn.	50	1st generation	10
	Lobesia botrana Schiff.	25-35	1st generation	4-5
Apples, pears	Cydia pomonella L.	25	last generation of last year	1 incl. windfall
	Adoxophyes orana, F.v R. Archips spp. and others	25	last generation of last year	1 fruit 5 shoots
Peaches	Cydia molesta Busck	30-35	last generation of last year	1 fruit incl. windfall 3 shoots

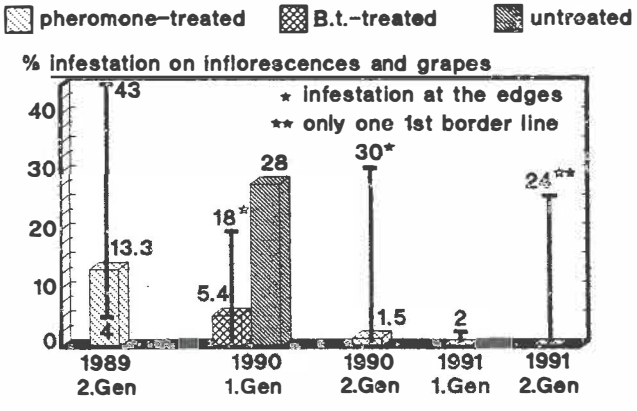
In viticulture such a behaviour could lead to the situation that from 6 individuals (three males and three females) during their 3 adult weeks still one successful mating would occur (fig. 2). This however could lead to a 100 % infestation. To illustrate: In 5 meters row of vine with 100 grapes, it could happen that 100 eggs laid by 1 female would become larvae.

Figure 2: Population dynamic



At Rothenberg (local area B) we found out how to sufficiently reduce a local population and gain successful disruption in the following years or generations. In the second generation of 1989, local infestations of up to 43 % were observed. Thus insecticides were applied to reduce local populations. In the following generation the situation appeared to be stable, and an average infestation level of 4.5 % was achieved (Kast et al. 1991). This result however can only be fully appreciated if we consider the reduction of the natural population as well, in the control an infestation level of 28 % was observed. In consecutive years (1991 and 1992) the effectiveness of the mating disruption technique in this area was confirmed and the population remained at a low, insignificant level (fig. 3).

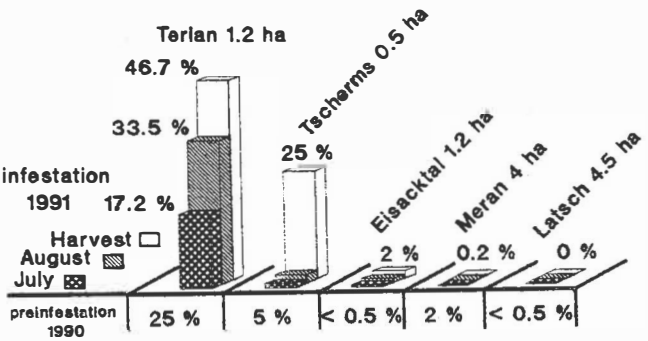
Figure 3: Effect of the population density of E. ambiguella on mating disruption



As I mentioned before, a low population density in the previous generation is of decisive importance for a fruitful application of the mating disruption technique. Only at a low population density we may be certain, that the female is unable to attract males.

With the codling moth we often started with acceptable infestation levels. However, later on the infestation levels might rise suddenly beyond 1 - 2 % (fig. 4, Waldner 1991).

Figure 4: Control of Codling moth by the mating disruption technique, South Tirol 1990/91



With an ever increasing number of newly emergeing moths (fig. 5), this increase of infestation might be attributed to the fact that an agreeable relationship between the concentration of pheromone and population density has been surpassed (fig. 6) and more and more random matings happen. Females really no more need the pheromones for mating (Neumann, 1992).

Figure 5: Influence of population density on efficacy of pheromones

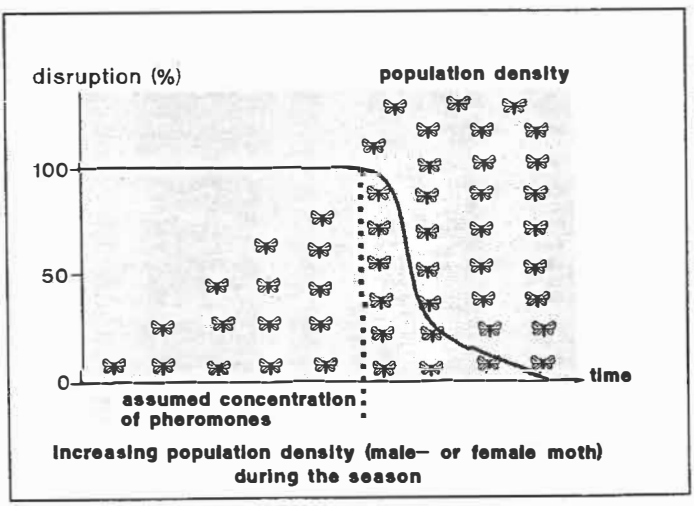
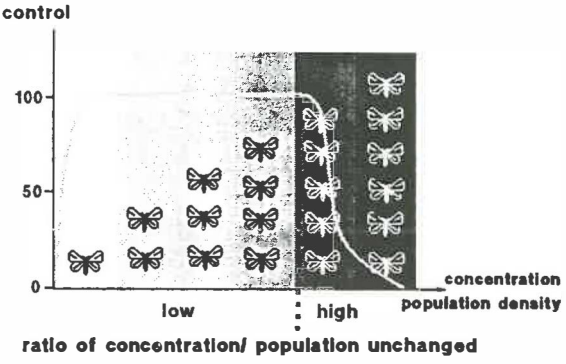


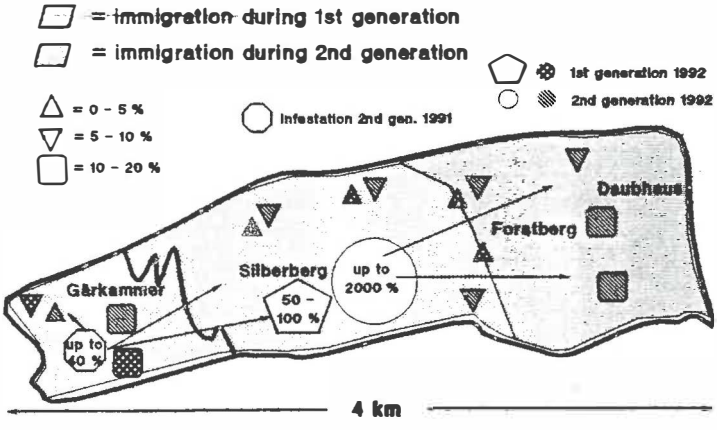
Figure 6: Influence of pheromone concentration- and population density with the efficacy of pheromones



FLIGHT ACTIVITY AND MIGRATION

In San Michele special attention was attributed to the migration of fecundated females over larger distances. As large distance migration has not often been observed in dedicated experiments, its effect are often not acknowledged. Outrageous, sudden infestation levels of Lobesia botrana or Cydia pomonella have however been observed both in vineyards and in apple plantations, which before had been virtually free of attack. At present we observe a spread of Lobesia into vine growing areas which were for many years exclusively infested by the grape berry moth (E. ambiguella). Such a takeover may take dramatic dimensions, as shown in an example of the Ahr-valley near Bonn in Germany in 1992 (fig. 7). Lobesia had infested for many years the area Gärkammer, but in 1992, during the flight period of the first generation in May, these animals were suddenly also found in distant pheromone treated areas, causing up to 100 % damage in the first generation, and up to 2000 % after an additional curative insecticide treatment in the second generation. Despite the fully developed dense vegetation, a further spread of this species into the adjacent untreated area Forstberg was observed during the flight period of the second generation, causing a rise in damage from 0 to 20 %.

Figure 7: Influence of immigration



The situation in the Ahr-valley demonstrates that long distance migration and the subsequent infestation may be such abundant, that several methods on control do not suffice any more.

CONCLUSION

In order to reach fair results with the mating disruption technique, the following special measures must be taken.

- The natural population density has to be sufficiently low or has to be reduced by one or more initial insecticide treatments.
- The formulation applied should be adapted for the requirements of the mating disruption technique. Their dissipation rate of pheromone should be rather constant at a level of 25-60 g/ha and month during the entire vegetation period.
- The treated area with one or several plantations should be sufficiently large. This will result in a rather uniform pheromone cloud and less boundary effects. Also the immigration of fecundated females may thus be reduced.
- A local organization of farmers should be set up in order to manage the uninterrupted treatment of a wide area.
- Care should be taken that also with time, no pause occurs in the application of the mating disruption technique. The omission of the treatment of one single generation will have vary negative effects.
  If the precautions mentioned above are considered, the mating disruption techniquemay lead to a very low, persistent level of damage, without the variations in efficacy found with insecticides.

REFERENCES

Kast, W.K.; Munder, H.; Gasser, A. (1991) Erfahrungen mit der Verwirrungsmethode gegen den Einbindigen Traubenwickler. Der Deutsche Weinbau, 10, 12. April, 374-377.

Neumann, U.; Harries, V.; Vogt, H.; Schropp, A.; Englert, W.D.; Schruft, G. (1986) RAK I - Eupoecilia ambiguella pheromone: The first mating disruption product for commercial use in German vineyards. Brighton Crop Protection Conference - Pests and Diseases 1986, 1., 371 - 375.

Neumann, U. (1992) Avoiding pitfalls in confusion: A review of mating disruption. Workshop on mating disruption in orchards and vineyards. S. Michele a/A (Italy), 30. August - 3. September 1992. In: IOBC/WPRS, Bulletin 1992 / XV/5.

Stüber, R.; Dickler, E. (1988) Untersuchungen zur Biologie und zum Verhalten des Apfelbaumglasflüglers Synanthedon (BORK.) (Lepidoptera: Sesiidae) als Grundlage für seine Bekämpfung mit Hilfe der Verwirrungsmethode. Mitt. Biol. Bundesanst. Land- u. Forstwirtschaft Berlin-Dahlem, 241, 1435.

Waldner, W. (1991) Lockstoffe gegen Apfel - und Fruchtschalenwickler in Südtiroler Obstbaubetrieben 1991. Südtiroler Beratungsring für Obst- und Weinbau, Lana, South Tirol, IT.

MATING DISRUPTION OF CODLING MOTH AND FRUIT TREE LEAFROLLERS IN APPLE ORCHARDS WITH TNO DISPENSERS

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Abstract In three consecutive years (1990, 1991 and 1992) TNO has tested the method of mating disruption against the codling moth (*Cydia pomonella*) in apple orchards in Spain (**1**) (0.7-2.6 ha) and in 1992 against the codling moth and fruit tree leafrollers in an apple orchard (1.0 ha) in the Netherlands (**2**), using a new, highly versatile controlled release dispenser.

Codling moth dispensers in the form of flat square wafers, each containing 80-240 mg of Codlemone (E,E-8,10-dodecadien-1-ol) were applied at densities of approximately 500/ha. Leafroller dispensers also were applied at densities of 500/ha and contained 650 mg of Z-11-tetradecen-1-ol acetate, the common component of the sex pheromone of various leafrollers, including *Adoxophyes orana, Archips podana* and *Pandemis heparana*. Whereas average temperature in the Netherlands is lower than that in Spain, dispensers of different composition (prepolymer mixture) were made, specifically adapted to local circumstances.

In areas with low population densities fruit damage was less than 1% in all plots. With higher densities (Spain, 1990 trials) damage at the end of the season could become as high as 3.4 % compared to 3.1% in the chemically treated plot.

In the Netherlands the mating disruption treatments resulted in season-long satisfactory control of both codling moth and leafrollers when compared with a chemical treatment. In all field tests the release of pheromone from the dispensers remained on a sufficiently high level during the whole season.

Introduction

A new type of controlled release dispenser for pheromones has recently been developed by TNO. This monolithic device is made of a crosslinked polymeric matrix prepared by radiation curing of acrylated prepolymers. The pheromone and protective compounds like UV-absorbers and anti-oxidants are added to the prepolymer mixture prior to polymerization. The release characteristics can be adjusted by varying the crosslink density of the matrix, the pheromone loading or the surface/volume ratio of the dispenser. Prototypes for a wide variety of semiochemicals were successfully made showing the very versatile nature of this type of dispenser. Here we report the results of mating disruption trials in Spain during three consecutive years (1990, 1991 and 1992) and in the Netherlands in 1992.

Materials And Methods

The tests in Spain in 1990 were conducted in two apple orchards: 1) Mas Badia, size 6000 m^2, provided with 324 codling moth dispensers (2.5 x 2.5 cm, 540 dispensers/ha) and 2) Cabanes, size 8925 m2, treated with 510 codling moth dispensers (572 dispensers/ha). Each dispenser contained 85 mg of E,E-8,10-12:OH. The number of dispensers set out along the border of the orchard had a double density compared to the centre of the field.

In 1991, experimental plots were also situated in Mas Badia (7800 m^2, 647 dispensers/ha) and Cabanes (9950 m^2, 550 dispensers/ha). Pheromone loading was 210 mg per dispenser.

In 1992, the pheromone treated plots were situated in Cabanes (27,000 m^2, 541 dispensers/ha) and Armentera (11,000 m^2, 528 dispensers/ha). Each dispenser contained 240 mg of E,E-8,10-12:OH.

Population development was monitored and damage assessments were made according to current experimental methods (trap catch reduction in monitoring traps, live female traps, numbers of infested fruits, larval band traps, yield damage). Pheromone treated plots were compared with untreated control plots and with plots under standardized IPM regimes.

In The Netherlands in 1992 a test was conducted near Wageningen (size of pheromone treated plot 10,000 m^2, 500 codling moth and 500 leafroller dispensers/ha). The codling moth dispensers contained 210 mg E,E-8,10-12:OH and the leafroller dispensers were loaded with 650 mg Z11-14:Ac. For comparison, population- and damage assessments were made in an IPM control plot.

The codling moth dispensers for this test were of different composition than those used in Spain.

During the season small numbers of pheromone dispensers were recollected from orchards at intervals for laboratory analysis of actual release rates and residual pheromone content. Release rates were measured by collecting volatiles on a polymeric absorbant in a temperature and air-speed controlled oven and subsequent GC analysis. Residual pheromone content was determined using Soxhlet extraction of dispensers.

Results And Discussion

In 1990 codling moth infestation levels were clearly different between the two experimental sites in Spain. At Mas Badia low numbers of moths were caught in all plots and fruit damage was below 1 % in both the pheromone plot and the untreated control.

At Cabanes trap catches were higher in the untreated control as well as in the chemical treatment. In the pheromone plot, however, no captures were found. At harvest fruit damage in the pheromone plot was at the same level as that observed in the chemically treated plot (3.1 % vs 3.4 % respectively). In the untreated control 7.6 % of fruits was infested by codling moth larvae.

Analyses of recollected pheromone dispensers showed a gradual decrease of pheromone content at both locations (Fig. 1, Left). After 20 weeks the pheromone residue was high enough to give sufficient release for effective mating disruption.

Trials in 1991 gave similar results: at Mas Badia population densities of codling moth were again low, leading to insignificant damage (less than 1 %) at harvest in all three plots. At Cabanes much higher densities were found. Trap catches at the end of the season were 239 (untreated plot), 102 (chemical treatment plot) and 40 (pheromone plot), respectively. Live female traps caught no males in pheromone treated plots, whereas in untreated plots more than 60 males were caught.

Captures in larval band traps were equal in the pheromone and chemically treated plot (1.5 larvae/trap). In the untreated plot almost 80 larvae per trap were observed. Severe fruit damage was observed during the season in the untreated plot: 1.4% at the first generation; 53.4 % at the second generation and 76.2 % at harvest.

Damage figures for the pheromone treated plot were: 0.2 %, 1.4% and 7.8 % at harvest; for the chemically treated plot these figures were: 0.1 %, 2.0 % and 2.1 %. Fruit damage thus showed a similar pattern in these two plots except for the last generation of the codling moth. Monitoring traps indicated significant invasion into the pheromone plot from adjacent fields. Given the very high population densities of moths at that time, the higher damage in the pheromone plot was presumably inflicted by larvae from immigrated mated females.

Analyses of field exposed dispensers again showed a gradual release of pheromone during the course of the season (Fig. 1, Right). This release again appears to be sufficiently high for effective mating disruption.

In 1992 the field trials in Spain suffered from severe bad weather in the beginning of the season. Because of this, population development of moths was almost absent at both locations and significant bio-efficacy data could not be determined.

From chemical point of view, however, the dispensers performed very well during the season as is shown in Fig. 2. After 21 weeks about 30-35 % of pheromone is still present in the dispenser and the release rate at the end of the season is still 50 % of the initial rate.

In contrast to the situation in Spain, 1992 was a very good experimental year in The Netherlands with high population densities of especially various leafroller species. Table 1 shows trap catches in sex pheromone loaded monitoring traps for the respective species. Trap catch reduction in pheromone treated plots is very high for all species.

Table 1. Trap catches and fruit damage in field test in Wageningen, 1992

	Trap catches		Fruit damage	
Moth Species	TNO plot	Control plot	TNO plot	Control plot
Cydia pomonella	0	7	0.25%	0.6%
Adoxophyes orana	1	132	z	z
Pandemis heparana	0	16	0.15%	0.4%
Archips podana	0	81	z	z

At harvest hardly any fruit damage was observed in the pheromone plot indicating the effectiveness of the new dispensers for mating disruption for both the codling moth and leaf roller species.

Figure 3 shows the release characteristics of codling moth and leaf roller dispensers. At the end of the season both types of dispensers still contain approx. 40 % of the active ingredient. During the season the decrease in actual release rate is rather low, indicating that also this type of dispenser, with a composition specifically adapted to moderate climatological conditions, is very suitable for successful mating disruption.

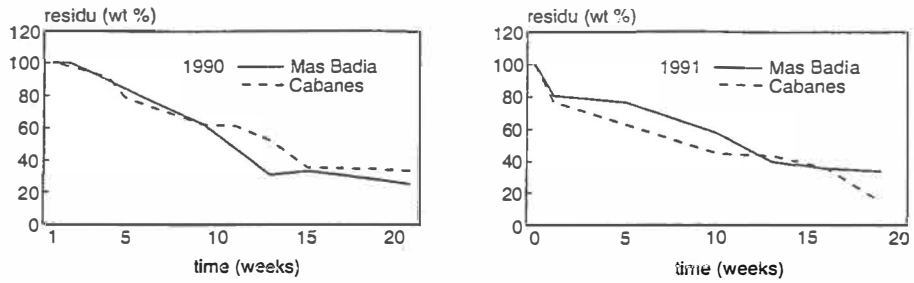

Figure 1. Residual pheromone content of field exposed dispensers in Mas Badia and Cabanes in 1990 and 1991

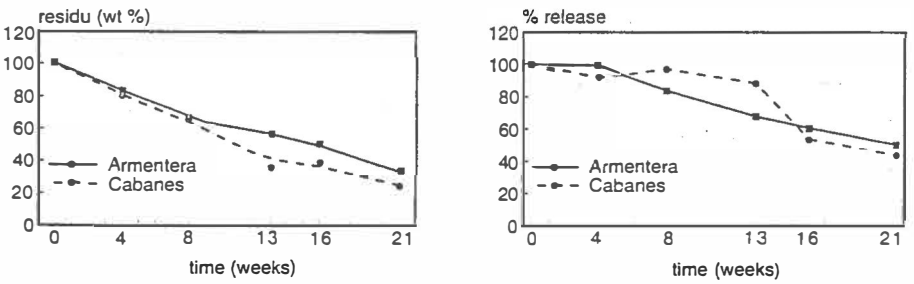

Figure 2. Residual pheromone content and release characteristics of field exposed dispensers in Mas Badia and Cabanes in 1992

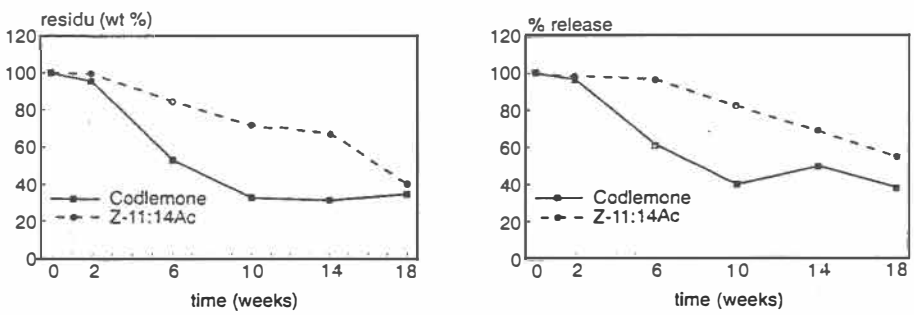

Figure 3. Residual pheromone content and release characteristics of field exposed dispensers in Wageningen in 1992

Conclusion

The results of field tests show that a newly developed controlled release dispenser can be successfully used for mating disruption against various insect species in orchards. Analytical measurements have shown that dispensers with various active ingredients and of various composition, can maintain a release rate sufficiently high for season-long mating disruption. This indicates the versatile nature of the TNO dispenser technology.

Acknowledgements

(1) Field tests were performed in collaboration with Servei de Proteccio dels Vegetals, Department Agricultura, Barcelona and Denka International, Barneveld

(2) Field tests were performed in collaboration with the Research Institute for Plant Protection, Wageningen and Denka International, Barneveld

INTEGRATION OF MATING DISRUPTION TO CONTROL LEPIDOPTEROUS PESTS OF CABBAGE

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Abstract The caterpillar complex on cabbage in Florida typically has consisted of cabbage looper, *Trichoplusia ni*, imported cabbage worm, *Artogeia rapae*, cabbage webworm, *Hellula rogatalis*, and diamondback moth, *Plutella xylostella*. Diamondback moth has progressed from being a very minor pest, easily controlled by insecticide programs aimed primarily at cabbage looper, to key pest status. Pyrethroid chemicals were introduced in 1980 and by 1985 they were failing to control diamondback moth in Florida and elsewhere in North America. Resistance of diamondback moth to all classes of insecticides, including *Bacillus thuringiensis* and growth regulators, has been documented worldwide and markedly increased the cost and risk of producing cabbage and other crucifers.

Management strategies being developed to control lepidopterous pests of cabbage include: 1) insecticide management, including the exclusion of pyrethroids and carbamates and emphasis on *B. thuringiensis*-based materials; 2) mating disruption (successful trials of mating disruption to control this pest have been conducted in Japan and Florida); and 3) conservation of native parasites, releases of *Cotesia plutellae*, an introduced species from S.E. Asia available from commercial insectaries, and development of methods to rear and release a native parasite, *Diadegma insulare*.

Our purpose is to develop a system for managing diamondback moth using mating disruption, parasites, and biological pesticides. Mating disruption is central to the project and we anticipate that this tactic also must be developed for cabbage looper which is, again, becoming a key pest.

Introduction

The caterpillar complex on cabbage in Florida typically has consisted of cabbage looper, *Trichoplusia ni* (Hübner), imported cabbage worm, *Artogeia rapae* (L.), cabbage webworm, *Hellula rogatalis* (Hulst), and diamondback moth, *Plutella xylostella* (L.). Cabbage looper has long been the key lepidopterous pest of cabbage in Florida. The other species were usually controlled by insecticide programs aimed at the looper. Pyrethriod chemicals were introduced to control cabbage pests in Florida in 1980 and by 1985 were failing to control diamondback moth there and elsewhere in North America. Thus, diamondback moth became the major yield limiting pest in cabbage in most

regions in the United States.

Resistance of diamondback moth to all classes of insecticides, including *Bacillus thuringiensis* toxins and insect growth regulators has increased markedly the cost and risk of producing cabbage and other cruciferous vegetables throughout the world (Talekar et al. 1985, 1990, Talekar & Griggs 1986,Tabashnik et al. 1990, Leibee & Savage 1992, Shelton & Wyman 1992). In the U.S., the problem of controlling diamondback moth economically and safely became so severe that many growers quit production of cabbage. Also, the market for cabbage sets produced in the southern U.S. to be planted in the northern U.S. and Canada was threatened because the importation of highly resistant larvae confounds control strategies in these areas.

Bacillus thuringiensis-based insecticides and growth regulators are effective control agents with minimal environmental impact, provided resistance can be avoided. Moreover, diamonback moth often is heavily parasitized. Combining pest control tactics may be the best approach for handling insect resistance. Mating disruption via permeation of the air with the odor of the female-produced sex pheromone of diamondback moth and cabbage looper seems feasible and would provide a basis for an integrated pest management system for the lepidopterous pests of cabbage.

We are engaged in a multi-year project to develop management strategies for lepidopterous pests of cabbage. Our approach includes: 1) insecticide management, including the exclusion of pyrethroids and carbamates and emphasis on *B. thuringiensis*-based materials; 2) mating disruption (successful trials of mating disruption to control this pest have been conducted in Japan and Florida); and 3) releases of *Cotesia plutellae* (Kurdjumov), an introduced species from S.E. Asia available from commercial insectaries, and development of methods to rear and release a native parasite, *Diadegma insulare* (Cresson). Mating disruption is central to the project and we anticipate that this tactic must also be developed for cabbage looper which is, again, becoming a key pest

Mating Disruption

Leibee & Savage (1992) found that differentials in resistance of diamondback moth larvae occurred in adjacent small plots treated with different insecticides for diamondback moth control. This suggests that movement by diamondback moths is limited, thus creating a situation favorable for mating disruption.

Nagata (1989), Nemoto et al. (1992), Ohbayashi et al. (1992), and Ohno et al. (1992) have reported successful trials of mating disruption to control diamondback moth in Japan on cabbage and radish using formulation technology developed by Shin-Etsu, Ltd. A field trial on 8 ha of commercial cabbage was conducted with this technology against diamondback moth in Florida (McLaughlin et al. in press). Commercially-grown cabbage was protected from damage with a single pheromone treatment and 3 insecticide treatments, despite heavy moth pressure from surrounding, conventionally-managed blocks of cabbage. There was a reduction in mating of sentinel females placed on mating tables and of native wild females collected from the pheromone-treated plot. Larval counts were comparable to those in conventional plots that received 13 or 15 insecticide treatments. The yield and quality of the cabbage harvested was sufficient to indicate that the development of a semiochemical-based control strategy in Florida is highly feasible.

Florida farmers do not consider the present continuous rope formulation of the diamondback moth pheromone acceptable. The method of application, interference with equipment, and need to remove the formulation after harvest add greatly to the cost. McLaughlin & Mitchell (unpublished) have successfully tested 20-cm segments of the formulation placed on stakes in arrays at several densities. Growers indicated a preference for this concept. The pheromone could be applied by

transplant crews at almost no additional labor cost. In one test they did not support the segments on stakes, but inserted one end about 3-4 cm into the ground. This method is still under investigation but appears promising. A formulation that could be applied to the plants as a dip or as a spray would greatly facilitate the use of mating disruption in cabbage.

Mating disruption technology is available for other lepidopterous pests that sometimes are damaging to cabbage in North America. Wakamura & Takai (1992) report suppression of beet armyworm, *Spodoptera exigua* (Hübner), on Welsh onion using mating disruption in Japan. Mitchell & McLaughlin (1982) suppressed egg laying and whorl damage by the fall armyworm, *Spodoptera frugiperda*, (J. E. Smith) in fields of corn treated with its sex pheromone. Disruption of beet armyworm, fall armyworm and corn earworm, *Helicoverpa zea* (Boddie), mating has recently been demonstrated in small plots of cotton in Florida (Mitchell & McLaughlin, unpublished). Moreover, a sprayable formulation suitable for use with acetate-based pheromones such as fall armyworm, beet armyworm, and cabbage looper has shown promise in trials against the tomato pinworm, *Keiferia lycopersicella* (Walsingham) (McLaughlin & Mitchell, unpublished).

Parasitoids

Numerous attempts have been made to introduce parasitoids for control of diamondback moth (Talekar et al. 1985, 1990, Talekar & Griggs 1986, Frank & McCoy 1993). *Diadegma insulare* (Ichneumonidae), often attacks diamondback moth larvae in North America. *Cotesia plutellae* (Braconidae) frequently is mentioned as a candidate biological control agent for diamondback moth. It is currently mass reared by IIBC in Switzerland for release in some of their tropical projects (IIBC 1991) and is sold by commercial firms. There have been sporadic releases of this parasitoid in Florida (Frank & McCoy 1993); however, little data is available on its recovery or efficacy. Insecticide resistant strains of this parasitoid are reportedly under assessment (IIBC 1991, Ke et al. 1991). We have released over 25,000 *C. plutellae* adults in experimental plots in Florida during the 1992-93 growing season.. The parasitoid has been recovered, but does not seem more effective than the resident native *Diadegma*.

Diadegma insulare is difficult to rear and is not commercially available. We are presently developing rearing methods for this parasitoid so that it can be evaluated in augmentative releases in Florida cabbage fields.

Pesticides

Brunner & Stevens (1986) found that *B. thuringiensis* does not harm the hymenopterous parasitoids of diamondback moth; thus, it can be used effectively in an integrated management system. Florida growers do not use well-developed scouting programs to determine the need for insect control. Leibee & Savage (University of Florida, Research Center, Sanford, unpublished) have developed a rating system for assessing insect damage to cabbage that we are utilizing in our program. Growers largely have ceased to use undesirable pyrethroid and carbamate pesticides in cabbage.

Cabbage Looper

The cabbage looper long has been the key pest of cabbage in Florida and is an important pest of vegetables and other crops elsewhere in North America. Integrated pest management has not been practiced in cabbage production in Florida, and the development of resistance in diamondback moth seems in large part to have been caused by increasing use of pesticides to control the looper. As growers expanded their chemical control programs to meet the threat from diamondback moth, cabbage loopers nearly disappeared from Florida agroecosystems. Field research on cabbage looper behavior at our laboratory had to be suspended in 1990 because of lack of suitable populations of the insect. As growers have begun to moderate their spray programs and avoid chemicals that trigger diamondback outbreaks, the cabbage looper has begun a return to its former status.

We are concerned that the increasing need to control cabbage looper will cause growers to repeat the mistakes that led to past outbreaks of diamondback moth. While *B. thuringiensis*-based pesticides are effective against cabbage looper, they require more effective scouting for maximum efficacy. *Bacillus* toxins do not immediately kill larvae and growers sometimes underestimate the effectiveness of applications of these products. Also, market tolerance for insects in the fresh product is very low. These concerns have led us to renew efforts to develop mating disruption as a control strategy for cabbage looper.

We have begun field experiments in small plots of cabbage using rubber septum formulations of the principal sex pheromone component, (*Z*)-7-dodecen-1-ol acetate, to establish the efficacious dosage for mating disruption of sentinel females. We also are seeking suitable commercial formulations for the pheromone and will expand our program to include evaluation of mating disruption of the cabbage looper as a primary component of an integrated pest management system for cabbage.

References

BRUNNER, E. & STEVENS, P.F.E. 1986. The control of diamondback moth with Thuricide, pp. 213-217 in Talekar, N. S. & T. D. Griggs [eds.], Diamondback Moth Management: proceedings of the first international workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

FRANK, J.H. & MCCOY, E.D. 1993. Introduction to behavioral ecology, 1992: Releases of biocontrol agents in Florida. Florida. Entomol. **76**: 1-53.

IIBC. 1991. Annual report, 1991. International Institute of Biological Control. CAB International.

KE, L.S., MOORE, D. & WAAGE, J.K.. 1991. Selection for fenitrothion resistance in *Apanteles plutellae* (Hymenoptera: Braconidae). J. Appl. Entomol. **12**: 107-110.

McLAUGHLIN, J.R., MITCHELL, E.R. &. KIRSCH, P. (in press). Mating disruption of diamondback moth in cabbage: Reduction of mating and suppression of larval populations. J. Econ. Entomol.

LEIBEE, G.L. & SAVAGE, K.E. 1992. Evaluation of selected insecticides for control of diamondback moth and cabbage looper in central Florida with observations on insecticide resistance in diamondback moth. Florida. Entomol. **75**: 585-591.

MITCHELL, E.R., & McLAUGHLIN, J.R. 1982. Suppression of mating and oviposition by fall armyworm and mating by corn earworm in corn, using the air permeation technique. J. Econ. Entomol. **75**: 270-274.

NAGATA, K. 1989. Pest control by mating disruption in Japan. Jpn. Pestic. Info. No. **54**: 3-6.

NEMOTO, H., YANO. E. & KIRITANI, K. 1992. Pheromonal control of diamondback moth in the management of crucifer pests, pp. 91-97 in Talekar, N.S. [ed.], Diamondback moth and other crucifer pests: proceedings of the second international workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center. AVRDC Publication No. **92**-368.

OHBAYASHI, N., SHIMIZU, K. & NAGATA, K. 1992. Control of diamondback moth using synthetic sex pheromones, pp. 99-104 in Talekar, N.S. [ed.], Diamondback moth and other crucifer pests: proceedings of the second international workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center. AVRDC Publication No. 92-368.

OHNO, T., ASAYAMA, T. & ICHIKAWA, K. 1992. Evaluation of communication disruption method using synthetic sex pheromone to suppress diamondback moth infestations pp. 109-114 in Talekar, N.S. [ed.], Diamondback moth and other crucifer pests: proceedings of the second international workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center. AVRDC Publication No. 92-368.

SHELTON, A.M., & WYMAN, J.A. 1992. Insecticide resistance of diamondback moth in North America, pp. 447-454 in Talekar, N.S. [ed.], Proceedings of the second international workshop: Management of diamondback moth and other crucifer pests. Asian Vegetable Research and Development Center Publication 92-368. Shanhua, Tainan, Taiwan.

TABASHNIK, B.E., CUSHING,N.L., FINSON, N. & JOHNSON, M.W. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. **83**: 1671-1676.

TALEKAR, N. S., YANG, J.C. & LEE, S.T. [eds.] .1990. Annotated bibliography of diamondback moth. Volume 2. Asian Vegetable Research and Development Center Publication 90-334 {ISBN 92-9058-044-5}. Shanhua, Tainan, Taiwan.

TALEKAR, N.S. & GRIGGS, T.D. [eds.]. 1986. Diamondback moth management: proceedings of the first international workshop: Diamondback moth management. Asian Vegetable Research and Development Center Publication 86-248 {ISBN 92-9058-0021-8}. Shanhua, Tainan, Taiwan.

TALEKAR, N.S., LEE, S.T., CHEN, B.S. & SUN, L.Y. [eds.]. 1985. Annotated bibliography of diamondback moth. Asian Vegetable Research and Development Center Publication 85-229 {ISBN 92-9058-0014-5}. Shanhua, Tainan, Taiwan.

WAKAMURA, S. & TAKAI, M. 1992. Control of the Beet Armyworm in open fields with sex pheromone, pp. 115-125 in Talekar, N. S. [ed.], Diamondback moth and other crucifer pests: proceedings of the second international workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center. AVRDC Publication No. 92-368.

SOME RECENT DEVELOPMENTS IN SEMIOCHEMISTRY

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Abstract GC with simultaneous MS and EAG detection would give the maximum amount of biological and chemical information from a single GC run. However this cocatenation of techniques has not been achieved because of the problems of running the EAG at atmospheric pressure and the MS at high vacuum. We have found that by using a post injector splitter and two capillary columns the pressure difference can be easily compensated. I will illustrate the application of this technique to the spiroketal pheromone of Olive fly, *Dacus oleae* and discuss our approaches to quantitative EAG.

Lepidopteran (butterflies and moths) pheromones, were the first semiochemicals to be isolated and a prodigous number of identifications have now been made. The structures typically consist of a linear even number carbon chain with a terminal oxygen function and from zero to four double bonds. Computer-aided analysis of the Arn *et al* database yielded 2292 semiochemicals from 1068 species but only 264 unique chemical structures. The chemicals used most frequently are Z-9:14Ac (168 cases), Z-11:14Ac (157), Z-11:16Ac (133), E11-14:Ac (124) and Z7-12:Ac (111). Taken together these are used by 30% of all species in the database. However 80% of species use at least two components. Approximately 40% of the structures have a terminal acetate group and the remaining 60% are evenly divided between aldehydes, alcohols and hydrocarbons with a few 2-ketogroups. The most common chain lengths are in C14, C12, C16 and C18. Approximately 40% are monoenes and 40% dienes with the double bonds located in the (ω-3) and (ω-5) positions. This is constant with the identification of ω-9 and ω-11 desaturases for dodecanoic and tetradecanoic acids from insects. Preliminary work on the analysis of multi-component pheromone bouquets will also be discussed.

COMMERCIAL ASPECTS

REGULATION OF SEMIOCHEMICALS - GLOBAL ASPECTS

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Abstract Data were obtained from twenty countries on their current regulations regarding the registration of semiochemicals, principally pheromones. The need for expediting and harmonizing semiochemical registration is discussed with consideration to [a] minimizing the risks to public health and safety without imposing crippling costs and restrictions on the industry, [b] ensuring the availability of efficacious and cost effective products and, [c] helping create a public climate in which pheromone commercialization and use can develop. By reviewing pheromone use, the history of pheromone regulation, the current requirements in those countries in which the regulatory authority differentiates between semiochemicals and conventional insecticides, a proposal is made for the use of a structure/activity based system to expedite the registration of a group of lepidopteran pheromones and the harmonization of regulation. The use of the system should be expanded to include other types of pheromones and semiochemicals as the database is enlarged.

Introduction

Concern for the impact of pesticides on health and the environment in almost all of the industrialized countries has resulted in increased regulatory action in those countries. For example, in Germany the Revised Plant Protection Act which came into effect in January 1987 has resulted in much stricter pesticide regulation, and as a consequence the number of registered products has been decreased from about 1700 based on >300 active ingredients to about 900 formulated products based on 200 actives. In the United States, Section 4 of the Federal Insecticide, Fungicide and Rodenticide Act [FIFRA] as amended in 1988 mandated the Environmental Protection Agency [EPA] to re-register all pesticides containing active ingredients that were first registered before November 1, 1984; reregistration must be completed by 1997. This requirement for accelerated reregistration of those pesticides for which the EPA has incomplete data on file, will for various reasons, ensure the withdrawal or cancellation of a large number of pesticide products. A similar scenario is certainly about to prevail in Europe as pesticide reregistration proceeds under the aegis of the European Community, and it is forecast that about 20% of the older pesticides will be canceled beginning June 1994. Through what is known in the Netherlands as the Multi-Year Crop Protection Plan it is hoped to reduce the annual pesticide usage, which currently stands at 700,000 kilograms of active ingredients, by 50% by the year 2000 (Anonymous, 1990).

Universally, there is much impetus to research, develop and commercialize alternative, environmentally benign, safer plant protection products. In May 1992 the United States Department

of Agriculture and the EPA jointly sponsored a forum to address the economic and environmental issues facing contemporary agriculture in the USA. Out of the discussions on products such as microbial insecticides and semiochemicals came a resolution that for the timely commercial implementation of such alternative pest management products there was an urgent need "to streamline the entire pesticide regulatory process."

To do this there has to be a recognition that semiochemicals are different from conventional insecticides. The EPA in 1982 classified certain materials, including pheromones, as "biochemical insecticides" and decided that the requirements for their registration should be different than those for conventional insecticides. A material, to qualify for the designation "biochemical insecticide" should be naturally occurring, have a unique mode of action (interpreted to mean a "non-toxic mode of action"), have target specificity and have low use rates. In 1988 FAO also published guidelines (Anonymous, 1988) recognizing the different nature of biological pest control agents and the need for different registration requirements " but the general principle that the product should demonstrate effectiveness and will not present unacceptable hazard to users, consumers of treated food, or the environment still applies."

Unfortunately, in Council Directive 91/414/EEC of the European Community there is no differentiation between chemical pesticides and biochemical pest control agents, all are classified as "chemical substances" and defined in part as "chemical elements and their compounds, as the occur in nature or by manufacture....." (Anonymous, 1991). Unless a very progressive waiver policy is adopted, registration data requirements will most certainly preclude the submission of applications to the EC for the authorization of pheromone products.

That the regulatory process, world-wide, needs to be revised is a view shared by the neophyte semiochemical industry which is made up, for the most part, by under-capitalized entrepreneurial companies, for whom the major hurdle to the market introduction of their products is the time and cost required to complete the registration process. This paper focuses on the regulation of semiochemicals, principally pheromones in the light of achieving three goals:

i) minimizing the risks to public heath and safety without imposing crippling costs and restrictions on the industry;
ii) ensuring the availability of efficacious and cost effective products;
iii) helping to create a public climate in which the technology can develop.

Pheromone use

Pheromones are used commercially in two ways [a] for indirect control (monitoring for quarantine, spray timing, *etc.*) and [b] as direct control agents. This latter application may be further divided into their use in mass trapping, and in area-wide dissemination. In area-wide dissemination there are three strategies, two of which, disruption and attracticide are widely used commercially, and bioirritation which saw limited use about five years ago.

On a worldwide basis, the number of insect species for which pheromones are commercially available and used in indirect control is estimated to be more than 200 (Inscoe *et al.*, 1990), whereas for direct control, there are less than twenty pheromone formulations registered and used commercially.

The 1990 U.S pesticide market was estimated to be of the order of $5.3 billion (Maxey, 1991), of that 22% or about $1.2 billion was the insecticide component. An estimate of the sales of all semiochemical products (with the exception of housefly scatter-baits) for the same year is

between $7-8 million or about 0.6% of the insecticide market. Since pheromone commercialization is further developed in the U.S. than in most countries, it is probable that pheromone sales are an even smaller percentage of the insecticide market in any other country.

Pheromone Regulation - Historical Perspective

In 1978 the EPA granted to Albany International a registration for Gossyplure HF, a pheromone product for control of pink bollworm on cotton. The registration costs for this, the first area-wide disseminatiuon product to be registered were in excess of $1 million (T.W. Brooks, personai communication). The same year, at a NATO conference in the Netherlands, the cost of registering a pheromone was estimated at $1.34 million (Siddall, 1979). One of the recommendations (Ritter, 1979) to come from this NATO meeting was that "regulatory agencies in all countries should publish special guidelines during 1979 for the registration of behaviour modifying chemicals for pest control. Such chemicals, which must provide insectistasis without insecticidal action, should be clearly distinguished from insecticides. Suitable terminology should be adopted internationally."

From 1980 through 1983 a pilot study was conducted under the auspices of the Committee on the Challenges of Modern Society of NATO. Unfortunately only four of the twelve countries which had participated in the original 1978 NATO conference took part in the study. None of the four [Netherlands, Britain, France and the United States] had really followed up on the 1978 recommendation although the U.S. had recognized the need to develop specific requirements for pheromones and other biorational insecticides (Anonymous, 1982). France, also about this time, appreciated the need to regulate differently from conventional insecticides, what they called "unconventional insecticides". When a pheromone was to be used in a control strategy "where the compound is not applied to the edible parts of plants and because of the extreme dilution of the product in the atmosphere," only basic toxicological data would be required. However, if an encapsulated form of the pheromone was to be used, it would be considered an insecticide (Hascoet and Hurpin, 1980). At this time in the U.S. the regulatory requirements were still viewed as a major disincentive to pheromone development, however the EPA issued four further pheromone registrations, and more importantly, having recognized the unique nature of what they called "biorational insecticides" issued special guidelines which are known as Subdivision M. (Anonymous, 1982). Two years later the Agency finalized the data requirements for biochemical pesticides and these were significantly different to those listed in Subdivision M. In July 1989 a substantially revised set of guidelines for microbial pest control agents were issued, however these new guidelines, also confusingly called Subdivision M, contain no requirements for biochemical pesticides. Earlier this year a congressional inquiry to the EPA regarding the requirements for biochemicals elicited the following response from the Acting Assistant Administrator - "Although the Agency is considering making changes in these testing requirements, this is a long term project and will not yield formal results quickly." (Wayland, 1993).

The 1990 "Brighton Conference" symposium on pheromones and other behaviour modifying chemicals re-focussed and re-defined both the regulatory problems of pheromones and the need for international cooperation to attempt harmonization of registration requirements *etc.*

Attendees at the IOBC/WPRS international conference held in 1991, at Veldhoven, to discuss environmentally safer agriculture were of the opinion that the registration procedures for all biocontrol agents are unclear and too time consuming, and that legislative measures should be adopted which will result in an easier and less expensive approach for selective and biopesticides (Van Lenteren *et al.*, 1992).

Pheromone Regulation - Current Status

Although most countries do not require regulation of pheromones when they are used in traps for monitoring purposes, there are some exceptions *eg*. Chile, the Czech Republic, Greece and Hungary. All of the countries which we surveyed required registration of pheromones and some other semiochemicals when used directly in pest control, but only Canada, Sweden and the United States, like FAO have adopted policies which differentiate between chemical toxicants and pheromones. In Denmark and Italy pheromones are not regulated, while in other countries such as Australia, South Africa and Switzerland the regulatory authorities have a flexible and pragmatic approach to their registration. In New Zealand, although all behavior-modifying chemicals are pesticides by definition, mating disruption formulations have been exempted from registration.

In 1990 Minks published a report in which he indicated that the EPA guidelines for the registration of biochemical insecticides should form the basis on which the regulatory authorities in all other countries should develop their requirements and guidelines. These requirements, which may be found at Title 40 of the Code of Federal Regulations §158.640 and detailed in Subdivision M far exceed those data actually required to obtain a pheromone registration. A complete product chemistry package is required, however in the U.S. no residue data are required provided certain toxicology and application rate conditions are met. Regarding mammalian toxicology, those studies required for a pheromone product to be used on a food or feed crop, include three acute toxicity studies, two irritation studies, a skin sensitization study and three genotoxicity studies including the Ames test. The cost to contract for such studies is about $32,000, however if the pheromone product has a non-food use or a forestry use, the genotoxicity studies are not required and hence the cost is reduced by $24,000. Mammalian toxicity data are required on the pheromone active ingredient and also the end-use product, however with certain formulations these end-use product data may be waived.

Non-target, fate and expression data required are all developed on the active ingredient only, and cost about $12,000 for the required avian acute toxicity, fish toxicity and aquatic invertebrate toxicity studies.

As stated above, Canada has recognized the need for separate requirements for semiochemicals; but at this time, the scope of the guidelines and the specific data requirements are still under discussion, hence a comparison of the requirements for biochemical pest control agents is restricted to those promulgated by the EPA, KEMI (the Swedish regulatory authority) and the FAO .

Considering first, the registration of the active ingredients, all three organizations have approximately the same product chemistry requirements although KEMI requests more physico-chemical data. KEMI and FAO have less stringent requirements for Tier I mammalian toxicology data, with KEMI not requiring acute dermal and acute inhalation toxicity data nor skin sensitization while FAO does not require either of the irritation studies nor the skin sensitization. Provided there are no problems in what the FAO calls "the primary toxicology data" a series of sub-chronic, chronic and carcinogenicity data are obviated. A somewhat analogous situation is in the KEMI requirements where further studies are keyed to problems in the Tier I genotoxicity data.

With regard to the nontarget organism, fate and expression data the FAO requirements are the most demanding, requiring avian acute and dietary toxicity studies, fish toxicity, and nontarget insect and plant studies. KEMI requires a fish toxicity study and an aquatic invertebrate reproduction study. As explained above, residue data is not usually required for biochemical insecticides under the EPA regulations however both KEMI and the FAO request residue, persistence and soil fate data.

For end-use products, KEMI and FAO require a discussion of the mode of action of the control agent while EPA asks for a discussion of the formation of unintentional ingredients. The

mammalian toxicity studies required by the EPA and FAO are almost identical and can include the three acute toxicity studies and the two irritation studies, while KEMI does not require the acute dermal nor the acute inhalation studies.

Environmental toxicology data are not required by the EPA nor FAO, but this presupposes that such data are available on the active ingredient.

Pheromone Regulation - The Future

Pheromones currently comprise but a small percentage of the insect control products available in the U.S. where there are about 50,000 insecticide products from 250 active ingredients (Maxey, 1991) and only about 12 pheromone products based on 5 actives. Arthropods elaborate many different types of chemicals as their pheromones, but those of commercial interest are restricted, at this time, primarily to lepidopteran pheromones, and in particular to unsaturated alcohol, ester and aldehyde pheromones with ten to eighteen carbons in the aliphatic chain.

The fact that such lepidopteran pheromones comprise but a minuscule percentage of the insect control products available, are much safer than conventional insecticides and due to their target specificity cannot command multi-million dollar markets, makes them an ideal case for the development of an expedited regulatory mechanism and global harmonization. The reasoning for this includes regulatory authorities would not be required to expend resources to register pheromones beyond their level of importance, pheromones are the safest of all currently registered insect control products and for every chemical toxicant removed from the market, there needs to be several pheromone products to replace it.

It is the unanimous opinion of those within the "pheromone industry" that there is an urgent need for the regulatory authorities in the various countries to acquire a mechanism for expediting registrations, which in turn will accelerate the rate at which pheromone products enter the market. A minority argue for the complete exemption of all pheromone products from regulation, contending that this is merely an extension of the current exemption, enjoyed in most countries, for the use of pheromones when employed in traps. In addition to being politically difficult to achieve, it is in the our opinion, not desirable since it would leave no mechanism for the exclusion of poorly manufactured active ingredients and end-use products from the market; inferior, ineffective products from a few "snake oil salesmen" will make it measurably more difficult to commercialize reputable, efficacious products. However, more importantly, it is possible that a pheromone or semiochemical may be discovered to have some hazards posing a risk to the public, and there needs to be a review process so that it cannot be used without the proper assurance and safeguards.

Although the majority of those involved in the pheromone industry favour regulation, there is almost no unanimity as to how this should be done. To expedite the registration of pheromones by reducing both the cost and the time-frame of the registration process, it is necessary to focus on how best the requirements may be reduced without reducing the public's confidence in the regulatory authorities' ability to assess the risks and benefits of the product under scrutiny. As has been seen, the primary basis for such decisions (EPA, KEMI & FAO) has been product chemistry, mammalian toxicology and ecotoxicology data. Considering the relative simplicity of the lepidopteran pheromone molecules under discussion and the innumerable synthetic routes possible for their production, the current product chemistry requirements are not unreasonable.

Studies considered by the EPA in determining the relative toxicity of compounds, including the causes of the toxic effects, are the acute oral, dermal and inhalation studies. It is known from toxicity data in the EPA files and in the public domain that alcohols, acetates and aldehydes with a chain length of 11-18 carbon atoms have LD_{50}'s for acute oral, acute dermal and acute inhalation

studies greater than the values of limit testing used by the EPA. The EPA guidelines define "limit testing" as "principles which provide that if no mortality is produced by administration of a specified dose level, no further testing is required." These dose levels are, for acute oral toxicity a LD_{50} of 5 g/Kg of body weight, for dermal toxicity a LD_{50} of 2 g/Kg and for acute inhalation toxicity the LC_{50} is 5 mg/l of air. In irritation studies, several of the compounds were slightly to moderately irritating to the skin, however none were classified as eye irritants. The registration of a pheromone, in the U.S., for use on a food crop requires an exemption from the requirement of a tolerance, this has a prerequisite of satisfying the battery of genotoxicity studies. No pheromone has ever given positive results in such studies, and all pheromones registered for use on food and feed crops have been granted tolerance exemptions.

A review of the ecotoxicity data for acetate and aldehyde pheromones again exemplifies the low toxicity of such compounds and the data generated is considered satisfactory according to EPA guidelines. The generation of most of the avian acute data was carried out at the "limit dose" of 2 g/Kg of test material. From available fish toxicity data, the LC_{50} values are greater than 100 ppm, the level established by the EPA indicating the data are satisfactory; this is not so for the aquatic invertebrate data, however the estimated environmental concentration in rivers, lakes *etc.* resulting from the use of hand applied pheromone devices will be immeasurable, while that from formulations broadcast area-wide will be very much less than the values found in the studies.

The data described above for the mammalian and ecotoxicity, together with other data compilations [*eg* Genotoxicity Database GEN (Würgler, 1991)] should form the nucleus of a data base for the evaluation of health and environmental risks. When this was first proposed (Weatherston, 1990) the EPA were not receptive but the situation has now changed.

The EPA, to satisfy the requirements of the Toxic Substances Control Act [TSCA] and the strict 90-day deadline for evaluation of new chemicals prior to their introduction into the market-place, has developed, over a period of several years, a computerized structure/activity based system for estimating the physical, chemical and toxicological properties of new chemicals. Using such a system to estimate the properties of a new compound when it is a member of a narrow class of closely related compounds for which actual data exist, as is the case with unsaturated C_{10} - C_{18} acetate, alcohol and aldehyde pheromones, is a simple and precise exercise. Although the system already exists and is fully operational in the Office of Toxic Substances [OTS], modification of the database will be necessary.

To achieve the goals set out in our introduction and to foster harmonization of the registration requirements for pheromones, the following is proposed:

i) A structure/activity database should be used for the evaluation of health and environmental risks;

ii) A computerized structure/activity database should be used for estimating the physical, chemical and toxicological properties of new pheromones;

iii) Other product chemistry requirements should remain "as is" for the time being;

iv) This approach should be initiated **now** for pheromones whose structures fall in a class of compounds defined by:

Carbon chain length 10C - 18C
Centers of unsaturation 0 - 3
Functional groups - Aldehydes, alcohols and esters

v) A 90-day evaluation deadline;

vi) That this approach should be expanded to other types of pheromones and semiochemicals as the data base is enlarged.

It is further recommended that the following action be implemented on the proposal. A submission be made, initially to the U.S. EPA then to other national regulatory authorities of:

i) Application(s) for one or more new pheromone active ingredient(s), preferably acetates and/or aldehydes:
   should contain no original lab data other than product chemistry information
   should contain requests to waive all toxicity and ecotoxicity data
   rationale for granting the registration
   justification for the continued use of this approach

ii) Formal request to the regulatory authority to adopt the structure/activity determinations and the 90-day time frame, as alternatives to new original studies for this class of lepidopteran pheromones.

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References

ANONYMOUS, 1988. Food & Agricultural Organization of the United Nations Guidelines on the Regulation of Biological Pest Control Agents. Rome.

ANONYMOUS, 1990. Multi-year Crop Protection Plan. Essentials - Public Information Booklet, published by the Ministry of Agriculture, Nature Management & Fisheries, The Hague. 14 pp.

ANONYMOUS, 1991. Council Directive 91/414/EEC, Official Journal of the European Communities, No. L230, pages 1-32.

HASCOET, M. & HURPIN, B. 1980. Proposed French Registration Guidelines for Unconventional Pesticides. in: *Environmental Protection and Biological Forms of Control of Pest Organisms*. B. Lundholm & M. Stackerud (*Eds*), Ecol. Bull.(Stockholm) *31*: 145-154.

INSCOE, M.N., LEONHARDT, B.A. & RIDGWAY, R.L., 1990. Commercial Availability of Insect Pheromones and Other Attractants. in: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein & M.N. Inscoe (*Eds*), Marcel Dekker Inc., New York. pp. 631-715.

MAXEY, F., 1991. Agricultural Chemicals, in: *U.S. Industrial Outlook 1991*. U.S. Department of Commerce, Washington, D.C. Document No. S/N003-009-00586-8.

MINKS, A.K., 1990. Registration for Pheromones In Europe. in: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein & M.N. Inscoe (*Eds*), Marcel Dekker Inc., New York. pp.557-568.

RITTER, F.J. 1979. Conclusions and Recommendations from the Advanced Research Institute on Chemical Ecology, Noordwijkerhout, The Netherlands 24-30 September 1978, in: *Chemical Ecology: Odour Communication in Animals*. F.J. Ritter (*Ed*), Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 403-410.

SIDDALL, J.B. 1979. Commercial Production of Insect Pheromones - Problems and Prospects. in: *Chemical Ecology: Odour Communication in Animals*. F.J. Ritter (*Ed*), Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 389-402.

VAN LENTEREN, J.C., MINKS, A.K. & DE PONTI, O.M.B., 1992. Biological and Integrated Crop Protection: Towards Environmentally Safer Agriculture. Proceedings IOBC/WPRS International Conference, Veldhoven, The Netherlands, September 8-11, 1991, Pudoc Scientific Publications, Wageningen, The Netherlands, 239 pp.

WAYLAND, S.H. 1993. Letter from EPA Acting Assistant Administrator to Senator Max Baucus.

WEATHERSTON, I., 1990. Regulatory Consideration - Perspectives from the Private Sector. Unpublished report given at the ARS Planning Workshop on Insect Semiochemicals: Opportunities and Constraints for Development and Use. Atlanta, Georgia, May 7-10, 1990.

WÜRGLER, F.E., 1991. Predicting Mammalian Mutagenesis by Submammalian Assays: An Application of Database GEN. Environmental Health Perspectives, **96**: 37-39.

COMMERCIAL ADVANCEMENT IN PHEROMONE RELATED MONITORING AND CONTROL TECHNOLOGY

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For more than two decades, arthropod pheromones have been viewed as having much promise for enhancing pest management strategies, yet the industry is, for the most part, still very small, and limited to small under-capitalized entrepreneurial companies. In horticulture and fruit production, pheromone-based monitoring programs are well established worldwide. However, usage of pheromone based monitoring systems is restricted on broadacre and vegetable crops, where considerable research efforts continue to be focussed on the development of labor intensive sequential sampling protocols for pest management decision making.With respect to control technology, management of the pink bollworm on cotton in the desert southwest, and of tomato pinworm, primarily in Mexico are the two most extensive uses of pheromones in agriculture. In addition, mating disruption has recently been adopted in three fruit production systems [peach, pome and grape] and a promising attracticide technology is in development for control of diabroticine species in North American corn production systems.

Indirect pheromone-based management is focussed on the development of monitoring tools, traps and lures, which when coupled with temperature driven predictive phenology models, permit considerable precision in the timing of control strategies. More sophisticated monitoring programs have been developed for some pests with the treatment decisions based on threshold captures in traps. The authors suggest that pest management decision making could be expanded to more pest species if treatment decisions were based on carefully researched threshold values, developed by correlating pheromone captures with data from sequential sampling protocols.

Direct pheromone-based management has focussed first on mating disruption and then on attracticide strategies. The authors believe that "a glass ceiling" is preventing progress in mating disruption, both from a research standpoint and in the commercialization of robust systems. Although a high degree of efficacy is often achieved, it is not consistent and hence commercialisation is difficult to realize. The possibility exists that mating disruption has reached the point where future significant breakthroughs will only occur with the infusion of new ideas and completely new perspectives (for review see Kirsch, 1992).

This paper will review current commercial applications of pheromone technology and highlight significant advancements made in the last two years. Finally, the authors will suggest priorities for ongoing research programs in both monitoring and control applications of pheromone technology.

Factors affecting commercial advancement.

Scientific and commercial advancement of pheromone technology is greatly affected by: corporate health, formulation development, commercial targets and distribution channels, product efficacy, availability of active ingredient, selectivity, scientific capacity, regulatory success and compatibility with conventional agriculture.

Corporate health

The worldwide pheromone industry has experienced significant upheaval in the past 24 months. Two well known companies, Agrisense and Scentry have changed hands: Agrisense being purchased by Biosys (Palo Alto California) from Philips Petroleum/Dow Corning and Scentry changing hands from United Agri Products (Greeley, Colorado) to Ecogen (Langhorne, Pennsylvania). To the studied observer, these acquisitions seem more related to enhancing corporate image of biotech companies than to the serious development of further commercial pheromone systems. For example, as part of their purchase agreement, Ecogen chose to terminate the Scentry director of registration, research and development. This has seriously curtailed Ecogen/Scentry product development.

The largest European player, BASF, has significantly downsized it's pheromone division and relocated staff to other sections of the company. Following significant downturns in their core silicon and polyethylene business, ShinEtsu Chemical Company (Tokyo, Japan) and the subsidiary, Pacific Biocontrol Corporation, have considerably reduced research and development support in North America. Further, ShinEtsu have implemented 'user-pays' programs when supplying research formulations in other parts of the world. In Australia's largest peach growing district, ShinEtsu subsidiary Biocontrol Limited has experienced widespread control difficulties for three consecutive seasons (90-91, 91-92, 92-93) with the oriental fruit moth disruption system, Isomate-M, leading to payment of significant compensation settlements in at least one of these seasons.

Formulation development

The majority of monitoring formulations are based on red rubber septa controlled release technology. Treatment thresholds are based on capture rates using these septa. Where economic thresholds are used, it is critical that comprehensive bridging studies are undertaken to validate any changes prior to implementation of new monitoring technology.

Today's commercial disruption formulations are mostly limited to technology that was developed over a decade ago, including the Hercon trilaminates, Scentry fibre, 3M microcapsules, ICI microcapsules, ShinEtsu rope, Consep membrane and beads, and BASF ampoules. There is a lack of comprehensive research and development. Companies do not commit resources to detailed investigation of release characteristics and product longevity as measured by on-time release rate (effluvial instead of residual analysis), with the result that most of this research is undertaken by public sector scientists. Further, companies do not seem interested in commiting the resources for development and commercialization of innovative new formulations that overcome the limitations of the existing technology.

Commercial targets and distribution channels

"If it walks like a duck, feeds like a duck, breathes like a duck and grows like a duck, it must be the golden goose."

Current advancements in disruption are limited by the majority focus on single highly competitive markets (pink bollworm, tomato pinworm, codling moth, oriental fruit moth). With

respect to monitoring, the focus remains on implementation of trapping systems in horticultural pest management programs. These markets are rapidly saturated, and of marginal profitability due to their highly competitive nature, inexperienced marketing strategies, and unrealistic pricing policies that risk any actual profitability.

Further, pheromone companies continue to attempt marketing of these products through conventional distribution channels. While this is feasible and very effective with respect to monitoring systems, experience indicates that a more direct scheme is considerably more effective with respect to information intensive disruption systems. Finally, conventional three to four tier marketing programs can lead to very high end-user product prices both for monitoring and control products. New distribution models may need to be developed.

Product efficacy

Pheromone technology is seldom robust due to the wide range of insect response to the same stimulus, and the interaction of this response with environmental conditions. Monitoring and control technology has been hindered through premature market introduction, with an inadequate supporting database, eg. early commercialization of the Consep Biolure, the BASF GBM disruption formulation (currently restricted to second generation), the BASF CM formulation (de-registered in Switzerland), and most recently recurrent control failures with respect to OFM mating disruption in Australia.

For reasons of both promotion and inadequate research and development programs, efficacy claims are often not supported by actual field data. This is especially problematic in countries that do not require submission and independent review of such data prior to issuance of regulatory approval. Further, there are often serious performance inconsistencies between different batches of the same formulation. Companies need to recognise the effect of inadequate quality control on product performance and credibility at the scientific and commercial level.

Availability of active ingredient

Pheromone companies are limited to 2-3 suppliers of bulk active ingredient. This situation is likely more limiting to rapid growth of this technology than issues of regulation, as purchasers are able to dictate terms of availability, cost, purity and quality. Further, purchasers are likely to reveal their control target to the supplier as soon as they request a quotation. This could present serious conflicts of interest, especially where the supplier maintains their own proprietary end use formulations. To facilitate further expansion, this industry requires an additional 3-4 specialist suppliers of bulk active ingredient.

Selectivity

Selective products by nature are limited in potential profitability. The high costs of research and development (both monitoring and control products, currently largely absorbed by the public sector) and registration (mostly control) need to be returned in profitability. Current public sector development of products with limited market sizes will be restricted with the reduction of public sector research budgets. Few pest management systems are focussed on only one insect, and selective pheromones need to compete with existing non-selective, relatively inexpensive pesticides. Insecticide based control tactics are relatively simple, whereas limited grower interest exists in selective products due to real or perceived increases in the direct and indirect cost (product, supplementary controls, increased losses) and management complexity of the control tactic.

Reduction in worldwide scientific capacity

There is presently an alarming worldwide trend in rapid downsizing of public sector entomological and agricultural field and research personnel. This will continue to create a vacuum in areas of commercial evaluation, independent field demonstration and supplementary product support. Companies will be required to fill this vacuum with their own extension programs. Are primary producers willing to carry upfront consulting costs, or do these programs need to be factored into the product pricing structure? Implementation and maintenance of selective programs is very labour intensive. Are there enough personnel in training to allow implementation of sophisticated information based IPM systems worldwide?

Registration success rate

The rate of corporate submission and regulatory approval of new or me-too pheromone formulations in North America seems to have slowed considerably in the past 24 months. Why has industry slowed down in seeking regulatory approvals? How reluctant is industry to commit resources to the registration game when uncertainty exists as to future changes in regulatory policy? Very few pheromone registrations have been approved in Europe. Regulatory structures in developing countries will not limit pheromone technology if they remain relaxed.

Compatibility with conventional agriculture

Insect pheromone based monitoring programs are very compatible with conventional agriculture and have become highly effective foundations to many horticultural IPM programs. Some agchem companies are now supplying pheromone traps to farmers as a component of new product launches (eg. fenoxycard introduction in Australia). Current monitoring programs need to be expanded firstly with the development of thresholds for more species, and secondly with the development of basic phenology information for more species, over a broad range of crops including vegetable and broadacre systems.

At present, many commercially available mating disruption products are not robust, not consistently effective and seldom economically attractive. Considerable basic research needs to be completed so that we can develop a greater understanding for factors that contribute to successful disruption and corresponding factors that lead to this techniques failure. Further considerable research needs to be conducted to determine monitoring and control strategies for all pests within a disruption system, especially those of economic importance. Finally, conventional pheromone based monitoring tools and phenology models are of only limited value in disruption orchards.

Recent advancements in pheromone technology

Area-wide control

The authors believe that the most significant advancements have been in the development and implementation of pheromone based strategies that coordinate tactics across an entire cropping region. This is demonstrated by the following examples from widely separated geographical regions:

Oriental fruit moth (OFM) in South Africa

OFM was first introduced to South Africa late last decade, and rapidly exploded into high populations that threaten the livelihood of the industry. This pattern is similar to that experienced when OFM was introduced to New Jersey/Maryland early this century and California and Australia in the late 1950's. Rather than immediately implement organophosphate controls, the industry

coordinated an area-wide 91-92 application of OFM mating disruption to the entire Tulbagh valley (approx 2000 ha mixed stonefruit). Despite the potential for very high damage levels (85-100% tip damage in the last generation of the previous season), complete control was achieved in one season with no supplementary applications of insecticide. Variable levels of control were experienced in neighbouring districts dependent on insecticide based control strategies. Tulbagh Valley pheromone treatment rates have been halved in 92-93 and populations have not recovered to damaging levels.

Boll-weevil trapping in the southeastern US

The successful US boll-weevil (BW) eradication program is highly dependent on semiochemicals for determination of both BW presence and population density. One to several fall (or autumn) applications of insecticide are aimed at reduction of diapausing populations. In the following spring, populations are monitored on an area-wide basis using pheromone traps placed at the rate of 1 trap/acre. These traps act to mop-up residual beetle populations and also provide a basis for treatment thresholds where higher populations are present. The program claims to have eradicated BW from the Carolinas, Georgia, Florida and parts of Kentucky, and West Virginia. In the state of Mississippi, an area-wide pheromone trapping network has been integrated with a computer based geographic information system (GIS) to provide a statewide graphic presentation of beetle trap capture. A weekly map is obtained clearly showing the relative pest density across the complete state. While still in development by USDA, it is expected that this program will enable introduction of well coordinated area wide IPM decision making and optimisation of control strategies. .

Management of Codling moth resistance to OP insecticides in California

CM resistance has been demonstrated by the University of California, Berkeley, in response to control failures in the field. CM populations are steadily increasing in pear growing districts. Neither maximum rate insecticide applications nor season long mating disruption programs are able to reduce damage to commercially acceptable levels under these high populations. Trials in 91 and 92 demonstrated commercially acceptable management of resistant populations using a combination program incorporating pheromone with insecticide. This program is being implemented this season in management of highly resistant populations on approximately 700 contiguous acres of pears in the Sacramento Delta.

Innovative new controlled release configurations

Commercial implementation of mating disruption is limited by difficulty in application of current controlled release technology. Application of commercially available dispensers often requires specialized equipment and is generally very time consuming requiring the use of both hands to complete the operation. Two new controlled release configurations should lead to increased application efficiency and commercial adoption for manually applied formulations.. These are the Scentry spiral and the Trécé invented puzzlepiece.

Puzzlepiece: Trécé have invented a new configuration for use with flat pheromone formulations. This shape, called the puzzlepiece, allows rapid one-handed application of a formulation that can be die-cut (eg. laminates, pvc or other plastic matrix wafers, cellulose flakes).

Spiral: Scentry have invented and extensively tested a pvc formulation that has a spiral configuration. This dispenser apparently has considerable field longevity for acetate compounds. For purposes of description, it is easiest to liken this to one coil in a telephone cord.

Pole applicator

One of the US regional Pacific Northwest distributors, Superior Ag Products, Yakima, Washington has developed a patented pole applicator and clip for fast and easy placement of dispensers in the upper canopy of fruit trees. This tool doubles the speed at which dispensers can be applied using conventional means at the same time as optimizing placement position.

Development of a semiochemical bait matrix for corn rootworm

The semiochemical bait for diabroticine beetles (SLAM_, Microflo Company) is an example of a selective control program that is currently in initial stages of commercialization. This tactic has been shown to be very effective in management of adult populations of corn rootworm and cucumber beeties. While quite effective in plots as small as 40 acres, the greatest impact will be had where large areas are treated. An area-wide grid of semiochemical traps should be established to map pest population densities and pinpoint high population centres.

Monitoring

i.) New stored product pest monitoring devices

USDA and Trécé have developed an innovative new pitfall trap for stored product pests. Further information is presented in reports from the two posters.

ii) 10 mg lures for monitoring of target pest populations in disruption orchards.

Researchers in British Columbia, Washington and California have demonstrated significant correlation between CM trap capture in disrupted orchards and likelihood for economic damage. This monitoring tool will considerably facilitate implementation of CM disruption technology.

iii) String technology

USDA Beltsville have patented pvc coated string as a new formlation for gypsy moth monitoring purposes. If demonstrated to be effective for other insects, this could be a possible successor to the rubber septa. Trécé has licensed this technology from USDA.

Conclusions and research needs

Monitoring

Pheromone based monitoring systems are the most effective tool for detection of insect pests while still at low population densities. Pest management becomes very sophisticated when trap data is coupled with temperature based computerized phenology models. Implementation of pheromone trap IPM treatment thresholds has led to wide-spread reduction in the quantity of insecticide applied in agriculture. Pheromone based pest management decision making should be expanded to more pest species with carefully researched threshold values, developed by correlating pheromone captures with data from sequential sampling protocols.

Sex pheromones are only a small component of the semiochemical information used by insects. Research must focus on development of other compounds, food attractants or host volatiles and also on the possible combination of such attractants with pheromone.

Area-wide monitoring programs will become increasingly more important as pest management shifts to use of pesticides with greater selectivity. As some of these strategies (eg.

mating disruption) rely on use of behaviour modification it is critical that pest managements decisions include information on pest pressure throughout the region. Area-wide trapping networks should act as early warning systems by identifying and enabling treatment of high populations that would otherwise become loci for re-infestation of areas with low populations.

Research should focus on developing more sophisticated uses of pheromone based monitoring systems for all key agricultural pests. Pheromone based IPM systems enable production of high quality food and fibre crops with selective new pesticides while addressing the demands for reduced pesticide usage, continuing development of insecticide resistance, and environmental hazards associated with pesticide usage. Due to shrinking public sector resources, it is likely that industry (primary producers, agchem suppliers) will need to coordinate and assume more responsibility for development, evaluation and implementation of these systems.

Mating disruption

Many of the research needs for mating disruption were highlighted and discussed in the previous presentation at the IOBC San Michele meeting (Kirsch, 1992). In brief summary, 'high density' research protocols need to be developed that maximize the amount of information obtained from each dispenser used. Of course this will require considerably more focus on actual insect behaviour, both male and female, and a higher component of human input. It is possible that future significant breakthroughs will only occur with the infusion of new ideas and completely new perspectives.

Reference

KIRSCH, P.A. 1992 Mating Disruption: Ideas on breaking through the glass ceiling?, p 18-26, in loriatti, C and H. Arn, Proceedings: Working Group Meeting, IOBC/WPRS Bulletin 1992/XV/5.

INTERNATIONAL COLLABORATION

PHEROMONES IN DEVELOPING COUNTRIES: THE 15-YEAR EXPERIENCE OF FRENCH RESEARCH ORGANIZATIONS (INRA-CIRAD)

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Abstract Since 1978, the "Laboratoire des Médiateurs Chimiques" has identified the pheromones produced by more than 25 moth or beetle species that are pests of crops in developing countries.

This paper tries to outline a framework of these cooperations in the general field of agricultural research, and then in the case of chemical ecology.

The tropical models studied by agricultural research share some features influencing the success of investigations. These characteristics arise from the **social and economical situation** of the developing countries, from the structure of **tropical crops and biotopes** and from the **possibilities of development** given by the basic discoveries.

The research on pheromones in tropical development programs proceeds from the above mentioned characteristics. From 25 species-based programs started in the laboratory, only 10 could be achieved by field tests in Africa, South America or Asia. The political ups and downs of the programs, or the removals of the scientist in charge of the field works were the main causes of these mishaps.

However the future of pheromones in developing countries remains promising. The expected increase of cash crops and the rejection by consumers of conventional insecticides will favour the use of semiochemicals instead of pesticides.

Introduction

Pheromones and other semiochemicals are now widely used in developed countries to monitor or control the populations of insect pests. In developing countries, the use of pheromones is very uncommon, although the insects cause more loss to agriculture than in temperate climate.

In France, the National Institute for Agricultural Research (INRA) is involved in pheromone research through the Laboratoire des Médiateurs Chimiques, founded in 1975 by Charles Descoins. This laboratory leads basic works on the pheromone biology of agricultural pests, and it is concerned in tropical insects through cooperation with organizations or persons working on applied entomology.

In this way, our main partner has been the CIRAD (International Centre for Agricultural Development) which supports entomologists in many developing countries (French-speaking Africa, South America and Far East Asia).

Since 1978, the Laboratoire des Médiateurs Chimiques has identified the pheromones produced by more than 25 moth or beetle species, pests of crops in developing countries (see Table). Some of these pheromones are really used in the field today, but many other species received little attention since the initial identification work.

Tropical models from developing countries-

Consequences of Tropical Climate

The influence of the tropical climate found in nearby all developing countries is highlighted by two components of the agricultural systems, the crops and the insects.

i) The Tropical Crops

The situation is different according as we consider a cash crop or a food crop. The cash crops are generally grown on large surfaces, by growers who show a fair technical competence. The cultivars used here are often susceptible to insects or diseases. On the contrary the traditional food crops are grown on small parcels in a mixed farming environment. The cultivars are more resistant in spite of their poor yields.

ii) The Tropical Insects

The warm and wet climate is propitious to the insects. The most conspicuous consequences may be shown by the high levels of population of some pest species, and by the number of species that may inhabit the same crop (it means a large number of candidate pests along with a large number of beneficial organisms).

The biological diversity of the tropical fauna may also offer good models for academic research (e.g., the relationships between *Passiflora* plants and *Heliconius* butterflies; or the study of courtship behaviour in *Eldana saccharina*). Furthermore, the occurrence of larval or adult stage throughout the year may quicken the field studies.

Characteristics of the Developing Countries

i) Social and Economical aspects

In the developing countries, the grants for cooperation programmes are found mainly through international projects (from FAO, EEC etc.), due to the critical financial situation of many local research organizations.

During field tests or material collections, some problems may arise owing to the poor equipment of the research stations. The long distances usually found in these countries between experimental sites and research centres may also restrain the size of the field tests, especially as the road infrastructure is very bad and petrol expensive. Some technical items like traps or recording devices left in open field for weeks are likely to be stolen.

The field test protocols in developing countries have to take into account all the above mentioned characteristics to avoid mishaps, but they may take advantage of the abundant labour to set up experiences on a large scale.

ii) Expected Development

The achievement of a pheromone identification work lasts two years at least, the final stage being the field tests of the candidate compounds. Here, our research work normally stops, but, if the results are conclusive, we try to encourage the development of monitoring systems, by supplying pheromone dispensers to the local entomologists.

This issue needs first a continuity of men and programmes for several years, which is not frequent in developing countries. Many projects are thrown over after one or two years without scientific justification, furthermore, the scientists themselves are often moved regardless of the evolution of their work.

These political mishaps occur specially when we work with scientists belonging to public research organizations. On the other hand, we have experienced the partnership with private companies involved in palm oil production in South America. In this case, the received funds were adjusted to the real importance of the insect, and we could develop a sustained programme for several years.

The political instability may reach the level of guerrilla or civil war, with the consequence of leaving the experimental sites in the field or closing the research stations. Such events occurred during our investigations on *Rhynchophorus palmarum* and *Stenoma cecropia* in Colombia; it also stopped the development of *Chilo* pheromones in Southern Senegal.

The Case of Chemical Ecology

i) Knowledge of the Fauna

The tropical fauna remains poorly known. Our experience shows that a study of pheromone biology is valid only for a limited population, although the use of attractants might be a good approach of the cartography of insects. For example, the sex pheromone of the Noctuid *Sesamia calamistis* was identified from and tested in Central Mali, but the synthetic attractant for *S. calamistis* never worked in Northern Senegal where it was tested for three years. In the same way, the pheromone of the Pyralid *Chilo zacconius*, identified in Southern Senegal, never attracted any males in Mali or Ivory Coast. Contrary to the European (or North American) fauna, the data about intermediate populations completely lack over a 1,500 Km distance. So, we cannot conclude on sibling species or pheromone polymorphism without further studies.

ii) Monitoring the Populations with Semiochemicals

The monitoring of pest populations with semiochemicals usually deals with, (a) the occurrence of the insect, (b) its phenology and (c) the population levels. The latter received little attention in temperate countries, as it is very difficult to predict an hypothetic larval population from a collection of caught male moths. Under tropical climates, the occurrence of adult insects throughout the year and the frequent overlapping of generations raises the question of the utility of insect trapping unless establishing correlations between catches and infestations.

Before to draw these correlations, the overall design of the traps should be evaluated, according to the behaviour, the season (sand and dust particles agglomerate on sticky traps in dry season) and the population levels.

One should keep in mind that the insect population levels can be very high in tropical monoculture. However, the efficiency of attractants decreases when the population increases, therefore results of monitoring programmes might be carefully checked before drawing conclusions.

iii) Control of Insect Pests with Pheromones

We are still far from a normal use of pheromones as pest control agents in the developing countries. Some trials of disruption have been performed with good results in Ivory Coast against pests of cotton (*Pectinophora gossypiella* and *Cryptophlebia leucotreta*). These trials have shown the efficiency of the method, but it will remain restricted to the research stations as long as companies do not invest in tropical crop markets.

The mass trapping of pests may be used, at least for the palm weevils *Rhynchophorus spp.*, which meet three conditions rarely combined in pests: (a): the adult is the most dangerous stage, (b) the pheromone attracts females and (c): it is a pest although the population levels are low. In this case the use of a synthetic attractant will simply replace a traditional trapping method using cut trees as bait.

Conclusions and Perspectives

The table shows that from more than 25 species that have been studied in our laboratory, only 10 programmes could be achieved by complete identification followed by field tests. My own statement of the future use of pheromones for these species is subjective, but it takes into account our experience of social reality of the country, along with a more realistic approach of the geographical and economical importance of the insect.

The future of pheromones in developing countries remains promising. The occurrence of very large surfaces in industrial crops, along with the increasing technical level of local agronomists will certainly promote more sophisticated methods of pest management like disruption, although the diversity of candidate pests in tropical crops is still a barrier for the development of species-specific control methods.

In developed countries, the emergence of "biological" or "natural" agriculture will favour the use of semiochemicals instead of pesticides. This may be perceived as a caprice of secured consumers, far from the African point of view, but at the same time, the products imported from developing countries are now submitted to severe analyses for pesticide residues, constraining the growers to moderate their classical approach of the pest control.

Partner	Species	Family	Crop	Origin	Laboratory Work	Field Tests	Expected Dev.
CIRAD-CTFT	*Hypsipyla robusta*	Pyralidae	Mahogany	W. Africa	☺		
CIRAD-CA	*Chilo zacconius*	Pyralidae	Rice	W. Africa	☺	☺	☹
(IRAT)	*Chilo diffusilineus*	Pyralidae	Rice	W. Africa	☺		
	Corcyra cephalonica	Pyralidae	Rice/stored prod.	Worldwide	☺		
	Eldana saccharina	Pyralidae	Sugarcane/maize	Africa	☺		
	Mussidia nigrivenella	Pyralidae	Stored products	W. Africa	in progress		
	Sesamia calamistis	Noctuidae	Graminaceae	W. Africa	☺	☺	☹
CIRAD-CA	*Cryptophlebia leucotreta*	Tortricidae	Cotton/cocoa	Africa	☺	☺	😐
(IRCT)	*Diparopsis watersi*	Noctuidae	Cotton	Africa	☺	☺	☹
	Earias biplaga	Noctuidae	Cotton/cocoa	W. Africa	☺		
	Heliothis armigera	Noctuidae	Cotton/vegetables	Old World	☺	☺	😐
CIRAD-SAR	*Ectomyelois ceratoniae*	Pyralidae	Dates/stored prod.	Worldwide	☺	☺	😐
CIRAD-CP	*Pimelephila ghesquieri*	Pyralidae	Oil Palm Tree	W. Africa	☺		
(IRHO)	*Rhynchophorus palmarum*	Col. Curculionidae	Oil Palm Tree	S. America	☺	☺	☺
Growers	*Rhynchophorus phoenicis*	Col. Curculionidae	Oil Palm Tree	Africa	☺	☺	😐
	Rhynchophorus vulneratus	Col. Curculionidae	Oil Palm Tree	Indonesia	☺	in progress	
	Sagalassa valida	Brachodidae	Oil Palm Tree	S. America	in progress		
	Sethotosea asigna	Limacodidae	Oil Palm Tree	Indonesia	☺	☺	☺
	Setora nitens	Limacodidae	Oil Palm Tree	Indonesia	☺	in progress	
	Stenoma cecropia	Stenomidae	Oil Palm Tree	S. America	☺	☺	☹
ORSTOM	*Hypothenemus hampei*	Col. Scolytidae	Coffee Tree	Worldwide	in progress		
INRA CRAAG	*Diaprepes abbreviatus*	Col. Curculionidae	Citrus spp.	S. America	☺		
	Metamasius hemipterus	Col. Curculionidae	Sugar-cane	S. America	in progress		
(ORSTOM)	*Spodoptera frugiperda*	Noctuidae	Graminaceae	America	☺	☺	😐

Table: programmes dealing with the pheromones of tropical pests at the *Laboratoire des Médiateurs Chimiques*, co-worked with French research organizations.

INDO-DUTCH COOPERATION ON PHEROMONES OF INDIAN AGRICULTURAL PEST INSECTS: SEX PHEROMONE COMPONENTS OF *DIACRISIA OBLIQUA* (ARCTIIDAE), *ACHAEA JANATA* (NOCTUIDAE) AND *AMSACTA ALBISTRIGA* (ARCTIIDAE)

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Abstract By means of HPLC, GC, EAG and GC-MS, the pheromone of *Diacrisia obliqua* could be identified as a mixture of (Z3,Z6)-cis-9,10-epoxy-3,6-heneicosadiene, (Z3,Z6)-cis-9,10-epoxy-1,3,6- heneicosatriene, Z9,Z12-octadecadienal, Z9,Z12,Z15-octadecatrienal and Z3,Z6,Z9-heneicosatriene in a ratio of 1.0 : 0.5 : 1.0 : 3.0 : 2.6. A synthetic blend in this ratio proved to be very attractive in windtunnel experiments. Field experiments still have to confirm these findings.

Along the same procedures the pheromone of *Achaea janata* was found to contain: heneicosane, Z6,Z9-heneicosadiene, Z3,Z6,Z9-heneicosatriene and Z9,Z12-octadecadienal in a ratio of 1.0 : 1.0 : 65 : 1.0. Due to lack of insects the functions of these compounds could not be determined yet.

The pheromone glands of *Amsacta albistriga* were found to contain octadecanal, Z9,Z12-octadecadienal, Z9,Z12,Z15-octadecatrienal and Z3,Z6,Z9-heneicosatriene in a ratio of 1.0 : 1.0 : 11.5 : 24.0. The functions of the isolated compounds still have to be determined.

DIACRISIA OBLIQUA (ARCTIIDAE)

Diacrisia (= Spilosoma) obliqua, is a polyphagous insect, attacking many crops in India and Bangladesh (Mathur, 1962). The presence of a female-secreted sex pheromone in this species has been reported by Islam and Alam (1980) and by Siddiqi (1985). The chemical identification of five pheromone components is described in this paper.

Extracts were prepared in hexane by dissecting pheromone glands of 1-2 days old virgin females. Injection of a crude extract on the gas chromatograph (GC), and monitoring of the effluent (2 min. fractions) by electroantennography (EAG) indicated the presence of one highly active fraction when a non-polar column was used (2 m x 1/8" column, 5% CPSil 5 on chromosorb WHP, 100-200 mesh, 195° C, He 33 ml/min.) and of two active fractions when a polar column was used (2m x 1/8" column; 5% CPWax 51 on chromosorb WAW, 100-200 mesh, 195° C, He 33 ml/min). Pheromones identified from *Arctiidae* so far are multi-component mixtures, containing among others C_{20} or C_{21} diene or triene epoxides (Descoins et al. 1989; Frérot et al. 1988; Millar et al., 1991; and Tóth et al., 1989). As *D. obliqua* belongs to this family, we concentrated on the presence of this type

of compounds in the extracts.

The retention times of synthetic (Z3,Z6)-cis-9,10-epoxy-3,6-heneicosadiene (= compound I) and (Z3,Z6)-cis-9,10-epoxy-1,3,6-heneicosatriene (= compound II) coincided with the EAG active fractions on the two GC columns. Subjection of these two compounds to EAG (10µg on the filter paper pad), showed these compounds to evoke high EAG responses: 4.0 mV by compound I and 5.7 mV by compound II (n=4). (Both compounds kindly provided by Nitto Denko Corp., Osaka, Japan).

High pressure liquid chromatography (HPLC) (column 25 cm, i.d. 1.0 cm; Alltech RSil, particle size 10 µm; flow rate 3.5 ml/min; UV detection at 254 nm; gradient: hexane/hexane-10% ethyl acetate) therefore focused on the presence of these two compounds in crude extracts. After determination of the elution times of the two synthetic compounds, a crude extract was subjected to HPLC under the same conditions. The fraction, suspected to contain compounds I and II, was submitted to GC-EAG. The results were as reported above: one EAG active fraction was detected when a non-polar column was used, whereas two EAG active fractions were found when a polar column was used.

GC-MS analysis (VG 70/250S instrument; fused silica column 50 m x 0,2 mm i.d.; CPWax 57 CB; initial temperature 120° C; gradient 10° C/min; final temperature 220° C) of the same HPLC fraction confirmed the presence of both I and II. The MS of I and II were identical to those of synthetic I and II. Based on these data it was concluded that the compounds isolated from *D. obliqua* are indeed (Z3,Z6)-cis-9,10-epoxy-3,6-heneicosadiene and (Z3,Z6)-cis-9,10-epoxy-1,3,6-heneicosatriene, respectively. As the synthetic compounds I and II, having the 9S, 10R configurations show high EAG activity, it is assumed that the compounds isolated from *D.obliqua* also have the 9S, 10R configuration. However, synthesis still has to confirm this.

Following the same procedure (GC, EAG, HPLC, GC-MS) we have isolated from the same HPLC fractions two additional compounds, viz. (Z9,Z12)-9,12-octadecadienal (compound III) and (Z9,Z12,Z15)-9,12,15-octadecatrienal (compound IV). However, the EAG responses evoked by these compounds, were far less intensive than those evoked by compounds I and II (1.6 mV and 1.5 mV for III and IV, respectively (n = 4). This may explain why we have overlooked this compounds initially.

Field test with these four compounds in various ratios did not give us any trap catches (Zoecon 1C and Delta traps fitted with rubber or polyethylene dispensers).

In the HPLC fraction, eluting just before the fraction containing compounds I-IV, another compound with moderate EAG activity was detected. This compound V was isolated along the same procedures and could be identified as (Z3,Z6,Z9)-3,6,9-heneicosatriene by comparison of its retention time on various GC-colums and by comparison of its MS with that of synthetic (Z3,Z6,Z9)-3,6,9-heneicosatriene (Kindly provided by Professor J.G. Millar, University of California, Riverside, USA).

Windtunnel experiments (Plexiglass cylinder; 55 cm diameter; length 150 cm; air velocity 0,5 m/sec.; tests were carried out in the middle of the scotophase) using calling virgin females (0-2 days old), attracted 60-80% of the males released. When using the 5-component blend (ratio I : II : III : IV : V=1.0 : 0.5 : 1.0 : 3.0 : 2.6), 40-60% of the males were attracted.

Field experiments still have to indicate the optimal blend (ratio of the various compounds and number of compounds strictly needed for attraction).

ACHAEA JANATA (NOCTUIDAE)

This is undoubtedly the most noxious insect in the castor culture in India, especially when young crops are attacked. It is an oligophagous insect. Its main damage is defoliation by the larvae (castor), but in grapes losses caused by piercing of the fruits by the adults are more important.

A good monitoring system would be of great value and identification of the pheromone was decided.

Presence of the pheromone could be demonstrated by releasing 20-30 males in a screened cage (23"x23"x23") in the corner of which a trap was installed, baited with one virgin female or a female extract. 95% of the males could be trapped.

Injection of crude extracts (1-2 day old virgin females in hexane) on two different GC colums (the same columns and conditions as used for *D. obliqua)* and monitoring of the effluent (2 min. fractions) by EAG, indicated the presence of one highly EAG active peak on both colums.

An extract of about 200 females was submitted to HPLC (conditions as mentioned for *D. obliqua*. Gradient: hexane/hexane-5% ethyl acetate) and 3 fractions were collected: fr. I 4-20 min. (56 ml); fr. II 21-28 min. (28 ml) and fr. III 29-52 min (84 ml). All 3 fractions were submitted to GC-EAG (CPSil 5; 2 m; 1/8"; 5% on chromosorb WAP; 100-200 mesh, He 33 ml/min; temp. 125° C, 3 min; 5° C/min 225° C, 10 min). Only fraction I revealed an EAG active GC fraction. GC-MS analysis (conditions as for *D. obliqua*) of fraction I indicated the compound to be a heneicosatriene. Based on its retention time and comparison of its MS with that of synthetic (Z3,Z6,Z9)-3,6,9-heneicosatriene, it was concluded that the natural compound also is (Z3,Z6,Z9)-3,6,9-heneicosatriene. Under these conditions a very small peak eluted just in front of the heneicosatriene. It could be identified by comparison of its MS with that of synthetic (Z6,Z9)-6,9-heneicosadiene (kindly provided by Professor J.G. Millar, University of California, Riverside, USA) as 6,9 heneicosadiene. Based on its retention time and biosynthetic considerations, this compound was assumed to have the all Z configuration. This minor compound therefore must be (Z6,Z9)-6,9-heneicosadiene.

Gland injection according to the method of Attygalle et al. (1987) on the GC-MS confirmed the presence of the above mentioned compounds. It also showed the presence of heneicosane, a compound that could not be detected in HPLC fraction I, from which the heneicosadiene and heneicosatriene were isolated.

An EAG active fraction, collected from the GC in the early stage of the project was submitted to GC-MS analysis. Apart from the three compounds mentioned above, this fraction contained an octadecadienal, that could be identified as (Z9,Z12)-9,12-octadecadienal.

The ratio of heneicosane: (Z6,Z9)-6,9-heneicosadiene : (Z3,Z6,Z9)-3,6,9-heneicosatriene : (Z9,Z12)-9,12-octadecadienal = 1.0 : 1.0 : 60-70 : 1.0. The functions of these compounds still have to be determined by windtunnel and/or field experiments.

AMSACTA ALBISTRIGA (ARCTIIDAE)

A. albistriga is a common polyphagous insect in South India, showing a strong preference for groundnuts. Especially young plantations can be completely defoliated. The highest population density of the insect coincides with the early growing stage of the crop. This often leads to complete destruction of the young crop and makes replanting necessary.It has only one generation per year, but under laboratory conditions a partly second generation can be reared. The extremely low supply of insects (17 and 5) seriously hampered progress of the work.

A crude extract (in hexane) was injected on the GC (CPSil 5; 2 m; 5%; temp. 150° C (5 min); 5° C/min 225° C; isotherm 10 min.) and 2 min. fractions were collected for EAG monitoring. EAG activity was confined to two fractions. Due to shortage of male insects, the GC effluent from one column only could be monitored.

As both *A. albistriga* and *D. obliqua* belong to the Arctiidae, it was assumed that they might have certain pheromone components in common. Therefore the compounds isolated from *D. obliqua* were injected on the same column using the same conditions as described above. Three of the *Diacrisia*-compounds, viz. (Z9,Z12)-9,12-octadecadienal, (Z9,Z12,Z15)-9,12,15-octadecatrienal and (Z3,Z6,Z9,)-3,6,9-heneicosatriene, eluted within the 4 min. area where the EAG active compounds of *A. albistriga* eluted. The remaining of the crude extract now was injected on the GC using the conditions described above, and three 2-minute fractions were collected. Subsequently these fractions (AM1-AM3) were submitted to GC-MS. In the first fraction (AM1) we could identify octadecanal, (Z9,Z12)-9,12-octadecadienal and (Z9,Z12,Z15)-9,12,15-octadecatrienal. (Z3,Z6,Z9)-3,6,9-heneicosatriene could be detected in fraction AM2. The epoxy-heneicosadiene and epoxy-heneicosatriene, identified in the pheromone blend of *D. obliqua,* could not be detected in the *A. albistriga* extracts.

The ratio octadecanal: (Z9,Z12)-9,12-octadecadienal : (Z9,Z12,Z15)-9,12,15-octadecatrienal : (Z3,Z6,Z9)-3,6,9-heneicosatriene was calculated as 1.0 : 1.0 : 11.5 : 24.0.

The functions of the individual compounds remain to be determined.

References

ATTYGALLE, A.B., HERRIG, M., VOSTROWSKY, O. AND BESTMAN, H.J., 1987. Technique for injecting intact glands for analysis of sex pheromones of Lepidoptera by capillary gas chromatography: Reinvestigation of pheromone complex of *Mamestra brassicae*. J. Chem. Ecol. **13** (5): 1299-1311.

DESCOINS, C., LALANNE-CASSOU, B., FRÉROT, B., MALLOSSE, C. ET RENOU, M., 1989. Étude comparative des sécrétions pheromonales émises par des femelles d'Arctiides et des Cténachides (Insectes, Lepidoptera) de la zone néotropicale. C.R. Acad. Sci. Paris, t. 309, Série III:577-581.

FRÉROT, B., POUGNY, J.R., MILAT, M.L., ROLLIN, P., ET MALLOSSE, C., 1988. Étude de la sécrétion pheromonale de *Cymbalophora pudica* (Esper) et de l'énantiosélectivité de la perception pheromonale. C.R. Acad. Sci. Paris, t. 306, Serie III:157-160.

ISLAM, B.N., AND ALAM, M.Z., 1980. Mating behaviour of the jute hairy caterpillar, *Diacrisia obliqua* Walker (Lepidoptera: Arctiidae). Rate of catch of male moths by virgin female traps. Appl. Ent. Zool. **15** (3):299-309.

MATHUR, A.C., 1962. Food-plant spectrum of *Diacrisia obliqua* Wlk. (Arctiidae, Lepidoptera). Ind. J. Ent. **24**:286-287.

MILLAR, N., GIBLIN, N., BARTON, D., AND UNDERHILL, E.W., 1991. Chiral Lepidopteran sex attractants: Blends of optically active C_{20} and C_{21} diene epoxides as sex attractants for Geometrid and Noctuid moths (Lepidoptera). Environ. Entomol. **20** (2):450-457.

SIDDIQI, J.I., 1985. Observations on the secretion of a sex pheromone in Diacrisia obliqua Walker (Lepidoptera: Arctiidae). Zool. Jb. Physiol. **89**:81-87.

TÓTH, M. BUSER, H.R., PE_A, A., ARN, H., MORI, K., TAKEUCHI, T., NIKOLAEVA,, L.N., AND KOVALEV, B.G., 1989. Identification of (3Z, 6Z)-1, 3, 6 - 9, 10 - epoxyheneicosatriene and (3Z, 6Z)-1, 3, 6 - 9, 10 - epoxyeicosatriene in the sex pheromone of *Hyphantria cunea*. Tetrahedron Letters **30** (26):3405-3408.

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CONTROL OF PINK BOLLWORM WITH GOSSYPLURE IN PUNJAB, PAKISTAN

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Abstract Sex pheromone *'gossypiure'* was used to monitor the activity of pink bollworm and to control it using mass trapping and mating disruption techniques. Moth activity was recorded throughout the year. Suicidal emergence took place from January to June. The activity increased gradually from July onwards. The increase in activity was related to increased infestation. Control with mass trapping and mating disruption proved very successful and the infestation in the pheromone treated area was significantly reduced. The mating disruption technique was, however, better than mass trapping. Single application of PB Rope/Tea bag of Mitsubishi Corporation of Japan provided 100% disruption and season long control of pink bollworm. Overall 50% reduction in insecticidal application was obtained and the yields were comparable with that of insecticides treated areas. However, the use of pesticides can be further reduced if pheromone of *Earias spp.* is also incorporated with *'Gossyplure'*. This will strengthen the IPM programme in Pakistan

Introduction

Cotton is very ancient to Pakistan. The name of Indus Valley is inextricably linked with cotton in both legend and history. The oldest cotton yarn and fabric known today were unearthed from the ruins of Moenjodaro, 200 miles North of Karachi. These fabrics have been estimated to be about 5000 years old. Very recently cotton seed from 5th millennium BC have been discovered at Mehargarh in Baluchistan.

Among various field crops cotton occupies a unique position in Pakistan because it provides food, feed, fibre and fuel. Cotton is the most important economic crop of Pakistan. It provides 60% of export earnings and over 55% of domestic edible oil production. It provides raw materials to about 300 textile mills, over 1000 ginning factories and 5000 oil expellers. As a result millions of people are employed in cotton based industries.

Pakistan ranks 5th in area and production in the world and has been an important exporter of raw cotton. Since the creation of Pakistan in 1947, the area under cotton has increased from 1.2 million to 2.8 million hectares, production from 1.1 million to 113 million bales and yield from 159 to 768 kgs of lint/ha. in 1991. .Increase in yield per hectare has contributed to nine-fold increase in production. However, the yield of lint/hectare is still quite low when compared to most of the other irrigated areas of the developed countries. One of the reasons related to low production is the heavy pest attack on the cotton crop.

The key pests which suck the sap of the cotton plant and affect the growth and induce shedding. are Cotton jassid (*Amrasca devastans, Dist.)*, Cotton whitefly (*Bemisia tabaci,*

(Gennadius)) and Thrips (*Thrips tabaci,(Lind.))* Important bollworms which attack cotton crop are Pink Bollworm (*Pectinophora gossypiella, (Saund.))*, Spiny and Spotted Bollworms (*Earias insulana(Boisd)*and *Earias vittella, F.)* and Cotton Bollworms (*Helicoverpa armigera, (Hb.))*. Pink bollworm is economic pest of cotton in Punjab and accounts for 10-20% loss in yield of seed cotton every year.

Insecticides are primarily used to control bollworms. The indiscriminate use of insecticides in Pakistan is resulting in the flare up of secondary pests like Teamite (*Polyphagotarsonemus latus*), *Helicoverpa armigera* and *Spodoptera litura*. With the discovery of gossyplure (Hummel et al 1973), it has become possible to suppress pink bollworm population with pheromones. Since then gossyplure is being used for the monitoring of pink bollworm in the field. In the Punjab, the studies were undertaken with gossyplure to monitor the activity of pink bollworm throughout the year and find methods for effective control of pink bollworm with gossyplure.

Materials And Methods

Monitoring

The pink bollworm sex attractant pheromone, gossyplure, a mixture of Z, Z and Z, E isomers of 7, 11 Hexadecadienyl acetate was used exclusively during these studies. All traps contained a rubber wick dispenser impregnated with 1 mg. of gossyplure at 1:1 ratio of isomers (Flint et al 1974). The Omni-Directional (Sharma et al 1973) and Delta traps were used to monitor the activity of pink bollworm. The pheromone was replaced every 3rd week. The studies were continued throughout the year since 1975 in Central Cotton Research Institute,(CCRI), Multan and at farmers' fields.

Mass Trapping

Ten delta traps/ha. were used throughout the season to control pink bollworm in 15 ha cotton fields. The traps were placed at squaring stage, at least 80 feet distance from each other. The traps were replaced after every 3 weeks. The control plot of the same size was maintained nearby. The pink bollworm infestation was determined by sampling 400 susceptible bolls (14-21 days old) at weekly interval. In the pheromone treated field one application of systemic granules, Disyston @ 12.5 kgs/ha. was applied with first irrigation to control sucking pests. Spray applications were planned for other bollworms, when required.

Mating Disruption

In nature male moths locate and mate with female moths by following a scent trail emitted by all calling vergin female moths of the same species. This behaviour can be interfered with, by creating false trails with synthetic formulation, thereby preventing mating, resulting in population decrease. Micro-encapsulated formulation (Pectone 2%) developed by ICI Agro-Chemical in U.K. was used for the experiment conducted in 1983 on 21 hectares in the farmers fields. The pheromone was applied with motorised knap-sack sprayer at pin square stage on 23rd August, 1983. Three more applications at about 15 days interval were given on 11th September, 28th September and 25th October. On simultaneous dates the control area was treated with insecticides for the control of bollworm complex. The mating disruption was assessed by placing 5 traps each in the treated as well as control plots.

In 1989 crop season twist tie dispensers developed by Shinetsu Company and marketed by Mitsubishi Corporation of Japan under the trade name of PB Rope were used on 11 hectares planted

field were very low as compared to insecticides treated fields. The pink bollworm infestation was significantly higher in the insecticides treated area compared to pheromone treatment as shown in Fig.2. The yield of seed cotton was higher in the pheromone treated field compared to insecticides treatment.

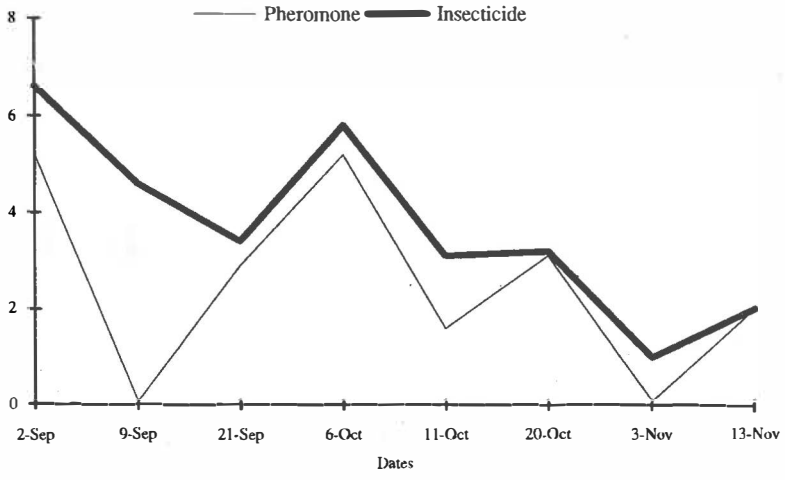

Fig: 2:- Pink Bollworm infestation in Pheromone and Insecticide treated areas in 1983

All the plots were treated with one spray of Dimethoate insecticides to control sucking pests. In the pheromone treated plot spot treatments with a combination of Cypermethrin + Profenofos and Methomyl were given to control the out-break of *Earias* species and *Spodoptera litura* species. In insecticides treated field four sprays were given in addition to Dimethoate i.e. two sprays of Carbryl 85% and 2 sprays of mixture of Cypermethrin + Profenofos.

In 1989 the control of pink bollworm was tried with PB Rope developed by Mitsubishi Corporation. The applications were made at pin head square stage (3.8.1989). The moth activity was observed with 5 Delta traps containing sex pheromones in both the plots. The results on mating disruption are given in **Table 2.** The bollworm infestation is given in **Table.3** The PB-Rope application provided 100% mating disruption because moth catches were negligible. The maximum bollworm infestation was only 6% in the pheromone treated field compared to 22% in the insecticides treated fields.

Table 2. Mating disruption in *P. gossypiella* following application of PB-Rope during 1989.

Weeks	Mean Weekly Catches per trap		
	Insecticide	Pheromone	% Disruption Treated
7/8/89	1.0	0	100
15/8/89	2.0	0	100
23/8/89	55.0	0	100
31/8/89	32.0	0	100
7/9/89	60.0	0	100
15/9/89	126.0	0	100
22/9/89	110 0	0	100
30/9/89	49.0	0	100
7/10/89	25.0	0	100
15/10/89	31.0	0	100
23/10/89	0	0	-

Table 3 Pink bollworm damage in the pheromone and insecticide treated area in 1989.

Date	Percentage Infestation	
	Insecticide	Pheromone
29/8/89	40	4
6/9/89	11	6
13/9/89	7	5
21/9/89	4	0
30/9/89	4	6
9/10/89	22	5
18/10/89	7	3
24/10/89	7	2
29/10/89	5	6
7/11/89	2	1

Chemical sprays: 7/8, 28/8, 13/9 and 2/10.

The pheromone treated field received four insecticide applications, two for sucking pests and two for *Earias* species and *Helicoverpa armigera* whereas 5 insecticides applications were given in the control. The yield of seed cotton was higher in the pheromone treated block.(3064 kg/ha) as compared with insecticide treated fields (2669 kg/ha).

In 1990 the Tea-bags and PB-Rope impregnated with pheromone were applied at pin head square stage (30.7.1990). The results on mating disruption are given in **Table-4.**

Table 4. Mating disruption in *P. gossypiella* following application of PB. Rope, large bag and small bag pheromone in 1990-91 crop season.

Dates	Percentage Infestation			
	Pheromone			Insecticide
	Large Bag	Small Bag	PB-Rope	
28/8/90	0.00	0.00	0.00	0.00
13/9/90	0.00	0.00	0.00	3.00
30/9/90	0.33	0.33	0.00	1.00
15/10/90	0.33	1.33	2.00	4.00
30/10/90	0.00	0.67	0.67	8.00
15/11/90	2.20	0.00	1.10	10.00
30/11/90	1.67	1.67	3.33	10.00

It may be seen from the results in Table-4 that the pink bollworm infestation to the bolls was higher in the insecticide treated plots. The results indicated that communication disruption with single application of PB Rope or Tea-bag dispensers at pin square stage provided season long control of pink bollworm. Potential for communication disruption of *P. gossypiella* in the U.S.A. was reported by Flint and Merkle (1983, 1984). Flint et al (1985) Staten et al (1987). Similar results in Egypt were reported by Critchley et al (1983) and McVeigh et al (1983). Critchley et al (1984) and Stone and Gutierrez (1986) indicated that mating disruption was most effective when early season applications were made which subsequently delayed population increase. Later season applications were in-effective. The same observation was made in Pakistan.

In Pakistan *Earias* species. attack cotton even before the fruiting starts. The initial attack is on the top shoots. The pink bollworm infests cotton when fruiting starts. The use of chemical sprays become necessary to control the *Earias* spp. The sprays against *Earias* spp. also help to check the attack of pink bollworm. Although the pink bollworm was controlled with sex pheromone, the use was limited because of infestation of *Earias* spp. The commercial formulation of pheromone of both species of *Earias* along with that of pink bollworm will help the IPM Programme on Cotton in Pakistan.

References

AHMAD, Z. (1979). Monitoring the seasonal occurrence of the pink bollworm in Pakistan with sex traps. FAO. Plant Protection.Bull. 27: 19-20.

CRITCHLEY, BR., CAMPION, D.G., MCVEIGH, L.J., HUNTER JONES, P., HALL, D.R., CORK, A., NESBITT, B.F., MARRS, G.J., JUTSUM, A.R., HOSNY, M.M., AND EL SAYED, NASR, A (1983) Control of pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera:Gelechiidae) in Egypt by mating disruption using an aerially applied micro-encapsulated pheromone formulation. Bull. Ent.Res.73: 289-99.

CRITCHLEY, BR., CAMPION, D.G., MCVEIGH, E.M., MCVEIGH, L.J.; JUTSUM, R.A. GORDON, R.F.S., MARRS, G.J., EL SAYED, .NASR, A AND HOSNY, M.M. (1984). Microencapuslated pheromones in cotton pest management. British Crop Protection Conference Vol.I (Pest and Diseases), 241-5.

FLINT., H.M., & MERKLE, J.R. (1983) Pink bollworm (Lepidoptera: Gelechiidae). Communication disruption by pheromone composition imbalance. J.Econ. Entomol.**76**: 40-46.

FLINT., H.M. AND MERKLE, J.R. (1984). Studies on disruption of sexual communication in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), with microencapuslated gossyplure or its component Z,S-isomer. Bull. Ent.Res.**74**: 25-32.

FLINT., H.M., MERKLE, J.R., AND YAMAMOTO, A (1985). Pink bollworm (Lepidoptera: Gelechiidae): Field testing a new polyethylene tube dispenser for gossyplure. J. Econ. Entomol.**78**: 1431-6.

FLINT., H.M., KUHN. S; HORN, B AND SALLAM, H.A., (1974). Early season trapping of pink bollworm with gossyplure J.Econ. Entomol.**67**: 738-40.

HUBER, R.T., MOORE, L AND HOFFMAN, M.P. (1979). Feasibility study of area-wide pheromone trapping of male pink bollworm moths in a cotton insect pest management programme. J.Econ. Entomol. **72**: 222-7.

HUMMEL, H.E., GASTON, L.K., SHOREY, H.H., RACE, R.S., BYRNE, K.H.AND SILVERSTEIN, R.M.(1973) Clarification of the chemical status of the pink bollworm sex pheromone. Science **181**: 873-5.

MCVEIGH, L.J., CRITCHLEY, B.R., AND CAMPION, D.G. (1983). Control of pink bollworm in Egypt by mating disruption using pheromone. In Proceedings of 10th Intern. Cong. of Plant Protection, held at Brighton, England, Nov.20-25, vol. 1, pp. 268.

SHERMA, R.K., RICE, R.E., REYNOLDS, H.T. AND HANNIBAL, R.M. (1973). Effect of trap design and size of hexalure dispensers on catches of pink bollworm males. J. Econ. Entomol., **66**: 377-379.

STATEN, R.T., FLINT, H.M., WEDDLE, R.C., HERNANDES, E., AND YAMAMOTO, A. (1987). Pink bollworm (Lepidoptera:Gelechiidae): Large-scale field trials with a high-rate gossyplure formulation. J. Eco. Entomol. **80**: 1267-71.

STONE, N.D. & GUTIERREZ, A.P. (1986). Pink bollworm control in south-western desert cotton.II. A strategic management model. Hilgardia **54**: 25-41.

PHEROMONE TRAPPING IN SOUTH ASIA: REVIEW AND REQUIREMENTS

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Abstract There has been considerable progress in the application of insect pheromone technology for the benefit of farmers in less developed countries. The best examples in Asia are *Spodoptera litura* and *Helicoverpa armigera* for which technology and industrial suppliers are available. The population densities developing countries in Asia once again approach levels at which the farming sectors will struggle to cope with the demand for food. Insect pests exert a considerable detrimental effect on several of the important food crops, especially the all important legumes. As some of these pest problems are induced by the over-application of insecticides there is a need to rationalise the insect management systems. Pest monitoring and forecasting will become increasingly important. Thus there is a considerable need to extend the range of pheromone trapping systems currently available to meet the anticipated demand.

Introduction

This Working Group meeting has covered matters relating to the sharp end of pheromone technology - for instance, the influence of molecules on discrete receptor cells. We have also heard about recent successes in various aspects of the application of pheromone technology in pest management, mainly in scenarios of western agriculture. We enlarge upon this theme and indicate some successes in developing countries in Asia. This is also an opportunity to indicate that there are pressing needs to expand the number of species considered.

The 'pressing need' is, in fact, the need to enable less developed countries to feed their populations. Green Revolution technology has provided some respite in these countries because the demand for cereal production often is actually less than the supply. However, in the face of continuing population growth, and, in some cases, a recent trend of diminishing agricultural productivity, this overproduction cannot be expected to continue. This is the position in Asia where most of the World's people live.

When we look at non-cereal crops, especially the pulses, the situation is considerably worse. Production and productivity have increased only slightly over the last 30 years, but have dropped markedly on a per capitata basis. Wightman (1993) provides statistical summaries in support of this statement. He also indicates that a cohort of legume pests and the insecticides applied to kill the insects are, to a large extent, responsible for this Asia-wide stagnation in production and the accompanying price hikes.

Our thesis is that the researchers and business people attending this Workshop have a

continuing role to play in alleviating this situation. Semiochemicals, especially sex attractant pheromones, have a key role to play in the sustainable management of the key pests of the prime protein and vitamin sources of the rural and urban poor of Asia.

Asia is a large place. As such, there is immense diversity in any concept one may care to consider - including opinions. Hopefully, it will be understood that we, as just two scientists working in an International Agricultural Research Centre, are giving an overview that reflects our activities. However, these are determined by the needs of the National Agricultural Research Programmes of the Region.

Our terms of reference are the major insect pests of agriculture in the arid and semi-arid agroecological zones, excluding those of wheat and rice. In terms of pheromone trapping, we are concerned primarily with monitoring the flight activity of pests that tend to be sporadic, with the intention of forecasting pest outbreaks.

To date, we have cooperated with other International Agencies to the extent of field verification of the activity of various formulations and testing methods of presenting pheromone (and kairomone) lures. If the opportunity to adapt a pest management procedure in which pheromones were involved as primary agents (mating disruptors etc.) we should certainly carry out experimentation that could lead to appropriate recommendations being made to the NARS.

We work with NARS by supplying their scientists with traps and septa and collating the data they send us. There is often a need to put them in contact with suppliers: hopefully this working group meeting will facilitate this procedure. We are pleased to initiate and then hand over networks once they are running effectively, as has happened with the *Helicoverpa armigera* network in India.

The current status of the various activities is summarised in Table 1.

Applications

Spodoptera litura

We are most advanced with *Spodoptera litura* in the context of including pheromone traps in pest management schemes. The male attractant pheromone ('litlure') was identified and tested by Youshima et al. 1974, but we have relied exclusively on colleagues in NRI (and its progenitors) for supplies. The presentation technology was formalised by Ranga Rao et al. (1991a and b) but we have yet to compare the 'Agrisense' bucket trap with our funnel trap. Our technology testing at ICRISAT Center allowed us to discriminate 10 peaks of activity during the year (Fig. 1), corresponding to generations, in terms of temperature (°D) summation exercises. The pheromone trap catches also showed us that *S. litura* is active in the hot season (April - May). We had suspected total aestivation because light traps catches did not include this species.

A comparatively small network is operating in India, and there is scope for extending it to other parts of Asia. The data so far indicate that flight activity builds up rapidly from late February. This has implications about pest mangement in postrainy season crops. The consolidated data from the two sites in northern India (Gwalior and Hisar) showed that the cool winter period (not so extreme at ICRISAT Center which is in southern India) had a marked effect on the catch (Fig. 1).

Our work outside ICRISAT Center (where *S. litura* is not habituated to groundnut plants) on farms leads us to believe that we shall be able to use pheromone trap catches to predict levels of defoliation in damage forecasting models.

Table 1. Current status of various pheromone activities in different species.

Species	Pheromone presence	Pheromone identification	Pheromones synthesis	Application monitoring	Application forecasting	Regional network	Control
Spodoptera litura	1	1	1	1	1	1	?
Helicoverpa armigera	1	1	1	1	1	1	?
Aproaerema modicella	1	1	1	1	*	*	?
Maruca testulalis	1	1	?	*	*	*	?
Contarinia sorghicola	1	*	*	*	*	*	?
Callosobruchus spp.	1	1	1	?	?	?	?
Etiella zinkenella	1	*	*	*	*	*	?
Diacrisia obliqua	1	1	*	*	*	*	?
Heteroptera	*	*	*	*	*	*	?
White grub	*	*	*	*	*	*	?

1 = Considerable progress made; * = Considerable effort needed;

? = Possible application.

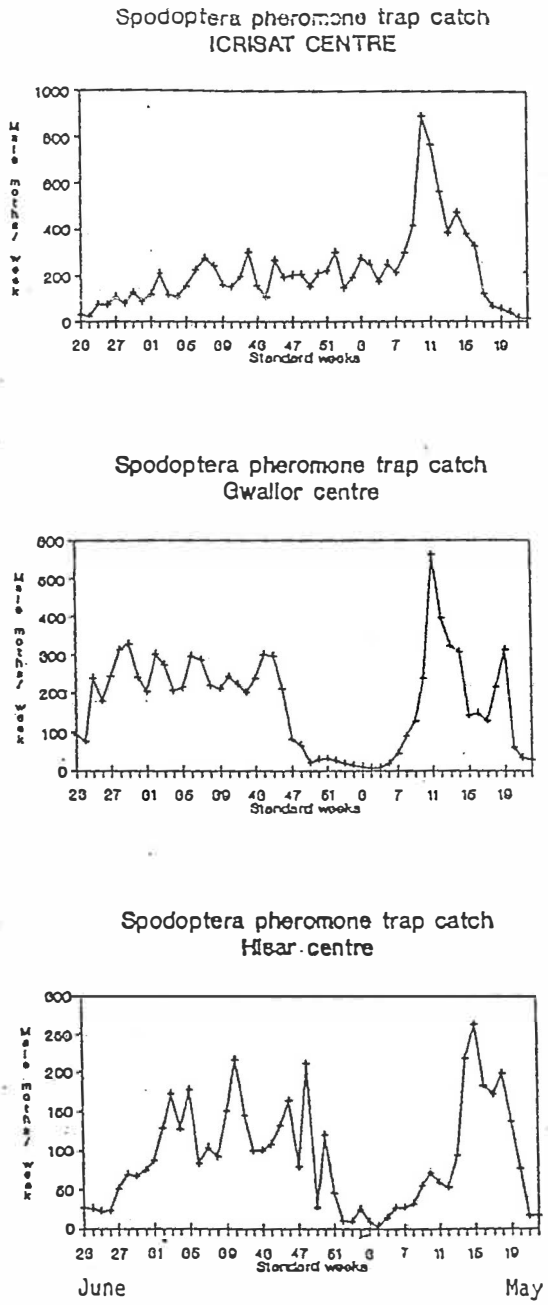

Fig.1. Monitoring Spodoptera litura adult populations using pheromone traps at ICRISAT Center (Hyderabad), Gwalior, and Hisar in India during 1985-90.

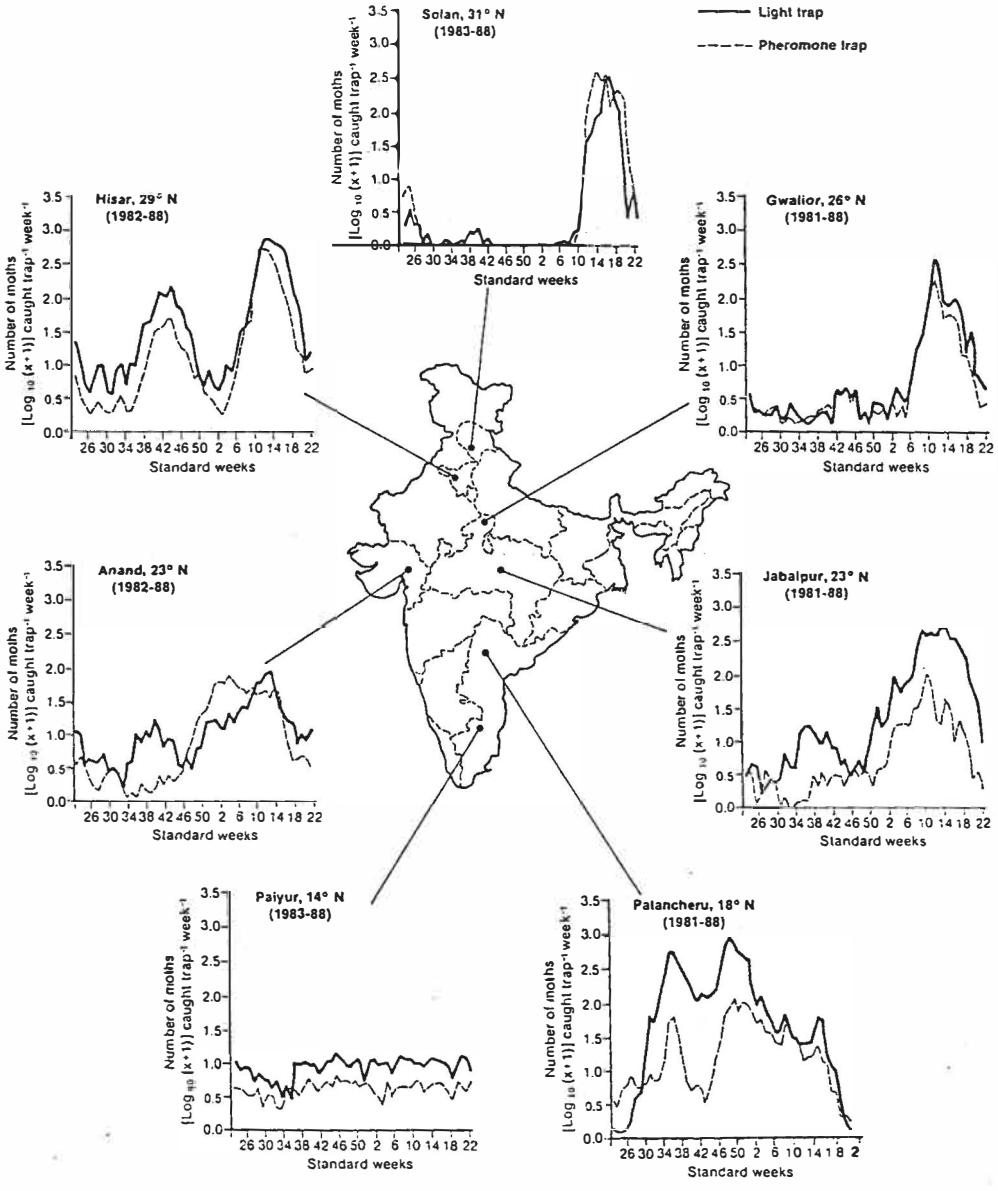

Fig.2. Mean weekly catches [$\log_{10}(x+1)$] per trap from standard week 23 (4 June) to week 22 (3 June) of *Helicoverpa armigera* moths in light and pheromone traps in India, June 1981-May 1988.

Helicoverpa armigera

Much time and effort has been devoted to the development of pheromone traps for *Helicoverpa armigera* (Pawar et al. 1988). Unfortunately, the effort has not been duly rewarded because the moths are not caught with the same efficiency as those of *S. litura*, for instance. We do not know if the blend is not quite right, whether the traps need to be redesigned or if there is too much olfactory competition from the environment. The net effect is that we have not been able to predict egg or larval densities from moth catches in such a way that they could be employed for pest-forecasting (Dent 1985). Srivastava et al (1992) have calculated regression equations by consolidating larval counts for the whole ICRISAT farm (1300 ha) and taking the total weekly pheromone trap catch. It is encouraging that that this exercise gave significant results, but we feel the need to be able to predict at least egg densities in a field complex of up to 5 ha from one or two traps, preferably on a daily basis.

A *Helicoverpa* trap network was run from ICRISAT for over a decade at >30 locations. We have a clear picture of the range of flight patterns of the moths throughout the India (Fig. 2). A similar network has operated in Pakistan (Srivastava et al. 1991).

Aproaerema modicella groundnut leafminer

The most recent addition is a trapping system for *Aproaerema modicella* the groundnut leaf miner. This species is a sporadic but devastating pest of groundnut and soybean in India and S.E. Asia. The synthetic pheromone is highly active biologically. We have found that a pheromone sepum placed above a water trap set 1 m above ground level is the most effective way of presenting the lure. Experiments in progress at present will give a relationship between male flight activity and larval density. We shall look at the possibility of confusing males by putting out high densities of traps.

Pending applications

There are a number of other species that are sporadic and damaging and for which we perceive the need for a pheromone trapping system. Research has started on three species - *Maruca testulalis, Callosobruchus maculatus,* and *Contarinia sorghicola*.

The latter, the sorghum midge, is a major, world-wide sorghum pest. The existence of a pheromome has been demonstrated by Sharma and Vidyasagar (1992).

Callosobruchus spp are major pests of stored pulses. Experimentation carried out at ICRISAT has demonstrated that a pheromone presentation system developed at NRI has potential for the early detection of infestations in warehouses. We will test further if required.

M. testulalis is a major legume pest in Africa and Asia. For instance, it is the major biotic constraint to the establishment of pigeonpea in Sri Lanka and Thailand. As 'avoidance' is one of the potential methods of managing this species we need to know more about its flight activity at a number of locations. Pheromone preparations have proved successful in laboratory conditions but have yet to be found successful in the field.

Potential applications

The hairy caterpillars (e.g. *Amsacta* and *Diacrisia* spp) are major pests of dry-land agriculture in S. Asia. Luckily there is usually only one generation a year (*Amsacta*) but in bad years

this alone is devastating, irrespective of the crops, over wide areas. Clearly, an early warning of flight activity could be helpful to farmers -who often have only one chance to sow the crop upon which their livlihood exists. The paper by Dr Persoons at this workshop indicates that collaborative research between Indian and Dutch scientists has lead towards the fulfillment of this necessity.

Etiella (zinckenella) is another widespread pest of leguminous crops. Its small size and cryptic nature mean that it can go undetected until harvest time. Early warning of its presence, via a pheromone trap system could be highly beneficial.

Some of the large and small Heteroptera - such as *Nezara viridula* and *Campylomma sp* can cause heavy losses to the seed yields of legume and other crops. We know little about their flight periodicity and behaviour in Asia. Again the development of specific trapping procedures preferrably involving kairomones would facilitate the filling of this void.

Conclusion

The international research community has made considerable progress in the development and application of techniques for monitoring the flight activity of several pests of essential food crops of the developing world. In view of the continuing pressure upon scientists to find ways of increasing the productivity of these crops a case can be made for increasing the number of taxa for which kairomone based trapping systems are available. This implies the need to concentrate funding on strategic and applied research. The agrochemical industry should be given every incentive to make the appropriate products available to the farmer support sectors of developing countries at realistic prices.

References

DENT, D.R., 1985. Population studies of *Heliothis armigera* Hubner (Lepidoptera: Noctuidae) through the analyses and interpretaton of lght and pheromone trap catches and larval counts. Pulses Entomology Departmental Progress Report 18, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh 502 324, India, pp.154.

PAWAR, C.S., SITHANANTHAM, S., BHATNAGAR, V.S., SRIVASTAVA, C.P. & REED, W., 1988. The development of sex pheromone trapping of *Heliothis armigera* at ICRISAT, India. Trop. Pest Management, **34**:39-43.

RANGA RAO, G.V., WIGHTMAN, J.A. & RANGA RAO, D.V. 1991a. Monitoring *Spodoptera litura* (F) (Lepidoptera: Noctuidae) using sex attractant traps: Effect of trap heght and time of the night on moth catch. Insect Sci. & Appl. **12**:443-447.

RANGA RAO, G.V., WIGHTMAN, J.A. & RANGA RAO, D.V. 1991b. The development of a standard pheromone trapping procedure for *Spodoptera litura* (F) (Lepidoptera: Noctuidae) population in groundnut (*Arachis hypogaea* L.) crops. Trop. Pest Management, **37**:37-40.

SHARMA, H.C. & VIDYASAGAR, P., 1992. Orientation of males of sorghum midge, *Contarinia sorghicola* to sex pheromones from virgin females in the field. Ent. Exp. & Appl. **64**:23-29.

SRIVASTAVA, C.P., PIMBERT, M.P., and KHALIQ AHMAD, 1991. Monitoring of *Helicoverpa armigera* (Hubner) in Pakistan with pheromone traps. Internl. Chickpea Newsl. **25**:20-22.

SRIVASTAVA, C.P., PIMBERT, M.P., and REED, W., 1992. Monitoring of *Helicoverpa armigera* (Hubner) moths wIth light and pheromone traps in India. Ins. Sci.& Appl. **13**:205-210.

WIGHTMAN, J.A., 1993. Towards the rational management of the insect pests of tropical legume crops in Asia: review and remedy. pp 233-250 in Proceedings of the Ciba Foundation Symposium No. 177, "World Food Production by means of sustainable agriculture: The role of crop protection" John Wiley, Chichester.

YOUSHIMA, T., TAMAKI, Y., KAMAMANO, S., and OYAMA, M., 1974. Field evaluation of synthetic sex pheromone 'Litlure' as an attractant for males of *Spodoptera litura* (F) (Lepidoptera: Noctuidae). Applied Entomology and Zoology, **9**:147-152.

PHEROMONE PRODUCTION AND APPLICATION IN THE COMMONWEALTH OF INDEPENDENT STATES (CIS)

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InterBav Ltd. (Kishinev, Moldova)

In the states of the former USSR insect sex pheromones are used successfully in large scale for monitoring development of pests and determination the accurate time and necessity of insecticide applications and with quarantine aims. There are a few places in the states of the former USSR where sets of traps and dispensers are produced.

Nowadays one can't imagine Integrated Pest Management (IPM) without pheromones. Narrow specificity, extraordinary high biological activity determing high sensitivity of the method, safety for the environment, handling simplicity, result obviousness and relative cheapness - these are the qualities which attracted attention of both scientists and plant protection specialists.

First of all sex pheromones as IPM components are already used to determine optimal time for chemical treatments or to optimize rates and time for biocontrol agents release (*Trichogramma*, *Habrobracon*, etc) as well as to make observation on the number of the pest population. Pheromone usage for quarantine purposes is also worth mentioning.

Great practical benefit considerably stimulated development of large-scale laboratory methods for the syntheses of these substances. In relatively short time commercial production of pheromones and associated attributes for their application (traps, glue) have been begun in the former USSR.

Sex pheromone sets are produced by the Research Production Amalgamation "Flora" (Tallinn, Estonia), pheromone production shop of the Institute for Biological Methods in Plant Protection (Moldavian Academy of Sciences, Kishinev) and "InterBav" Ltd company (Kishinev, Moldova). Some quantities of pheromones are produced by the experimental plant of the Russian Institute of Chemical Means in Plant Protection (Moscow, Russia), by the experimental plant of the Institute of Bioorganic Chemistry of the Uzbek Academy of Sciences (Tashkent, Uzbekistan) and by the technological laboratory of the Institute of Chemistry (Ufa, Bashkortostan). Pheromones of Elateridae were produced by the experimental plant of the Chemical Institute of Estonian Academy of Sciences (Tallinn, Estonia). In order to imagine the scales of pheromone production and therefore application I'll demonstrate it on example of "InterBav" activity. "InterBav" Ltd was organized at the beginning of 1989. Dynamics of pheromone selling by "Interbav" in thousands of dispensers is given in Table 1. As it is seen from Table 1 some decrease of pheromone production and realization took place in 1992 what can be explained by economical difficulties which has appeared in all republics of the former USSR.

Pheromones turned out to be of special value for revealing and establishing distribution boundaries of quarantine pests which recently penetrated into the territory of the states of the former USSR. They changed radically existing methods for revealing of the quarantine pests. The probability to find the quarantine pest at low infestation density is extremely small when visual observation methods are used. That's why in the past the first revealing of a pest took place only 3-5

Table 1. Dynamics of pheromone selling (Thousands of dispensers) by "InterBav" in 1989-1992.

No	Species	1980	1990	1991	1992
1	*Lobesia botrana*	22.1	120.1	661.0	333.3
2	*Heliothis armigera*	1.2	121.1	514.4	321.4
3	*Cydia pomonella*	29.6	112.6	214.5	255.8
4	*Grapholita molesta*	27.1	25.5	54.7	37.8
5	*Dispidiotus perniciosus*	62.9	50.9	42.6	34.4
6	*Phthorimaea operculella*	40.8	23.0	34.5	10.4
7	*Trogoderna granarium*	19.5	23.4	17.9	9.4
8	*Pseudococcus comstocki*	8.6	8.6	12.1	13.4
9	*Grapholita funebrana*	1.6	21.5	25.0	5.4
10	*Agrotis segetum*	1.3	18.9	6.4	15.7
11	*Archips rosana*	!	8.7	3.9	15.6
12	*Synanthedon tipuliformis*	!	5.5	7.8	4.7
13	*Mamestra brassicae*	1.0	2.3	11.7	0.7
14	*Carposina niponensis*	3.1	3.4	3.7	1.6
15	*Archips podana*	-	1.0	6.1	1.4
16	*Hedya nubiferana*	-	4.0	4.5	0.9
17	*Pandemis cerasana*	-	5.1	2.6	1.3
18	*Adoxophyes orana*	!	1.0	0.9	!
19	*Spilonota ocellana*	-	0.7	0.8	10.1
20	*Archips xylosteana*	-	0.6	2.5	!
21	*Spodoptera litura*	2.1	1.0	0.9	0.5
22	*Phyllonorycter corylifoliella*[3]	-	5.0	4.5	1.6
23	*Phyllonorycter blancardella*	-	-	1.0	0.6
24	*Anarsia lineatella*	-	0.5	0.7	0.6
25	*Spodoptera exigua*	-	1.7	0.9	!
26	*Leucoptera scitella*	-	-	0.5	3.7
27	*Eupoecilia ambiguella*	-	-	0.5	5.1
28	*Synanthedon myopaeformis*	!	2.1	!	1.1
29	*Porthetria dispar*	0.7	!	0.8	!
30	*Lymantria monacha*	0.8	0.6	!	!
31	*Pandemis heparana*	-	-	0.5	0.5
32	*Planococcus citri*	-	!	0.7	!
33	*Plodia interpunctella*	-	!	!	!
34	*Ephestia kuehniella*	-	!	!	!
35	*Panolis flammea*	!	!	!	!
36	*Dendrolimun pini*	-	!	!	!
37	*Cydia nigricana*	-	!	!	!

(!) - produced small amounts: (-) - not produced

years after its penetration to the territory of the USSR. Pheromone traps allow to find pest foci at the lowest density when visual observations are absolutely ineffective. New revealing techniques promote finding ways of pest distribution and evaluating possibilities for its establishing in a new region.

The State plant Quarantine Services of the Commonwealth Independent States at present widely applies synthetic pheromones of the following species: *Grapholita molesta, Carposina niponensis, Phthorimaea operculella, Diaspidiotus perniciosus, Pseudococcus comstocki, Pectinophora gossypielle, Spodoptura litura, Trogoderma granarium.*

About 300000 pheromone traps are used annually. It resulted in establishing distribution boundaries of such pests as *Grapholita molesta, Carposina niponensis, Phthorimaea operculella, Diaspidiotus perniciosus.*

More than 30000 ha under fruit trees have been examined to reveal infestation by *Diaspidiotus perniciosus.* And what is the most important the pheromone of this pest was used to analyze quarantine situation in 135 fruit crop nurseries where visual observations failed to reveal the pest.

Over 10000 hectares of plants have been examined by means of *Pseudococcus comstocki* pheromone and its new foci have been revealed in Transcaucasian states, in Moldova and in the south of Russia.

During 1984-1985 single individuals of *Spodoptera litura* were found in seven points of Primorskii region (the Far East).

Insect sex pheromones are simple, reliable, exact and economical means to observe appearance of the imaginal pest stage, adult flight dynamics and generations number. These parameters are taken into account when sex pheromones are used to determine time for treatments. The data are calculated. To do it (depending on pest species) the number of days for mating, ovipositing and embrional development are added to the date of flight beginning or mass flight beginning. The figures are obtained experimentally and they vary depending on the region and climatic conditions of the year. It was done for *Lobesia botrana, Eupoecilia a,biguella, Cydia pomonella, Mamestra brassicae.*

Methodical guidances were worked out on the use of traps baited with sex pheromones to calculate pest number and to determine time for pest control. There are rather detailed instructions on the use of the sex pheromones for this purpose against *Cydia pomonella, Cydia nigricana, Heliothis armigera* and rates of *Trichogramma* releasing against *Mamestra brassicae, Heliothis armigera, Scotia segetum, Ostrinia nubilalis Agrotis exclamationis.*

In the former USSR the method of mate interruption has been developed for *Grapholita molesta* and *Grapholita funebrana.*

At present "Interbav" Ltd produces the sex pheromones shown in table 2

Table 2. List of Pheromones Produced by "InterBav".

No	Species	No	Species
1	*Adoxophyes orana*	35	*Mithima unipunctata*
2	*Agrotis exclamationis*	36	*Orgyia antiqua*
3	*Agrotis ypsilon*	37	*Ostrinia nubilalis*
4	*Amathes c-nigrum*		*(E- and Z-Strain)*
5	*Anarsia lineatella*	38	*Pandemis heparana*
6	*Apamea anceps*	39	*Pandemis cerasana*
7	*Archips crataegana*	40	*Panolis flammea*
8	*Archips podana*	41	*Pectinophora gossypiella*
9	*Archips rosana*	42	*Phthorimaea operculella*
10	*Archips xylosteana*	43	*Phyllonorycter corylifiliella*
11	*Argyrothenia pulchelana*	44	*Phyllonorycter blancardella*
12	*Autographa gamma*	45	*Planococcus citri*
13	*Carposina niponensis*	46	*Plodia interpunctella*
14	*Cnephasia pascuana*	47	*Plutella xylostella*
15	*Cydia nigricana*	48	*Prays olea*
16	*Cydia pomonella*	49	*Pseudaulacaspis pentagona*
17	*Dendrolimus pini*	50	*Pseudococcus comstocki*
18	*Dendrolimus sibiricus*	51	*Ptycholoma lecheana*
19	*Enarmonia formosana*	52	*Quadrospidiotus perniciosus*
20	*Ephestia elutella*	53	*Rhyacionia buoliana*
21	*Ephestia kuehniella*	54	*Scotia segetum*
22	*Eupoecilia ambiguella*	55	*Scotogramma trifoli*
23	*Grapholita molesta*	56	*Scrobopalpa ocellatella*
24	*Grapholita funebrana*	57	*Sitotroga cerealella*
25	*Hedya nubiferana*	58	*Spargonotis pilleriana*
26	*Heliothis armigera*	59	*Spilonota ocellana*
27	*Hyponomeuta malinellus*	60	*Spodoptera exigua*
28	*Leucania loreyi*	61	*Spodoptera litura*
29	*Leucoptera scitella*	62	*Synanthedon myopaeformis*
30	*Lobesia botrana*	63	*Synanthedon tipuliformis*
31	*Lymantria dispar*	64	*Tortix viridana*
32	*Lymantria monacha*	65	*Trogoderma granarium*
33	*Mamestra brassicae*	66	*Operophthera brumata*
34	*Mamestra oleracea*	67	*Tribolium castaneum*
		68	*Tribolium confusum*

POSTERS

EXPERIMENTS ON MATING DISRUPTION OF GRAPE VINE MOTH, *LOBESIA BOTRANA* DEN. ET SCHIFF., IN SARDINIAN VINEYARDS

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Abstract Over a three-year period, trials were carried out to evaluate the efficiency of the mating disruption technique for grape vine moth control in three vineyards in the north of Sardinia. Approximately 500-600 BASF ampoules per ha were used releasing an average of between 29 and 99 mg/ha/h of pheromone. In the diverse conditions in which the experiments were performed, damage to the cultivations was almost always higher than the economic thresholds for this region, even reaching very high levels. Furthermore, in certain cases there was no difference observed between damage in pheromone-treated and untreated vineyards. The mating disruption technique, therefore, did not adequately protect the vineyards, especially where there was a high grape vine moth population density.

Introduction

The grape vine moth, *Lobesia botrana* (Den. et Schiff.), is the main wine-grape insect pest in Sardinia (Delrio *et al.*, 1987). Among methods used in the control of this insect, the mating disruption technique has been experimented in various European countries (Roehrich *et al.*, 1981; Schmid *et al.*, 1990; Stockel *et al.*, 1992). In order to evaluate its efficiency in a Mediterranean environment where the grape vine moth produces 3 or 4 generations a year, disruption trials were undertaken in 3 different vineyards situated in the north of the island (Bonnanaro, Sennori and Olmedo) over 3 consecutive years.

Materials And Methods

The vineyards where the tests were carried out were different in surface area and isolation as well as in grape variety and form of culture (Tab. 1). In particular the pheromone treated fields at Bonnanaro and Sennori bordered on one side with other vineyards.

Table 1. Characteristics of the vineyards where mating disruption trials were carried out.

locality	form of culture	cultivars	treated vineyard area in ha	control vineyard area in ha	year	dispensers per ha
Bonnanaro	espalier	Pascale Sangiovese	4.0	1.0	1990 1991	625 475
Sennori	arbour	Vermentino	1.2	0.5	1991 1992	500 600
Olmedo	espalier	Vermentino Malvasia	4.5	1.0	1992	624

BASF ampoules were used in order to hinder communication between sexes. They were applied at the latest at the beginning of the first grape vine moth flight (end of April), to the second and third wire of the espalier or to the supporting wires of the arbour-trained vineyard. They were distributed with one dispenser to every 20-35 mq, and this density was generally intensified on the outer planting rows in order to reduce the risk of possible immigration. In 1990 and 1992, 3 delta pheromone traps (Agrimont) per ha, plus another one for each additional ha, were used to monitor adult flights. In 1991 one BASF trap per ha was used. All traps were checked every week. Normal fungicidal treatments were carried out in all the vineyards.

Infestation (n° of larvae / 100 clusters) and damage (percentage of clusters damaged by larvae) were estimated on a random sample of 100 clusters per ha, 20 from each of 5 inter-rows. In 1992 evaluation was carried out to ascertain whether there was a reduction in mating in the disruption fields as against the untreated fields, by observing the spermatheca of females captured by bait traps every week. The traps were distributed 6 in the treated and 6 in the untreated vineyard at Sennori, and 13 in the treated and 7 in the untreated vineyard at Olmedo. The traps were containers made of PVC filled with a mixture of water, vinegar and sugar, and were hung on the espalier wires or on a branch of the plant. The average quantity of pheromone released over the whole season was evaluated by the monthly weighing of a random sample of 20 dispensers.

Results

Bonnanaro

A reduction of captures for all three grape vine moth flights was observed in the pheromone-treated field during the two trial years (Tab. 2). However, the damage to both vineyards was similar and at the end of the 1990 season, approximately 50 % of the clusters were damaged whereas in 1991 the figure was over 90 %, with about 3 larvae per cluster (Tab. 3 and 4). In 1990 two Carbaryl treatments were carried out on second generation larvae in both vineyards, whereas in 1991 there

was only one treatment. In 1990 the pheromone diffusion rate was 99 mg/ha/h and in 1991 it was 28 mg/ha/h.

Sennori

Four flights were observed in both 1991 and 1992 (Fig. 1). The captures in the treated vineyard were reduced to practically nil (Tab. 2) and damage and infestation was half of that in the untreated vineyard (Tab. 3 and 4). In 1991 there were 34 % damaged clusters in the treated field as opposed to 72 % in the control field and, in 1992, 25 % as opposed to 64 %. However the infestation in the pheromone-treated vineyard was above the regional economic thresholds (5-10 % damaged clusters in the compact-cluster varieties and 10-15 % in the loose-cluster varieties) (Delrio *et al.*, 1987). Captures in bait traps were very low (24 females in the treated vineyard and 36 in the untreated one during the trial period) and indicated a reduction in mating in the treated field (50 % mated females in the pheromone treated vineyard and 70 % in the untreated one). The monthly pheromone diffusion rate was very irregular throughout the trial period. In 1991, the average figure was 34 mg/ha/h and in 1992 the average was 65 mg/ha/h (Tab. 5).

Olmedo

Four grape vine moth flights (Fig. 2) and a marked reduction in captures in the treated vineyard were observed (Tab. 2). A low level of infestation and damage was maintained throughout the season, although the percentage of attacked clusters did not vary between the pheromone-treated and the control vineyards at harvesting. As the captures in the bait traps were sporadic, it was impossible to acquire significant data on the percentage of mated females. The pheromone diffusion rate was very variable during the experiment, averaging 68 mg/ha/h.

Table 2. Grape vine moth captures per generation by pheromone traps.

	Bonnanaro				Sennori				Olmedo	
	1990		1991		1991		1992		1992	
generation	m.d.	c.	m.d.	c.	m.d.	c.	m.d.	c.	m.d.	c.
1st	4	70	7	482	0	40	0	66	2	3
2nd	12	64	94	290	1	36	1	163	1	31
3rd	9	53	47	517	0	53	1	88	0	15
4th					0	108	2	323	1	18

m.d.: mating disruption; c.: control

Table 3. Number of grape vine moth larvae per 100 clusters in the trial vineyards.

	Bonnanaro				Sennori				Olmedo	
	1990		1991		1991		1992		1992	
generation	m.d.	c.	m.d.	c.	m.d.	c.	m.d.	c.	m.d.	c.
1st	-	-	32	21	0	2	11	31	4	1
2nd	5	17	101	135	2	24	12	67	12	1
3rd	65	72	284	292	37	64	34	48	10	4

Fig. 1. Grape vine moth captures by pheromone traps in 2 arbour-trained vineyards (Sennori, 1992)

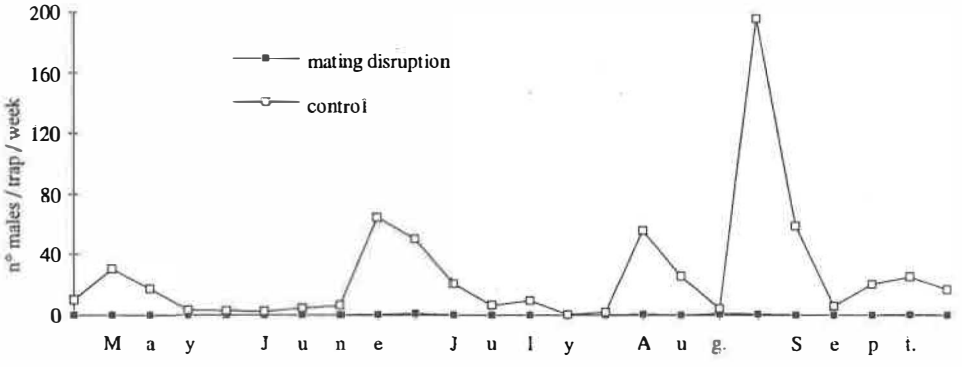

Fig. 2. Grape vine moth captures by pheromone traps in 2 espalier-trained vineyards (Olmedo, 1992).

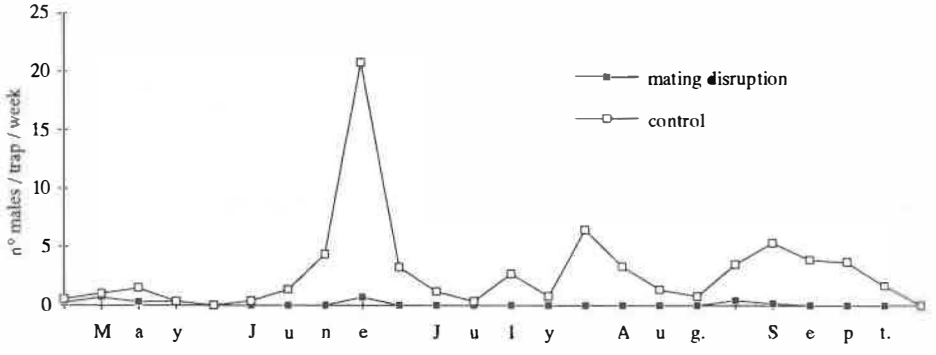

Table 4. Percentage of damaged clusters in the trial vineyards.

	Bonnanaro				Sennori				Olmedo	
	1990		1991		1991		1992		1992	
generation	m.d.	c.	m.d.	c.	m.d.	c.	m.d.	c.	m.d.	c.
1st	-	-	-	-	-	-	15	33	4	1
2nd	7	18	59	71	4	28	14	57	17	2
3rd	45	51	91	93	34	72	25	64	13	10

Table 5. Pheromone emission rate per hectare per hour (mg/ha/h).

	Bonnanaro	Sennori		Olmedo
month	1991	1991	1992	1992
May	4	14	64	80
June	38	1		
July	10	41	84	36
August	53	58	14	29
September	35	58	113	105
average	29	34	65	68

Discussion

The results of the mating disruption trials are difficult to interpret because it is not easy to find two separated, isolated vineyards, having a similar grape vine moth population density.

In our experiments, the confusion technique did not exert sufficient control over grape vine moth infestations, although the pheromone trap captures were considerably reduced in the treated vineyards. The fact that a reduction in captures does not correspond to a reduction in infestation has already been noted (Charmillot, 1992). In spite of this, the most positive results were obtained in our experiments at Sennori, where the captures were practically nil. The reduction of mating in the pheromone-treated vineyard at Sennori, evaluated on the percentage of unmated females captured by bait traps, was lower than that in others experiments (Stockel *et al.*, 1992).

The failure of the mating disruption technique could be due to various factors: 1) the high number of grape vine moth generations (3-4) in Sardinia; 2) a high population density that increased the possibility of mating; 3) a low or irregular pheromone diffusion which was not strong enough to compete against the female trails or to create a background noise louder than the natural pheromone signals in the vineyards (Schmitz, 1992); 4) the effects of high temperature and strong winds on pheromone concentration in the vineyards. It is also possible that there was a significant immigration of adults due to the close proximity of other vineyards.

The diffusion of pheromone varied greatly from month to month and from year to year. In 1991 the quantity of diffused pheromone, 30 mg/ha/h, was lower than some authors retain necessary for mating disruption (Stockel *et al.*, 1992). The yearly variation could be attributed to the use of a different type of dispenser.

Conclusions

The mating disruption experiments were carried out in varying microclimatic and cultural conditions (cultivars, form of culture, surface area and distance between vineyards) and with differing grape vine moth population density levels. In such conditions, which, however, reflect the Sardinian viticulture, the confusion technique was not sufficiently reliable. However this technique could be used in a different way by installing the dispensers in the field at the end of the first flight, having already evaluated and, if necessary, reduced the pest population density by the use of insecticide treatments.

References

CHARMILLOT, P.-J., 1992. Mating disruption technique to control grape and wine moths: general considerations. IOBC/WPRS Bull., "Use of pheromones and other semiochemicals in integrated control" Proc. working group meeting, San Michele all'Adige 31 August-3 September 1992: 113-116.

DELRIO, G., LUCIANO, P. & PROTA, R., 1987. Researches on grape-vine moths in Sardinia. In: Integrated Pest Control in Viticulture. Proc. meeting EC. Experts' Group, Portoferraio 27-28 September 1985: 57-67.

ROEHRICH, R. & CHARLES, J. P., 1982. Essai de confusion sexuelle en vignoble contre l'Eudémis de la vigne, *Lobesia botrana* Schiff. Les Médiateurs Chimiques, Versailles 16-20 Nov. 1981: 365-371.

SCHMID, A. & ANÇAY, A., 1990. Essai de confusion contre l'Eudémis (*Lobesia botrana*) dans le vignoble valaisan. IOBC/WPRS Bull., Working group "Integrated control in viticulture", Sion 28 February 1989: 76-79.

SCHMITZ, V., 1992. Contribution à l'étude du mecanisme de la confusion sexuelle des mâles chez l'insecte. Application à la mise au point du procéde biotechnique de lutte contre l'Eudémis de la vigne *Lobesia botrana* Den. et Schiff. Doctoral thesis 44-66.

STOCKEL, J.P., SCHMITZ, V., LECHARPENTIER, P., ROEHRICH, R., NEUMANN, U. & TORRES-VILA, M., 1992. Three years experience in the control of the grape moth *Lobesia botrana* using mating disruption technique in a Bordeaux vineyard. IOBC/WPRS Bull., "Use of pheromones and other semiochemicals in integrated control" Proc. working group meeting, San Michele all'Adige 31 August-3 September 1992: 117-120.

SELECTIVE ATTRACTION OF ALFALFA GEOMETRIDS TOWARDS PURE ENANTIOMERS OF THEIR SEX PHEROMONE COMPONENTS

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Abstract The presence of 3(*Z*),6(*Z*),9(*Z*)-3,6,9-heptadecatriene (Z3Z6Z9-17Hy) and 6(*Z*),9(*Z*)-6,9,-cis-3,4,-epoxiheptadecadiene (Z6Z9-3,4-epo-17Hy) was indicated in pheromone extracts of female calling *Chiasma clathrata* L. (Lepidoptera:Geometridae), a defoliator pest of alfalfa. Co-injection of the extract with synthetic racemic Z6Z9-3,4-epo-17Hy indicated an 3R,4S enantiomeric configuration for the natural epoxide. In field trapping tests, only the pure 3R,4S enantiomer of the epoxide attracted males. The addition of the triene component was synergistic.

The same epoxide, Z6Z9-3,4-epo-17Hy, has recently been identified from the pheromone of another alfalfa pest, *Tephrina arenacearia* Hbn. (Lepidoptera:Geometridae). In the present study, males of this species were trapped only in traps with baits containing the 3S,4R enantiomer (together with the previously described minor component 6(*Z*),9(*Z*)-6,9,-cis-3,4,-epoxiheptadecadiene).

The pheromone of *Abraxus grossulariata* L. (Lepidoptera:Geometridae), a third species inhabiting a different biotop, has also been know to contain Z6Z9-3,4-epo-17Hy. In trapping tests with the pure enantiomers of Z6Z9-3,4-epo-17Hy, males were caught in traps baited with the 3S,4R enantiomer, whereas the 3R,4S enantiomer attracted a close relative *Abraxas sylvata* Scop. (Lepidoptera:Geometridae).

The present results suggest that seemingly in the cases of both the alfalfa geometrids and the two *Abraxas* species occurring in their respective biotops, one key mechanism maintaining reproductive isolation may be the production of different enantiomers of the same mother epoxide in the sex pheromone. Very recently we found evidence for similar reproductive isolation mechanisms in a group of late autumn/early spring-flying geometrids. So far few other examples have been described due to the difficulties in the preparation and analysis of pure enantiomers of epoxides. It is probable that the present isolation mechanism is more widespread among moths utilising epoxide pheromone components than thought before.

SYNTHETIC PHEROMONES IN INTEGRATED PEST MANAGEMENT IN PEACH AND PLUM ORCHARDS IN ITALY

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Abstract The application status of synthetic pheromones in the control of peach and plum pests in some of the most important cultivation areas is reported: in northern Italy, Emilia Romagna and Piemonte and in southern Italy, Calabria.

Monitoring by pheromone traps is widely used for *Cydia molesta* (Busck), *Anarsia lineatella* Zeller and *Cydia funebrana* (Treitschke). Male catches give reliable information on population density, especially for the former species.

The large scale adoption of a threshold based on weekly catches has made a significant reduction in chemical applications possible during the last 15 years; in Emilia Romagna the I.P.M. Regional Project involved, in 1991, over 3000 orchards and more than 7000 hectares.

Traps for *A. lineatella* and *C. funebrana* are as well used, but a deeper knowledge is required.

Mating disruption has proved to be a valuable means for controlling *C. molesta*, after application on more than 3000 hectares in 1992.

The application of synthetic pheromones of diaspidid *Pseudaulacaspis pentagona* (Targ.) and *Comstockaspis perniciosa* (Comst.) is investigated since some years.

Trimedlure, a parapheromone for teprhitid flies, is used for monitoring *Ceratitis capitata* Wied., to determine the starting moment of the hazardous period for peach damage in southern areas.

Introduction

Synthetic pheromones have found an important place in pest control in stone fruit orchards: the most widespread use of these substances is monitoring adult flight catching males of some lepidoptera in sticky traps, and recently mating disruption has been applied to control *Cydia molesta* and *Anarsia lineatella* on a large scale; attractants for Rhynchota and Diptera have been tested as well.

The great diffusion of Integrated Pest Management has been possible within coordinate programmes, in which the presence of well trained technicians who can rely upon research Institutes allows a continuous large-scale ceck of experimental data.

The Institute of Entomology of Piacenza has given, since the mid seventies, its scientific support to several programmes of Integrated Pest Management in some of the main fruit-growing areas in Italy.

In Emilia Romagna, the regional Project for Integrated Pest Management involved, in 1991, 6772 hectars peach and 411 plum orchards.

In Piemonte, a fruit-grower association, Asprofrut, coordinates the activity of technicians on some 3500 hectares peach orchars.

In Calabria, agricultural Cooperative OSAS in Castrovillari, province of Cosenza, has about 1000 hectars peach orchards.

Monitoring

Oriental Fruit Moth (*Cydia molesta* (Busck)) and Peach twig borer (*Anarsia lineatella* Zeller) are the stone-fruit pests in the fight against which synthetic pheromones have determined the most striking changes.

Field trials and laboratory data indicate a reliability of trap catches in estimating actual population, particularly for *C. molesta*; a less sound relation has been seen for *A. lineatella*.

After these remarks economic thresholds based on male captures have been proposed, that have been largely tested within IPM programmes.

For *C. molesta*, an economic threshold of 10 males per trap per week, using 2-3 traps per orchard, is adopted. Treatments against *A. lineatella* are applied when an average of 7 males per trap per week are recorded, or when 5 males per trap are caught for two weeks in succession.

The lower threshold for *A. lineatella* has its reasons both in the preference of Anarsia larvae for fruits, and in the poor trustworthiness of trap catches: the behaviour of *A. lineatella* males in the presence of pheromone are not as deeply studied as *C. molesta*: finally, monitoring is less reliable.

Traps for *Cydia funebrana* are fairly used, but sometimes their indications are uncertain; some recent researches on the life-cycle of the plum moth in Italy will allow a better understanding of trapping data.

Mating Disruption

After some years, during which researches and trials were carried out, and several practical aspects of mating disruption method on peach were pointed out (Molinari and Cravedi, 1988, 1989) in 1990 commercial application was possible in Italy, increasing up to 3000 hectares peach orchards in 1992.

Commercially available dispensers were RAK 5-6, for Oriental fruit moth and peach twig borer, Elios Pesco and Ceckmate for oriental fruit moth.

Peach growing areas in Italy can differ greatly from one another for environmental, agronomic and phytopatological conditions; such differences are to be considered when applying pheromones for mating disruption.

In some orchards in Emilia-Romagna, where pest population was particularly high, as much as 60-80 mg pheromone per hectare per hour was necessary to avoid damage (Molinari and Cravedi, 1989, 1992; Pari *et al.*, 1990).

In many other cases lower amounts of pheromone were required. A progressive reduction of the release rate has been tried in peach orchards were no damage had been detected after two years

of mating disruption application: in 1991 and 1992 only a small number of dispensers were applied on the border trees (Molinari and Cravedi, 1992).

In Piemonte, a single district near Saluzzo, in the province of Cuneo, including 172 peach orchards on 300 hectares, was treated for the first time in 1990 with pheromones for mating disruption (Cravedi *et al.*, 1991).

Experimental Applications

Synthetic sexual pheromones of two scale insects, the diaspidid *Pseudaulacaspis pentagona* (Targ.) e *Comstockaspis perniciosa* (Comst.) are the object of researches to assess the possibility of their utilization in Integrated pest management (Cravedi and Molinari, 1988).

The parapheromone *trimedlure* is used to attract adult Mediterranean fruit fly *Ceratitis capitata* Wied.: the aim is to determine the starting moment of the hazardous period for peach damage in southern areas.

References

CRAVEDI P., MOLINARI F. 1988. "Efficacité de la phéromone de synthèse de Pseudaulacaspis pentagona". *WPRS Bulletin*, XI/7:24-25

CRAVEDI P., MOLINARI F., ARZONE A., ALMA A., GALLIANO A. 1991. "Applicazione sperimentale su base comprensoriale del metodo della confusione sessuale contro *Cydia molesta* (Busck) su pesco. Inf. Fitopat. **12**:27-31.

CRAVEDI P., MOLINARI F., GUARINO F. and COSENTINI F.. 1992. "Esperienze di applicazione del metodo della confusione contro Cydia molesta (Busck) in pescheti della Calabria." Atti Giornate Fitopatologiche, Copanello (CZ) 21-24 aprile 1992 **1**:115-122.

MOLINARI F. and CRAVEDI P. 1988. "Esperienze sul metodo della confusione nella lotta contro *Grapholita molesta* Busck (Lepidoptera, Tortricidae)." Atti XV Congr. naz. ital. Ent., L'Aquila 1988 :965-972.

MOLINARI F. and CRAVEDI P. 1989. "Applicazione dei feromoni con il metodo della confusione contro *Cydia molesta* (Busck)(Lepidoptera Tortricidae) e prove preliminari su *Anarsia lineatella* Zell. (Lepidoptera Gelechiidae)." Boll. Zool. agr. Bachic. Ser. II, **21**:163-182.

MOLINARI F. and CRAVEDI P. 1988. "Synthetic pheromone and control of *Grapholita molesta* and *Anarsia lineatella*". *WPRS Bulletin*, XI/7:39-40

MOLINARI F. and CRAVEDI P. 1989. "Applicazione dei feromoni con il metodo della confusione contro *Cydia molesta* (Busck) (Lepidoptera Tortricidae) e prove preliminari su *Anarsia lineatella* Zell. (Lepidoptera Gelechiidae)." *Boll. Zool. agr. Bachic.* Ser. II, **21**:163-182.

MOLINARI F. and CRAVEDI P. 1990. "Applicazione dei feromoni secondo il metodo della confusione nella lotta contro *Cydia molesta* (Busck)(Lepidoptera Tortricidae) in Piemonte." Redia, **73**:381-395.

MOLINARI F. and CRAVEDI P. 1992. "The use of pheromones for the control of *Cydia molesta* (Busck) and *Anarsia lineatella* Zell. in Italy". *Acta Phytopatologica et Entomologica Hungarica* **27** (1-4): 443-447.

MOLINARI F. and CRAVEDI P. 1992. "Application of mating disruption method in peach orchards in Italy." Working group "Use of pheromones and other semiochemicals in integrated control" 31 august-3 september 1992, San Michele all'Adige (Italy) IOBC/WPRS Bulletin, 1992/15/5: 52-55.

NICCOLI A., SACCHETTI P. and LUPI E. 1990. "Il metodo della confusione nel controllo di *Cydia molesta* (Busk) e *Anarsia lineatella* Zell. in un pescheto della Toscana." Redia LXXIII(n. 2):531-541.

PARI P., SPADA G., GARAFFONI M., GUARDIGNI P., CANESTRALE R., MINGUZZI R., RAVAIOLI M. and CARLI G. 1990. "Il metodo della confusione sessuale nella difesa contro *Cydia molesta* Busk. ed *Anarsia lineatella* Z. nei pescheti dell'Emilia Romagna." Inf.tore fitopatol. **10**:35-42.

REGIONE EMILIA-ROMAGNA, 1992. Lotta Integrata. Supplemento al n.3 di Agricoltura. 36 pp

ROTUNDO G. and VIGGIANI G. 1989. "Esperienze sul controllo dell'Anarsia e della Tignola orientale con il metodo della confusione sessuale." L'Informatore Agrario **40**:67-68.

PERIODICITY IN THE FREQUENCY OF CALLING AND QUALITY OF PHEROMONE IN VOLATILE EMISSIONS OF THE SPOTTED STALK BORER, *Chilo partellus*

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Abstract The Spotted stalk borer, *Chilo partellus* (Swinhoe) is an important pest of maize, sorghum, rice, sugarcane, pearl millet, wheat and various other grasses. The major components of *C. partellus* sex pheromone are (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-11-hexadecen-1-ol (Z11-16:OH). We investigated the periodicity of calling and pheromone emission of virgin *C. partellus* females. The calling behaviour of female *C. partellus* moths was observed every 30 minutes throughout their whole life. The quantity of pheromone in the volatile emissions of female *C. partellus* was determined by measuring the quantity of Z-11-16:Ald emitted from forcibly exuded glands of live females during the night of eclosion and on the first, second, third and fifth day after emergence. Calling occurred between the sixth and tenth hour of the scotophase while pheromone emission was mainly between the fourth and twelfth hour of the scotophase. Both maximum calling and maximum pheromone emission was observed between the seventh and ninth hour of the scotophase. However, in older females maximum calling occurred slightly earlier than in the younger females. For the 1-5-day-old females, the percentage of females that called during the maximum calling period was fairly constant.

COMMERCIAL USE OF PINK BOLLWORM PHEROMONE IN EGYPT

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Abstract Two slow release pheromone formulations were applied commercially to a total of 50,000 feddans (about 20,000 ha) of cotton in four governorates in Egypt during the 1992 cotton growing season as a part of integrated pest control programmes. Two to three applications of a microcapsulated formulation or one application of a twist-tie formulation of pink bollworm pheromone, followed by one to two applications of conventional insecticides, gave control of the total pest complex equal to that achieved by a conventional broad spectrum insecticides alone.

Cotton yields averaged from 6.36, 7.52, 6.51 and 5.4 kantars (1 kantar = 157.5Kg) in the pheromone treated areas compared to 5.47, 6.55, 6.22 and 5.07 Kantars in the insecticide treated areas in Dakahlia, Sohag, Minia and Assiut governorates, respectively.

The mean total cost of the pheromone-plus-insecticide treatments was same as insecticide-only treatments.

SEX ATTRACTANTS FOR CHESTNUT TORTRICID MOTHS *PAMMENE FASCIANA* L., *CYDIA FAGIGLANDANA* Z. AND *CYDIA SPLENDANA* (Hb.) FOUND BY ELECTROPHYSIOLOGICAL AND FIELD STUDIES.

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Abstract Sexual response of chestnut tortricid male moths, *Pammene fasciana* L., *Cydia fagiglandana* Z. and *Cydia splendana* Hb. (Lepidoptera:Totricidae), was tested to several synthetic compounds (saturated or unsaturated aldehydes, alcohols and acetates) having 12 carbon atoms, by electroantennography and field evaluation in several chestnut areas of Southern Italy.

Electroantennograpic study showed high responses to Z8-12:Ac for *P. fasciana* and field evaluation confirmed that it is an important component of the sex pheromone blend of this species. The compound, mixed with Z8-12:OH, increased its attractant power when the ratio of binary mixture was 3:1. Increased amounts of alcohol generally led to a decreased capture of *P. fasciana* and *Cydia funebrana* Tr. Besides, this binary mixture showed a greater selectivity eliminating the captures of *Pammene gallicolana* Z.

The inclusion of 12:Ac (0,8-1,6) in the binary mixture (3:1) led, in the chestnut areas of Montella (AV) and of Mendicino (CS), to a notable increase of capture and to slight reduction in Melfi (PZ), but the selectivity towards *C. funebrana* was not improved.

Acetates with two double bonds (Z8,Z10-12:Ac, E8,E10-12:Ac, Z8,E10-12:Ac, E8,Z10-12:Ac) highly stimulated the antennae of *C. fagiglandana* and *C. splendana*. Field study showed that only E8,E10-12:AC and Z8,E10-12:Ac were attractive for *C. fagiglandana*.

The E8,E10-12:Ac, mixed with the respective alcohol in ratios between 2:1 and 3:1, slightly increased its capture power towards *C. fagiglandana* but showed better specificity in reducing the captures of *Cydia succedana* D&S., *Cydia coniferana* Sax. and *Cydia aurana* F. The capture power was notably increased when the compound was mixed with Z8,E10-12:Ac in the ration 1:10. Besides this mixture improved the specificity removing captures of *C. coniferana* and *Cydia gemmiferana* Tr. The attractive power towards *C. fagiglandana* was removed when Z8-12:Ac was present in the blend.

The flying activity of *P. fasciana* occurs between May and September with the greatest capture peak between mid-June and July. A second flying peak between mid-August and September was observed in different Southern Italian chestnut areas (Soveria, Mannelli, Mendicino and Mintella).

The flight period of *C. fagiglandana* ranges between June and October, in all areas, with the highest peak in the second week of August.

The flight peak of *C. splendana* monitored by light traps occurs within the second week of September while the flying activity starts at the end of August and finishes at the end of September.

FIELD EVALUATION OF THE SEX PHEROMONE COMPONENTS OF CORN STALK BORER, *SESAMIA NONAGRIOIDES* (LEF.) (LEPIDOPTERA:NOCTUIDEA)

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Abstract Field responses of *Sesamia nonagrioides* (Lef.) males to traps baited with different blends of the sex pheromone components were evaluated in corn fields. Different types of dispensers and traps were tested. Captures were compared to those obtained by virgin females and light traps.

In six years of experiments (1985 to 1990), using rubber septa dispensers, the best results were achieved by utilising two blends of Z11-16:Ac + Z11-16:OH + Z11-16:Ald + 12:Ac (85/10/10/20 and 136/16/16/32µg) whose attractant power was not significantly different from that of two *S. nonagrioides* virgin females. Different tested blends also attracted males of other noctuid species, *Mythimna unipuncta* (Hw.), *Dicestra trifolii* (Hfn.) and *Trachea atriplicis* L.

Virgin females of *S. nonagrioides* also attracted a large number of males of *M. unipuncta.* However, *M. unipuncta* males seem to prefer higher doses of the above mentioned blend or blends containing 14:Ac or 16:Ac.

The highest capture of *D. trifolii* males was obtained by blends containing only Z711-16:Ac and Z11-16:OH at the ratio 9:1, 8.5:1; adding the aldehyde at the same quantity of alcohol caused the captures to drop significantly.

Rubber septa and rubber stopper (for vials) dispensers captured more than sandwich dispensers, both for *S. nonagrioides* and *M. unipuncta.* The two types of rubber dispensers showed similar results at different doses of the same blend.

Oil traps captured more than funnel traps: Unitrap (Biol. Contr. Syst. Ltd, UK) and Mastrap (I.S.A.G.R.O., Italy)

PHEROMONES AND PHEROMONE-RELATED VOLATILES OF FOUR *RHYNCHOPHERINAE* WEEVILS (COLEOPTERA: CURCULIONIDAE)

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Abstract (*E*)-2-methyl-5-hepten-4-ol (Rhyncophorol I), major volatile emitted by *Rhyncophorus palmarum* males, was previously identified as the aggregation pheromone of this species. We showed that males of *R. palmarum*, *R. phoenicis*, *R. vulneratus* (Palm weevils) and *Metamasius hemipterus* (Sugercane weevil) produce several other pheromone-related volatiles. The compounds were disclosed by volatile collections and gas chromatography (GC), then identified by GC-mass spectrometry and comparisons to synthetic reference compounds. (*R**,*R**)-3-methyl-4-octanol (rhyncophorol II) was the sole compound emitted by *R. phoenicis*, and was emitted by *R. vulneratus* as a minor compound. (*R**,*R**)-4-methyl-5-nonanol (rhyncophorol III) was identified as the major compound in both *R. vulneratus* and *M. hemipterus*. 2-methyl-4-heptanol, 2-methyl-4-octanol and 5-nonanol were identified as minor components in *M. hemipterus*. 2,3-epoxy-6-methyl-4-heptanol was identified as a minor volatile in *R. palmarum*. *R. vulneratus* and *M. hemipterus* both produced another minor unidentified volatile. Laboratory bioassays showed that the male effluvia of *M. hemipterus* had a sex pheromone activity. EAG and field trapping showed that rhyncophorol II was the aggregation pheromone of *R. phoenicis*. EAG and pitfall bioassay indicated that *R. vulneratus* male effluvia had an aggregation pheromone activity. Rhyncophorol III alone proved to be a key compound of the aggregation pheromone of *R. vulneratus* in laboratory bioassay. EAG, pitfall bioassay and field trapping indicated that epoxy-rhyncophorol I had no pheromone activity in *R. palmarum*, either alone or combined with rhyncophorol I. investigations are in progress to precise the enantiomeric composition of rhyncophorols II and III and to study the activity of all the weevil-produced volatiles. The discovery of rhyncophorol I has led to a great improvement in controlling *R. palmarum* with synthetic pheromone. The identification of pheromones in *R. phoenicis*, *R. vulneratus* and *M. hemipterus* should lead to a similar progress in controlling these pests.

Introduction

Palm weevils of the genus *Rhyncophorus* are tropical pests of coconut and oil palm crops: *e.g.* *R. palmarum* (L.) in Latin America, *R. phoenicis* (F.) in Africa and *R. vulneratus* (Panzer) in Southeastern Asia. Damage is direct, due to the larvae or indirect, caused by the adults vectoring pathogens to the trees (*e.g.* Red ring disease, transmitted by *R. palmarum*). *Metamasius hemipterus* (L.) is a pest of sugercane, mainly in the Caribbean and Latin America.

In 1991 we reported that (*E*)-2-methyl-5-hepten-4-ol (rhyncophorol I), major volatile produced by *R. palmarum* males was the aggregation pheromone of this species (Rochat *et al.* 1991a, b). Oehlschlager *et al.* (1992, 1993) showed that synthetic rhyncophorol I was a potent lure to trap *R. palmarum* in the field. These results led us to search for new pheromones in other Rhyncophorinae pests. Here we report the identification of a minor volatile from *R. palmarum* and of the new Coleopteran pheromone compounds from *R. phoenicis*, *R. vulneratus* and *M. hemipterus*.

Materials and methods

The weevil-produced volatiles were isolated and identified by volatile collections on Supelpak^{tm}-2 (Rochat *et al.* 1991b); GC, GC-MS, GC-FTIR (Rochat *et al.* 1993); and organic synthesis (Rochat *et al.* 1993, Mori I *et al.* 1993).

The biological activity of natural or synthetic molecules was studied by EAG (*Rhyncophorus* spp.); laboratory bioassays; and field trapping with synthetic compounds (*Rhyncophorus* spp.) The laboratory bioassays were performed with a 2-choice pitfall olfactometer for the *Rhyncophorus* spp. (Rochat *et al.* 1991a) or a 4-way airflow olfactometer (Vet *et al.* 1983) for *M. hemipterus* (Ramirez-Lucas *et coll.*, to be published).

Results

Chemical data

GC analyses revealed that the males of the four species released volatile compounds that were not produced by females nor sugercane. Nine compounds were disclosed in the 4 species. Seven of them were identified (Table). While males of *R. phoenicis* produced a sole volatile, males of the other species released blends of 2 to 5 compounds. The blends varied in ratios but one major compound did always represent more than 80% of the blends.

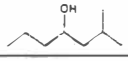
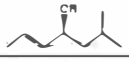
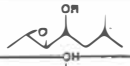
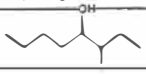
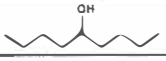
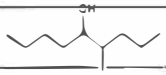
Compounds		Rhynchophorinae species			
		R. palmarum	*R. phoenicis*	*R. vulneratus*	*M. hemipterus*
2-methyl-4-heptanol					●
(4S,5E)-2-methyl-5-hepten-4-ol (Rhynchophorol I)		●			
2-methyl-4-octanol					●
2,3-epoxy-6-methyl-4-heptanol		●			
(R*,R*)-3-methyl-4-octanol (Rhynchophorol II)			●	●	
5-nonanol					●
Rv	?			●	
(R*,R*)-4-methyl-5-nonanol (Rhynchophorol III)				●	●
Mh4	?				●
Major (●) and minor (●) compounds: respectively up to 80% and less than 20% of the total amounts of male-produced volatiles within a species.					

Table: Structure of the volatile compounds emitted by *Rhynchophorus palmarum*, *R. phoenicis*, *R. vulneratus* and *Metamasius hemipterus* males

Biological data

Previous data (Ramirez 1990) and the present results of behavioural bioassays suggested that *M. hemipterus* males produced a sex pheromone. Only virgin females of the Sugercane weevil showed a significant attraction to the male-produced volatiles, exploring the zone with male effluvia during 52% of the test time (Figure 1).

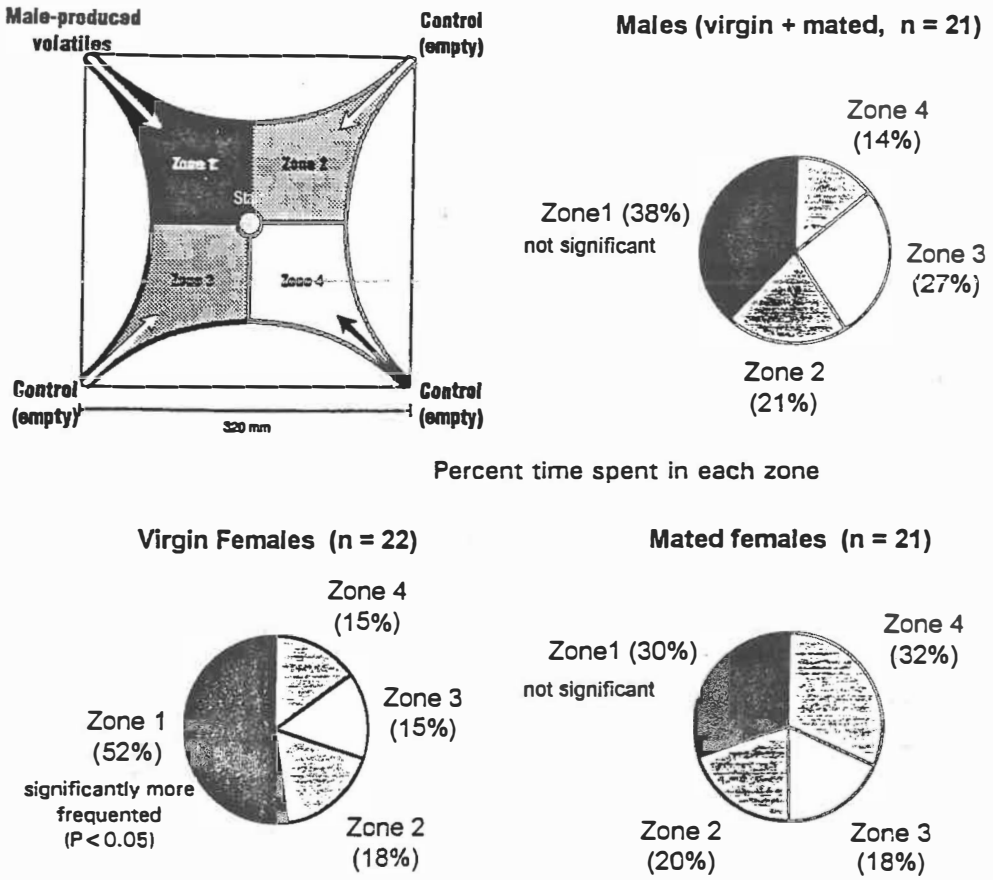


Figure 1: Responses of *Metamasius hemipterus* to conspecific male effluvia (2 male.day.equivalents) in a 4-way airflow olfactometer

In *R. phoenicis*, field trapping results corroborated EAG data obtained from the natural male volatiles. 3-methyl-4-octanol, for which we propose the trivial name rhyncophorol II, was the aggregation pheromone of *R. phoenicis*. The pheromone has strong synergistic effect with host-plant volatiles for weevil attraction in the field (Figure 2).

In *R. vulneratus*, the male-produced volatiles showed to have an aggregation effect in pitfall olfactometer, in agreement with their EAG activity in both sexes. 4-methyl-5-nonanol (200ng of diastereoisomeric blend) reproduced this effect, indicating that this compound was a key-compound of the aggregation pheromone of *R. vulneratus*. We propose the trivial name of rhyncophorol III for this molecule.

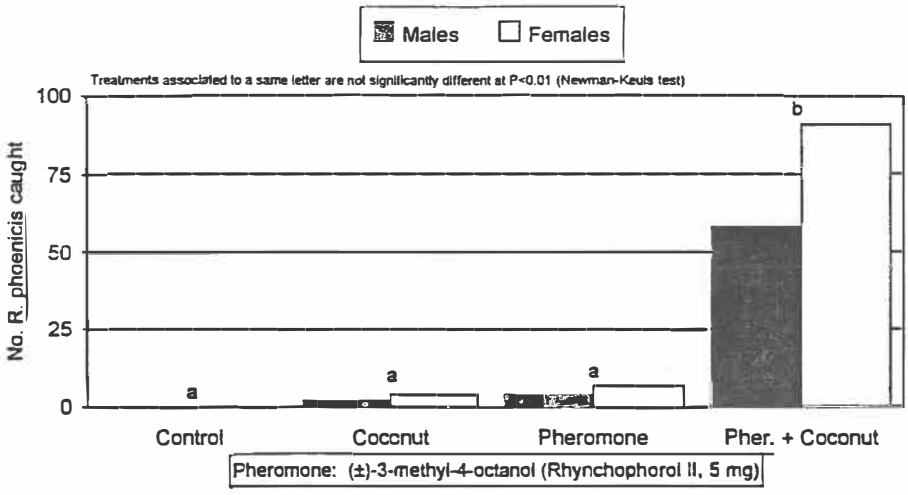


Figure 2: Field responses of *Rhynchophorus phoenicis* to (±)-3-methyl-4-octanol (rhynchophorol II) in Ivory Coast

EAG showed that(±)-epoxyrhyncophorol I was detected at 1000-fold higher doses than (±)-rhyncophorol I by both sexes of *R. palmarum*. Neither in field trapping nor in pitfall olfactometer, epoxyrhyncophorol I exhibited any biological effect on *R. palmarum* (alone or combined with rhyncophorol I). These results indicated that epoxyrhyncophorol I, produced by *R. palmarum* males, has no pheromone activity in this species at physiological doses.

Discussion - Perspectives

The male produced volatiles identified here are compounds newly identified from insects according to the pheromone literature. 3-methyl-4-octanol proved to be the aggregation pheromone of *R. phoenicis*. Our present data indicated that 4--methyl-5-nonanol was an essential component of *R. vulneratus* aggregation pheromone and might be the key compound of *M. hemipterus* pheromone. Epoxyrhyncophorol I, which is inactive in *R. palmarum* may be a by-product of rhyncophorol I biosynthesis or an oxidative metabolite of the pheromone.

The male-produced compounds identified from the 4 Rhyncophorinae species belong basically to two structural groups: a `rhyncophorol I group' and a group of α-methyl-hydroxylated compounds (rhyncophorols II and III). These 2 later molecules are closely related to previously reported pheromones, identified from Scolytid beetles (Pearce *et al.* 1975, Blight *et al.* 1977 and Rhyncophorineae weevils (Schmuff *et al.*- 1984). Thus the pheromone compounds identified to date in the Rhyncophorinae present a clear structural relationship in agreement with the taxonomy of this homogeneous group of weevils, which has been elevated to a family rank (Rhyncophoridae) by

Thompson (1992). Complementary chemical investigations are underway to identify the 2 weevil-produced volatiles not identified yet. Enantioselective synthesis is in progress to precise the entiomeric composition of Rhyncophorols II and III.

The pheromone role of all the volatiles identified here is presently investigated using synthetic compounds. additional data should give further evidence for the exact nature of *M. hemipterus* male pheromone, which might be an aggregation pheromone.

The recent discovery of rhyncophorol I has led to a great improvement in controlling *R. palmarum* using synthetic pheromone. The identification of pheromones in *R. phoenicis*, *R. vulneratus* and *M. hemipterus* should lead to a similar progress in controlling these tropical pests.

Acknowledgements

We thank our co-workers: D. Mariau and the CIRAD-CP (Division Palmier - Cocotier); F. Akamou, A. Sangaré and the IDEFOR/DPO (Ivory Coast); Fedapalma (Colombia); F. Bernal. R. Herrera and Palmar de Oriente (Colombia); R. Desmier de Chenon (INRA/CIRAD-CP, Indonesia and C. Pavis (INRA, Guadeloupe FWI).

References

BLIGHT, M.M., MELLON, F.A. WADHAMS, L.J. and WENHAM, M.J. 1977. Volatiles associated with *Scolytus scolytus* beetles on English elm, Experientia **33**: 425-427.

MORI, K., KIYOTA, H. and ROCHAT, D. 1993. Pheromone synthesis, CLIV. Synthesis of the stereoisomers of 3-methyl-4-octanol to determine the absolute configuration of the naturally occurring (3*S*,4*S*)-isomer isolated as the male-produced aggregation pheromone of the African palm weevil, *Rhyncophorus phoenicis*. Leibigs Ann. Chem. in press

OEHLSCHLAGER, A.C., PIERCE, H.D. Jr, MORGAN, B., WIMALARATNE, P.D.C., SLESSOR, K.N., KING, G.G.S., GRIES, G., GRIES, R., BORDEN, J.H., JIRON, L.F., CHINCHILLA, C.M. and MEXZAN, R.G. 1992. Chirality and field activity of rhyncophorol, the aggregation pheromone of the American palm weevil. Naturwissenschaften **79**: 134-135.

OEHLSCHLAGER, A.C., CHINCHILLA, C.M., GONZALEZ, L.M., JIRON, L.F., MEXZAN, R.G. and MORGAN, B. 1993. Development of a pheromone-based trapping system for the American palm weevil, *Rhyncophorus palmarum* (L.) (Coleoptera: Curculionidae). J. Econ. Entomol. in press.

PEARCE, G.T, GORE, W.E., SILVERSTEIN, R.M., PEACOCK, J.W., CUTHBERT, R.A., LANIER, G.N. and SIMEONE, J.B. 1975. Chemical attractants for the smaller European elm bark beetle, *Scolytus multistriatus* (Coleoptera: Scolytidae). J. Chem. Ecol. **1**: 115-124.

RAMIREZ, P. 1990. Mise en évidence d'une phéromone sexuelle chez *Metamasius hemipterus* L. (Coleoptera: Curculionidae). Master report, Université Paris 6, 22 pp.

ROCHAT, D., GONZALEZ, V.A., MARIAU, D., VILLANUEVA, G.A. and ZAGATTI, P. 1991a. Evidence for male-produced aggregation pheromone in American palm weevil, *Rhyncophorus palmarum* (Coleoptera: Curculionidae). J. Chem. Ecol. **17**: 1221-1230.

ROCHAT, D., MALOSSE, C., LETTERE, M., DUCROT, P.H., ZAGATTI, P., RENOU, M. and DESCOINS, C. 1991b. Male-produced aggregation pheromone of the American palm weevil *Rhyncophorus palmarum* (Coleoptera: Curculionidae); collection, identification, electrophysiological activity, and laboratory bioassay. J. Chem. Ecol. **17**: 2127-2141.

ROCHAT, D., MALOSSE, C., LETTERE, M., RAMIREZ-LUCAS, P., EINHORN, J. and ZAGATTI, P. 1993. Identification of new pheromone-related compounds from volatiles produced by males of four Rhyncophorinae weevils (Coleoptera: Curculionidae). C.R. Acad. Sci Paris Ser II **316** in press.

SCHMUFF, N.R., PHILLIPS, J.K, BURKHOLDER, W.E., FALES, H.M., CHEN, C.W., ROLLER. P.P. and MA, M. 1984. The chemical identification of the rice weevil and maize weevil aggregation pheromone. Tetrahedron Lett. **25**: 1533-1534.

THOMPSON, R.T. 1992. Observations on the morphology and classification of weevils (Coleoptera: Curculionidae) with a key to major groups. J. Nat. Hist. **26**: 835-891.

VET, L.E.M., VAN LENTEREN, J.C., HEYMANS, M. and MEELIS, E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiol. Entomol. **8**: 97-106.

MATING DISRUPTION TRIALS FOR CONTROLLING *SESAMIA NONAGRIOIDES* LEF. IN MAIZE

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Abstract Efficacy of mating disruption for the control of the Mediterranean corn stalk borer, *Sesamia nonagrioides*, was evaluated. Results obtained with monitoring traps in 1990 indicated a level of disruption near to 100% in the treated plot. Larval density and occupancy percentage in the treated plot were significantly lower than in the control only after the second flight but not at the end of the season just before harvesting. Higher pheromone dose and earlier applications should be investigated in order to improve the effectiveness of mating disruption.

Introduction

Sesamia nonagrioides (Lep.:Noctuidae) is one of the most important maize pests in the Mediterranean countries (Castañera, 1986). Mating females lay their eggs between the stem and one of the lower sheaths of maize plants and neonate larvae bore very quickly into the stem. There, they complete their development,only going out to disperse into nearby plants. This endophytic behaviour difficults the accessibility of insecticide sprays.

The pheromone of *S. nonagrioides* identified by Sreng et al. (1985) and Mazomenos et al. (1989) has allowed to monitor the flights in the Ebro Valley (Riba, et al., 1992). In this area *S. nonagrioides* has 2 or 3 generations depending on the year's weather.

Due to little effectiveness of chemical treatments, the use of synthetic pheromone to monitor populations and, eventually, to control the pest by applying mating disruption techniques, seemed to be a good option. First trials on mating disruption were carried out in 1989 (Perdiguer et al., 1992). In these tests different factors such as pheromone dose, formulation, application rate and dispenser separations were conveniently estimated.

In 1990, on basis of those preliminary results, the efficacy of mating disruption as a control method of *S. nonagrioides* was evaluated in a commercial plot in Lleida (Catalonia, Spain).

Material And Methods

A synthetic pheromone blend, composed of four products: Z11-16:Ac (69%), Z11-16:OH (8%), Z11-16 Ald (8%) and 12:Ac (15%), was used. PVC-resin dispensers charged with 700 mg of the lure (4% w/w) (BCS Ltd) were placed twice equally spaced (12 m x 12 m) over a 5 ha commercial plot. First application was conducted in early June and dispensers were hung up in a

wooden stick support (1 m height, 6 mm diam). Second application was carried out in early August and dispensers were placed in the maize plant near the ear. For all season the average release rate of attractant was evaluated to be 19 mg/ha/h.

An untreated 5 ha plot was employed as a control area.Mating disruption efficacy was measured by:

a) Male catches in funnel monitoring traps baited with 0.2 mg of sex lure: 3 traps in mating disruption plot and 3 traps in the control plot. The disruption efficacy (E) was measured as follows:

$$E = 100 - \frac{\text{Trapped Males Disruption plot}}{\text{Trapped Males Control plot}} \times 100$$

b) By counts of the number of individuals by plant and occupied plants (1) after the second flight of adults and (2) before the harvest (**Tab 1**). The number of plants observed on each date, both in the treated and in the control plots, was 180 . Larval density comparison was made by Student's "t" test.

The release rate of pheromone components throughout the trial was determined collecting three dispensers from the field every three weeks, extracting the lures with hexane and analyzing them by gas-liquid chromatography.

Results And Discussion

The efficacy of mating disruption in monitoring traps was 100% (**Tab.1**) throughout the season. That indicated that the separation between dispensers and the quantity and rate of disruptant per ha were adequate.

After the second flight of adults the number of larvae per plant (first sampling date) in the treated plot was significantly lower ($p<0.05$) than in the control plot. However, in the second sampling date, before the harvest, the number of larvae per plant was not significantly different ($p>0.05$). Higher *S. nonagrioides* populations registered in August/September than in July could be the cause of the lack of efficacy late in the season.

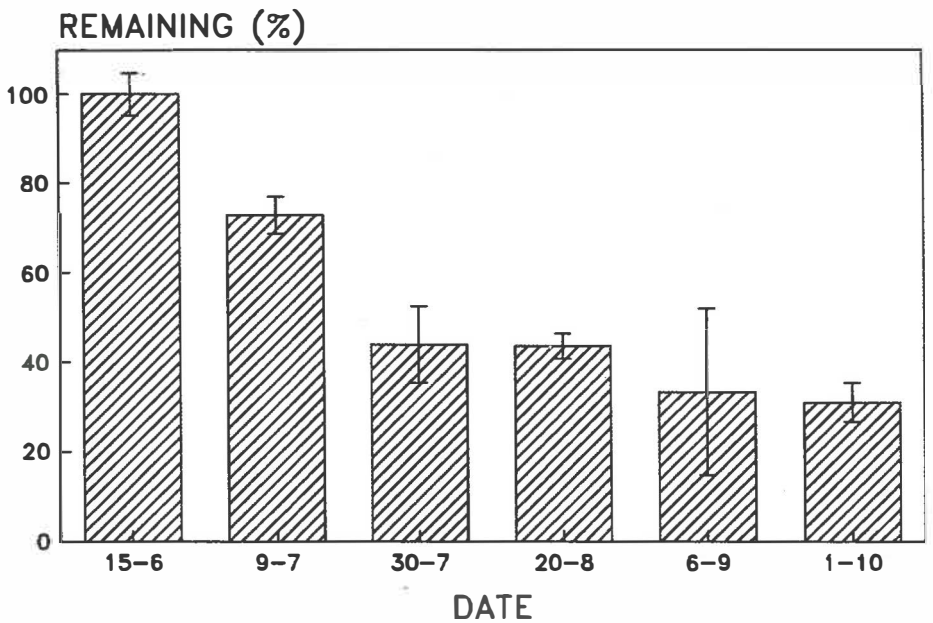
Release rate was correct during the experimental period (**Fig. 1**)

Higher pheromone doses and earlier applications should be investigated in order to improve the effectiveness of mating disruption.

Table 1. Results of mating disruption experiences to control *Sesamia nonagrioides* in Lleida (Catalonia), in 1990, in a commercial plot with one dispenser every 12 m x 12 m and an attractant dose of 49 g/ha.The number of sampled plants was, as in treated (T) as in control plot (U), 180. Means followed by the same letter were not significantly different (Student's "t" test, $p < 0.05$).

Mating disruption efficacy (%)	Sampling dates	% Occupied Plants	Number of Larvae/Plant
T 100	8/30	T 36.0	1.50 ± 0.29 b
U -		U 54.0	3.50 ± 0.67 a
T 100	10/3	T 76.0	1.90 ± 0.12 a
U -		U 75.0	2.10 ± 0.67 a

Figure 1. Evolution of the major component of the pheromone from the initial dispensers throughout the trial. Average (± S.D.) of three dispensers collected in the field every three weeks, from June to October in 1990.



References

CASTAÑERA, P., 1986. Plagas del maíz. IV Jornadas técnicas sobre el maíz, Lérida. Plagas: 1-24. MAPA, Madrid.

MAZOMENOS, B.E., BARDAS, D. & PANTAZI-MAZOMENOU, A., 1989. *Sesamia onagrioides* sex pheromone: field attractiveness use of pheromone traps for monitoring and control. Ac. Phytopatol. Entomol. Hungarica **24**: 153-156.

PERDIGUER, A., GIMENO, F., AGUILAR, L., EIZAGUIRRE, M., RIBA, M. & SANS, A,. 1992. Ensayos de confusión sexual en *Sesamia nonagrioides*. Investigación Agraría Produc. Protec. Vegetal **2**: 253-260.

RIBA, M., SANS, A., EIZAGUIRRE, M., PERDIGUER, A., GIMENO, F. & AGUILAR, L., 1992. Optimización de la composición del atrayente sexual de *Sesamia nonagrioides* Lef. Bol. San. Veg. Plagas **18**: 193-200.

SRENG, I., MAUME, B. & FREROT, B., 1985. Analyse de la sécrétion phèromonale produite par les femelles vierges de *Sesamia nonagrioides* (Lef.) (Lépidoptére, Noctuidae). C.R. Acad. Sc. París, **t 301**, Série III, nº 9.

ZEUZERA PYRINA L. (LEPIDOPTERA, COSSIDAE): RESULTS OF FIVE YEAR RESEARCHES ON SEX ATTRACTANT. (1)

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Preface From 1988 to 1990, using the synthetic sex-pheromone compounds E2-Z13-18Ac (95%) + E3-Z13-18Ac (5%) (at 1 mg dose), were carried out some trials toimprove the Z. *pyrina* males catches in the fields (apple and pear orchards). The trials included performance studies of:

1) a funnel (with buffles) against a glue trap (Mastrap L® and Traptest®);
2) a polyethylene against rubber dispensers (IPO and Isagro);
3) a trap position with respect to plant height (low, top or above canopy) (Fig. 1);
4) the effects of orchards features on capture rates (rootstock, training system, plant age, etc.) (Fig. 2,3,4)

Table 1.- Blends compared in 1991.

lure	blends	ratio %	dose (mg)	dispenser size	factory
1	E2-Z13-18Ac	95	0.1	polyeth.	Isagro
	E3-Z13-18Ac	5		small	
2	E2-Z13-18Ac	95	1	"	"
	E3-Z13-18Ac	5			
3	E2-Z13-18Ac	95	10	"	"
	E3-Z13-18Ac	5			
4	E2-Z13-18Ac	90	1	"	"
	E3-Z13-18Ac	10			
5	E2-Z13-18Ac	95	1	"	"
	E3-Z13-18Ac	5			
	Z2-Z13-18Ac	5			
6	E2-Z13-18Ac	95	1	"	"
	E3-Z13-18Ac	5			
	Z13-18Ac	5			
7	E2-Z13-18Ac	95	1	"	"
	E3-Z13-18Ac	5			
	Z2-Z13-18Ac	2			
	Z13-18Ac	1			
8	E2-Z13-18Ac	95	1	polyeth.	Russell
	E3-Z13-18Ac	5		interm.	

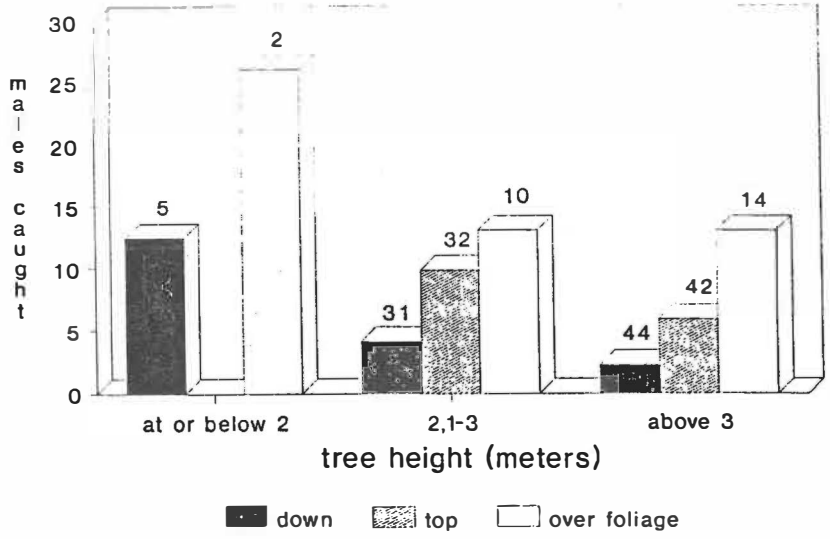


Fig. 1.- Average yearly captures per trap in orchards of varying tree height. The number of cases is shown above each column.

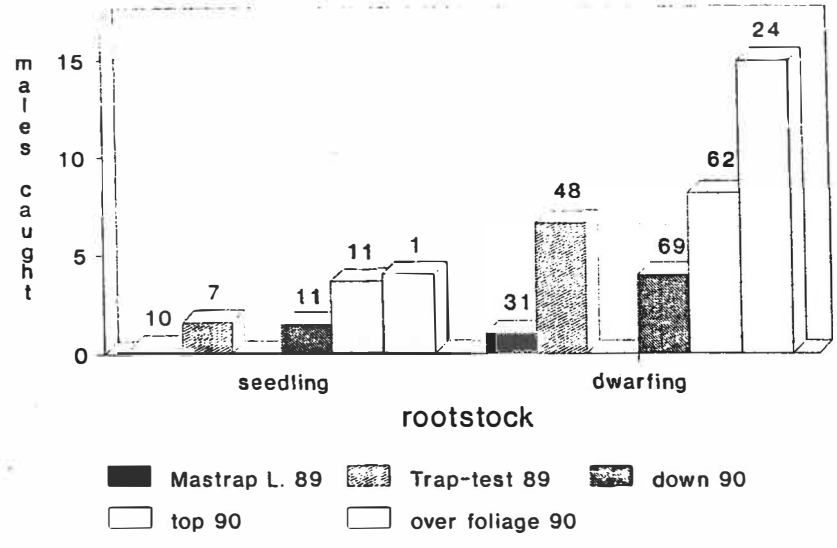


Fig. 2.- Comparison of average yearly captures per trap in orchards with seedling and dwarfing rootstock. The number of cases in shown above each column.

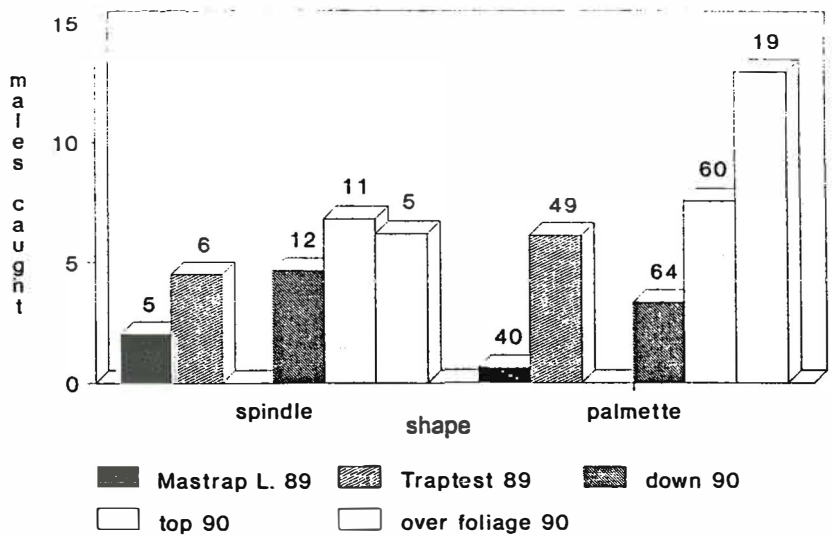


Fig. 3.- Relationship between average yearly captures per trap and training system. The number of cases is shown above each column.

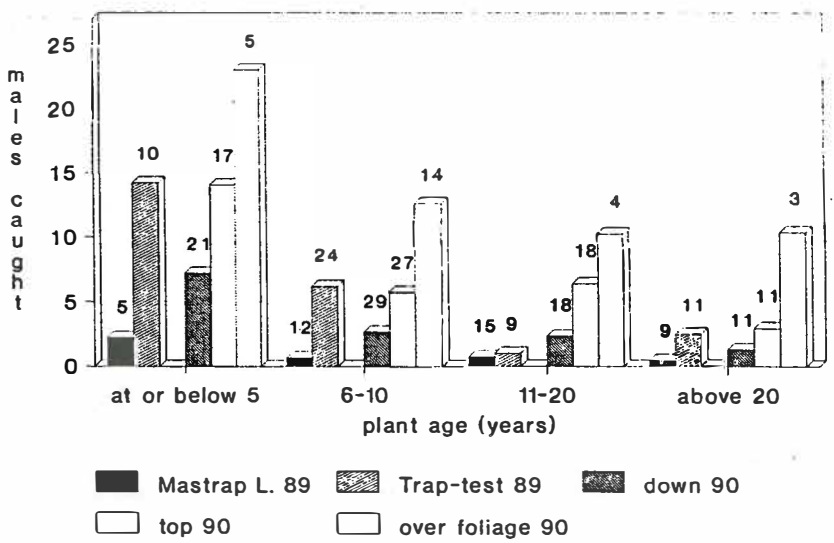


Fig. 4.- Average yearly captures per trap compared to tree age. The number of cases is shown above each column.

Table 2.- Lures (blends, doses and dispensers) compared in 1992.

lure	blends	ratio %	dose (mg)	dispenser size	factory
1	E2-Z13-18Ac	95	0.1	polyeth.	Isagro
	E3-Z13-18Ac	5		large	
	Z2-Z13-18Ac	5			
2	E2-Z13-18Ac	95	1	"	"
	E3-Z13-18Ac	5			
	Z2-Z13-18Ac	5			
3	E2-Z13-18Ac	95	10	"	"
	E3-Z13-18Ac	5			
	Z2-Z13-18Ac	5			
4	E2-Z13-18Ac	95	0.1	polyeth.	"
	E3-Z13-18Ac	5		small	
	Z2-Z13-18Ac	5			
5	E2-Z13-18Ac	95	1	"	"
	E3-Z13-18Ac	5			
	Z2-Z13-18Ac	5			
6	E2-Z13-18Ac	95	10	"	"
	E3-Z13-18Ac	5			
	Z2-Z13-18Ac	5			
7	E2-Z13-18Ac	95	1	polyeth.	Russell
	E3-Z13-18Ac	5		interm.	

During 1991 and 1992 (Tab. 1-2) were tested the performance of different lures (blends, doses) and dispensers size (Tab. 3) and the attractivity of natural females.

Table 3 Dispensers

		Dispenser				
factory	size	height (mm)	external diameter (mm)	internal diameter (mm)	volume (ml)	ratio sup. int./vol. int.
Isagro	large	31	14	12	3, 5	0,4
Isagro	small	31	8	6	0,8	0,73
Russell	intermediate	27	11	9	1,7	0,52

Results

Traps: at this moment the glue trap was better than funnel one.
Dispensers: the more attractive were the polyethylene than rubber;.
Trap position: the captures improves markedly with trap high (the ratio was about 1:2:4 from the lower to the higher).
Orchard parameters: in general the higher captures were obtained in young orchards, as well as dwarfing rootstock and palmette training.
Blends: the higher captures (but not significant) were obtained by the blend (1 mg in total) with the main compounds E2-Z13-18Ac and E3-Z13-18Ac (95% and 5%, respectively) + Z2-Z13-18Ac (5%)(1 mg in total) (Tab. 4-5).

Table. 4.- Results obtained in 1991.

Lure	n	Mean ES
1	18	8.89 (1.22)a
2	18	8.94 (2.20)a
3	18	17,33 (3.79)b
4	18	7.22 (2.06)a
5	18	11.00 (3.06)a
6	18	9.39 (1.87)a
7	18	9.17 (1.96)a
8	18	21.89 (4.75)b

Table 5.- Results obtained in 1992.

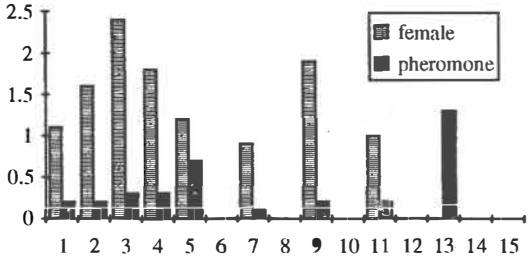
Lure	n	Mean ES
1	15	6.73 (1.97) a
2	15	7.33 (2.71) a
3	15	10.07 (3.00) a
4	15	7.47 (1.97) a
5	15	7.80 (2.77) a
6	15	57.73 (14.65)b
7	15	41.93 (7.44)b

Doses: the captures increase with dose but not proportionally (Tab. 4-5).
Dispensers size: the small Isagro dispenser (10 mg tri-compound) works better than large one and in the same way of Russell (1 mg only) (Tab. 5).
Females: the natural females were more attractive than the synthetic lure compared (Russell) (ratio 5,33:1) (Fig. 5).

Conclusions

All trials carried out are not sufficient to start on the large scale mass-trapping that is the main aim. However it will be possible to improve the lure by other screening of blends and doses. Also the baiting of dispensers and their size seem to have a great importance to improve the ratio female/pheromone captures. The glue traps work well for a monitoring technique, but for mass-trapping method it will be necessary to develope a funnel performance traps because of its easier manipulation.

Fig. 3.-Results of comparison female/pheromone



(1) A first part of this paper was published on Boll. Ist. Ent. G. Grandi, Univ. Bologna,46: 101-108. More detailed paper on the second part will be published on the same Bulletin (47).

Poster presented at OILB-SROP/IOBC-WPRS. Working group: Use of pheromones and other semiochemicals in integrated control. Chatam (UK), 10-14 May 1993.

A REVIEW OF THE SEMIOCHEMICALS USED BY THE NEW WORLD VECTOR OF LEISHMANIASIS, *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) DURING OVIPOSITION.

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ABSTRACT

The sandfly *Lutzomyia longipalpis* Lutz and Neiva (Diptera: Psychodidae) is the vector of visceral leishmaniasis in Central and South America. The causative agent is the protozoan flagellate *Leishmania donovani chagasi*, the disease is fatal if not treated. Recent investigations into the oviposition behaviour of *L. longipalpis* have shown that female flies utilize semiochemicals in the site selection process. An oviposition pheromone is known to attract gravid females and induce them to oviposit an average of 24h earlier than control flies. Apneumones from non-living organic material are also known to attract and stimulate ovipositing females. Furthermore, the oviposition response when both the oviposition pheromone and apneumones were used, was highly targeted and flies were stimulated to lay their eggs earlier, lay more eggs and survive oviposition in greater numbers than those with no semiochemical stimulus. This evidence has been used in the development of a laboratory oviposition trap, that successfully captured an average of 31 out of 50 (62%) gravid flies, over a 72h period.

INTRODUCTION

Little is known regarding the oviposition behaviour of *L. longipalpis* in the field and most of the research in this area has been carried out in the laboratory. The transmission of leishmaniasis is gonotrophically concordant and the females pass through more than one gonotrophic cycle (Guilivard *et al.*, 1980; Dye *et al.*,1987). A major problem with the study of *Leishmania* transmission in the laboratory is the very high mortality rates at oviposition. Females are known to lay up to 100 eggs in a single oviposition but the average number of eggs 40. It is known that temperature, relative humidity and photoperiod all play a role in the selection of oviposition site for sandflies (Foster *et al.*, 1970; Ready, 1976; Chaniotis, 1986). Furthermore, the physical nature of the substrate stimulates a thigmotropic response in gravid flies (ElNaiem and Ward, 1992a).

In the wild, the eggs of *L. longipalpis* are thought to be laid in microhabitats such as cracks and crevices, leaf litter, animal faeces, rodent burrows and other media rich in organic nutrients (Young *et al.*, 1926; Lewis and Kirk, 1954; Ward, 1974; Bettini and Mellis 1988). Sites containing larvae are difficult to find and contain very low numbers (Rutledge and Mosser, 1972). However, evidence for the aggregation of eggs at specific oviposition sites was given by Bettini (1988), who caught 27,405 emergent flies in one year from a $25m^2$ area.

This present report will review the major recent advances in the investigation of semiochemicals in the mediation of sandfly oviposition behaviour.

OVIPOSITION PHEROMONE

The first evidence for the presence of an oviposition pheromone associated with the eggs of *L. longipalpis* was given by ElNaiem and Ward (1990). They found that an area used previously as an oviposition site

was more attractive to gravid flies than an untreated surface.

L. longipalpis females laid more eggs onto 3mm deep groves, in the plaster of Paris base of a larval rearing pot, that contained conspecific eggs, than grooves without eggs (ElNaiem and Ward, 1991) (See Tables 1 and 2). When the same number of eggs were first washed in a cocktail of hexane, diethyl ether and distilled water, the oviposition response was lost. It was found that a minimum of 80 eggs was necessary to induce a positive oviposition response at the test site.

Table 1. Oviposition response of gravid *Lutzomyia longipalpis* to different treatments of conspecific eggs

Population	Age of eggs (days)	Treatment of eggs	Oviposition response (No. of eggs/8 cm²) (Mean±SE) Test	Control	P
L'Aguila	1-2	Unwashed	50.90±11.70	7.53±1.37	0.001
L'Aguila	1-2	Washed	17.27±1.56	16.0±2.00	0.470*
Jacobina	1-2	Unwashed	34.53±5.01	2.53±0.66	0.001
Jacobina	5-6	Unwashed	28.70±4.39	13.60±2.19	0.006

P = Probability level of Wilcoxon's signed-rank test; * = non-significant difference between test and control. Number of replicates carried out in each experiment was fifteen, except the response to unwashed eggs, which was repeated ten times. Produced with the pennission ElNaiem and Ward (1991).

Table 2. Oviposition response of gravid *Lutzomyia longipalpis* females to different numbers of conspecific eggs.

No. of Eggs placed on test site	Eggs laid by females (Mean±SE) Test	Control	Oviposition rate on test site (%)	P
20	12.80±02.47	13.20±3.34	49.83±5.37	0.609*
40	24.67±03.82	18.80±12.0	57.22±4.31	0.244*
80	20.00±02.01	10.87±1.75	67.92±3.45	0.001
160	50.90±11.70	07.73±1.37	82.27±3.66	0.001
320	46.83±03.79	25.83±1.17	63.97±2.64	0.036

P = Probability level of Wilcoxon's signed-ranked test; * = non significant difference between test and control. Number of replicates carried out in each experiment was fifteen, except the response to 320 eggs which was repeated six times. Produced with the pennission of ElNaiem and Ward (1991)

An increase in the percentage of eggs laid at the test site by gravid females was recorded, when up to 160 eggs were used as the test. When 320 eggs were placed on the test site, the oviposition response started to decline. This activity may be as a result of a pheromone concentration effect, with the pheromone at higher concentrations being repellent instead of attractive.

A hexane extract (1000 eggs/1.6ml hexane) was prepared and found to be an attractant and/or stimulant to gravid *L. longipalpis* (ElNaiem and Ward, 1991). Gas chromatography mass spectrometery revealed only cholesterol and squalene. Neither of these compounds induced the observed oviposition response, although the authors suggested that more compounds may be present.

Table 3. Attraction to and oviposition on rabbit faeces by gravid *L. longipalpis* in an olfactometer.

	Chamber with rabbit faeces				Chamber without rabbit faeces			
	No. eggs laid			No.	No. eggs laid			No.
	Unit A	Unit B	(A+B)	Fem's	Unit C	Unit D	(C+D)	Fem's
Mean	192.2	21.7	213.0	21.8	17.2	16.3	33.5	10.8
SE	±16.2	±1.0	±16.3	±2.1	±11.4	±9.8	±21.1	±1.9

Units A, B, C, and D are oviposition units in two olfactometer chambers. Unit A was treated with rabbit faeces; units B, C and D were left untreated. No. Fem's, number of females attracted to the chamber (n=50). Produced with the permission of ElNaiem and Ward (1992b).

SEMIOCHEMICALS FROM ORGANIC SOURCES

An olfactometer was used to investigate the oviposition attractants and stimulants from apneumones, semiochemicals from non-living organic matter (ElNaiem and Ward, 1992b). In an oviposition choice chamber, significantly more eggs were laid on sites containing colony frass, larval rearing medium or rabbit faeces. Experiments using unwashed and washed materials indicated the presence of chemical oviposition attractants and stimulants in larval rearing medium. The olfactometer investigation showed rabbit faeces was a strong attractant to gravid *L. longipalpis* over a minimum distance of 26cm (See Table 3.) A water extract of rabbit faeces was attractive to gravid flies, whereas, a hexane extract was not. Individually tubed female flies exposed to a water extract of rabbit faeces laid significantly more eggs than those exposed to a solvent control.

OVIPOSITION PEROMONE, SITE OF PRODUCTION AND CHEMISTRY

The solubility of the oviposition pheromone in hexane was confirmed by Dougherty *et al.*, (1992). High performance thin layer chromatography and gas chromatography showed that two compounds were present in both female accessory glands and eggs (100 egg equivalents/10 ul of hexane), along with

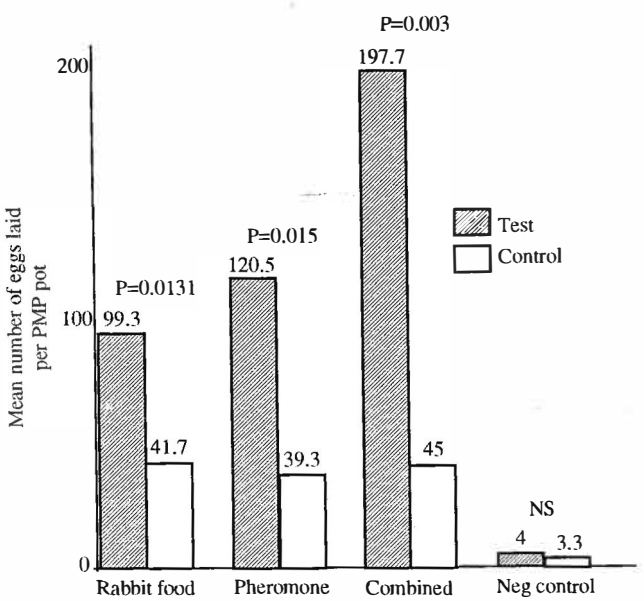

Fig. 1. The number of eggs laid by *L. longipalpis* in response to extracts of rabbit food, oviposition pheromone, a combination of these extracts and a negative control of solvents only. P= Probability. Ns = non-significant using the Wilcoxon matched pair sign rank test.

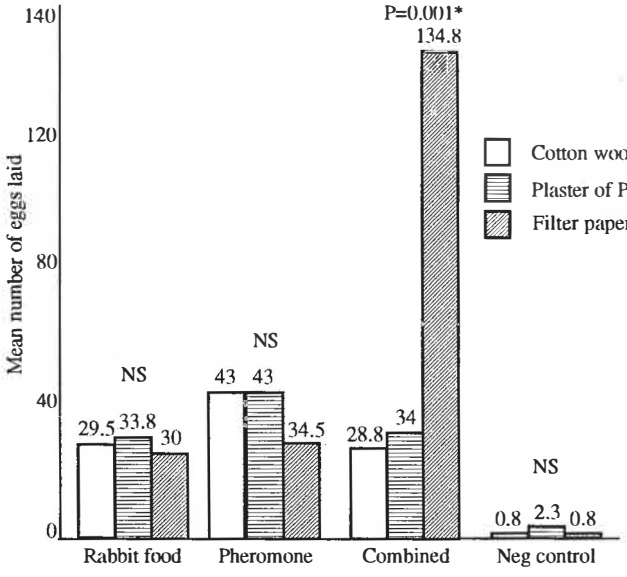

Fig. 2. Effect of extracts of rabbit food, oviposition pheromone, a combination of the above and a negative control of solvents only, on the choice of oviposition media by *L. longiplapis* in the test pots of the barruad cage bioassay. P = Probability of one media being preferentially oviposited on. * = Highly significant difference using analysis of variance.

several other compounds. When bioassayed, a hexane extract of female accessory glands induced an oviposition response equal to that of whole eggs. No other part of the male or female sandflies attracted or stimulated gravid flies. It is known that sandflies secrete a sticky substance onto the eggs, which is used to adhere them to the oviposition surface (Wu and Tesh, 1989). This evidence would suggest that the oviposition pheromone is produced in the accessory glands and secreted onto the eggs during egg laying. The sticky secretion may form a stable matrix from which the pheromone is slowly released. An active component from the hexane extract of sandfly eggs was isolated by column chromatography and

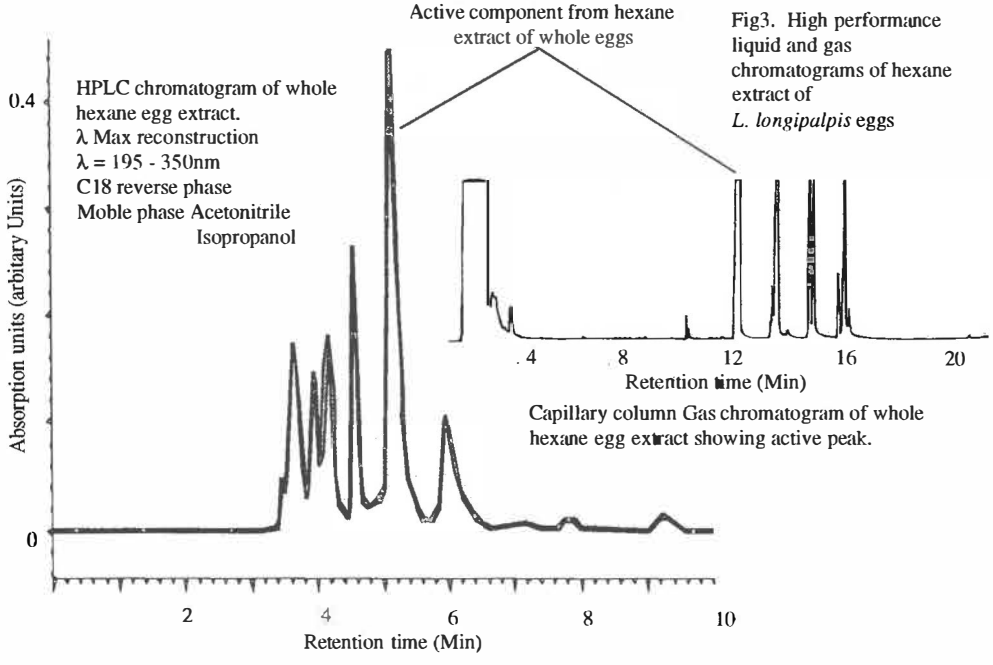

Fig3. High performance liquid and gas chromatograms of hexane extract of *L. longipalpis* eggs

high performance liquid chromatography and quantitatively monitored by gas chromatography (Dougherty *et al.*, 1993). A single peak appears to be contributing the total activity associated with the presence of whole eggs (See Fig. 3.). In a bioassay, this peak is both attractive and induces gravid flies to lay earlier than control flies.

SYNERGY BETWEEN OVIPOSITION SEMIOCHEMICALS

Evidence that rabbit food, rabbit faeces and hay contained oviposition attractants and stimulants was given by Dougherty *et al.*, (1993). All of the components gave a positive oviposition response, showing that the digestive process of the rabbit did not alter the semiochemical nature of the extracts. Solvents of polarity range water (9) - diethyl ether (3.6) are able to extract the semiochemicals from rabbit faeces. When the oviposition pheromone and apneumones from rabbit food were used in conjunction, a synergistic effect on sandfly egg laying was recorded. (See Figs. 1 and 2). The number of eggs laid was greatly increased and oviposition was highly targeted. Flies that were tubed individually with the combined semiochemicals were 3.5 time more likely to survive oviposition and laid 2.5 times more eggs than control flies. Using combined oviposition pheromone and apneumones in a laboratory trap, an average 31 out of 50 (62%) gravid flies were caught over a 72h period.

CONCLUSION

A theory for the behaviourial mechanism of oviposition site selection by gravid *L. longipalpis* was given by Dougherty *et al.*, (1993). At a distance the gravid flies are attracted and stimulated by the chemical odour cues from the oviposition substrate (apneumones). The physical nature of the organic material provides the correct tactile and thigmotropic cues and the microclime is an important factor. At a closer range, the oviposition pheromone synergises the stimulant and attractant cues and oviposition occurs. The synergy between the oviposition pheromone and apneumones on the oviposition behaviour of *L. longipalpis* could be a first step in the development of an oviposition trap.

The stimulation of gravid *L. longipalpis*, is not a classical response. Flies will not immediately oviposit when exposed to the semiochemicals. Rather, it appears that oviposition is being made more efficient. Flies exposed to the semiochemicals are approximately 24h younger when they oviposit. The flies may spend less time searching for an oviposition site and be physically fitter when they lay. This results in more eggs being laid and more flies surviving oviposition. The use of semiochemicals more closely mimics the oviposition environment found in the wild.

Further work is under way to reveal the relative contribution of each factor affecting the overall oviposition response of *L. longipalpis*. The isolated oviposition pheromone component from whole hexane egg extract is under investigation to characterize the chemical structure. Furthermore, the semiochemical component of rabbit faeces extract is to be fractionated and the chemical constituents characterized. These steps are seen as essential prior to the further development of an oviposition trap for *L. longipalpis*.

ACKNOWLEDGMENTS

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REFERENCES

BETTINI, S. C. (1988) Sandfly breeding sites, pp. 179-188, in D. T. Hart (ed.). Leishmaniasis, the Current Status and New Stratergies for Control. Plenum Press, New York and London.

BETTINI, S. C. & MELLIS, P. (1988) Leishmaniasis in Sardinia. III. Soil analysis of a breeding site of three species of sandflies. Med. Vet. Ent. **2**: 67-71.

CHANIOTIS, B. H. (1986) Successful colonization of the sandfly *Lutzomyia trapidoi* (Diptera: Pschodidae) , with enhancement of gonotrophic activity. J. Med. Ent. **23**: 163-166.

DOUGHERTY, M. J., WARD, R. D. & HAMILTON, G. (1992) Evidence for the accessory glands as the site of production of the oviposition attractant and/or stimulant of *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Chem. Ecol. **18**: 1165-1175.

DOUGHERTY, M. J., HAMILTON, J. G. C. & WARD, R. D. (1993) Semiochemical mediation of phlebotomine sandfly (*Lutzowyia longipalpis)* Oviposition. Med. Vet. Ent. **7:** (In Press).

DOUGHERTY, M. J., HAMILTON, J. G. C. & WARD, R. D. (1993) Isolation and identification of oviposition pheromone from eggs of *Lutzomyia longipalpis* (Diptera: Psychodidae). In Prep.

DYE, C., GUY, M. W., ELKINS, D. B., WILKES, T. J. & KILLICK-KENDRICK, B. (1987) The life expectancy of phlebotmomine sandflies: first feild estimate from Southern France. Med. Vet. Ent. **1**: 417-425.

ElNAIEM, D. A. & WARD, R. D. (1990) An oviposition pheromone on the eggs of sandflies (Diptera: Psychodidae). Trans. R. Soc. Trop. Med. Hyg. **84**: 456-475.

ElNAIEM, D. A. & WARD, R. D. (1991) Response of the sandfly *Lutzomyia longipalpis* to an oviposition pheromone associated with conspecific eggs. Med. Vet. Ent. **5:** 87-91.

ElNAIEM, D. A. & WARD, R. D. & REES, H. H. (1991) Chemical factors controlling oviposition of *Lutzomyia longipalpis* (Diptera: Psychodidae). Parassit. **33**: (suppl. 1), 217-224.

ElNAIEM, D. A. & WARD, R. D. (1992a) The thigmotropic oviposition response of the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae) to crevices. Annal. Trop. Med. Parasit. **86**: 425-430.

ElNAIEM, D. A. & WARD, R. D. (1992b) Oviposition attractants and stimulants for the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Ent. **29**: 5-12.

FOSTER, W. A., TESFA-YOHANNES, T. M. & TECKLE, T. (1970) Studies on leishmaniasis in Ethiopia. II. Laboratory culture and biology of *Phlebotomus longipes* (Diptera: Psychodidae). Annal. Trop. Med. Parasit. **64**: 403-409.

GUILIVARD, E., WILKES, T. J., KILLICK-KENDRICK, R. & RIOUX, J. A. (1980) Ecologie des leishmanioses dans le sud de la France, Developmet des cycles gonotrophique chez Phlebotmous ariasi Tonnoir, 1921 et Phlebotomous mascati Grassi, 1908, en Cenennes Corollaire epidemioloque. Annals of Parasitolog. **55**: 659-664.

LEWIS, D. J. & KIRK, R. (1954) Notes on the phlebotominae of the Anglo Egyptian Sudan. Annals. Trop. Med. Parasitol. **48**: 33-45.

READY, P. D. (1976) Studies on the biology of phlebotomid sandflies. Ph. D. thesis, University of London, London.

RUTLEDGE, L. C. and MOSSER, H. L. (1972) Biology of phlebotomine sandflies on the open forest floor in Panama. Environ. Entomol. **1:** 300-309.

WARD. R. D. (1974) Studies on the adult and immature stages of some phlebotomid sandflies (Diptera: Psychodidae) in Northern Brazil. Ph. D. thesis, University of London, London.

WU, W.K. & TESH, R. B. (1989) Experimental infection of old and new world phlebotomine sandflies (Diptera: Psychodidae) with *Ascogregarina chagasi* (Eugregarinarida: Lecudinidae). J. Med. Ent. **26**: 237-242.

YOUNG, T.C.M., RICHMOND, A. E. & BRENDISH, G. R. (1926) Sandflies and sandfly-fever in the Peshwar district. Indian J. Med. Res. **13**: 961.

BASIC AND APPLIED STUDIES ON THE PHEROMONES OF THE ALMOND SEED WASP, *EURYTOMA AMYGDALI* ENDERLEIN (HYMENOPTERA, EURYTOMIDAE)

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Abstract The almond seed wasp, *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae), is a serious pest of almonds in several Mediterranean countries, destroying up to 90% of the crop of certain susceptible varieties. It is a univoltine species the female of which oviposits singly inside the endosperm of the unripe, green almonds.The larva develops feeding on the endosperm and destroys it completely. With the aim to develop modern methods for controlling this serious almond pest we initiated studies on its biology, ecology and behaviour. The studies demonstrated the existence of a sex pheromone, that is produced by virgin females and attracts males, and of a host-marking pheromone, that is deposited by the females on the fruit surface and deters oviposition on already oviposited fruit.

Based on preliminary laboratory and field experiments with the sex pheromone, we developed a method with the help of which we monitored the flight of the wasp in an almond orchard, in the region of Thessaloniki, for 7 consecutive years. The method consisted in baiting Pherocon 1C traps (of the Zoecon Corp.) with 25 virgin females that released sex pheromone, and suspending them in the trees to attract and capture the males. The emergence of adults from infested almonds of the previous year was also monitored in cages with infested almonds placed in the field, as well as also the progress of fruit infestation and egg hatching.

In another study it was found that the optimal time for chemical control with the application of a systemic insecticide was when the percentage of egg hatching is between 10% and 50%. The results of 7 years of observations (1986-1992) showed that 10% and 50% of egg hatching in the area of Thessaloniki occurred with high consistency approximately 21 and 27 days respectively after the first captures in the sex pheromone traps.

Laboratory and field results showed that, as long as the level of infestation is not very high, the females of the wasp distribute their eggs more or less uniformly among the available fruit. This behaviour is mediated by a host-marking pheromone that is applied on the fruit surface by females immediately after an oviposition.

The responses of individual females to different treatments of almonds, in a series of laboratory experiments, revealed that the pheromone can by perceived by the females on direct contact and, when at high concentrations, also olfactorily from a short distance. The pheromone was present in the feces and inside the abdomen and thorax of females but not of males. Although water soluble, the pheromone could not

be entirely removed from heavily infested almonds when rinsed with water and persisted under laboratory conditions for at least 8 days.

In 1992, a first attempt to deter oviposition in the field by spraying a water solution of female feces was not very successful. This was probably due to the fact that the experiment was conducted under unfavorable, extremely high population densities.

It is anticipated that when isolated, identified and synthesized, both pheromones will prove very useful for monitoring the population of the wasp and for controlling it. Chemical work concerning the sex pheromone of the wasp is in progress.

General Information On The Insect

Geographic distribution

Eurytoma amygdali occurs in Greece, Turkey, Cyprus, former Yugoslavia, Syria, Lebanon, Israel, Jordan, France, Armenia, Georgia, and Azerbaijan.

Biology

It is a monophagous species which attacks only cultivated and wild almonds. It has one generation per year, and overwinters as fully developed larva inside the infested almonds that remain mummified on the trees. Adults emerge from these almonds in spring and the female oviposits singly inside the endosperm of unripe fruit. The larva develops within the endosperm and destroy it.

Damage

Frequently more than 90% of the fruit of certain susceptible almond varieties are destroyed.

Conventional population monitoring:

i) By exposing mummified almonds into cages in the field and monitoring adult emergence. However, field-cage data are not precise because they are affected by certain factors, such as the date and place of cage installation.

ii) By monitoring the progress of infestation, i.e. egg deposition and egg-hatching.

Chemical control

Optimal time for chemical treatment with a systemic insecticide (phosphamidon), was found to be at about 10% to 50% of egg-hatching.

For additional details and related references see Katsoyannos et al. 1992.

Studies On The Sex Pheromone

Demonstration of its existence and biological characteristics

It was reported by Pittara & Katsoyannos (1985). Chemical work concerning its identification is in progress.

Use of sex pheromone traps in forecasting optimal time for chemical treatment

In an orchard in the area of Thessaloniki (northern Greece), we monitored an adult male population for 7 years (1986-1992) with the use of sex pheromone traps (Pherocon 1C), baited with 25 living virgin females that were replaced every 5 days. The trap data were correlated with the progress of infestation and egg hatching in order to determine the optimal time for chemical treatment.

It was found that the 10 and 50% of egg-hatching (optimal time for chemical treatment) occurs about 21 and 27 days respectively after the first captures in the traps (Table 1). The maximum of captures can be also used for this purpose, but with less precision (see also Katsoyannos et al. 1992).

Provided that the pheromone is isolated, identified and synthesized, it can be possibly used for the mass trapping and the mating disruption technique.

Table 1: Approximate number of days from respectively the beginning and the maximum of captures of *E. amygdali* males in sex pheromone traps in spring (April, May), until 10% and 50% of egg hatching. Data were collected in the years 1986-1992 in the area of Thessaloniki, and concern the Retsou variety.

	Number of days from:			
	Beginning of trap captures until:		Maximum of trap captures until:	
Year	10% egg hatch	50% egg hatch	10% egg hatch	50% egg hatch
1986	22	25	16	20
1987	23	28	6	10
1988	24	29	10	15
1989	16	25	12	21
1990	18	24	12	18
1991	23	30	10	18
1992	24	26	11	13
Mean:	21.4	26.7	11.0	16.4
SD:	3.2	2.3	3.3	4.0

Studies On The Host Marking Pheromone (HMP)

Demonstration of its existence and ecological function

It was reported by Kouloussis & Katsoyannos (1991; 1993).

Basic bioassay used

Confinement of individual females with susceptible almonds of various treatments and recording of female visits and ovipositions on the fruit, for 1-3 hours (see some of the results obtained in Table 2).

Some attributes of the HMP:

Females deposit the pheromone after oviposition by dragging the tip of the abdomen over the fruit surface. The pheromone deters repeated oviposition in already oviposited fruit, thus contributing to the uniform egg distribution among available fruit. It is present in the abdomen and, at lower concentrations, in the thorax of the females but not of the males. It is present in the feces of the females but not of the males. At low concentrations it is perceived by conspecific females after direct contact. At higher concentrations it is perceived also olfactorily, from a short distance. It is water soluble but it cannot be removed easily from the fruit surface by rinsing with water. Its biological activity persists for at least 8 days under lab conditions.

Table 2 HMP: Some examples of results obtained in 2-choice tests.

Treatments	N	Mean number of	
		Visits	Ovipositions
Infested almonds (1 egg/fruit)	14	8.5a	0.9a
Uninfested (control)		9.2a	3.4b
Highly infested (14 eggs/fruit)	19	5.3a	0.2a
Uninfested (control)		13.0b	5.0b
Water with faeces of females	8	5.0a	0.4a
Water (control)		11.3b	4.4b

Means followed by different letters are significantly different at 0.01 level.

First attempt for field application of the HMP

In an experiment conducted in 1992, almond tree branches were sprayed with a water solution of female feces. The solution (collected from ca. 2.000 females) did not produce satisfactory results in the field, although it showed a high oviposition deterring activity under laboratory conditions. However, it should be considered that in the particular year the population of the insect in the field was extremely high. Females oviposited an average of 9.0 eggs in the control fruit and of 6.1 eggs in the fruit treated with the highest concentration of the pheromone-water solution (1:10). At lower population densities usually only 1 egg per fruit is deposited. The experiment will be repeated in 1993.

References

KATSOYANNOS, B. I., N. A. KOULOUSSIS, and A. VASSILIOU, 1992.- Monitoring populations of the almond seed wasp, *Eurytoma amygdali,* with sex pheromone traps and other means, and optimal timing of chemical control. Entomol. Exp. & Appl. **62**: 9-16.

KOULOUSSIS, N. A., and B.I. KATSOYANNOS, 1991.- Host discrimination and evidence for a host marking pheromone in the almond seed wasp, *Eurytoma amygdali*. Entomol. Exp. & Appl. **58**: 165-174.

KOULOUSSIS, N. A., and B.I. KATSOYANNOS, 1993. Egg distribution patterns in the almond seed wasp, *Eurytoma amygdali*. Entomol. Exp. & Appl. **66**: 31-38.

PITTARA, I.S. and B.I. KATSOYANNOS, 1985. Male attraction to virgin females in the almond seed wasp, *Eurytoma amygdali* Enderlein (Hymenoptera: Eurytomidae). Entomologia Hellenica, **3**: 43-46.

FEMALE SEX PHEROMONE GLANDS IN GALL MIDGES: ANATOMO-PHYSIOLOGICAL AND BEHAVIOURAL EVIDENCE

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Abstract Sex calling of males by females in Gall Midges (Diptera, Cecidomyiidae) has been reported from long ago, and also the releasing of a female sex pheromone has been proven in several species, including serious pests such as the Sorghum Midge (*Allocontarinia sorghicola* Coq.) and the Brassica Pod Midge (*Dasineura brassicae* Winn.). Thus far, the female sex pheromone glands of only these two species of the family have been identified. An illustration of the relative anatomo-physiological and behavioural proofs is reported here through scanning and transmission electronmicrographs of the glands, as well as macrophotographs of adult midges displaying mating behaviour. In brief, in both species investigated, the pheromone gland lies in the ovipositor and consists of the hypertrophied epidermis of the intersegmental membrane between 8th and 9th uromeres. The said epidermis appears quite different in virgin than mated females. In the former, the epidermis is made of cylindrical-cuboidal cells displaying ultrastructural features typical of pheromone-producing gland cells; whereas in the latter, the cells in question undergo atrophy following copulation. In the resting virgin female the pheromone(s) is stored in the fold formed by the cuticular outer surface of the mentioned intersegmental membrane (ovipositor retracted). The pheromone(s) is released when the membrane is extended during sex calling behaviour, i.e., ovipositor extension-retraction. Mated females do not display calling behaviour nor attract males any more.

Introduction

Gall midges, are a very important dipteran family (Cecidomyiidae) consisting of some four thousand species (Skurava', 1986) that display the most various feeding habits, from mycophagy and saprophagy to phytophagy (most species are gall makers: hence the familiar name) and to zoophagy. Many species are serious pests of cultivated plants or mushrooms, some of them, such as the Hessian fly (*Mayetiola destructor* Say) and the Sorghum midge (*Allocontarinia sorghicola* (Coq.) Solinas, 1986), even on a world-wide scale, while the larvae of zoophagous species, such as *Aphidoletes aphidimyza* Rond., are important biological control agents of harmful insects.

Sex calling of males by females in Cecidomyiidae has been reported from long ago (Enoch, 1891; Cartwright, 1922; Barnes, 1932 and 1935).

Recently, the existence of a female sex pheromone in gall midges (Miller & Borden, 1981, in *Contarinia oregonensis* Foote; Wall *et al.*, 1985, in *Contarinia pisi* Winn.; Garthwait *et al.*, 1986, in *Dasineura tetensi* Ruebs.; Isidoro & Bin, 1988, in *Dryomyia lichtensteini* Fr. Lw.) has been

demonstrated, while in a few species (McKay & Hatchett, 1984, in *M. destructor*; Miller & Borden, 1984, in *C. oregonensis*;Williams & Martin, 1986, in *Dasineura brassicae* Winn.) also the release site of the pheromone, i.e. the ovipositor, has been located. Lately (Foster *et al.*, 1991), the first identification of a gall midge sex pheromone, i.e., that of the Hessian fly, has been carried out.

For the Sorghum midge and the Brassica pod midge (*Dasineura brassicae* Winn.), major pests of cultivated *Sorghum* spp. and *Brassica* spp., respectively, the presence of a female sex pheromone has been proven (Moura *et al.*, 1988; Sharma, 1988; Williams & Martin, 1986), and also the identification of the relative glands has been performed (Solinas & Isidoro, 1991; Isidoro *et al.*, 1992) through anatomo-physiological and behavioural investigations.

The aim of this presentation was to give an illustration of Cecidomyiidae sex pheromone glands and mating behaviour, in order to get people of complementary expertise involved in discussion on and further research for chemical identification and synthesis of the pheromones in question, and about the possible strategies of application of the same in integrated control programmes.

Results and discussion

Anatomo-histological, ultrastructural, and physiological evidence

In both species investigated, *A. sorghicola* (Solinas & Isidoro, 1991) and *D. brassicae* (Isidoro *et al.*, 1992), the ovipositor is telescopic and consists of the last three abdominal segments (8th + 9th + 10th), of which the 8th uromere is caudally tapering and forms the ovipositor base, while the 9th + 10th uromeres make up the true ovipositor connected to the base by a well-developed intersegmental membrane. The epidermis of this membrane (i.e., the 8th-9th intersegmental membrane) represents the sex pheromone gland for the reasons below explained.

In newly emerged virgin females, the 8th-9th intersegmental membrane epidermis consists of a single layer of cylindrical-cuboidal cells, quite varied in shape and size , resting on a well-developed, rather thick basement membrane. These cells possess a large nucleus with features typical of intense activity, and cytoplasm containing: extensive smooth endoplasmic reticulum; a moderate amount of granular reticulum confined around the nucleus; abudant ribosomes isolated or in groups randomly distributed throughout the cell; well-developed mitochondria frequently displaying whorled cristae; obvious Golgi apparatus; numerous electronlucid and electrondense secretion vesicles. The cuticle overlying the cells in question does not show any obvious porosity.

At low magnification, the outer surface of the 8th-9th intersegmental membrane looks quite smooth, but at higher magnification it can be seen to consist of a continuos series of alternate irregular minute protuberances and irregular grooves, the former bearing microtrichia.

Features such as extensive smooth endoplasmic reticulum and numerous and modified mitochondria are typical of pheromone producing cells (Solinas & Isidoro, 1991,and references therein).

Furthermore, the observed ultrastructure of the cuticular outer surface may form a special device for storing the pheromone by retaining it within the grooves when they are closed in the folded (ovipositor retracted) intersegmental membrane. Also, the said cuticular features may regulate the pheromone evaporation during ovipositor extension by the calling female midge.

In 24 h old virgin females, the epidermis of the 8th-9th intersegmental membrane, i.e. the pheromone gland, has the same ultrastructural features observed in the newly emerged virgin females. Whereas, in 25 h old mated females, fixed 24 h after copulation, the gland cells show an obvious atrophy as a consequence of copulation, thus confirming that it is the sex pheromone gland.

Behavioural evidence

Direct observations and videotape recordings carried out both in nature and in the laboratory confirmed previous knowledge about the reproductive behaviour of the Sorghum midge (Isidoro, 1987) and the Brassica pod midge (Williams & Martin, 1986). About 30' after emergence, the females start calling behaviour, i.e., they repeatedly extend the ovipositor almost completely and slowly retract it, sometimes waving it back and forth but remaining almost immobile for the rest. Soon after the ovipositor extension, conspecific males are attracted to the females and copulation takes place without any courtship: male just mounts female which immediately retracts her ovipositor, only leaving its posterior end exposed to allow the male to clasp this with his forceps copulatrix and then to inseminate; the whole sequence takes about 10". Females are strictly monogamous (males usually mate several times with different females) and, after copulation, they repeatedly fully extend and retract the ovipositor a few times , but without accepting males any more; then they wait almost immobile in the same place for about 1h and finally they fly in search of oviposition sites. Virgin females display calling behaviour and attract males all their life span. Males are attracted only to virgin females when exhibiting ovipositor extended.

Traps baited with virgin females (both newly emerged or 24h old) always catch conspecific males in the fields and in the laboratory as well. Whereas traps baited with mated females or virgin females ovipositor-ectomized do not catch males either in the fields or in the laboratory.

Male midges exposed to extended ovipositors, freshly excised from virgin females, immediately exhibit sexual excitation: they fan their wings, crawl around the ovipositors until they touch one of them, and then they stop and try to copulate with it.

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References

BARNES, H.F., 1932. On the gall midge injurious to the cultivation of willows. I. The bat willow gall midge (*Rhabdophaga terminalis* H.Lw.). Ann. appl. Biol. **19**: 243-252.

BARNES, H.F., 1935. On the gall midges injurious to the cultivation of willows. II. The so-called "shot hole" gall midge (*Rhabdophaga* spp.). Ann. Appl. Biol. **22**: 86-105.

BILLEN, J., 1991. Ultrastructural organization of the exocrine glands in ants. Ethology, Ecology & Evolution, special Issue. **1**: 67-73.

CARTWRIGHT, W.B., 1922. Sexual attraction of the female Hessian fly (*Phytophaga destructor* Say). Can. Ent. **54**: 154-155.

ENOCH, F., 1891. The life history of the Hessian fly. Trans. Entomol. Soc. London. **39**: 329-366.

FOSTER, S.P., BERGH, J.C., ROSE, S. & HARRIS, M.O., 1991a. Aspects of Pheromone Biosynthesis in the Hessian Fly, *Mayetiola destructor* (Say). J. Insect Physiol. **37**: 899-906.

FOSTER, S.P., HARRIS, M.O. & MILLER J.G., 1991. Identification of the sex Pheromone of the Hessian Fly, *Mayetiola destructor* (Say). Naturwissenschaften. **78**: 130-131.

GARTHWAIT, D.G., WALL, C. & WARDLOW, L., 1986. Further evidence for a female sex pheromone in the black currant leaf midge *Dasineura tetensi*. -British Crop Prot. Conf., Pests and Diseases, 3C. **18**: 355-357.

HARRIS, K.M., 1979. Description and host ranges of the sorghum midge, *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae), and of eleven new species of *Contarinia* reared from Gramineae and Cyperaceae in Australia. Bull. ent. Res. **69**: 161-182.

ISIDORO, N., 1987. *Allocontarinia (Contarinia) sorghicola* (Coq.) (Diptera, Cecidomyiidae): morfologia, biologia, danni, controllo. Entomologica. **XXII:** 35-73.

ISIDORO, N. & BIN, F., 1988. Attività cairomonale del feromone sessuale di *Dryomyia lichtensteini* Fr. Lw. (Dipt.: Cecidomyiidae) sul parassitoide oo-larvale *Platygaster dryomyiae* Silv. (Hym.: Platygastridae). Atti XV Congr. naz. ital. Entomol., L'Aquila, pp. 939-944.

ISIDORO, N., WILLIAMS, I.H., SOLINAS & M., MARTIN, A., 1992. Mating behaviour and identification of the female sex pheromone gland in the brassica pod midge (*Dasineura brassicae* Winn.: Cecidomyiidae, Diptera). Boll. Ist. Ent. "G. Grandi" Univ. Bologna. **XLVII**: 1-22.

KIEFFER, J.J., 1900. Monographie des Cecidomyides d'Europe et d'Algerie. Ann. Soc. Entom. France. **LXIX**: 181-472.

MCKAY, P.A. & HATCHETT, J.H., 1984. Mating Behaviour and Evidence of a female sex Pheromone in the Hessian Fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. **77**: 616-620.

MILLER, G.E. & BORDEN, J.H., 1981. Evidence for a sex pheromone in the Douglas-fir cone gall midge. Can. For. Serv. Res. Notes. **1**(2): 9-10.

MILLER, G.E. & BORDEN, J.H., 1984. Reproductive behaviour of the Douglas-fir cone gall midge, *Contarinia oregonensis* (Diptera: Cecidomyiidae). Can. Ent. **116**: 607-618.

MOURA, J.I., VILELA, E.F. & WAQUIL, J.M., 1988. Atraçao sexual na mosca no sorgo, *Contarinia sorghicola* (Coquillett, 1899) (Diptera; Cecidomyiidae) no campo. An. Soc. Ent. Brasil. **17** (supl.)

NOIROT, C. & QUENNEDEY, A., 1974. Fine structure of insect epidermal glands. Ann. Rev. Entom. **19**: 61-80.

PERCY, J.E. & WEATHERSTON, J., 1974. Gland structure and pheromone production in insects. In: Birch M.C., Pheromones. Frontiers of Biology, Noprth Holl. Pub. Comp., Amsterdam. **32**: 11-34.

PETTERSSON, J., 1976. Ethology of *Dasineura brassicae* Winn. (Dipt., Cecidomyiidae). I. Laboratory Studies of Olfactory Reactions to Host-Plant. Symp. Biol. Hung. **16:** 203-208.

SHARMA, H.C., 1988 - I.C.R.A.S.A.T. - Annual Report : 8.

SKURAVA', M., 1986. Family Cecidomyiidae. In: Soos A & Papp L. (Eds), Catalog of Palaearctic Diptera, Elsevier, Amsterdam. **4**: 72-297.

SOLINAS, M., 1986. *Allocontarinia n.g., A. sorghicola* (Coq.) n. comb. (Diptera, Cecidomyiidae). Entomologica. **XXI**: 23-35.

SOLINAS, M. & ISIDORO, N., 1991. Identification of the female sex pheromone gland in the Sorghum Midge, *Allocontarinia sorghicola* (Coq.) Solinas (Diptera, Cecidomyiidae). Redia. **LXXIV** (2): 441-446.

SYLVÉN, E., 1970. Field Movement of Radioactively Labelled Adults of *Dasineura Brassicae* Winn. (Dipt., Cecidomyiidae). Ent. Scand. **1**: 161-187.

TAMAKI, Y., 1985. Sex Pheromones. In: Kerkut G.A. & Gilbert L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology. vol. **9**: 145-191.

WALL, C., PICKETT, J.A., GARTHWAIT, D.G. & MORRIS, N., 1985. A female sex-pheromone in the pea midge, *Contarinia pisi*. Entomol. exp. appl. **39**: 11-14.

WILLIAMS, I.H. & MARTIN, A.P., 1986. Evidence for a female sex pheromone in the brassica pod midge *Dasineura brassicae*. Physiol. Entomol. **11**: 353-356.

MATING-DISRUPTION OF *SPODOPTERA LITTORALIS* IN EGYPT

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Abstract Recent advances in pheromone formulation technology have permitted a re-assessment of mating-disruption using controlled-release pheromone formulations as a technique to control the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) in cotton. Results show that significant mating-disruption of *S. littoralis* can be achieved using both PVC (40 g a.i. per ha) and twist-tie (60 g a.i. per ha) formulations. At these application rates, evidence accumulated through a wide variety of evaluation methods such as pheromone trap catch suppression, suppression of mating in tethered virgin female moths, direct observation of behaviour with low light-level video, bait and light trap catches and numbers of egg-massess in treated and untreated areas, shows that mating-disruption is maintained for at least 40 days. The data accumulated further suggested that both formulations are capable of maintaining mating-disruption for longer periods.

Results also suggest that the level of control could be enhanced if application was made to a larger area, thus reducing levels of immigration by gravid females into the treatment area. Earlier application of the pheromone formulations before insect populations have built up could also be expected to enhance the effectiveness of the treatments.

LARGE SCALE USE OF PINK BOLLWORM SEX PHEROMONE FORMULATIONS INTEGRATED WITH CONVENTIONAL INSECTICIDES FOR THE CONTROL OF COTTON PESTS IN EGYPT

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Abstract. Two slow release pheromone formulations were applied commercially to a total of 7354 feddans of cotton in four governorates in Egypt as part of integrated pest control programmes in 1991. These programmes gave control of the total pest complex at least equal to that achieved by a conventional pesticide programme. Estimated yield losses were lower in the pheromone treated areas. Higher boll weights in the pheromone treated districts in Dakahlia governorate were attributed to enhanced hybrid vigour as the result of cross-pollination by bees.

Introduction

Following several years of large-scale testing (Critchley *et al.*, 1983 and 1985, El-Adl *et al.*, 1988) four synthetic pheromone formulations were registered in Egypt for the control of pink bollworm *Pectinophora gossypiella* (Saunders); a hollow-fiber formulation from Sandoz, a microencapsulated formulation from ICI, a laminate flake formulation from BASF and the twist-tie or rope formulation supplied by Shin Etsu.

Commercial applications of all four formulations took place in the years 1984-85 during which time the area treated increased from 12,500 feddans to 50,000 fed. (1 feddan = 0.42 ha). However, in 1986 pink bollworm infestation in some pheromone-treated areas were reported to be higher than those in areas treated solely with conventional insecticides and yields of seed cotton were claiming to be lower. It was not possible to establish what, if anything, went wrong with the 1986 pink bollworm control programme. It has been suggested that pheromones may have been less effective under high population pressures or alternatively that lower yields might have resulted in the absence of conventional pesticides from early season damage of the Egyptian cotton leafworm Spodoptera littoralis (Boisd.) combined with late season attacks by spiny bollworm Earias insulana (Boisd.).

When pests other than Pectinophora occur in damaging numbers, Pectinophora pheromone must be integrated into a conventional pesticide programme with the aim of controlling the other pests while preserving beneficial insects as much as possible (El-Adl *et al.*, 1988). Trials of this kind were first conducted in Egypt in 1983 in a series 100 fed. cotton blocks (Critchley *et al.*, 1984). Various combinations of pheromone and insecticide applications were tested. The most effective involved two early season applications of pheromone followed by two to three later applications of conventional insecticides. Subsequent small-scale trials using rope pheromone were conducted in a

total of 2500 fed. cotton during the 1990 cotton growing season. The large-scale trials reported here were carried out in 1991 to confirm the earlier results.

Materials And Methods

Details of the two commercially registered pink bollworm pheromone formulations used in the 1991 trials are shown in Table (1). Analysis by D. Hall (Natural Resources Institute) showed that storage of the microencapsulated pheromone used in the trials under local, unrefrigerated, conditions for nearly two years, had not resulted in any breakdown or loss of the active ingredient. The rope pheromone was freshly formulated each season.

Table 1: Commercially registered pink bollworm, *Pectinophora gossypiella* pheromone formulation application in Egypt in 1991.

Product trade name	Formulation	Supplier	Common name	Recommended Dose
P.B. Rope	Stiffened 20cm polythene tube 300 rope per feddan 72mg a.i. per rope 21.8g per feddan Hand	Mitsubishi	P.P. Rope Gossyplure	300 rope per feddan
Pectone 69	2% a.i. microcapsules diluted in water 4g per feddan Application equipment	ICI/PPD (UK)	Gossyplure	200ml format per feddan

The insecticide products used in the first spray round were a mixture of compounds containing an organo-phosphate and an insect growth regulator used for controlling larval stages of the cotton leafworm (*Spodoptera littoralis*). Materials used are listed in Table (2). Choice and timing of all the insecticide treatments were under the control of the chief pest control officer for each governorate following instructions from the Ministry of Agriculture in Cairo.

Up to six sites were chosen to represent a variety of cotton-growing areas with differing climatological and agronomic conditions. Two of the sites were in the Delta (Dakahlia and El-Beheira) where the majority of cotton in Egypt is grown, while the others were in Upper-Egypt (in El-Minia and Assiut) (Table 3). The total area of cotton treated was approximately 4934 fed. for the rope pheromone and 2420 fed. for the microencapsulated pheromone.

Control of pink bollworm using pheromones should start when the first flowers are observed on the plants. Micro-encapsulated formulations are applied aerially and delays in the timing of the first application occurred, as indicated in Table 3.

Table 2: List of insecticides used in treatment and control areas in the large-scale PBW pheromone trials in Egypt 1991

Production trade name	Formulation	Supplier	Common name
Tamaron Combi	30 + 3 EC	Bayer	Methamidophos (OP) + triflumuron
Empire	48 + 3 FL	DOW	Chlorpyrifos + dimilin
Primicid	50EC	ICI	Primiphos ethyl
Dursban	48EC	DOW	Chlorpyrifos ethyl
Larvin	50DF	Union Carbide	Thiodicarb
Baythroid	5EC	Bayer	Cyflutrhin

Table 3: Details of areas treated, dates of the first application and stage of cotton growth in large-scale PBW pheromone trials in Egypt 1991

Governorate	Locality	Area treated (Fed.)	Method of application	Stage of crop growth at first application	Date of 1st application
Rope					
Dakahlia	Miniet El-Nasr	493	Hand applic.	Before pin squares	2-3/6/91
	El-Manzala	507	Hand applic.	Before pin squares	14-16/6/91
Assiut	Dayrout	1000	Hand applic.	Before pin squares	8-11/6/91
El-Minia	Bani Mazar	2000	Hand applic.	Before pin squares	11-15/6/91
El-Beheira	Damanhour	934	Hand applic.	Before pin squares	14-20/6/91
Micro-encapsulated					
Assiut	El-Qusiea	1019	Cessna	2nd flowers	1st & 2nd Sp 30/6-14/7
El-Minia	Magaga	1000	Cessna	2nd flowers	1/7-15/7 3/7-16/7
Dakahlia	El-Manzala	401	G. motors	2nd flowers	4/7 1/7-19/7

Sp. = spray Fed. = Feddan G. = Ground

The rope formulation was applied only once, by hand, using groups of children. By using the variation in planting dates to time applications, it was possible to complete a given area in two to ten days, with the applications made at the correct stage of plant growth.

Delta type pheromone traps were deployed (one per 50 fed.) to monitor pink bollworm moth numbers in all the treatment areas and the number of pink bollworm caught recorded daily.

Application of the microencapsulated formulation was aimed at achieving complete trap catch suppression and hence mating disruption for a period of two weeks. A minimum of two applications was therefore required to cover the critical six week period from the appearance of the first pin-squares to that of the first susceptible boll. Only one application of the rope formulation is required for season-long mating disruption. Conventional pesticide were applied for the control of pests other than pink bollworm in both the pheromone (at 5% boll infestation) and conventionally treated areas (every 15 days from 1 July).

Estimates of seed cotton yields were made prior to the first pick after more than 60% of the bolls on each plant had opened. The yield loss was calculated using the standard equation in El-Deeb and Critchley (1986). The numbers of fully and partially opened bolls, mature green bolls and dried brown bolls, both healthy and infected, were counted from a total of 80 plants per treatment by examining ten plants from eight 2-metre sections of cotton row in four different fields.

Results

Application of rope formulation in Assiut, El-Minia and Dakahlia resulted in 100% delta trap catch suppression for the seven weeks before insecticide applications were made. In El-Beheira 93% suppression relative to the insecticide area was achieved. In El-Minia, Assiut and Dakahlia the suppression due to the presence of microencapsulated pheromone was 75%, 65% and 54% respectively.

The levels of boll infestation recorded from weekly samples in each area are shown in Table 4. In three governorates the rope formulation performed better than the microencapsulated pheromone when results are averaged over all boll inspections. In the fourth governorate the reverse was true. In all areas the pheromone treatments were at least as effective as the full conventional insecticide schedule and in most cases much more effective.

Estimates of yield losses are presented in Table 5. In all governorates the yield loss estimates for the pheromone treated areas were lower than in the comparable insecticide only treated areas. No differences in boll weights were recorded from insecticide and pheromone areas except in Dakahlia (Meniet El-Nasr and El-Manzala) where the boll weights in the rope treatment (but not the microencapsulated treatment) were 16% heavier than in the corresponding conventional insecticide plots.

Table 4: Pink bollworm pheromone treatments (Egypt, 1991) Percentage boll infestation by pink bollworm as the result of pheromone treatments compared with conventional insecticides

	Sampling Date										
ElMinia	4/7	11/7	18/7	25/7	1/8	8/8	15/8	22/8	29/8	5/9	Mean
Rope T1	0	0	0	0	0.2	0.8	0.2	0	0	0.4	0.2
Microencapsulated T2	3.3	2.5	2	1.5	2	5.5	4.7	2.2	2.2	5.5	3.2
Insecticides	1.5	2.5	1.5	1.5	4	4.5	5	5	4.5	-	3.2
Assiut	9/7	14/7	22/7	29/7	5/8	12/8	19/8	26/8	5/9		Mean
Rope T1	0	0	0.3	0.2	0.5	0.7	2.5	1.8	5.8		1.3
Microencapsulated T2	0	0.3	0.5	1	1.3	1.5	2	3.4	6.5		1.8
Insecticides	1	2.5	3	3	5	5.7	8.5	9.5	23		6.8
El-Beheira	17/7	24/7	29/7	5/8	12/8	20/8	27/8	4/9	10/9		Mean
Rope T1	0.3	0.6	0.3	0.5	0.6	2.3	3.6	1.1	1.1		1
Insecticides	-	2.5	3.1	2.8	5.3	2.5	6.7	1.2	22		2.9
Dakahlia	24/7	1/8	7/8	13/8	20/8	27/8	3/9	10/9	17/9	25/9	Mean
Rope* T1	0	0.2	1.3	1.6	2.3	6.1	11.8	14.7	-	-	4.7
Rope** T1	0	0.6	0.9	1.1	2.3	8.7	5.5	5.7	8.8	14	4.6
Microencapsulated T2	0	0.8	0	1.2	5	2.7	4.9	4.9	4	2.1	2.8
Insecticides	13.8	2.8	1.5	2.2	2.9	3.6	2.7	4.8	5.3	6.2	4.5

* Miniet El-Nasr District ** El-Manzala District

Table 5: Mean yield loss estimates and average weight of seed cotton in pheromone and insecticide treated areas in the Governorates of El-Minia, Assiut and Dakahlia in Egypt, 1991

Governorate	Treatment	%Estimated yield loss	Average weight of seed cotton per boll (gm)
El-Minia	Ropeph.	5.8	2.5
	Microencapsulated ph.	5.2	2.6
	Insecticides	9.0	2.5
Assiut	Rope ph.	6.2	2.4
	Microencapsulatedph.	7.3	2.3
	Insecticides	13.0	2.4
El-Beheira	Ropeph.	4.6	2.8
	Insecticidesph.	5.5	2.6
Dakahlia	Ropeph.	4.8	2.9
	Microencapsulatedph.	5.1	2.6
	Insecticides	6.7	2.5

ph. = pheromone

Discussion And Conclusions

No problems were encountered in applying the microencapsulated formulation since conventional spray equipment is used. It was found that one person can treat from 1.5 to 2 feddans of cotton with the hand applied rope formulation each day. As planting dates varied by up to ten days within a small area, judicious timing of the use of the application teams could ensure that the rope formulation was applied at the correct point is the cotton flowering phenology (Table 3).

In large areas of three environmentally distinct governorates two applications of microencapsulated pheromone followed by two or three applications of conventional insecticide, or the single application of the rope formulation was followed by three conventional insecticide applications, provided adequate protection against the entire sucking pest, leafworm and bollworm complex. This compares with the four leafworm and bollworm insecticide treatments normally applied, with the additional benefits of the preservation of beneficial fauna and the reduction of undesirable environmental impact.

Estimated yield losses in pheromone treated areas were generally lower than in the comparable conventional treatment areas. This may be associated with the early season protection of the fruiting bodies against pink bollworm attack provided by the pheromone.

The heavier boll weights reported in the Dakahlia pheromone areas may be attributed to greater hybrid vigour resulting from cross-pollination by bees from commercial bee hives kept in the pheromone treated areas. Bee hives were not maintained to the same extent, if at all, in the other pheromone treated areas.

References

CRITCHLEY, B.R.; CAMPION, D.G.; MCVEIGH, L.J.H.; HUNTER, JONES, P.; HALL, D.R.; CORK, A.; NESBITT, B.F.; MARRS, G.J.; JUSTIN, A.R.; HOSNY, M.M. AND NASR, EL-SAYED A. (1983) Control of pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera:Gelechiidae) in Egypt by mating disruption using an aerially applied microencapsulated pheromone formulation. Bulletin of Entomological Research **73,** 289-299.

CRITCHLEY, B.R.; CAMPION, D.G.; MCVEIGH, E.M.; MCVEIGH, L.J.; JUSTIN, A.R.; GORDON, R.F.S.; MARRS, G.J.; NASR, EL-SAYED, A. AND HOSNEY, M.M. (1984) Microencapsulated pheromone in cotton pest management in Proceedings of British Crop Protection Conference of Pests and Diseases, Brighton, November, pp. 241-245.

CRITCHLEY, B.R.; CAMPION, D.G.; MCVEIGH, L.J.; MCVEIGH, E.M.; CAVANGH, G.G.; HOSNY, M.M.; NASR EL-SAYED, A.; KHIDR, A.A. AND NAGUIB, M. (1985) Control of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera:Gelechiidae), in Egypt by mating disruption using hollow-fiber laminate-flake and Pectone formulations of synthetic pheromones. Bulletin of Entomological Research **75,** 329-345.

EL-ADL, M.A.; HOSNY, M.M. AND CAMPION, D.G. (1988) Mating disruption for control of pink bollworm *Pectinophora gossypiella* (Saunders) in the Delta growing region of Egypt. Tropical Pest Management **34,** 210-214.

EL-DEEB, Y.A. AND CRITCHLEY, B.R. (1980) An improved pheromone trap for mass trapping of cotton leafworm moths in Egypt. Proceedings of the 1st Conference of Plant Protection Research Institute, Cairo, Egypt 13-15 Dec., 1980, Vol. **3,** pp. 391-394.

THE RELATIVE EFFICACY OF DIFFERENT CAPSULES OF SEX-PHEROMONES IN ATTRACTING THE PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) MALE MOTHS IN EGYPT

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Abstract. Two sex pheromone traps of the yellow plastic funnel type, each was baited with one of two different capsules [British (NRI) and American (Feromone Crop.)] were set up at Fayoum and Qalyubia Districts from May 1 til September 30, 1991. The results revealed that the American capsules attracted the largest number of males (2088,1181) followed by the British (818,457) during the whole period. These results might be attributed to the best concentration of the pheromone in the American capsules than in the British ones.

Introduction

Nowadays pheromone traps have been used as means of determining the seasonal fluctuations of nocturnal moths (Campion, 1972). Nasr *et al.* (1978) reported that the pheromone traps revealed great superiority over light traps in attracting the male months of the PBW. The findings of Hosny *et al.* (1978) was in agreement with the results obtained by Nasr *et al.* in 1978. Campion (1974) stated the pheromone traps gave very promising results for control by eliminating large numbers of males from the population thus disrupting mating. El-Deeb and Nasr (1988) indicated that pheromone traps are effective methods for reducing the cotton leafworm moth population. Therefore, it is interesting to study the effectiveness of two different pheromone capsules manufactured in UK and Feromone Corporation in America in attracting the male moths of bollworm, *Pectinophora gossypiella* (Saunders) under Egyptian environmental conditions.

Materials And Methods

The experimental part of the present study was conducted at Fayoum (Sinnuris) and Qalyubia (Bahtim farm) from 1st of May til the 30th of September, 1991.

Two pheromone traps of the yellow plastic funnel type were set in the fields, with a distance of about 250 metres between each one and the other. The first trap was baited with a 1 mg. polythene vial of the attractant (as 7, cis trans 11-hexadeca-diemyl acetate). This formulation was created by NRI, London. The second trap was baited with 1 mg rubber of same formulation (cis 7, cis trans 11-hexadecadiemyl acetate) formulated by Feromone Corporation Company in America.

The captured moths were removed from the polythene bags every morning and counted. The monthly totals of the catch, in each case, are shown in Table (1) and Fig. (1).

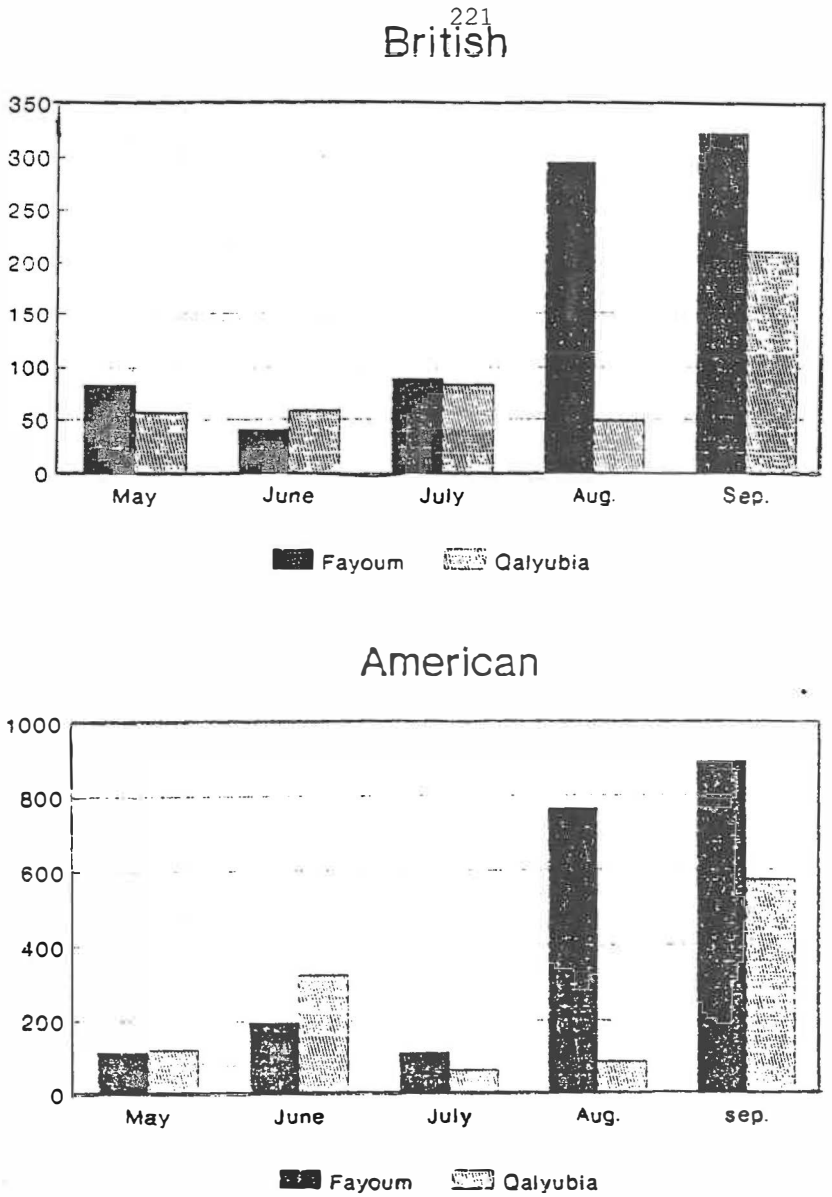

Fig. 1: Number of male moths of *Pectinophora gossypiella* in different lure products at Fayoum and Qalyubia in 1991 season.

Results And Discussion

Data in Table (1) indicate that the rubber attracted the largest number of males (1088, 1181) than polythene (828, 457) were captured in funnel traps at the two different locations. Now rubber pheromone PBW using at Egyptian system IPM control pests to surveying and controlling the PBW, *Pectinophora gossypiella* (Saunders) male moths because are very cheap and easy to operate in the fields than the British polythene vials.

Table 1: Total monthly catches of males of *P.gossypiella* in two pheromone traps each baited with a different capsule (Fayoum, Qalyubia, 1991).

Month	Total monthly catch			
	British		American	
	Fayoum	Qalyubia	Fayoum	Qalyubia
May	83	57	118	126
June	41	59	195	323
July	89	83	116	68
August	294	49	766	90
September	321	209	893	574
Total	828	457	2088	1181

References

CAMPION, D.G. (1972) Some observations on the use of pheromone traps as a survey tool for *Spodoptera littoralis*. Center for Overseas Pest Research Misc. Rep. No. **4:** 10 pp.

CAMPION, D.G. (1974) The use of sexpheromones in the control of *Spodoptera littoralis* (Boisd.). EPPO Bull. **4:** 357-362.

CAMPION, D.G. (1976) A comparison of the catches of moths of the cotton leafworm, *Spodoptera littoralis* (Boisd.) in light traps and pheromone traps. Center for Overseas Pest Research. Miscellaneous Report No. **21:** pp. 4.

EL-DEEB, Y.A. AND NASR, E. (1988) The relative efficacy of different formulations of sex pheromones in attracting the cotton leafworm *Spodoptera littoralis* (Boisd.) male moths. Ph.D. Thesis, Fac. of Agric., Al-Azhar University, Cairo, Egypt.

EL-DEEB, Y.A. AND CRITCHLEY, B.R. (1980) An improved pheromone trap for mass trapping of cotton leafworm moths in Egypt. Proceedings of the 1st Conference of Plant Protection Research Institute, Cairo, Egypt 13-15 Dec., 1980, Vol. **3:** pp. 391-394.

HOSNY, M.M.; NASR, EL-SAYED, A. AND EL-SHAFEI, S.M. (1978) Catches if *Spodoptera littoralis* (Boisd.) male moths in light and pheromone traps in the Delta and in Middle Egypt. Fourth Conf. Pest Control, NRC, Caiso, **1:** 6.

NASR, EL-SAYED, A.; KHATTAB, A.A.S. AND SAOUD EL-SHAFEI, M. (1978) A comparison of light and pheromone trap catches of *Spodoptera littoralis* in Egypt. PANS **24 (3):** 290-293.

EFFICIENCY OF MITE PHEROMONE WITH SOME PESTICIDES AGAINST *TETRANYCHUS URTICAE* KOCH

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Abstract The acaricide (kelthane) 18.5% EC, Antimoulting agent (cascade) 5% EC and Juvenile Hormone Analogue (JHA, S-31183) 10% EC sprayed alone and in combination with Mite-pheromone (Stirrup-M) 1.76% EC were investigated for controlling the phytophagous mite, *Tetranychus urticae* Koch on apple trees in the field. The Mite pheromone (Stirrup-M) at rate 50 cc mixed with recommended dose of kelthane 250 cc/100 litre of water induced 99.36% significant reduction in the population of *T.urticae* followed by 97.51% reduction when the mixture of Stirrup-M at 50 cc with Kelthane at 175 cc/100 litre of water. Also, the partial spray for the tree induced high percentages of reduction in population of spider mite, *T.urticae* on apple trees on both treated and untreated part of the trees. Therefore, the partial spray for the trees with acaricides could be used by mixing with Mite-Pheromone to avoid the bad application of acaricides, hazardous on the environment and phytotoxicity.

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch is the major pests causing damage to apple orchards trees in Egypt (Zaher *et al.*, 1973 and El-Halawany *et al.*, 1990). Several authors in the world concerned pheromone in mites comes from studies on phytophagous species which are pests of agricultural crops. When mixed acaricides with pheromone could be useful in attracting individuals of mites and bringing them into contact with the killing agent (Regev and Cone, 1975 & 1976; Cone, 1979, and Kuwahara *et al.*, 1979, 1980 & 1982). The present work aims to study the effect of acaricide (kelthane), antimoulting agent (cascade) and Juvenile hormone analogue (JHA, S-31183) alone and in different concentration combination with Mite-pheromone (Stirrup-M) against *T.urticae* on apple trees in the field.

Materials and Methods

Experiments were carried out to evaluate the effect of the mixture of Mite-pheromone (Stirrup-M) 1.76% EC with kelthane (Dicofol) 18.5% EC; Antimoulting agent (Cascade) Flufenoxuron 5% EC; and Juvenile Hormone Analogue (JHA, S-31183) and these compounds alone by using full covering trees and partial spray technique (spray one side of the free and left the other

side without spraying). One feddan of apple Anna variety orchard of six years old was highly infested with the spite Mite, *T.urticae*. These experiments designs were randomised in complete blocks. Treatments were conducted with four replicates of four trees in each. Samples of 80 apple leaves from each treatment were taken and examined immediately in the field each week intervals at Tokh, Qalubiya Governorate by using a stereoscopic microscope. All sprays were applied by utilising a motor sprayer of 600 litres capacity. Equation of Henderson and Tilton (1955) was applied to calculate the percentages reduction in the population of *T.urticae*.

Results and Discussion

Data in Table 1 indicated that, the recommended dose (250 cc/100 litre of water) of Kelthane gave 88.94% reduction in population of *T.urticae*. The mixture of each 250, 175 and 125cc of Kelthane with (50 cc/litre of water of Mite-pheromone (Stirrup-M) resulted in 99.36, 97.51 and 92.98% reduction in the population density of *T.urticae* respectively.

Table 1: Effect of mite pheromone (Stirrup-M) 1.76% EC on efficiency of Kelthane, Anti-moulting (Cascade) and JHA, S-31183 against *Tetranychus urticae* Koch on apple trees at Tokh, Qalubiya Governorate.

Treatments	Rate of application per 100 litres of water	Reduction % of moving stages of mites/80 leaves				Average reduction percentages
		1st week	2nd week	3rd week	4th week	
Kelthane 18.5% EC alone	250cc	89.57	88.74	89.17	88.29	88.94
Kelthane + Stirrup-M 1.76% EC	250+50cc	100.00	99.78	98.56	99.11	99.36
Kelthane + Stirrup-M	175+50cc	98.97	98.74	96.75	97.88	97.51
Kelthane + Stirrup-M	125+50cc	94.46	93.94	90.85	92.65	92.98
Anti-moulting (Cascade 5% EC)	100cc	82.90	90.58	91.57	92.85	89.48
Cascade + Stirrup-M	100+50cc	83.33	93.12	95.03	96.97	92.11
Cascade + Stirrup-M	50+50cc	81.02	90.40	92.61	96.76	90.20
Cascade + Stirrup-M	25+50cc	80.01	91.39	91.30	94.96	89.42
JHA, S-31183 10% EC	100cc	70.11	80.20	82.22	84.33	79.22
S-31183 + Stirrup-M	100+50cc	82.39	89.01	92.50	96.26	90.04
S-31183 + Stirrup-M	50+50cc	79.86	88.15	92.85	96.60	89.37
S-31183 + Stirrup-M	25+50cc	73.86	84.18	86.74	93.65	84.61

When each of 100, 50 and 25 cc of Cascade was mixed with Stirrup-M at 50 cc/100 litre of water gave 92.11, 90.20 and 89.42% reduction on *T.urticae* compared with 89.48% reduction when Cascade was applied alone with 100 cc/100 litre of water. Juvenile Hormone Analogue (JHA, S-

31183) at each of 100, 50 and 25 cc when mixed with Stirrup-M at 50 cc/100 litre of water produced 90.04, 89.37 and 84.61% reduction in the population of *T.urticae*, respectively compared with (JHA) was applied alone at 100 cc/100 litre of water gave 79.22% reduction on *T.urticae*. These results are in agreement with Moussa (1987) who indicated that the effect of Mite-pheromone (Stirrup-M) and (JHA) R0-10-3108 mixture was significantly higher than (JHA) when tested alone against *T.urticae* under field conditions.

From the previous results revealed that in combination 50 cc of Mite-pheromone (Stirrup-M) with Kelthane 250 cc/100 litre of water gave 99.36% significant reduction in the population of the *T.urticae* followed by 97.51% reduction when mixed Mite-pheromone at 50 cc with Kelthane at 175 cc/100 litre of water.

Also, results in Table (2) showed that the mixture of 50 cc of Stirrup-M with Kelthane at each of 250 and 125 cc/100 litre of water gave 99.44 and 93.70% reduction in the treated half tree, while the reduction in the untreated half tree was 92.82 and 72.71% reduction for each mixture, respectively. In conclusion, the partial spray technique of application revealed that the bad application of acaricides could be avoided when the Mite-pheromone (Stirrup-M) at 50 cc/litre of water mixed with the recommended dose of acaricide under field conditions. These results go in line with several findings such as Regev and Cone (1975 and 1976), Cone (1979) and Kuwahara *et al.* (1979, 1980 and 1982). They indicated that when mixed acaricides with pheromone could be useful in attracting individuals of mites and bringing them into contact with the killing agent.

Table 2 : Effect of partial spray technique by Kelthane and its mixtures with Mite pheromone against *Tetranychus urticae* Koch on apple trees at Tokh, Qalubiya Governorate.

Treatments	Rate of application per 100 litres of water	Reduction % of moving stages of mites/80 leaves				Average reduction percentages
		1st week	2nd week	3rd week	4th week	
A)Kelthane 18.5% EC alone (the whole tree treated)	250cc	89.57	88.74	89.17	88.29	88.94
b) Kelthane + Stirrup-M (half the tree treated)	250 + 50cc	100.00	99.80	98.80	99.16	99.44
- The other side of the tree untreated	-	92.72	94.01	91.59	92.95	92.82
C) Kelthane + Stirrup-M (half the tree treated)	125 + 50cc	96.03	94.04	91.64	93.07	93.70
- The other side of the tree untreated	-	83.81	77.99	64.09	64.95	72.71

References

CONE, W.W. 1979. Pheromones of *Tetranychidae* in Recent Advances of Acarology, Vol. 2, Rodrigues, J.G. (ed.), Academic Press, New York, 309 pp.

EL-HALAWANY, M.E.; R.G. ABOUT-EL-ELA AND H.M. ISMAIL, 1990. Population dynamics of mites and their natural enemies on apple and appricot trees, Agric. Res. Rev., Egypt, **68** : 59-66.

HANDERSON, C.F. AND E.W. TILTON, 1955. Test with acaricides against the brown wheat mite. J. Econ. Entomol., **48** : 157-161.

KUWAHARA, Y.; H.FUKAMI; S.ISHII; K.MATSUMOTO AND Y. WADA, 1979. Pheromone study on acarid mites. II- Presence of the alarm pheromone in the mold mite, *Turophagus putrescentiae* (Schrank) (Acarina : Acaridae) and the site of its production. Eisei Dobutsu, **30** : 309 pp.

KUWAHARA, Y.; K. MATSUMOTO AND Y. WADA, 1980. Pheromone study on acarid mites. IV. - Citral : Composition and function as an alarm pheromone and its secretory gland in four species of acarid mites. Eisei Dobutsu, **31** : 73 pp.

KUWAHARA, Y.; L.T. MY YEN; Y.TOMINAGA; K.MATSUMOTO Y. WADA, 1982. 1,3,5,7-tetramethyldecyl formate, lardolure: Aggregation pheromone of the acarid mite, *Lardoglyphus Konoi* (Sasa et Asanuma) (Acarina : Acaridae). Agric. Biol. Chem., **46** : 2283 pp.

MOUSSA, H.Y.M., 1987. Toxicological studies on certain acaricides PhD. Thesis, Fac. of Agric., Zagazig Univ., Egypt.

REGEV, S. AND W.W. CONE, 1975. Evidence of Farnesol as a male sex attractant of the two-spotted spider mite, *Tetranychus urticae* Koch (Acarina : Tetranychidae). Environ. Entomol., **4** : 307 pp.

REGEV, S. AND W.W. CONE, 1976. Analysis of pharate female two-spotted spider mites for nerolidol and geraniol : Evaluation for sex attraction of males. Environ. Entomol., **5** : 133 pp.

ZAHER, M.A.; Z.R. SOLIMAN AND G.S. EL-SHAFI, 1973. Survey and population studies on mites associated with deciduous trees in Egypt. Bull. Soc. Ent., **4** (111) : 425-433.

FLIT-TRAK M^{2TM}: THE NEW HIGH EFFICIENCY PITFALL TRAP STORGARD® MONITORING SYSTEM FOR FLOUR BEETLES

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Abstract *Tribolium* beetles are major worldwide pests of stored, packaged and bulk food commodities.

Insects' secretion of benzoquinones imparts a nauseous smell and taste to infested grain. (esp. Confused Flour Beetle, *Tribolium confusum* and Red Flour Beetle, *T. castaneum*)

Conventional monitoring systems are made primarily of corrugated cardboard, plastic and paper. An aggregation pheromone lures the insects to capture, either on a sticky surface or in an oil filled pit.

Savannah Trap: A new pitfall trap developed by USDA in Savannah, Georgia, with cooperative re-design, joint patenting, licensing and commercialization under the trade name FLIT-TRAK M^{2TM} by Trécé, Inc. (U.S. Patent 4,090,153).

Purpose of research

Development of a new trap that enhances convenience and efficacy while overcoming some conventional trap disadvantages.

i. Development and evaluation of the new trap configuration and newly developed food attractant.
ii. Improve durability, insect access and dust resistance.
iii. Comparison of new FLIT-TRAK M^2 trap with conventional monitoring systems (Storgard® pitfall, PT 6 Allure® and Trappit®)
iv. Demonstration of trap efficiency in capture of other species, weevils (*Sitophilus*), and grain borers (*Oryzaephilus surinamensis*).

Monitoring history

DeCoursey (1931, *J. Econ. Ent.* **24**: 1079) reported development of a corrugated cardboard trap using wheat flour bait.

Barak and Burkholder (1984, *Agric. Ecos. Environ.* **12**: 207) reported development of a system combining a food attractant with the aggregation pheromone for synergistic attraction of Tribolium spp. This trap contained a plastic oil-filled reservior for attraction and annihilation.

The trap developed by Nishimure, et al (1975, United States Patent 3,913,259. 25 October) was modified by Whitmire (U.S.) and marketed as PT 6 Allure.

Wyatt and Wynn have developed the Trappit system, marketed by Agrisense BCS (UK), as reported in Barak, et al (1990, *J. Kansas Ent. Soc.* **63**: 466)

Limitations of current pitfall traps

Corrugated pitfall traps are not escape proof.
Insects hiding in the corrugations are difficult to see and count.
Traps are not durable and are often crushed.
Oil-based food attractants are often spilled, reducing the attractancy and drowning efficiency of the oil filled pit.

Design ingenuity

The USDA Savannah pitfall trap (The prototype for FLIT-TRAK M^2)

The prototype was constructed from a plastic tennis ball canister.

i. The upper 2.5 cm of the can removed, four slits were cut in the plastic sheeting for insect access.
ii. Concave base of the can was inverted and a hole drilled through the center.
iii. Upward slope of the base was roughened to allow insects to crawl upwards, downward slope left smooth to cause insects to slide into trap.
iv. Tabs at top glued to outer roughened surface, trap is 2.5 cm tall by 6 cm in diameter.
v. Holes punched in original lid of trap to allow suspension of pheromone lure directly over the pit. Trimmed canister lid for base to minimize vertical surfaces encountered by insects attempting to gain entry.
vi. Sticky surface in pit captured the insect.

i. Comparison of Savannah trap with Storgard pitfall and Trappit system.

Researcher
Dr. Michael Mullen

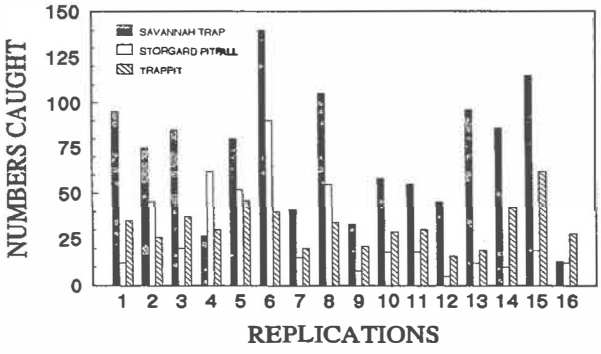
Method
Warehouse size: 227 m^3
Direct competition between three trap systems:
i. Savannah trap
ii. Storgard pitfall
iii. Trappit (glued surface trap)
The same type of pheromone lure was used in both the Storgard and Savannah traps.
Further protocol as published by Mullen (1992, *J. Stored Prod. Res.* **28**: 245)

Results

Presented in Figure 1.

In summary: The Savannah trap was generally more efficient in capturing *T. Castaneum* adults than either of the other traps (f = 19.41; df = 2; P 0.0001).

FIGURE 1



ii. Comparison of Savannah trap with Storgard pitfall, Trappit and Allure systems

Researcher

Dr. Michael Mullen

Method

Warehouse size: 19,233 m^2

Direct competition between four trap systems:

i. Savannah trap
ii. Storgard pitfall
iii. Trappit (glued surface)
iv. Allure

The same pheromone lure was used in both Storgard and Savannah traps. Trappit and Allure systems were baited with lures supplied by the manufacturer. (Note: Allure was dropped from the test at eight weeks due to lack of availability.)

Further protocol as published by Mullen (1992).

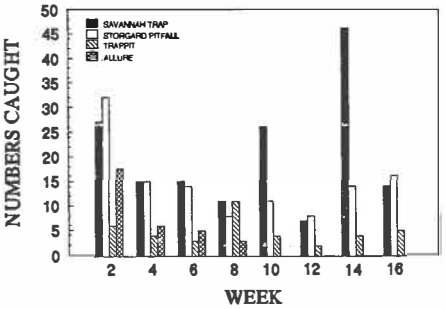
Results

Presented in Figure 2.

In summary: The Savannah trap was consistently more effective than either the Allure or the

Trappit and generally more effective than the Storgard pitfall for capturing *T. castaneum* ($F = 5.8$; $df = 3$; $P > 0.0040$).

FIGURE 2



Commercial re-design

Cooperation between industry and government:

Redesign was necessary to facilitate manufacturing, packaging and shipping.
Critical features of the prototype were retained:

- 360° insect access
- dust resistance
- suspension of lure above pitfall
- presentation of climbing surface

Two holes above pitfall enable simultaneous monitoring of different species with each species specific pheromone.
Airflow through the commercial trap is maximized to enable optimum dispersal of pheromone plumes.
Product name: FLIT-TRAK M^2

iii. Validation: FLIT-TRAK M^2 vs. Storgard pitfall

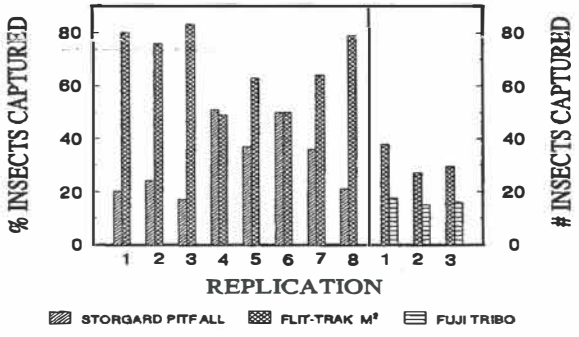
Method

Room size: 171 m^2
Direct competition between trap systems, separation = 10 m.
Lure: Aggregation pheromone + improved food attractant
1,000 unsexed adult beetles released, counted at 48 hours.

Results

Presented in Figure 3 (left side of graph)
In summary: The FLIT-TRAK M^2 was consistently more efficient than the Storgard pitfall ($x^2 = 27.93$; $P > 0.01$)

FIGURE 3



iv. Validation: FLIT-TRAK M^2 vs. Fuji Tribo

Method

Test performed at USDA-ARS, Madison Wisconsin, USA
Tray size: 1 m^2
Direct competition between trap systems, one trap of each type placed on top of monolayer of infested wheat kernels.
Lure: Hercon Environmental aggregation pheromone
Adult beetles counted at 24 hours

Results

Presented in Figure 3 (right side of graph).
In summary: The FLIT-TRAK M^2 was more efficient than the Fuji Tribo pitfall (mean difference = 11.3; SE diff +0.9)

v. Evaluation of FLIT-TRAK M^2 for other species

Test 1: Savannah trap for Sitophilus zeamais

Protocol: Infested shed that had been emptied and swept clean of infested corn.
Lure: Crude corn oil extract

Replication: Three traps placed on floor
Results: 450 weevils captured over 10 days.

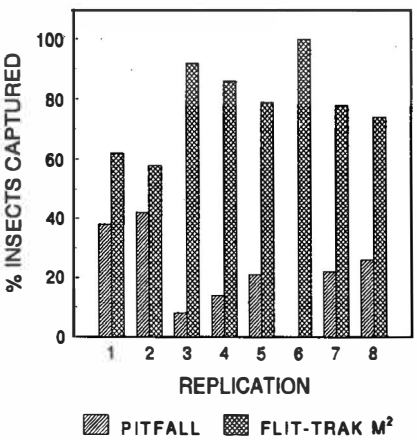
Test 2:FLIT-TRAK M^2 for Oryzaephilus surinamensis

Cooperator: Michael Mullen
Room size: 171 m^2
Trap type: i. FLIT-TRAK M^2
ii. Storgard pitfall standard
Lure: Food oil attractant
Replication: i. Four traps of each type
ii. Spaced at 10 m
Counted at 24 hours

Results

Figure 4
In summary: FLIT-TRAK M^2 captured a consistently higher number of beetles than the standard Storgard pitfall ($x^2 = 95.16$; $P > 0.01$)

FIGURE 4



Conclusions

Independent USDA studies demonstrate:

1. FLIT-TRAK M^2 is more sensitive than other commercially available trapping systems for monitoring of stored grain beetles in the genus *Tribolium* and *Oryzaephilus.*
2. The FLIT-TRAK M^2 trap is also an effective tool for monitoring grain weevils in the genus

Sitophilus.

In summary, the cooperative USDA-Trécé development program has led to the development of a new trap with the following advantages:

Effective in capture of multiple species
Higher sensitivity, and faster location of "hot-spots"
Dust proof
Escape proof
Increased efficiency and consistency

This development program is an excellent example of effective technology transfer possible in collaberative relationships between government and industry.

FLIT-TRAK CB^{3TM}: THE NEW HIGH EFFICIENCY LURE/TRAP STORGARD® MONITORING SYSTEM FOR CIGARETTE BEETLE.

Joan Fisher, Philipp Kirsch, Bill Lingren

Trécé Incorporated, Salinas California USA

Abstract Cigarette beetle (CB) *Lasioderma serricorne*: A serious pest of tobacco, cocoa, spices, dried fruit and nuts.

Conventional monitoring systems, eg. Sanitrap™, consist of placing a pheromone lure in a sticky trap.

FLIT-TRAK CB^3: A new pitfall trap developed by USDA in Savannah, Georgia, with joint patenting, licensing and commercialization by Trécé, Inc (U.S. Patent 5,090.153)

Purpose of research

i. Comparison of a new rubber septa lure, with two commercially available lures.
ii. Comparison of the new pitfall trap, FLIT-TRAK CB^3, with commercially available traps, Serico™ and Sanitrap™.

Introduction and background

Need for highly sensitive cigarette beetle monitoring systems

Most of the tobacco and food processing industry have a zero tolerance for cigarette beetle. Regulatory actions are limiting the use of fumigant control measures such as methyl bromide.

Monitoring history

Serricornin, the female produced sex pheromone (Chuman, 1979, *Tetrahedron Lett.* **25**, 2361 and Chuman, 1979, *Agric. Biol. Chem.* **43**, 2005) has been widely used as the basis for monitoring over the past decade.

Current traps consist of a glued surface, a pheromone lure, and occasionally a food lure derived from tobacco volatiles (Chuman, 1981, *Yukigoseikagaku*, **39**: 1183).

Limitations of current traps

Capture efficiency is dependent on efficacy of a glued surface in very dusty environments
Capture efficiency of glued surfaces can be inconsistent between batches and products.

i.Comparison of different lure types

Cooperator

Dr. Michael Mullen, USDA-ARS Stored Products Insects Research and Development Laboratory, Savannah, Georgia, USA

Method

Direct competition between three lure systems:

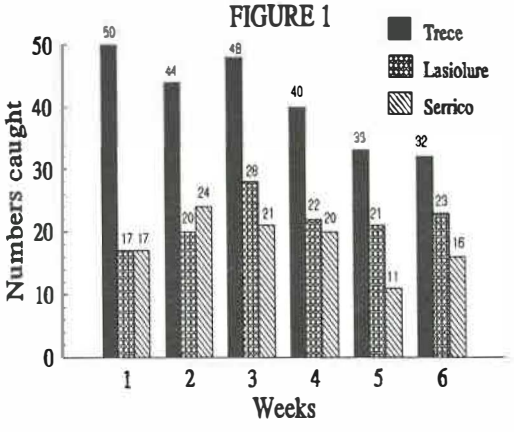
i. Trécé rubber septa (Serricornin @ 10mg/septa)
ii. Lasiolure
iii. Serrico lure

Same trap type used for all lures
6 week trial, 96 m^3 rooms
500 unsexed adult beetles introduced into the rooms each week.

Results

Presented in Figure 1
In summary: Trécé rubber septa consistently captured more insects than Lasiotrap or Serrico lures.

FIGURE 1



The USDA Savannah pitfall trap (The prototype for FLIT-TRAK CB^3)

USDA Savannah pitfall trap prototype development (FLIT-TRAK M^2) described in previous paper, was developed for use against cigarette beetle and given the product name FLIT-TRAK CB^3

ii. Comparison of different traps

Test 1: FLIT-TRAK CB^3 vs. Serrico

Cooperator

Dr. Michael Mullen

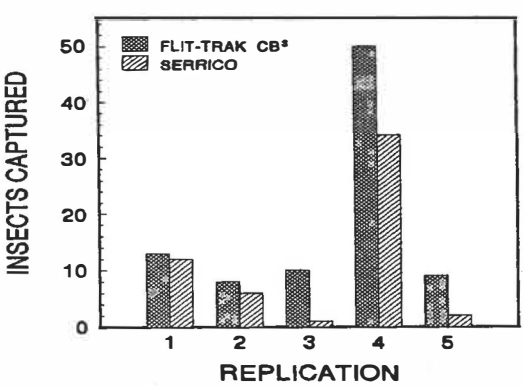
Method

Warehouse size: 2,718 m^3
Test design: Two trap types:
i. FLIT-TRAK CB^3
ii. Serrico Trap
Lure: Rubber septa with serricornin
Replication:
i. Five traps of each type
ii. Randomized in two rows
iii. Re-randomized at 24 hours
4,000 beetles released at four locations (1000 beetles per location)

Results

Figure 2
In summary: FLIT-TRAK CB^3 is more efficient than Serrico

FIGURE 2



iii. Comparison of different traps

Test 2: FLIT-TRAK CB^3 vs. Sanitrap

Cooperator

Dr. Dennis Keever, USDA-ARS, Crops Research Laboratory, Oxford, North Carolina, USA

Method

Warehouse size: 1,022 m^3

Test design: Two trap types:

i. FLIT-TRAK CB^3

ii. Sanitrap trap

Lure: Rubber septa with serricornin

Replication:

i. Eight traps of each type

ii. Randomized

iii. Re-randomized at 7 days

Placement:

i. FLIT-TRAK CB^3 on floor

ii. Sanitrap wall-mounted

iii. Within 4.5 m of each other

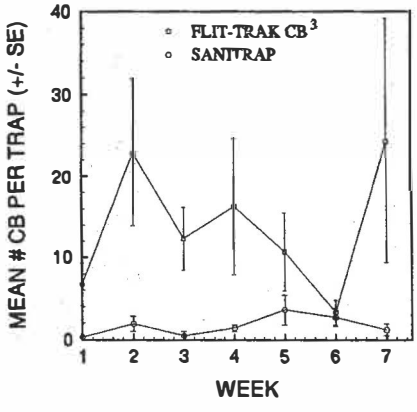
No release: Resident population of beetles

Results

Figure 3

In summary: FLIT-TRAK CB^3 captured significantly more beetles than Sanitrap (mean difference = 12.08 ± 2.61)

FIGURE 3



Conclusions

Independent USDA studies demonstrate:

1. FLIT-TRAK CB^3 is more sensitive than other commercially available trapping systems for cigarette beetle.
2. The new Trécé serricornin rubber septa lure is a more sensitive attractant than other commercially available lures.

Trécé has designed several wall mounting devices for the FLIT-TRAK CB^3 trap. These will be tested in 1993.

The pitfall trap is almost non-saturating. As many as 750 insects have been captured in a single trap over 36 hours. USDA advised no difficulty in counting these insects (D. Keever, pers. comm).

In summary, the cooperative USDA-Trécé development program has led to the development of a new trap with the following advantages:

Higher sensitivity
Increased durability
Dust proofing
Increased efficiency and consistency

This development program is an excellent example of effective technology transfer possible in collaboration between government and industry.

SEMIOCHEMICALS PRODUCED BY CARPET BEETLES OF THE GENUS *ANTHRENUS* (COLEOPTERA: DERMESTIDAE)

John CHAMBERS and David B. PINNIGER

Central Science Laboratory (MAFF), London Road, Slough, SL3 7HJ, UK.

Abstract *Anthrenus* species are pests of economic significance in the woollen textile industry and pose a serious threat to irreplaceable specimens in museums. Trapping efficiency must be improved to establish the range of an infestation, pinpoint its source and assess the effectiveness of control measures. The purpose of this paper is to consider the need for work on semiochemicals in the genus *Anthrenus* which might help improve trapping efficiency.

During a recent trial of traps to monitor *A. verbasci* in a museum store, the majority of adults caught were those of the closely related species, *A. sarnicus*, the Guernsey carpet beetle, which had been relatively rare in the UK previously. Laboratory tests have confirmed the release of material by the adult female *A. sarnicus* and not the males. EAG and behavioural tests have shown that males respond more to the material than females. The major component has been identified as decyl butyrate by GC-MS and comparison with an authentic synthetic sample.

The present report demonstrates the increasing importance of *A. sarnicus*. Concern about damage by *Anthrenus* spp in other countries shows that the abundance of existing species may be changing and new species are being identified. Expertise to identify and exploit pheromones of this genus is important. The availability of pheromones for a variety of *Anthrenus* spp would be of considerable value in both the developed and developing world.

Introduction

Carpet beetle larvae of *Anthrenus* spp consume proteinaceous material such as wool, fur and feathers. Damage can be severe in the woollen textile industry, where these species are pests of economic significance (Veer *et al*, 1991). Arguably of greater concern is the threat they pose in museums where they can seriously damage specimens which are irreplaceable (Pinniger, 1990).

Finding *Anthrenus* spp by visual inspection is difficult due to their small size and habit of infesting dark and inaccessible areas. First signs of damage may reveal the presence of larvae but these can be difficult to identify to species. Unbaited sticky traps are useful for collecting the short-lived adult stage and enable the species to be identified. However, trapping efficiency must be improved as much as possible to establish the range of the infestation, pinpoint its source and assess the effectiveness of control measures.

Sex pheromones have proved useful for the monitoring of other species such as the cigarette beetle, *Lasioderma serricorne* (Gilberg and Roach, 1991). Pheromones have already been identified

in *A. flavipes* (the furniture carpet beetle; (*Z*)-3-decenoic acid; Fukui *et al*, 1974) and *A. verbasci* (the varied carpet beetle; a mixture of (*Z*)- and (*E*)-5-undecenoic acids; Kuwahara and Nakumura, 1985). There are as yet no other reports of identification of pheromones in other *Anthrenus* species. The purpose of this paper is to consider the need for further work on semiochemicals in this genus.

Observations, Results and Discussion

The abundance of individual species of *Anthrenus* can change from year to year dependent on various factors such as climate and availability of food. During a recent trial of traps to monitor *A. verbasci* in a museum store, the majority of adults caught were those of the closely related species, *A. sarnicus*, the Guernsey carpet beetle, which was thought to be relatively rare in the UK previously (Hillyer and Blyth, 1992). The brief period of adult emergence emphasises the need to make traps as effective as possible (Figure 1).

It is known that adult male *A. verbasci* are attracted by n-undecanoic acid in addition to the acids which comprise its pheromone (Adams *et al*, 1975). In view of this lack of specificity, it was thought that the presence in some of the traps of the *A. verbasci* sex pheromone might have been responsible for attracting the *A. sarnicus* but the numbers caught were insufficient to determine with any statistical significance whether this was so. Subsequent behavioural tests in the laboratory have shown conclusively that *A. sarnicus* is not attracted to the *A. verbasci* pheromone (C. P. Morgan, personal communication).

Detailed observations elsewhere have given strong evidence that adult female *A. sarnicus* release a sex pheromone (Armes, 1985). By a combination of passive volatile collection, aeration and extraction of homogenised beetles, we have confirmed the release of material by the adult females and not the males. EAG tests showed that males respond more to the material than females. In behavioural tests with males, the material elicited increased turning, attraction, examination of the source with the antennae and repeated visits to the source. In contrast, females showed none of these features. The major component of the material has been identified by GC-MS and comparison with an authentic synthetic sample, as decyl butyrate (Figure 2; Finnegan and Chambers, 1993). It is interesting that this structure is markedly different in both polarity and functional group from the previously identified pheromones in this genus. Work is underway to establish the role of a second chemical, decanol, which was found in addition to decyl butyrate in those experiments with females which were conducted at higher humidity.

Conclusions

While ecological factors can cause real changes in the abundance of *Anthrenus* spp, the evidence can become confused due to mis-identification of specimens. Until recently, the most widespread species in the UK has been thought to be *A. verbasci* yet the present report demonstrates the increasing importance of *A. sarnicus*. Concern about damage by *Anthrenus* spp in other countries shows that the abundance of existing species may be changing there too and new species are being identified, for example *A. coloratus* (Ansari and Basalingappa, 1986) and *A. oceanicus* (Veer *et al*, 1991).

These points further illustrate the importance of having the expertise to identify and exploit pheromones of this genus. There is no doubt that the availability of pheromones for a range of

Figure 1: Trap catches of adult *A. sarnicus* in a museum store in London

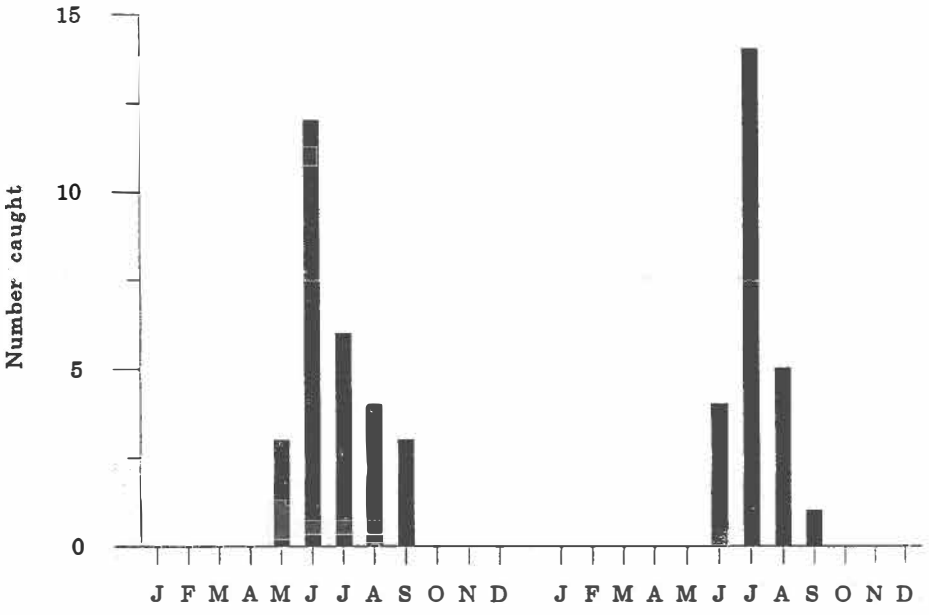


Figure 2: Response of virgin adult *A. sarnicus* in laboratory bioassays using arenas with two filter paper disks

Sex Tested	Filter Paper Test Disk		Filter Paper Control Disk
	Test	% Response	% Response
Males (n = 40)	disk exposed for 30 min to one virgin female in headstand posture	73	0
Females (n = 40)	disk exposed for 30 min to one virgin female in headstand posture	8	6
Males (n = 35)	10μg decyl butyrate	82	0
Females (n = 35)	10μg decyl butyrate	6	10

Anthrenus spp would be of considerable value in both the developed and developing world to assist in control strategies to minimise damage.

Acknowledgments

Professor Kenji Mori, Department of Agricultural Chemistry, University of Tokyo for the sample of *A. verbasci* pheromone and Pesticides Safety Directorate of the Ministry of Agriculture, Fisheries and Food, UK for financial support in the development of techniques used in this study.

References

ADAMS P.H., WARREN R. and HORLER D.F. (1975). Attractancy of long chain fatty acids to *Anthrenus verbasci* in PICL Report 1974-77, HMSO, p207.

ANSARI M.F. and BASALINGAPPA S. (1986). Infestation of stuffed birds and mammals by the larvae of *Anthrenus coloratus* Reitter (Dermestidae: Coleoptera). *J. stored Prod. Res.* **22**, 131-134.

ARMES, N.J. (1985). Biology and control of *Anthrenus sarnicus* Mroczkowski (Coleoptera: Dermestidae). PhD Thesis, University of London.

FINNEGAN D.E. and CHAMBERS J. (1993). Identification of the sex pheromone of the Guernsey carpet beetle, *Anthrenus sarnicus* Mroczkowski (Coleoptera: Dermestidae). *J. Chem. Ecol.* **19**, 971-983.

FUKUI, H., MATSUMURA, F., MA, M. and BURKHOLDER, W.E. (1974). Identification of the sex pheromone of the furniture carpet beetle *Anthrenus flavipes* (Leconte). *Tetrahedron Lett.* **40**, 3563-3566.

GILBERG M. and ROACH A. (1991). The use of a commercial pheromone trap for monitoring *Lasioderma serricorne* (F.) infestations in museum collections. *Studies in Conservation* **36**, 243-247.

HILLYER L. and BLYTH V. (1992). Carpet beetle - a pilot study in detection and control. *The Conservator* No.16, 65-77.

KUWAHARA, Y. and NAKAMURA, S. (1985). (*Z*)-5- and (*E*)-5-Undecenoic acid: identification of the sex pheromone of the varied carpet beetle *Anthrenus verbasci* (L.)(Coleoptera: Dermestidae). *Appl. Entmol. Zool.* **20**, 354-356.

PINNIGER, D.B. (1990). Insect pests in museums. Archetype Publications, Denbigh, Clwyd, UK, 45pp.

VEER V., PRASAD R. and RAO K.M. (1991). Taxonomic and biological notes on Attagenus and Anthrenus spp (Coleoptera: Dermestidae) found damaging stored woollen fabrics in India. *J. stored Prod. Res.* **27**(3), 185-198.

ELECTROANTENNOGRAM RESPONSES OF THE SAW-TOOTHED GRAIN BEETLE *ORYZAEPHILUS SURINAMENSIS* (L.), TO COMPONENTS OF ITS AGGREGATION PHEROMONE.

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[2]Department of Chemistry, Southampton University, 509 5NH, UK.

Abstract. Three components of the male produced aggregation pheromone of *Oryzaephilus surinamensis* have been identified previously as macrolide lactones II, III and IV. These have now been synthesized by new routes which are considerably more efficient than previous routes and make the lactones available at a commercially acceptable price for the first time. As part of our program to test the biological activity of the products from the new synthetic routes, electroantennogram (EAG) responses have been recorded. Using a laboratory strain of *O.surinamensis*, the pure enantiomers of lactone II, (II*R* and II*S*) have been assessed for the first time using the EAG technique and compared with III and IV. Significantly greater EAG responses were produced by lactone IV compared with III and II. Lactone III produced significantly greater responses than lactone II*R*. II*R* and II*S* produced positive responses which were not significantly different, thus indicating no enantio-specificity for component II.

Introduction

The saw-toothed grain beetle, *Oryzaephilus surinamensis* is an international pest of stored grain, ranked as one of the most important pests of stored products and processed foods (Prickett and Muggleton, 1991). Using current methods however it is still difficult to detect this pest at low population densities. The use of pheromones to improve the detection and monitoring of pests by trap enhancement has been proposed (Burkholder and Ma, 1985), though previously this has been impractical for some species due to the expense of pheromone synthesis. This has certainly been the case for *O. surinamensis*, whose male produced aggregation pheromone components have been identified as macrolide lactones II (*Z,Z*)-3,6-dodecadien-11-olide, III (*Z,Z*)-3,6-dodecadienolide and IV (*Z,Z*)-5,8-tetrahdecadien-13-olide, (Pierce *et al.*, 1984; White *et al.*, 1989). However, the development of new synthetic routes (Boden, 1992; Boden *et al*, 1993) has now made available at commercially acceptable prices a range of pheromone components of five cucujid species of *Oryzaephilus* and *cryptolestes*.

Before being employed for the detection of infestations, it is essential that these lactones are screened to ensure that they are not only structurally the same, but also as biologically active as the naturally produced components. For this purpose the electroantennogram (EAG) responses of *O. surinamensis* have been measured to components of its aggregation pheromone, produced by the

new synthetic route. Previously only a racemic mixture of macrolide lactone II has been assessed using the EAG technique (White and Chambers, 1989). The ability to undertake stereospecific synthesis by the new routes has enabled the screening of the pure enantiomers of lactone II. This provides a greater understanding of the mode of action of these complex semiochemicals and may improve their usage.

Methods and Materials

Insects

An insecticide susceptible strain of *O. surinamensis*, cultured at an average population density of 1500 insects per 50 gms of a 5:5:1 mixture of rolled oats, whole wheat flour, and brewers yeast respectively was used. The insects were maintained at 25 ^{o}C in a reversed 8 hr : 16 hr light-dark regime. Adults of 4-5 weeks post eclosion were removed and conditioned in darkness for 24 hrs without food prior to testing, held in a 3" x 1" glass tube.

Materials

Samples of macrolide lactones III, IV, II*R* and lactone II*S* synthesised by the new routes (Boden, 1992; Boden *et al*, 1993) were checked by GC and found to have purity > 96%. Logarithmic dilutions were made up for each component in HPLC-grade hexane. Carob distillate a food attractant for this species (Stubbs *et al*, 1985), was used as a standard stimulus, to control for variation between insects and within the life of a preparation (White and Chambers, 1989).

EAG Responses

The EAG system used was based on that of White and Birch (1987). Each preparation was presented with five doses of each pheromone component in 10 µl of HPLC-grade hexane, in ascending order of concentration, interspaced with a blank (10 µl of solvent only) and a standard of 10 µl of carob distillate. Two series of log concentrations were used (-5 $\log_{10}$ µg to -1 $\log_{10}$ µg and -3 $\log_{10}$ µg to 2 $\log_{10}$ µg). This was necessary because insect deterioration occurred if the whole concentration range was used for one preparation. This method also provided two 'mid range' reference points to account for insect population variability over the course of the experiment. Five insects of each sex were used for each concentration range, 20 insects in total. The results were normalised as a percentage of the standard response of carob distillate, and subjected to one-way analysis of variance to determine sexual differences. Differences between respective pheromone components were analyzed by arcsin transformation of the data, and then subjected to t-tests.

Results

One-way analysis of variance showed no significant difference between the sexes in response to any of the pheromone components for any concentration. For this reason, the data for both males and females were pooled (Figs: 1-4). There was no significant difference between the total response of the separate pheromone components over both concentration ranges apart from lactone IV being greater than lactone II*R* ($T = 2.95$, $P = 0.0036$, d.f. = 189). When only the higher concentration range was considered, lactone IV produced a significantly greater response than all of the other components, against II*S* ($T = -2.18$, $P = 0.032$, d.f. = 94), against II*R* ($T = 4.61$, $P = 0.0000$, d.f. = 92), against III ($T = -2.81$, $P = 0.006$, d.f = 89). Lactone III also produced significantly greater

Fig. 1 EAG response to lactone IV by Oryzaephilus surinamensis

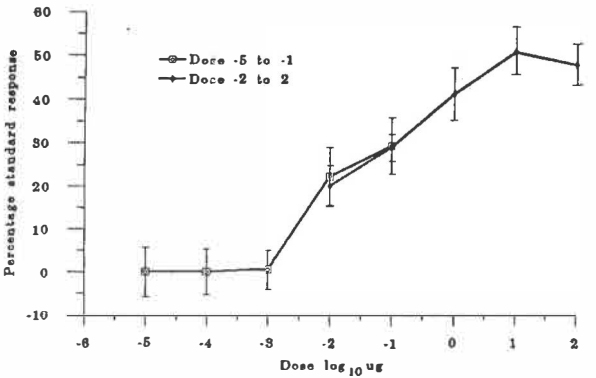


Fig. 2 EAG response to lactone III by Oryzaephilus surinamensis

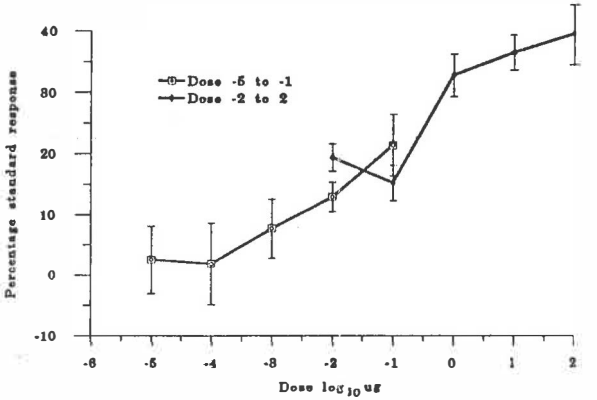


Fig. 3 EAG response to lactone IIR by Oryzaephilus surinamensis

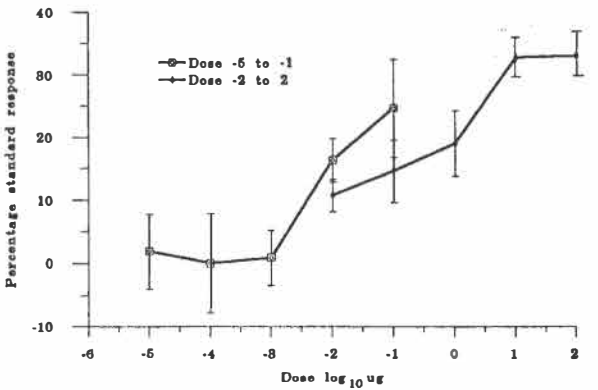
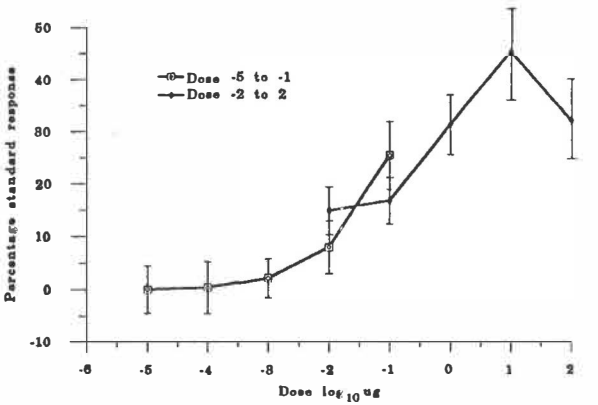


Fig. 4 EAG response to lactone IIS by Oryzaephilus surinamensis



responses than lactone II*R*, (T = 2.24, P = 0.028, d.f. = 97). Lactones II*R* and II*S* produced positive responses which were not significantly different. The mean response to 10 µl of carob distillate was 475.8 µV +/- 13.5 µV, N=20.

Discussion

It is clear from the EAG results that the products of the new synthetic routes for the production of the macrolide lactones are biologically active. To get the full picture of biological activity it is important to cover a full range (Chambers, 1990). This can be difficult however if the insect cannot survive the entire dose ranges. To overcome this problem in this study there are two overlapping dose ranges. Considering firstly both ranges together; there are few differences between the lactones which is in good agreement with White and Chambers, 1989.

However several of the doses in the lower dose range were below the threshold for response. Therefore the upper doses are of greater importance. Considering the results from the upper dose range seperately, lactone IV produced the significantly highest response. This contrasts with previous EAG work with the *O. surinamensis* aggregation pheromone, in which lactone III had produced the significantly largest response (White and Chambers, 1989). This could be attributed to the different synthetic routes: the purity of the samples used in this study was greater than that of the lactones used previously, (lactone IV, >96% Vs 86% purity, and lactone III, >96% Vs 89% purity).

An interesting aspect of the results, is that positive responses were recorded for the II*S* lactone. It has been reported with Canadian strains of *O. surinamensis*, that this enantiomer is inactive as a component of the aggregation pheromone, and that only the II*R* enantiomer contributes (Pierce *et al.*, 1987; Oehlslager *et al.*, 1987). The results of the present study indicate that both the *R* and *S* enantiomers of lactone II have EAG activity. This complies with the attractancy of both these enantiomers in this strain reported by Boden (1992). Differences in response to pheromone components been demonstrated in other species as shown with *Cryptolestes pusillus* and *C. ferrugineus* (Chambers *et al* 1990). Strains of the same species have also been known to use different blends of optical isomers, for example *Ips pini*, (Birch, 1984). To date this has not been reported with *O. surinamensis.*

This study has shown that the macrolide lactones produced by new routes are as biologically active as those tested previously (White and Chambers 1989). Also demonstrated is the rapidity and effectiveness of the EAG technique for the evaluation of these macrolides. It is clear from the responses of this species to macolide lactones which comprise, or are closly related to, its aggregation pheromone are complex. This emphasises the necessity of thorough screening procedures for these chemicals against numerous strains before a universally functional pheromone lure can be developed.

Acknowledgement

Pesticides Safety Directorate of the Ministry of Agriculture, Fisheries and Food, UK for financial support in the development of techniques used in this study.

References

BODEN, C.D.J. 1992. Towards a viable grain beetle lure: practical syntheses of Cucujolides and behavioural studies with *Oryzaephilus surinamensis*. PhD Thesis, Southampton University.

BODEN, C.D.J., CHAMBERS, J. and STEVENS, I.D.R. 1993. A concise, efficient and flexible stratergy for the synthesis of the pheromones of *Oryzaephilus* and *Cryptolestes* grain beetles. *Synthesis*. (in press).

BURHOLDER, W.E. and MA, M. 1985. Pheromones for monitoring and control of stored product insects. *Ann. Rev. Entomol.* **30**: 257-272.

BIRCH, M.C. 1984. Aggregation in bark beetles. In: Chemical Ecology of Insects, ed W.J. Bell and R.T. Carde. Chapman and Hall. London. pp331-354.

CHAMBERS, J. 1990. Overview on stored product insect pheromones and food attractants. *J. Kansas Entomol. Soc.* **63**(4): 490-499.

CHAMBERS, J.; MORGAN, CP.; WHITE, P.R.,MORI, K.; FINNEGAN, D.E. and PINNIGER, D.B. 1990. Rust red grain beetle, *Cryptolestes ferrugineus*, and flat grain beetle , *Cryptolestes pusillus*: Antennal and behavioural responses to synthetic components of their aggregation pheromones. *J. Chem. Ecol.* **16**: 12: 3353-3372.

OEHLSCHLAGER, A.C., KING, G.G.S., PIERCE, J.R., PIERCE, A.M., SLESSOR, K.N., MILLAR, J.G. and BORDEN, J.H. 1987. Influence of Pheromone chirality on response by *Oryzaephilus surinamensis* and *Oryzaephilus mercator* (Coleoptera: Cucujidae). *J. Chem. Ecol.* **13**: 6, 1543-1554.

PIERCE, A.M., PIERCE, H.D., Jr., MILLAR, G.J., BORDEN, J.H. and OEHLSCHLAGER, A.C. 1984. Aggregation pheromones in the genus Oryzaephilus (Coleoptera: Cucujidae). In. Proc. Third International Working Conf. on Stored-Product Entomology. Kansas State Uni. Manhattan, Kansas. pp. 107-120.

PIERCE, A.M., PIERCE, H.D., Jr., MILLAR, G.J., BORDEN, J.H., OEHLSCHLAGER, A.C., CZYZEWSKA, E. and BORDEN, J.H. 1987. Influence of pheromone chirality on response by *Oryzaephilus surinamensis* and *Oryzaephilus mercator* (Coleoptera: Cucujidae).*J. Chem. Ecol.* **13**: 1525-1542.

PRICKETT, A.J. and MUGGLETON, J. 1991. Commercial grain stores 1988/89, England and Wales, pest incidence and storage practice. *Home-Grown Cereals Authority Report* 29, pp218.

STUBBS, M.R., CHAMBERS, J., SCHOFIELD, S.B. AND WILKINS, J.P.G. 1985. Attractancy to *Oryzaephilus surinamensis* (L.) of volatile materials isolated from vacuum distillateof heat treated carobs. *J. Chem. Ecol.* **11**: 565-581.

WHITE. P.R. and BIRCH M.C. 1987. Female sex pheromone of the common furniture beetle *Anobium punctatum* (Coleoptera: anobiidae): Extraction, identification and bioassay. *J.Chem. Ecol.* **13**:1695-1706.

WHITE, P.R. and CHAMBERS, J. 1989. Saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera : Silvanidae). Antennal and behavioural responses to individual components and blends of aggregation pheromone. *J.Chem. Ecol.* **15**: 1015-1031.

WHITE, P.R., CHAMBERS, J., WALTER, C.M., WILKINS, J.P.G. and MILLAR, J.G. 1989. Saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera : Silvanidae): Collection, identification, and bioassay of attractive volatiles from beetles and oats. *J.Chem. Ecol.* **15:** 999-1013.

THE BEHAVIOURAL ACTIVITY OF THE ENANTIOMERS OF THE MALE PRODUCED *SITOPHILUS GRANARIUS* AGGREGATION PHEROMONE 2*S*,3*R*-SITOPHILATE.

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Abstract 2*S*,3*R*-Sitophilate (1-ethylpropyl 2*S*,3*R*-3-hydroxy-2-methylpentanoate) is produced by male *Sitophilus granarius* and has been identified previously as the aggregation pheromone in this species. In making the stereochemical assignment, NMR evidence demonstrated that the naturally produced material has the *erythro* configuration, and since that time little attention has been given to the *threo*-isomers. In our recent experience with other stored product beetles, behavioural activity is not confined to the naturally produced isomer. For this reason, samples of all four isomers of sitophilate have been tested separately for behavioural activity. Additionally, bioassays were also undertaken on two synthetic samples of the natural isomer prepared by different synthetic routes.

Introduction

The granary weevil *Sitophilus granarius* is a major storage pest of high economic importance in cereals (Prickett and Muggleton, 1991), particularly so in temperate climates. The development of its immature stages unseen within the kernels means that its presence may go unnoticed until serious physical and economic damage has been caused. Early recognition of an infestation at low population densities, can reduce the amount of pesticide use and minimise crop loss. For such detection, the use of insect pheromones to enhance traps is a possible solution, as they are now becoming available at commercially acceptable prices, lowering the number of traps and sampling required. However, for a pheromone lure to be efficient, it is essential to have a full understanding of these chemicals and the role they play in the natural chemical communication system.

2*S*,3*R*-Sitophilate (1-ethylpropyl 2*S*,3*R*-3-hydroxy-2-methylpentanoate) has been identified as the male produced aggregation pheromone of *S. granarius*, (Phillips *et al*, 1987; 1989). Since sitophilate has two chiral centres, there are three other stereoisomers in addition to the one which is produced naturally. Phillips *et al* (1987) reported that the synthetic racemic *threo* sitophilate was significantly less attractive than the racemic *erythro* material which includes the natural isomer, however no experimental details, doses used or numerical results were quoted. It has been demonstrated that other isomers of a naturally produced pheromone can also elicit behavioural activity, as shown with *S. oryzae* and *S. zeamais*, (Walgenbach *et al*, 1987). Similarly S. granarius has been attracted to chemicals other than its own naturally produced pheromone, Phillips *et al*,

(1985) demonstrated this with female *S. granarius* which were strongly attracted to the *R**,*S**-diastereoisomer of sitophilure.

The main purpose of this study was to investigate if any of these other enantiomers of 2*S*,3*R*-sitophilate produced any behavioural activity. In our experience there is growing evidence for unexplained variability in the behavioural results obtained with pheromone lures of various species. It is essential to ensure that pheromone samples of different synthetic origin are proved to be of comparable biological activity before embarking on large testing programmes. The opportunity was taken in this study to compare two samples of the 2*S*,3*R*-isomer prepared by different routes.

Methods and Materials.

Insects

An insecticide susceptible strain (Windsor) of *S.granarius*, cultured at an average population density of 500 insects per 50 g wheat grain and at 25°C and 70% relative humidity, was used. Test insects were conditioned in darkness, without food for 24 hrs. Mixed sexed adults of 4-5 weeks post eclosion were used for all experiments. Five replicates of ten insects were used for each concentration and for the control.

Bioassays

A standard single-choice pitfall bioassay technique was used (Boden 1992). All bioassays were conducted simultaneously to minimise any variability within or between days. The results were subject to an arcsin transformation and analysed by t-test.

Materials

2*S*,3*R*-Sitophilate (97% purity), 2*R*,3*S*-sitophilate (97% purity), 2*R*,3*R*-sitophilate (92% purity), and 2*S*,3*S*-sitophilate (85% purity) were synthesized by A.M. Moiseenkov in Moscow. The other sample of 2*S*,3*R*-sitophilate (85% purity) was synthesized by K. Mori in Tokyo (Mori and Ishikura, 1989). These were tested at doses of 100ng, 10ng and 1 ng on a 2cm diameter filter paper disc in 5µl of solvent, HPLC grade hexane. The doses that were used were based on electroantennogram responses of 2*S*,3*R*-sitophilate and 2*R*,3*S*-sitophilate (Chambers *et al*, 199X).

All purities and concentrations were checked by GC. The papers were aired for 1 minute to allow solvent to evaporate before being placed in the pitfall tube.

Results

Control mean % response 26+/- 4. 2*S*,3*R*-Sitophilate (fig.1) elicited a statistically significant response at the two higher doses tested ($p<0.05$). There was no significant difference between the two independent sources of 2*S*,3*R*-sitophilate ($p > 0.05$) at all three concentrations when compared by t-test. The antipode 2*R*,3*S*-sitophilate (fig.2), produced a significant behavioural response at 100 ng dose ($p,< 0.05$). Neither of the *threo* isomers produce a significant behavioural response (fig.2).

Figure 1 Pitfall bioassay results with 2S,3R-Tokyo and 2S,3R-Moscow.

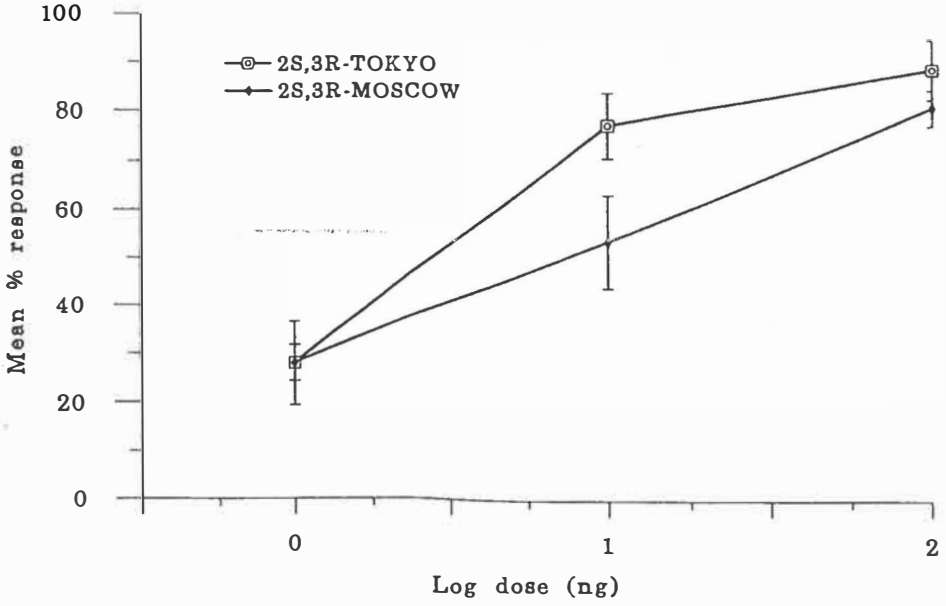
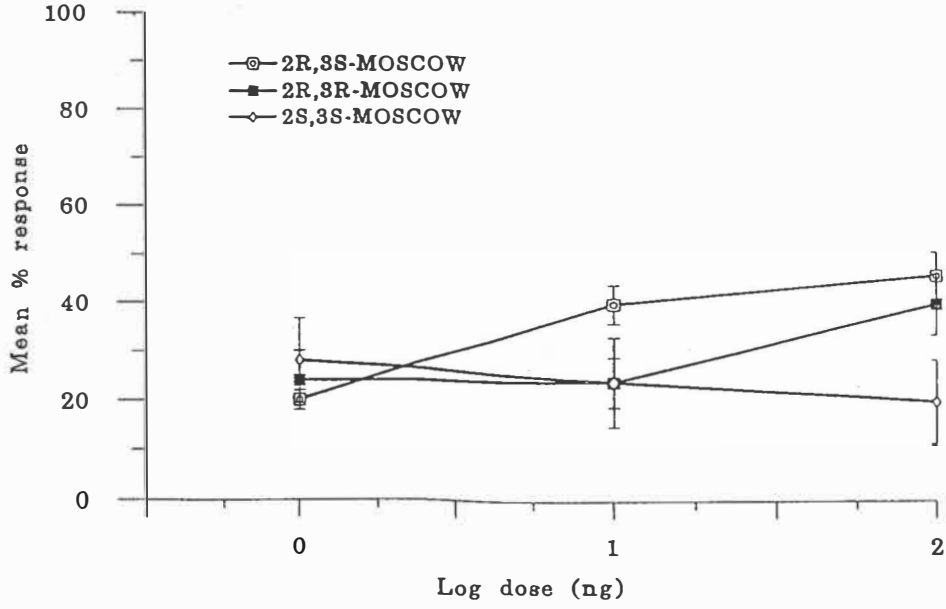


Figure 2 Pitfall bioassay results with 2R,3S-Moscow, 2R,3R-Moscow and 2S,3S-Moscow.



Discussion

As anticipated, the 2*S*,3*R*-sitophilate elicited a high behavioural response in our laboratory strain as seen in the strains of Phillips *et al* (1989) and Levinson *et al* (1990). This behavioural result is in good agreement with EAG responses for this isomer (Chambers *et al*, 199X) which were found to be small for the lowest dose but significant at the other doses. The antipode 2R,3S-sitophilate produced a low but statistically significant attractancy at the highest dose. This parallels the EAG results (Chambers *et al*, 199X) which showed a significant response at 100ng but not at the two lower doses tested here. Attractancy to this isomer was not observed by Phillips *et al* (1989) but this may not be surprising since that testing was undertaken at a single dose of only 22.5ng. Testing over a range of doses is advisable (Chambers, 1990) and might establish whether there is any difference in response between the strain tested in this study and that tested earlier. Such differences have been reported previously with aggregation pheromones of other stored product beetles such as the flat grain beetles *Cryptolestes ferrugineus* and *C. pusillus* (Chambers *et al*, 1990). Low but significant responses have been demonstrated in other *Sitophilus* species in response to the other isomers of the naturally produced aggregation pheromone component (Walgenbach *et al* 1987). Both *S. zeamais* and *S. oryzae* are strongly attracted to 4*S*,5*R*-sitophilure the naturally produced, but *S. oryzae* also responds to 4*R*,5*S*-sitophilure, and *S. zeamais* to 4*R*,5*R* and 4*S*,5*S*-sitophilure. In the present study significant attraction was not obtained with the 2*R*,3*R*-sitophilate or 2*S*,3*S*-sitophilate which implies that *S. granarius* is more related to *S. oryzae* than *S. zeamais* in terms of its aggregation pheromone action.

Of the three isomers of the naturally produced sitophilate, only the 2R,3S-isomer was attractive and this was only at the highest dose. This suggests that its use as an attractant is unlikely to be economically worthwhile. It is encouraging that the samples of the 2S,3R-isomer produced by the different synthetic routes gave similar biological activity. Additionally, this study has shown the value of the EAG technique as a rapid screening method for suggesting threshold doses below which it is not worth attempting behavioural studies.

Acknowledgement

Pesticides Safety Directorate of the Ministry of Agriculture, Fisheries and Food, UK for financial support in the development of techniques used in this study.

References

BODEN, C.D.J. 1992. Towards a viable grain beetle lure: practical syntheses of Cucujolides and behavioural studies with *Oryzaephilus surinamensis*. PhD Thesis, Southampton University.

CHAMBERS, J. 1990. Overview on stored product insect pheromones and food attractants. *J. Kansas Entomol. Soc.* **63**(4): 490-499.

CHAMBERS, J., MORGAN, C.P., WHITE, P.R., MORI, K., FINNEGAN, D.E. and PINNIGER D.B. 1990. Rust red grain beetle, *Cryptolestes ferrugineus*, and flat grain beetle, *Cryptolestes pusillus:* Antennal and behavioural responses to synthetic components of their aggregation pheromones. *J. Chem. Ecol.* **16**: 12: 3353-3372.

CHAMBERS, J., VAN WYK, C.B., WHITE, P.R., GERRARD, C.M. and MORI, K., 199X. The grain weevil *Sitophilus granarius* (L.): Antennal and behavioural responses to male produced volatiles. (In preparation.)

LEVINSON, H.Z., LEVINSON, A., REN, Z. and MORI, K. 1990. Comparative olfactory perception of the aggregation pheromones of *Sitophilus oryzae* (L.), as well as the stereoisomers of these pheromones. *J. Appl. Ent. 110:* 203-213.

MORI, K. and ISHIKURA, M. 1989. Synthesis of sitophilate, the aggregation pheromone of *Sitophilus granarius* (L.), and its antipode. *Liebigs Ann. Chem.* 1989:1263-1265.

PHILLIPS, J.K., WALGENBACH, C.A., KLEIN, J.A., BURKHOLDER,W.E., SCHMUFF, N.R. and FALES,H.M. 1985. (R*,S*)-5-Hydroxy-4-methyl-3-heptanone, a male-produced aggregation pheromone of *Sitophilus oryzae* (L.) and *S.zeamais* Motsch. *J. Chem. Ecol.* **11**, 1263-1274.

PHILLIPS, J.K., CHONG, J.M., ANDERSEN, J.F., BURKHOLDER, W.E. 1989. Determination of the enantiomeric composition of (*R**,*S**)-1-ethylpropyl 2-methyl-3-hydroxypentanoate, the male produced aggregation pheromone of *Sitophilus granarius*. *Entomol. exp.appl.* **51**:149-153.

PHILLIPS, J.K., MILLAR, S.P.F., ANDERSON, J.F., FALES, H.M. and BURKHOLDER, WE.1987. The chemical identification of the granary aggregation pheromone. *Tetrahedron Lett.* **28**:6145-6146.

PRICKETT, A.J. and MUGGLETON, J. 1991. Commercial grain stores 1988/89, England and Wales, pest incidence and storage practice. *Home-Grown Cereals Authority Report* **29**, pp218.

WALGENBACH, C.A., PHILLIPS, J.K., BURKHOLDER, W.E., KING, G.G.S., SLESSOR, K.N. and MORI, K. 1987. Determination of chirality in 5-hydroxy-4-methyl-3-heptanone, the aggregation pheromone of *Sitophilus oryzae* (L.) and *S. zeamais* Motschulsky. *J.Chem. Ecol.* **13:**2159-2169.

THE ASSESSMENT OF POTENTIAL ATTRACTANTS AGAINST BEETLE PESTS: IMPROVEMENTS TO THE LABORATORY PITFALL BIOASSAY.

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Abstract The assessment of potential insect attractants under laboratory conditions is often a crucial stage in the development of a successful lure. At the Central Science Laboratory, a single pitfall bioassay has been adopted as a standard method for use when testing a variety of small beetle pests. Detailed studies have led to some fundamental improvements to the bioassay technique and of its implications for studying insect behaviour. In particular, the response of the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) to a pheromone mixture was used to investigate aspects likely to influence the sensitivity of the bioassay. Results demonstrated that it was possible to improve both the sensitivity and consistency of response, whilst still maintaining a method which was simple and easy to use.

Introduction

The use of attractants, incorporated into a trap design, can often lead to significant improvements in the detection of insect infestations. Increasing the efficacy of traps by adding a lure based on either pheromones or food aromas, may provide several benefits (Chambers, 1990: Cogan and Wakefield, 1987). These largely derive from the ability to detect infestations at lower insect densities. However, the development of a suitable lure often involves considerable laboratory testing. Typically, this will include the determination of the chemical nature of the attractant, both in terms of composition and dosage, followed by tests to determine the best method for its controlled release. Studies of this nature can be labour intensive, particularly in the case of food based aromas which often contain components of widely differing volatility. Clearly, as the assessment of attractants under laboratory conditions represents a crucial stage in the development of a successful lure any improvements in the bioassay method used will be beneficial.

At the Central Science Laboratory (CSL), a single pitfall bioassay has been in use for a number of years to investigate potential attractants against a variety of small beetle pests. It was originally developed from the two-choice pitfall bioassay described by White and Birch (1987). However, despite the advantages of the single pitfall bioassay over the statistically complex two-choice method (Chambers et al, 1990), previous studies have suggested that there was potential for improvement and a need for a better understanding of the methodology: the variations in insect response both to control (blank) replicates and those containing an attractant have sometimes defied explanation and at their worst have led to inconsistent conclusions. This study was therefore designed to investigate aspects of the bioassay methodology and its ability to distinguish the effect of a semiochemical.

Materials and methods

Unsexed adult *Oryzaephilus surinamensis* L. (Laboratory Susceptible strain) 1-3 weeks post eclosion were used in this study. Tests were carried out in arenas measuring 100mm (standard) or 200mm diameter (Figure 1), each containing either an untreated filter paper disc (20mm diameter) or one treated with a pheromone mixture. The size of the pitfall tubes (75 x 25mm diameter) and the number of *O. surinamensis* tested in each replicate (20) were the same in both arena sizes. Bioassays were run for 3 hours starting immediately after introduction of the filter paper disc and test insects. The number of insects found in individual pitfall tubes was recorded at periods of 1, 2 and 3 hours using a torch which was red filtered in order to minimise disturbance of the test insects. Testing was carried out weekly over a period of 3 months and at test conditions of 20°C 50% rh and darkness.

Results were pooled and compared using ANOVA and t-tests where appropriate.

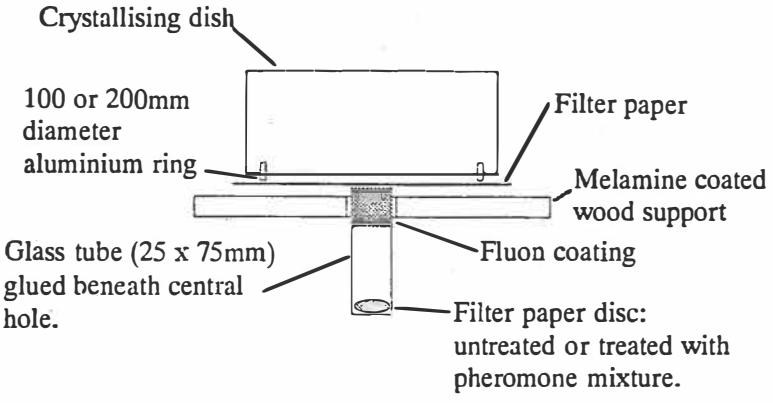


Figure 1. Diagram of pitfall bioassay arena with components exploded for clarity.

Results

Results were initially analysed to determine the optimum assessment periods for each of the arena sizes. Optimum assessments were considered as those where the difference between the response to pheromone treated replicates and control replicates was greatest. For the purposes of this work the term 'response differentiation' has been used to define the response to pheromone minus the response recorded for control replicates.

The response differentiation in both arena sizes is shown in Figure 2. For tests carried out in the larger arena size there was a progressive increase in response differentiation for successive assessments. Furthermore, the variation between the tests over a 3 month period showed that response differentiations were more likely to be statistically significant ($p<0.05$) at the 3 hour assessment than at the 1 or 2 hour assessments. By comparison, tests in standard size arenas generally produced the greatest response differentiation at the 1 hour period despite a relatively high response at 2 and 3 hours for replicates containing the pheromone treatments.

Variation was assessed using the results from the standard bioassay (1 hour assessment) and modified bioassay (200mm arena and 3 hour assessment) for the 3 month period over which tests were carried out. These showed significant variations in the responses of replicates with pheromone mixture and controls when using either bioassay method (see Figure 3). Moreover, there was no apparent correlation between the variation in response to pheromone and control replicates. However, variation in response across tests was lower when using the modified technique . In the example shown in Figure 3 shows that only one test in ten failed to show a statistically significant ($p<0.05$) increase in response to the pheromone over control replicates. This compared favourably with the standard bioassay where four out of ten tests failed to detect a significant difference.

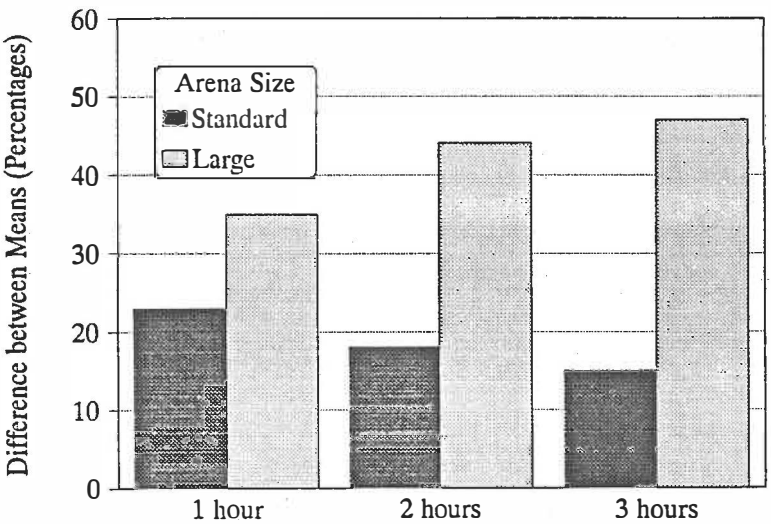
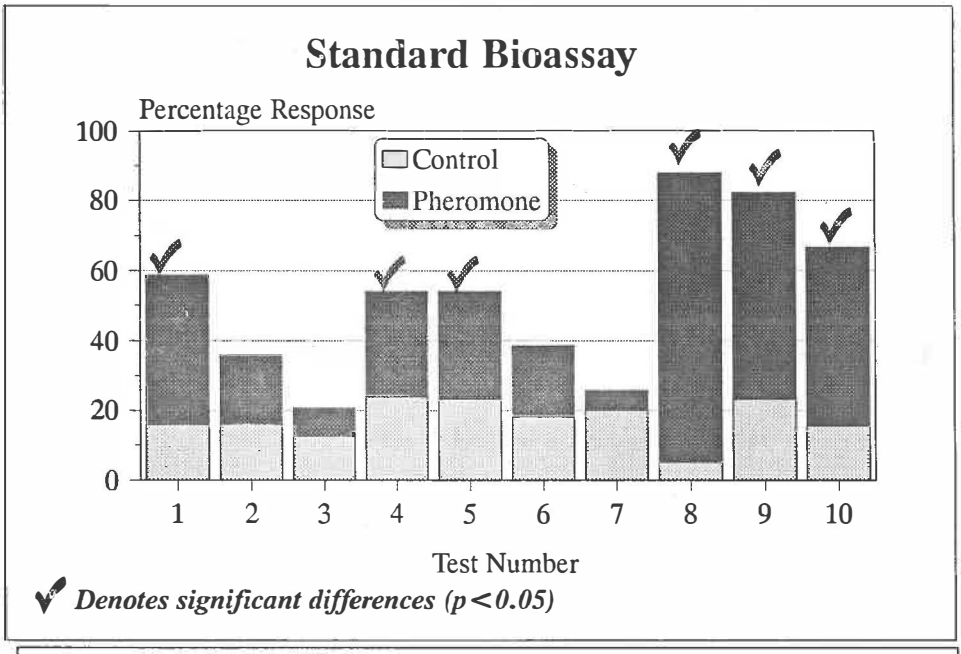


Figure 2. Response differentiation (response in pheromone replicates minus response in control replicates) of *O. surinamensis*, Laboratory Susceptible strain, in standard and modified arenas.

Conclusions

This study clearly shows that changes made to the standard pitfall bioassay have resulted in a more reliable and sensitive technique for the assessment of attractants against the saw-toothed grain beetle. The improved technique, using a larger 200mm diameter arena and 3 hour assessment, was particularly successful in reducing the level of response recorded for control replicates while the extended assessment period resulted in a higher response to the attractant. Results also suggest that the variation in response recorded across tests has been reduced. Given the variable nature of insect behavioural tests, the use of the improved technique with tests carried out over a number of days will help to increase the reproducibility of results and, thereby, reduce the risk of incorrect conclusions. Further work will now be carried out to investigate the suitability of the pitfall bioassay as a new standard technique for use with other strains of *O. surinamensis* and beetle pest-species.



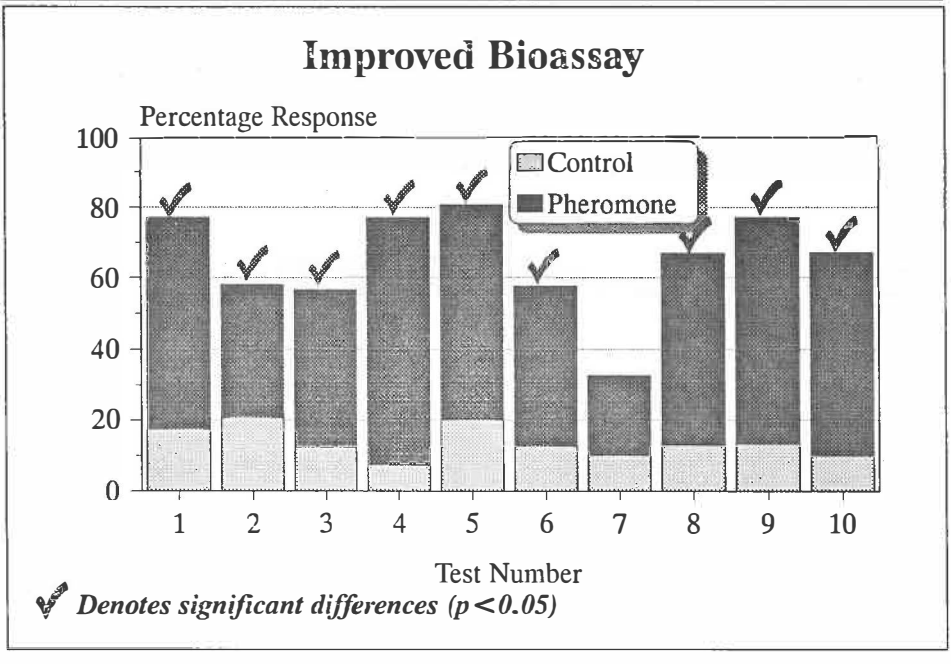


Figure 3. Response of *O. surinamensis* Laboratory Susceptible strain to pheromone and control replicates in standard and modified (200mm diameter arena and 3 hour assessment) pitfall bioassays.

Acknowledgment

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References

CHAMBERS, J., 1990. Overview on stored-product insect pheromones and food attractants. *J. Kansas Ent. Soc.* **63** (4), pp. 490-499.

CHAMBERS, J., MORGAN, C.P., WHITE, P.R., MORI, K., FINNEGAN, D.E. AND PINNIGER, D.B., 1990. Rust-red grain beetle, *Cryptolestes ferrugineus*, and flat-grain beetle, *Cryptolestes pusillus*: antennal and behavioural responses to synthetic components of their aggregation pheromones. *J. Chem. Ecol.* **16** (12), pp. 3353-3372.

COGAN, P.M. AND WAKEFIELD, M.E., 1987. Further developments in traps used to detect low-level infestations of beetle pests in bulk stored grain. BCPC Mono.No. 37 Stored Products Pest Control, pp 161-167.

WHITE, P.R. AND BIRCH, M.C., 1987. Female sex pheromone of *A. punctatum*. Extraction, identification and bioassays. *J. Chem. Ecol.* **13** (7).

THE USE OF A MARKING METHOD TO STUDY THE BEHAVIOURAL EFFECTS OF TWO FOOD LURES IN THE PC TRAP ON MARKED *SITOPHILUS GRANARIUS* (L.).

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Abstract. The use of a marking method in experiments to improve trap efficiency was evaluated. A colour code was used with poster paints applied to the insect's dorsal surface and the behaviour of individually marked *Sitophilus granarius* on the PC trap in the presence of two potential food lures, kibbled carob and cocoa, was examined. The presence of kibbled carob resulted in a significantly decreased time that *S. granarius* spent stationary on the trap when compared to controls and enhanced the effectiveness of the trap with a 75% increase in beetles caught. The presence of cocoa resulted in a 60% decrease in the number of beetles caught. It also caused a significant decrease in the total time the beetles were on the trap and the time spent walking upon the trap top compared to controls. The marking method provided an inexpensive, fast and convenient way to examine the effect of lures in traps on beetle behaviour.

Introduction

Behavioural bioassays may involve many insects of the same species in the same test if a simple behaviour such as the number entering a trap is to be examined or a single insect if the complex behavioural patterns of an individual are investigated. In the field, insects of the same species may interact and this could alter their behaviour. To mimic this effect in the laboratory, several insects must be used in behavioural bioassays and if a detailed analysis of behaviour is undertaken, particularly in the presence of pheromone or food lures, the individual insects must be identified.

Insect behavioural bioassays such as pitfall bioassays (Pierce *et al*, 1981; Phillips and Burkholder, 1981) are generally undertaken in the absence of a food source for the species under examination. If a food source is present in the observation arena it is possible that insects may become hidden from view. Repeat visits to a trap can only be assessed in these circumstances if an individual is identifiable.

Methods previously used for marking insects involve the use of paints (often with complex symbols or application to precise areas of the body), tagging or mutilation (Southwood, 1978). Storage pest beetles are small, generally less than 4mm in length, and therefore tagging or mutilation are inappropriate. A marking method was developed using poster paints to identify up to 50 individual beetles (Wakefield, in prep.) for use in behavioural bioassays using insect traps. The purpose of the present study was to evaluate the marking method for use in experiments to improve trapping efficiency. The addition of lures may improve the effectiveness of traps by attracting the

insects to the trap or by increasing the chance of insects entering the trap on arrival. In this study the marking method has been used to examine the behaviour of the grain weevil *S. granarius* on the PC trap (Cogan *et al*, 1990) in the presence of two potential food lures, kibbled carob and cocoa powder.

Method

Adult *S. granarius* (2-4 weeks old) were marked using the method of Wakefield (In prep.). Fifty adults were used for each test.

Tests were carried out using a PC trap in a plastic container (diameter 26cm, height 23cm) holding 8kg of wheat. Traps were placed in the wheat so that the outer row of holes was level with the grain surface.The marked beetles were left to settle in a room at 20°C, 30-50% r.h. for one hour with the trap covered with a paper cone before observations began. Insects were observed for 0.5 hour after removal of the trap cover and activities were recorded using a modified version of the Observer software package (Noldus *et al*, 1989) on a Psion organiser (model LZ64). The activities recorded were:-

Touch - the beetle made contact with the PC trap.

Away - the beetle left the PC trap.

In - the beetle entered the PC trap.

Walk - the frequency and duration that the beetle walked on the PC trap.

Dangle - the frequency and duration for which the beetle hung through a hole in the PC trap holding on to the lid with its hind legs.

Stop - the frequency and duration that the beetle remained stationary on the PC trap.

The six activities were recorded using the keys A-F. The software only permitted a maximum of 50 assigned keys and therefore with six activity keys it was only possible to record the activities of insects 1-44. The activities of beetles numbered 45-50 inclusive, were recorded manually and only the number of visits and whether the insects entered the trap was recorded.

To investigate the behavioural effects of food lures, a 0.7g piece of kibbled carob or 0.7g of cocoa powder, secured in a square of muslin, were attached to the lid beneath the central solid area of the PC trap. Due to the possibility of differences in behaviour from day to day, controls were undertaken for each series of experiments.Tests using traps with no lure and traps with a lure were performed at randomly assigned times on the same day. Tests with and without carob were conducted over a 16 day period and tests with and without cocoa over a 13 day period.Ten replicates with and without kibbled carob and 10 replicates with and without cocoa were undertaken.

After each test the wheat was placed in an oven at 70°C for 7 hours to kill the *S. granarius* before being used for subsequent tests. Dead beetles were not removed from the wheat. Each batch of grain was used approximately five times before it was discarded.

Results for *S. granarius* behaviour at traps with and without lures were analysed using one way analysis of variance. Oneway ANOVAR was also used to compare the two sets of results for traps without lures.

Results and Discussion.

No significant difference ($p>0.05$) was found for the two sets of data where no lure was present. These results provide the base-line level of activity for *S. granarius* on the PC trap and it was against this data that the change in behaviour with food lures was assessed.
Results for the behaviour of *S. granarius* at traps with and without kibbled carob are shown in Table 1. The data for beetle numbers 45-50 inclusive are not included.

Table 1. The behaviour of *S. granarius* on the PC trap with and without carob.

Behaviour	without carob	with carob
Number of visits[1]	51.4 +/- 4.1	55.3 +/- 6.1
% time on trap	71.8 +/- 6.8	74.4 +/- 8.5
Number of beetles caught	0.4 +/- 0.2	0.7 +/- 0.3
Mean time walking (sec)	24.4 +/- 1.0	23.0 +/- 1.0
Mean time dangling (sec)	4.9 +/- 0.6	11.2 +/- 5.1
Mean time stationary (sec)	8.7 +/- 1.5*	5.8 +/- 0.5*

[1]Number of visits includes both beetles that touched the trap and eventually walked away and those that touched the trap and fell in.
In each row means followed by *are significantly different ($p<0.05$).

The results for traps without a lure present showed that for approximately two-thirds of the duration of each test an insect was present on the trap top but very few of these insects actually entered the trap. The major activity on the trap was walking.

The behaviour of several stored product beetles including *S. granarius* at pitfall traps in millet was studied by Obeng-Ofori (1993). He concluded that the frequency of encounters with the trap is unrelated to capture rate. He also noted that the activity he referred to as 'probing' and which in this paper is referred to as dangling was common for *S. granarius* and was one of the avoidance behaviours that prevented capture. He noted that the beetle would often become unbalanced during this activity and fall into the trap.

The presence of carob appeared to improve the effectiveness of the PC trap for *S. granarius* as there was a 4% increase in the time spent on the trap but a 75% increase in the number caught. When kibbled carob was present there was a significant decrease ($p<0.05$) in the mean time spent stationary on the trap top.There was a 130% increase in the mean time spent dangling and it was observed in the present study that an insect which spends a long time dangling will often fall into the trap.

The results for the behaviour of *S. granarius* at traps with and without cocoa are shown in Table 2.

Table 2. The behaviour of *S. granarius* on the PC trap with and without cocoa.

Behaviour	without cocoa	with cocoa
Number of visits	41.8 +/- 4.1	33.3 +/- 2.9
% time on trap	62.3 +/- 7.4*	42.5 +/- 4.2*
Number of beetles caught	0.5 +/- 0.3	0.2 +/- 0.1
Mean time walking (sec)	26.5 +/- 1.3*	23.1 +/- 0.9*
Mean time dangling (sec)	8.5 +/- 2.8	6.2 +/- 1.6
Mean time stationary (sec)	6.0 +/- 0.7	6.6 +/- 0.7

[1]Number of visits includes both beetles that touched the trap and eventually walked away and those that touched the trap and fell in.
In each row means followed by * are significantly different ($p<0.05$)

Cocoa has been shown to be as attractive as kibbled carob in single pitfall bioassays using another storage pest species, the saw-toothed grain beetle, *Oryzaephilus surinamensis* (C. P. Morgan, personal communication).

When cocoa was present in the experiment reported here there was a significant decrease ($p<0.05$) in the time spent on the trap and the mean time spent walking. The number of visits fell by 20% and the number caught by 60% when cocoa was present. Cocoa did not therefore improve the effectiveness of the PC trap for *S. granarius* and may even repel this species.

Conclusion

The marking method provides a fast and convenient way to examine the effect of lures in traps. The direct observation of identified individuals enables the potential of lures to be determined. The response of an individual beetle to a lure can only be observed if the beetle is identified. It is then possible to determine the actual number of individuals visiting the trap and the number of repeat visits. The method can be adapted for other insect traps where behaviour can be recorded.

The test provided the base-line level of activity for *S. granarius* against which tests involving lures can be compared. The results show that the overall level of activity of *S. granarius* may vary slightly from day to day but these variations are not significantly different and thus a base-line level of activity can be determined.

The presence of kibbled carob did not increase the number of *S. granarius* approaching the trap but did increase the chance of these beetles entering the trap.

Cocoa did not improve the effectiveness of the PC trap for this species. Fewer beetles approached the trap and far fewer entered the trap when cocoa was present.

References

COGAN P.M., WAKEFIELD M.E. AND PINNIGER D.B., 1990. PC, A novel and inexpensive trap for the detection of beetle pests at low densities in bulk grain. Proc. 5th Int. Working Conf. Stored Prod. Prot., Bordeaux. **Volume II** :1321-1330.

NOLDUS L.P.J.J., VAN DE LOO E.L.H.H. AND TIMMS P.H.A., 1989. Computers in behavioural research. Nature **341**: 767-768.

OBENG-OFORI D., 1993. The behaviour of 9 stored product beetles at pitfall traps arenas and their capture in millet. Ent. exp. app. **66**: 161-169.

PIERCE A.M, BORDEN J.H. AND OEHLSCHLAGER A.C., 1981. Olfactory response to beetle-produced volatiles and host food attractants by *Oryzaephilus surinamensis* and *O. mercator*. Can. J. Zoo. **59**: 1980-1990.

PHILLIPS J.K. AND BURKHOLDER W.E., 1981. Evidence for a male-produced aggregation pheromone in a rice weevil. J. Econ. Ent. **78**: 539-542.

SOUTHWOOD T.R.E., 1978. Ecological methods with particular reference to the study of insect populations. 2nd edition. London: Chapman and Hall Eds.

ECONOMICAL CONSIDERATION OF THE STRATEGY IN APPLYING PHEROMONE FOR CONTROLLING THE PINK BOLLWORM (*PECTINOPHRA GOSSYPIELLA* SAUNDERS) ON COTTON

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Abstract Four formulations of pheromone gossyplure were applied for controlling the pink bollworm on cotton in 1986-1992. The effectiveness of all pheeromone formulations showed better control than the conventional pesticides. For the purpose of reducing the input cost of control a lower dosage of pheromone was tested and 10-15 g ai/h was proved to be comparable with conventional dosage (over 30 g ai/h). The lower dosage was considered more liable to accept by farmers.

Trials of applying pheromone in controlling the pink bollworm on cotton have been conducted from 1986-1992 in China. The results showed that the effectivenessof pheromone applied in mating disruption form can be comparable with conventional pyrethroids application. But due to other pests such as mirids, mites, corn borer and sometimes bollworm usually occurring through the cotton growing season pheromone should be applied with other pesticides for maintaining a normal growth of cotton. Since the price of pheromone formulation is expensive as comparing with conventional pesticides so we try to develop a strategy of reducing the dosage of pheromone to keep a reasonable level of total pesticides input for the cotton production. This was accomplished by delaying the time of application and reducing the dosage of pheromone from over 30 g ai per hectare in usual to 10-15 g ai per hectare.

Samples of pheromone formulations:

Micro-encapsule 2%	from ICI 1986-1989
Agricon G 2%	from IPS 1990-1992
Fiber plat 1.3 mg/plate	from Shanghai Entomological Institute
PVC tape 6 mg/tape	from NRI, Agrisense

The results of the trials are summarised in the tables 1-3.

Table 1: Results from the trials of pheromone formulations in controlling the PBW from 1986-1989

Formulations & Treatments	Dosage g ai/h	Flower inf. %	Boll Damage %	worms / B	worms in seed cotton (100 bolls)	
Micro-encap.	51.0	2.40	2.27	0.014	2.80	1986
CK conv. cont.	-	4.37	15.80	0.136	12.80	
Micro-encap.	15.1	0.023	0.68	0.006	0.60	1987
Fiber plate	11.7	0.29	2.26	0.005	1.00	
CK conv. cont.	-	1.10	12.76	0.096	9.80	
Micro-encap.	4.9	0.20	1.60	0.002	4.10	1988
Fiber plate	4.9	0.09	4.20	0.020	6.30	
CK conv. cont.	-	1.81	10.10	0.080	25.00	
Micro-encap.	12.0	0.10	2.40	0.016	1.50	1989
Fiber plate	3.3	0.70	7.50	0.021	2.00	
CK conv. cont.	-	1.68	31.15	0.340	40.30	
Micro-encap.	4.5	0.65	3.00	0.010	19.60	1990
Fiber plate	-	0.63	4.00	0.02	20.4	
CK conv. cont.	-	1.46	20.00	0.07	58.4	

Table 2: The effectiveness of pheromone compared with pesticides in controlling the PBW in 1991

Formulations &Treatments	Dosage gai/h	Boll damage % Inf.	worms/ B	worms in seed cotton /100 B	
PVCtape (1)	60	5.30	0.048	0	Dafeng
CKconv.cont. (2)	-	43.60	0.541	26.3	
AgriconG (3)	50	7.73	0.800	3.20	
CKconv.cont. (3)	-	43.60	0.540	18.15	
PVCtape	60	19.06	0.410	2.16	Nantong
PVC+Pyre (4)	60	8.93	0.041	0.60	
Pyrethroids (4)		14.90	0.012	2.37	
CKpoor cont.		78.00	1.190	40.00	

(1) For other pests using 0.45 l pyrethroids and 4.35 l OP
(2) Pesticides 0.825 l pyrethroids and 5.25 l OP
(3) For other pests using 3.55 l pyrethroids and 5 l OP
(4) For other pests using 3.75 l pyrethroids

Table 3: The effectiveness of pheromone compared with conventional pesticides in controlling PBW in 1992

Formulations & Treatments		Dosage g ai/h	Boll damage % inf.	worms / B	Worms in seed cotton / 100 B	
PVCtape	(1)	62.5	1.06	0.0013	0	Dafeng
CKconv.cont.	(1)	-	3.80	0.0160	0	
AgriconG	(2)	12.0	0.33	0.0017	0.037	Dongtai
Paste	(2)	20.0	0.42	0.0017	0.012	
CKconv.cont.	(2)	-	0.58	0.0050	0.005	
AgriconG	(3)	24.0	10.5	0.0180	0	Yanchen
CKconv.cont.	(4)	-	25.5	0.125	0.6	

(1) For other pests using 1.90 l/h pyrethroids and 8.475 l OP
(2) For other pests using 2.25 l/h pyrethroids and 5.475 l OP
(3) For other pest susing 2.25 l/h chlordimeform and 7.125 l OP
(4) For other pests using 4.65 l/h pyrethroids 3.75 l chlordimeform

As indicated from the trials it is possible to reduce the dosage of pheromone gossyplure in less than 15g ai per hectare to give a satisfactory control of pink bollworm. The benefits of applying pheromone are easy manipulating, long sustaining, high effective, and environmental safty. If the cost of input of pheromone is reasonable it will of course be likely to be accepted by farmers to constitute an IPM system of cotton pests with other control methods.

MODELLING PINK BOLLWORM MATING DISRUPTION IN EGYPTIAN COTTON

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Abstract: A version of a Californian cotton plant growth and pest model has been modified for Egyptian cottons and is being used to time pheromone applications against pink bollworm *(Pectinophora gossypiella*, Saunders). Release rates of pheromone (sprayable and 'twist-tie' formulations) per degree day depend on formulation specific decay coefficients- and delays in commencing decay. Adult moth numbers, mating status and fecundity are obtained on a daily basis from the insect model. The maximal mating disruption considered to occur above a field derived threshold is reduced by increasing population density. Below the threshold, disruption is decreased proportionately as the pheromone concentration decreases. The system is being used for the first time this season to time pheromone applications in the Nile Valley and Delta.

Introduction

Commercial application of pink bollworm pheromone in Egypt, bought and applied by the Ministry of Agriculture, commenced in 1984. The area applied expanded rapidly but high pink bollworm numbers throughout the Egyptian cotton growing area in 1986 led to a decline in confidence in pheromones which has been slowly overcome, with the pheromone treated area reaching a record 50,000 ha in the 1993 cotton season. Over the years flake and chopped fibre applications have been made but the current materials of choice are the long persistence, twist-tie formulation, 'PB-Rope' and the sprayable, microencapsulated formulation 'Pectone'.

An average of around 2 insecticide applications are now used for bollworm (mainly *Earias insulana* and pink bollworm) control in the second half of the growing season in the pheromone treated areas as opposed to an average of around 4 insecticide applications in the conventionally treated area. Pink bollworm control by pheromones could be approached with more confidence and pheromonal protection allowed to be effective for longer in the season if bollworm numbers and the level of mating suppression by pheromone could be predicted accurately. This is particularly true of the shorter persistence, sprayable formulations.

Cotton/Pest model

Partly to address these questions, the distributed delay mathematical model of cotton (*Gossypium hirsutum*) development and pink bollworm population dynamics developed by A. Gutierrez (Gutierrez et al 1975), and provided with a management model by N. Stone (Stone and Gutierrez 1986), has been modified by the authors to accurately mimic the growth and development of the main Egyptian (*Gossypium barbadense*) cotton variety - Giza '75. The cotton model, (structure shown in outline in Fig 1) requires abiotic data such as weather, planting date, plant density and the dates and quantities of fertiliser and water applied in addition to a detailed parameter list describing the growth form of the particular cotton variety. Optimal plant growth is restricted in the model by deficiencies in any of inputs and by plant density effects. The insect model, which is also based on supply/demand restrictions on optimal growth, has insects passing from one life stage to another via a series of distributed delays to take account of the variance in individual development rates, controlled by temperature and food source dependant variables. Fecundity is controlled by the feeding history and diapause status of the individual insect, modifying a user entered average oviposition schedule.

Pheromone routine

The pheromone management routine of this Fortran'77 model (modified from Stone and Gutierrez 1986) uses the number of pheromone sources applied, pheromone quantity per source, temperature-dependant release rates and time since application in heat units, to calculate the total pheromone released per unit area per day. For the application of sprayable materials the source number is modified by the leaf-area-index calculated for the application day in the plant model, to exclude material not adhering to the foliage. Important features of materials used in cotton are given in Table 1. Release rates vary depending on the formulation used and differ widely between, for example 'rope' and sprayable formulations. These differences have been modelled flexibly here by introducing formulation-specific decay coefficients- and delays in commencing decay.

Table 1: Features of pink bollworm pheromone products.

Dispenser Types	Application type	No. sources/ha	a.i./ha	a.i./source
Microencaps.	Aerial	1,000,000 (nominal)	9.5g	0.001mg
Flake	"	180,000	53g	0.3mg
'Twist-tie'	Manual	714	52g	73mg
PVC laminate	"	524	78g	149mg

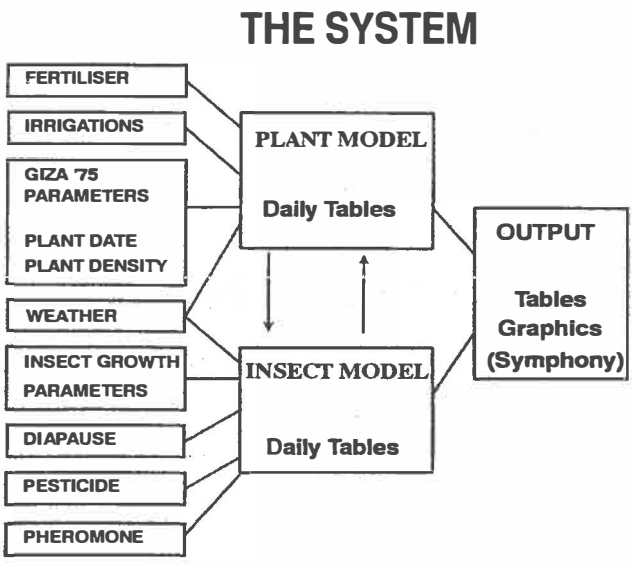


Fig. 1 Cotton Model Structure

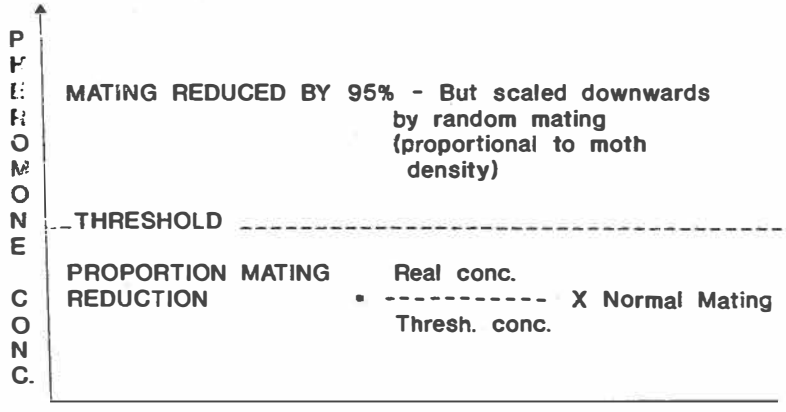


Fig.2 Pheromone Concentration Threshold

The effect of pheromone on the number of eggs resulting from pink bollworm mating depends on:

a) The density of moths and the age structure of the population present in the field.
b) Age specific and larval feeding effects on individual fecundity.
c) The mating status of females present, incorporating their post-emergence biology.

All of these figures are obtained from the insect model by the pheromone routine. New mating is maximally suppressed when aerial pheromone levels rise above a threshold value, but the suppression is scaled downwards by a level of random mating proportional to moth density (from the pink bollworm model). Beneath the threshold the maximum efficacy of mating disruption is reduced in inverse proportion to the ratio of the real aerial concentration to the threshold concentration (Fig. 2).

We have attempted to measure the aerial concentration of pheromones and the effective mating suppression thresholds directly using known volumes of air pumped over pheromone absorbing chemical filters in the field in Egypt. Similar work in Arizona (Flint et al 1990 and 1993) has suggested a mating suppression threshold of c.1.3 ng/m^3. Despite careful collection and analysis at NRI's laboratories we have been unable to quantify pink bollworm pheromone levels in the field, though levels of 1.3 ng/m^3 were easily quantified in laboratory wind-tunnel experiments using the same equipment. We have therefore relied on measured release rates and comparison with the literature to parameterise the system.

For microencapsulated 'Pectone' (applied at 9.5g/ha) the initial 'plateau' period of release is modelled as only one day, with a temperature dependant exponential decline in quantity released from the end of day 1 following application, to 325 day degrees >5^{o}C, with the maximum mating disruption efficacy threshold crossed at 250 day degrees under Egyptian conditions (Fig 3).

The 'PB-Rope' twist-tie in current use, applied at 52g/ha a.i. in 700 dispensers is modelled as having a high and steady 'plateau' release rate of about 35% of the initial material over the first 650 day degrees followed by an exponential decay to effectively zero over the succeeding 1125 day degrees. In practice about 30% of the liquid remains in the dispenser at this point. This needs to be borne in mind when calculating release rates. From computation of the field release rates and comparison with the work of Flint et al, (1993) the threshold is taken to be crossed at 1550 day degrees following application. This corresponds to a point when there is roughly 20g a.i./ha remaining with a release of less than 1% of the remaining material per day. Low though the figure is, it is still implies about 5 times the aerial pheromone density at which multiple dispersed point sources have been shown to operate as normal lures in an attracticide formulation in Egypt (commercial data unpublished).

Usefulness

The efficacy of the system in predicting pink bollworm larval numbers seen in non-insecticidal fields is high provided that the total bollworm numbers emerging in the area in the spring can be estimated from pheromone trap catches prior to fruit formation. An example contrasting model and field data for an area where microencapsulated pheromone was used (3 applications) is given in Fig. 4 and for a single 'twist-tie' application in Fig. 5.

Recommendations are being tested by manipulating pheromone application dates within the commercially applied areas in Egypt in this season. Applications are being made at the date when the model predicts (using pheromone trap catch data from the areas and average weather data to predict

Fig. 3 Microencapsulated Pink Bollworm Pheromone Threshold

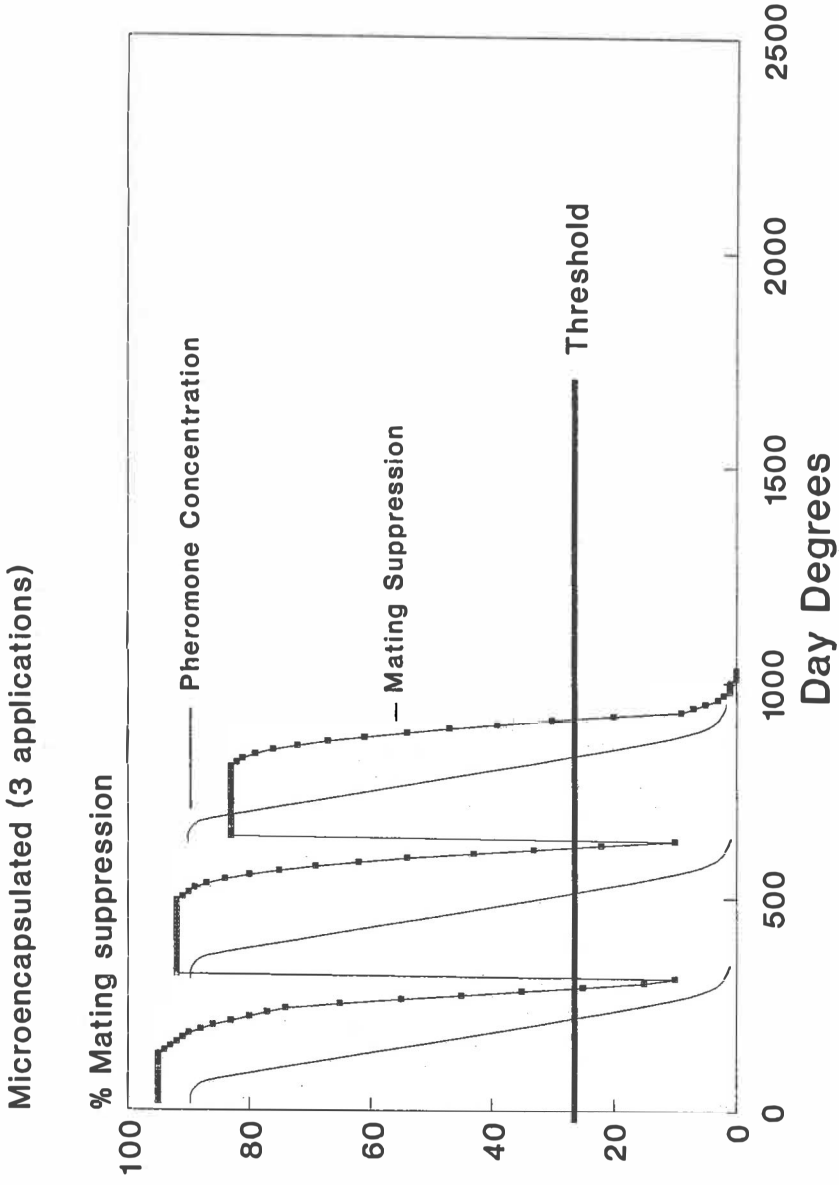


Fig. 4 Microencapsulated Pink Bollworm Pheromone (3 Applications) Predicted Larval Numbers.

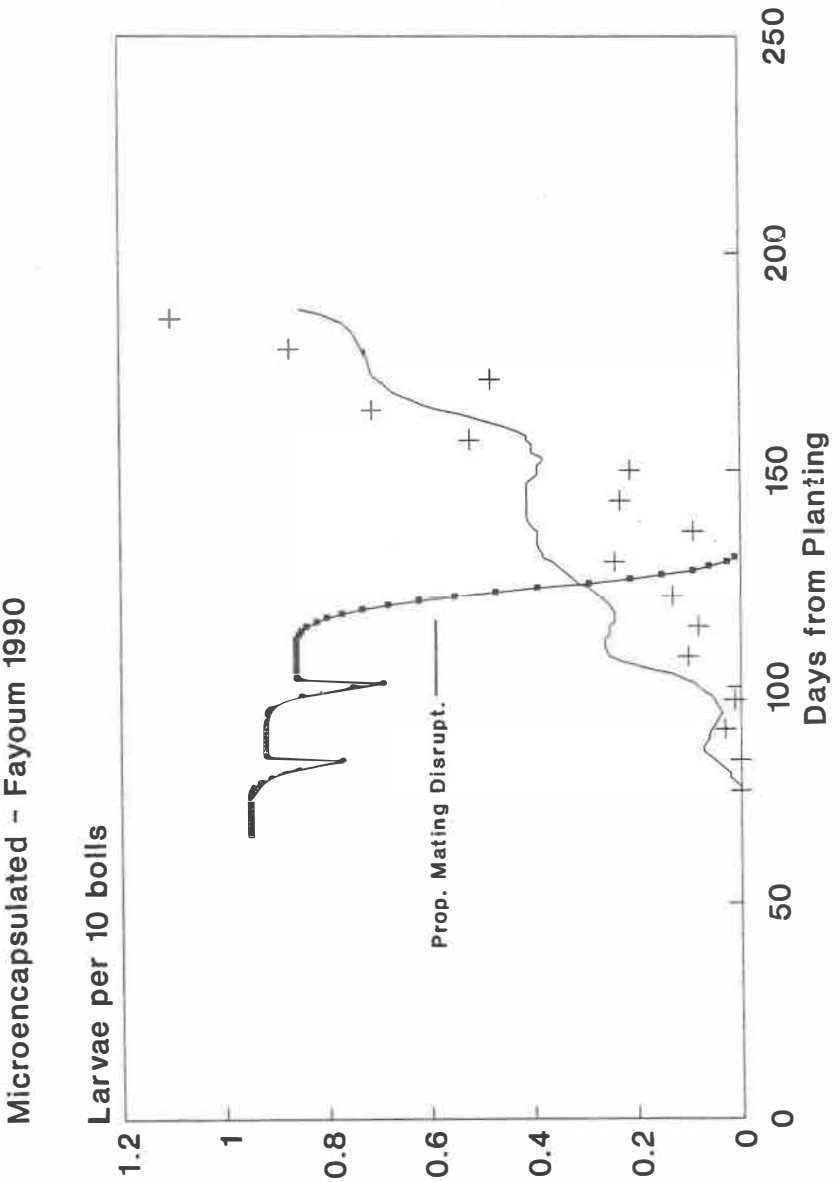
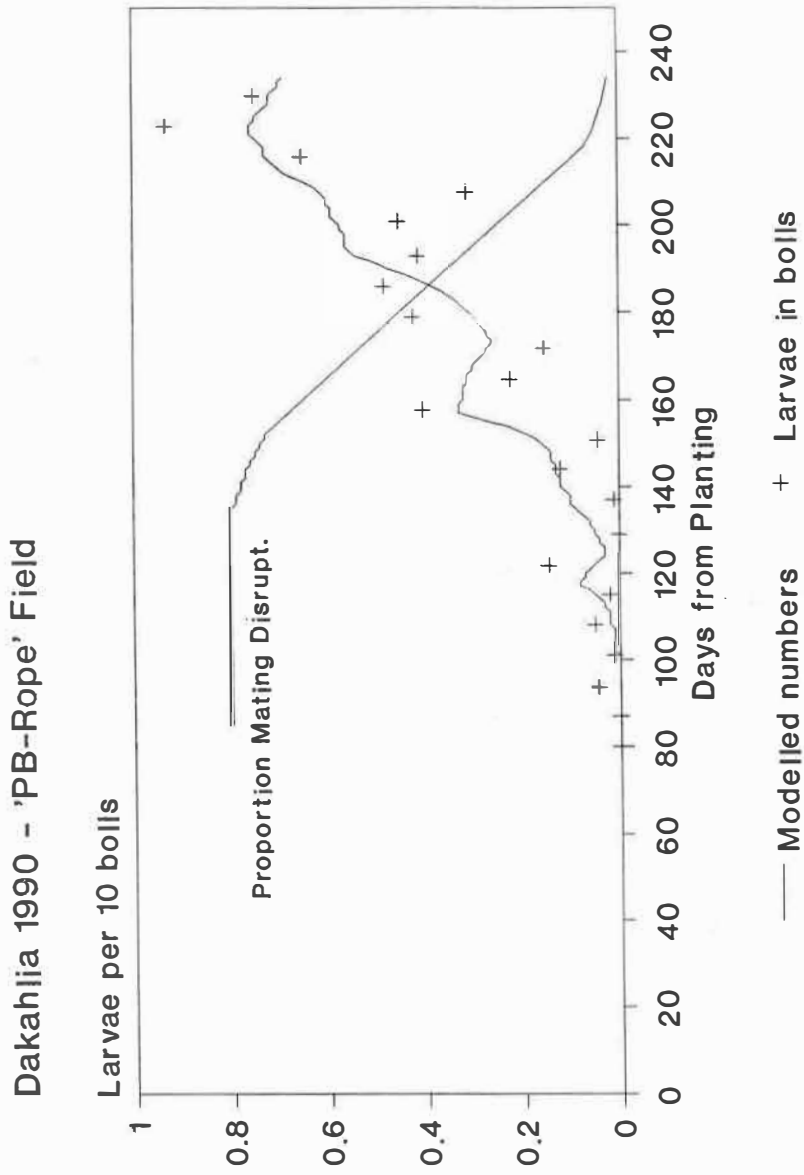


Fig. 5 PB-Rope Pink Bollworm Pheromone - Predicted Larval Numbers.



forward) that delaying application will result in increased boll infestations. Dates significantly different from those recommended by the Ministry of Agriculture have been generated. The effect on total pest management costs and eventual yield loss due to pink bollworm damage remain to be seen.

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References

FLINT, H.M., YAMAMOTO, A.K., PARKS, N.J. & NYOMURA, K. 1990: Aerial concentrations of gossyplure, the sex pheromone of the pink bollworm (Lepidoptera: Gelechiidae), in cotton fields treated with long lasting dispensers. Environ. Entomol. **19**: 1845-1851.

FLINT, H.M., YAMAMOTO, A.K., PARKS, N.J. and NYOMURA, K. 1993: Aerial concentration of Gossyplure, the sex pheromne of the pink bollworm (Lepidoptera: Gelechiidae), within and above cotton fields treated with long lasting dispensers. Environ. Entomol. **22(1)**: 43-48.

GUTIERREZ, P., FALCON, L.A., LOEW, W., LEIPZIG, P.A. & VAN DEN BOSCH, R. 1975: An analysis of cotton production in California: a model for Acala cotton and the effects of defoliators on its yields. Environ. Entomol. **4**: 125-36.

STONE, N., GUTIERREZ, P. 1986: Pink bollworm control in Southwestern desert cotton. I. A field-oriented simulation model. II. A strategic management model. Hilgardia **9**: 1-41.

BEHAVIOURAL REPRODUCTIVE ISOLATION AMONG FOUR PLUSIINAE SPECIES IN ISRAEL MEDIATED BY SEX PHEROMONE

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Abstract The role of the sex pheromone components in the inter- and intra- relationships between four sympatric species in Israel was studied. The four species, were: *Chrysodeixis chalcites*, *Autographa gamma*, *Cornutiplusia circumflexa* and *Trichoplusia ni*.

The chemical composition of the four pheromone blends was determined by chemical analysis of gland extracts and in *C. chalcites* and *T. ni* also of airborne collections. The analyses were conducted by GC, GC-MS and dimethyl disulfide derivatization for location of the double bonds. A relatively small number of monounsaturated acetates and alcohol were found in the various pheromone blends. Z7-12:Ac was the major component in three of the species., whereas Z7-12:OH was the major component in *C. circumflexa*. Behavioural studies in a wind tunnel and field tests confirmed the identity of the pheromone components.

Two factors affect the behavioural reproductive isolation among sympatric moth species based on sex pheromone blends: 1) optimal attraction to the species-specific pheromone emitted by conspecific females; 2) inhibition by certain pheromone components emitted by heterospecific females. Both aspects were demonstrated in this research.

C. circumflexa males were attracted by a binary blend of Z7-12:OH and Z7-12:Ac in a 5 to 1 ratio. This moth is reproductively isolated from the three other species by its major pheromone component Z7-12:OH, which has an inhibitory effect on the three other species.

C. chalcites males were attracted only to a multicomponent blend, which included the major component Z7-12:Ac and the minor components Z9-12:Ac and Z9-14:Ac. The relatively higher amount of Z9-14:Ac emitted by the *C. chalcites* females as compared to the *T. ni* females , and the Z9-14:Ac are inhibitory to *T. ni* and *A. gamma* males.

T. ni males may be attracted by high doses of Z7-12:Ac alone, however addition of the minor components Z7-14:Ac and Z9-14:Ac enhanced the response significantly. Conversely, the minor component Z5-12:Ac present in the *T. ni* pheromone blend is strongly inhibitory to *C. chalcites* and *A. gamma* males. In addition *A. gamma* males are also inhibited by Z9-14:Ac.

The only case of some cross-attraction was found between *T. ni* and *A. gamma* moths. The sex pheromone of *A. gamma* comprises Z7-12:Ac and Z7-12:OH in a 10 to 1 ratio. The total amount of the pheromone per female was very low. Since extremely low amounts of the alcohol did not inhibit *T. ni* males, they responded to some extent to *A. gamma* lures and females.

MATING DISRUPTION WITH SEMI-PERMEABLE MEMBRANE DISPENSERS FOR THE CONTROL OF *CYDIA POMONELLA* L.

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Abstract The use of synthetic pheromones, both for mating disruption and mass trapping methods, has given variable results.

Regarding *Cydia pomonella* L., the reasons of the failures are due to the inadequate knowledge of the specific insect behaviour related to the pheromones and to the complex interactions between the synthetic molecules and the surrounding environment. With regard to this, the climatic conditions (temperature and direct insolation of the dispensers in particular) play a relevant role, as they have a direct impact on the pheromone release. This should be constant and uniform or vary only on a vary limited range of value in order to assure the expected mating disruption effect.

In the present work, the results of the field-trials carried out with in 3 main Italian fruit-growing regions (Piemonte, Lombardia and Trentino) will be presented. In these tests, the rates of pheromones have been rather low, 2 applications of 31.5 g/ha, thanks to the use of dispensers based on the semi-permeable membrane technology.

Materials and Methods

The trials have been carried out in the Italian provinces of Torino Sondrio and Trento, using three apples orchards representative of the different regional fruit growing areas.

Checkmate CM, the patented system of Concep Membranes Inc. (Oregon/U.S.A.) for diffusing pheromones, has been used in these trials. In these dispensers, loaded with 104 mg of Codlemone (E,E-8,10-dodecadien-1-ol), the release of the pheromone occurs by diffusion through a rate-controlling membrane. This system should allow a more consistent and constant release of the pheromone in the environment.

In the trials areas, *Cydia pomonella* has two generations and its activity normally requests 2-3 insecticide treatments. Both in 1991 and 1992, the dispensers have been applied twice, the first application early May, the second one after 60 days, in the first half of July. The dispensers have been placed in the orchards in the customary manner. They have been homogeneously distributed inside the orchard (a dispenser each 4-6 trees) and their number has been increased along the borders (a dispenser each 2-3 trees). Totally, in each of the two applications, 300 dispensers/ha equivalent to

about 31,5 g of codlemone per hectare, have been placed in the orchards. The principal features of the experimental field-trials are shown in the table 1.

Table 1 - Main features of the trials.

Region	Surface	Variety	Training system	Height of tree	Number of dispensers	Date of application
Torino	0.7	Ozark Gold	palmette	4.5	270	05.15; 06.26.91 05.11; 07.06.92
Sondrio	1.3	Stark Gold	palmette	4.5	360	05.20; 07.09.91 05.12; 07.08.92
Trento	3.2	Hi Early G.delicious Summered	spindle	3.0	1050	05.13; 07.04.91 05.05; 06.30.92

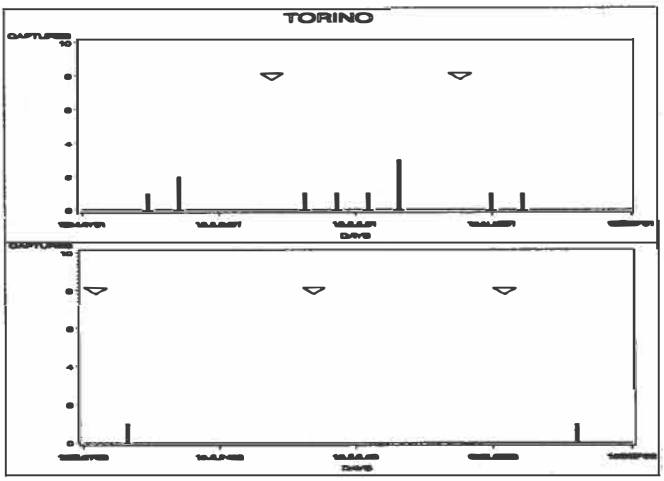
The effectiveness of the method has been assessed according to the pheromone traps catches (the biolure wing-type pheromone traps of Concep Inc. were used) placed in both pheromone treated orchards and control orchards treated with standard insecticide programs. A direct control, made after the flight, in order to check the presence of penetration holes due to the larvae of *Cydia pomonella*, has been performed assessing a minimum of 1000 fruits of 50 randomly selected trees per hectare.

Results

I) TORINO

Figure 1 shows the catches of *Cydia pomonella* made in the orchard used as control. In this block, both in the first and in the second year, the control of this insect needed 2-3 specific treatments with insecticides. In the orchard treated with pheromones we did not observe any captures and the orchard did not need any insecticide treatments for the codling moth - control. Comparing the percent of attacked fruits at the harvest, we assessed that the damage was very low, below the economic threshold of this pest.

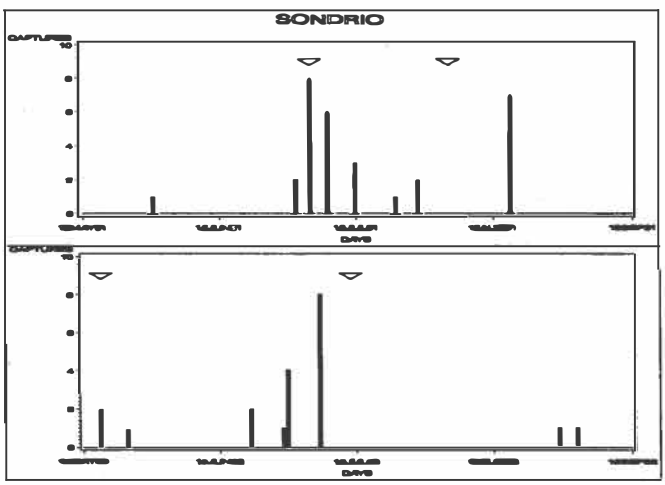
Figure 1 - Torino. Codling moth captures in the control orchard in 1991 and 1992.



II) SONDRIO

Also in this case, both in 1991 and 1992, the catches of male in the pheromone traps located in the middle of the trial orchards were completely inhibited. The apple production has been obtained

Figure 2 - Sondrio. Codling moth catches in the control orchard in 1991 and 1992.

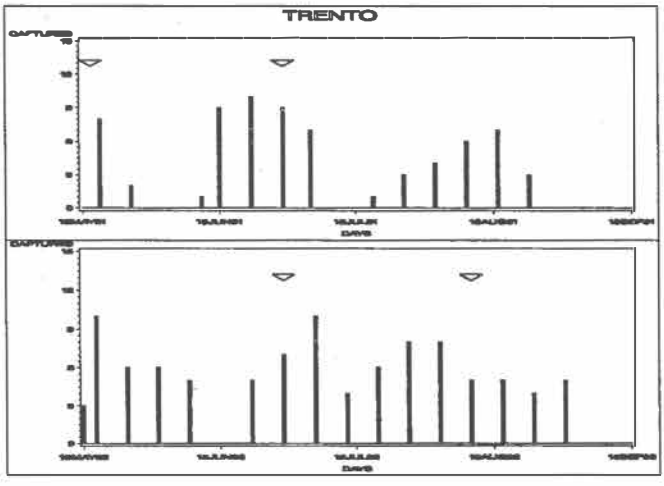


without any specific chemical treatment. In the conventional orchards the control of *Cydia pomonella* has demanded two insecticide treatments (fig.2). At the harvest, the damage in the control orchard has been near to 10% in 1991 and 2% in 1992, while in the pheromone treated orchard the damage was around 1% in both years.

III). TRENTO.

The region selected for the test presented high levels of codling moth infestation. In the same orchard used in the trial, the standard chemical in the past years consisted of three treatments (2 with diflubenzuron and 1 with organophosphate). The figure 1 shows an example of the period and the intensity of the codling moth flights. In this figure there have been reported the catches recorded during the two years of experimentation in a check orchard located close to the orchard treated with the mating disruption method. In this orchard, as already mentioned for the whole region, the codling moth has been controlled with three treatments (2 diflubenzuron and 1 organophosphate). The fruit damage at harvest has been 0.1% in 1991 and 0.5% in 1992. These values stand widely below the economic threshold of this pest. The application of mating disruption system with the Concep dispensers has given different results in the two years of experimentation. In 1991 the codling moth has been controlled using the sole mating disruption method.

Figure 3 - Trento. Codling moth catches in the control orchard in 1991 and 1992.



The average fruit damage at harvest has been 0.75% it was higher on the Summered (3.0%) variety located on one border of the block, lower in the centre of the block (0.5%) and on the Hi-Early variety (0.4%). The pheromone diffusion has achieved an almost complete inhibition of catches in the pheromone traps. In 1992, the disruption method failed to control sufficiently the codling moth and one pesticide treatment with diflubenzuron has been necessary. This treatment has been made on July 30 on the whole surface of the block excepted 6 central rows. In the insecticide-treated area the average fruit damage at harvest has been 2.9%. As in the previous season, the variety Hi Early has

been less attacked (2.3%) while in Golden delicious the fruit damage at harvest has been 4.2% in the insecticide-treated area and 2.8% in the central area non-treated with chemical insecticides. During this year only a partially inhibition of the sex traps has been recorded as well as an increase of the number of larvae per cardboard-bands from 0.7, in 1991, to 2.5 in 1992. The variable results obtained in the two years of experimentation can be partially explained observing the graphs illustrating Checkmate CM's pheromone release rate analysed by Gas Chromatography (fig. 4 and 5). In 1991 codlemone has been consistently released, with a calculated rate of 11.7 mg/ha.h in the first application and 13.8 mg/ha.h in the second application. In 1992 the pheromone has been released very slowly, particularly in the first two weeks after the first and second application, with a calculated rate of 3 and 7 mg/ha.h respectively during these two periods. These value are below the minimum release rate of 10 mg/ha.h indicated by Charmillot (1987) and Audemard (1988). Then the release rate increased rapidly, particularly in the second generation, and the dispensers were even voided too early in respect to the second insect flight.

Conclusions

In the three Italian fruit-growing regions chosen for this test, the results of the two years experimentation have been variable, but on the whole rather positive. The fruit damages at the harvest have resulted below the economic threshold in the provinces of Torino and Sondrio, while in the Trento province the fruit damage in the second year experimentation has been higher.

The reason of this failure are probably due to the low release of codlemone with the fresh temperature during May. That has caused an increase of first generation population with a direct influence on the second one. Moreover, the pheromone release in the second application has not completely covered the summer flight. The preliminary results with the newly designed Consep "dual release area dispenser" tested in 1992 are encouraging. They indicate that with this dispenser a better codlemone release can be achieved also under cool spring temperatures (fig. 6).

In general, due to low rates, sometime below to the accepted threshold of 10 mg/ha.h, of pheromone released in the orchards during the trials, we believe that the mating disruption has operated through a "false-trial-following" (Arn, 1992; Bartell, 1982) rather than the sensory overload, that normally occurs when high rates of codlemone are used.

With regard to this, the results obtained recommend an implementation of the knowledge regarding the subtle relationships among the rates of synthetic pheromones released into the environment, the semio-chemicals surrounding the plants, and the receptivity of the individual, both males and females, belonging to the targeted species.

Figure 4 - Diffusion rate of pheromone from Checkmate CM assessed by GC-analyses in 1991.

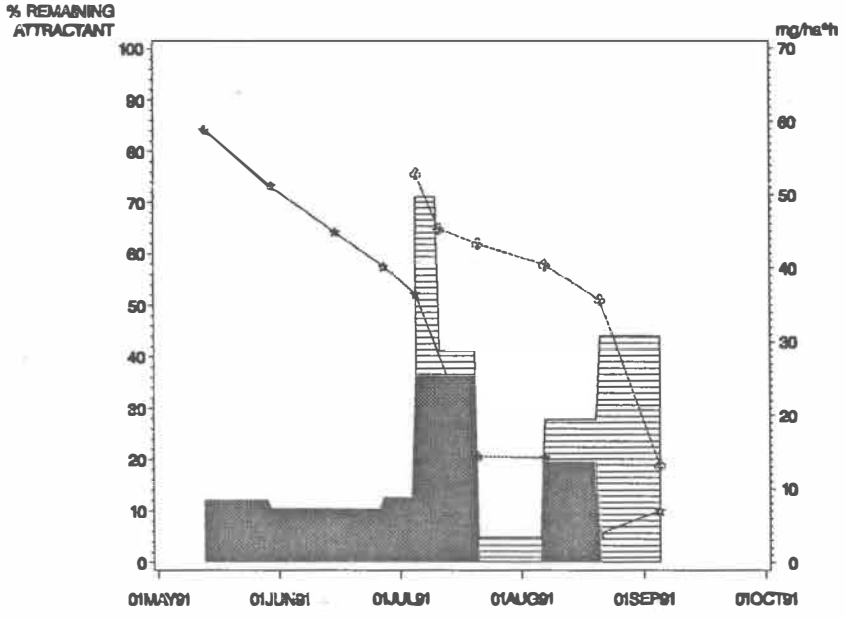


Figure 5 - Diffusion rate of pheromone from Checkmate CM assessed by CG-analyses in 1992.

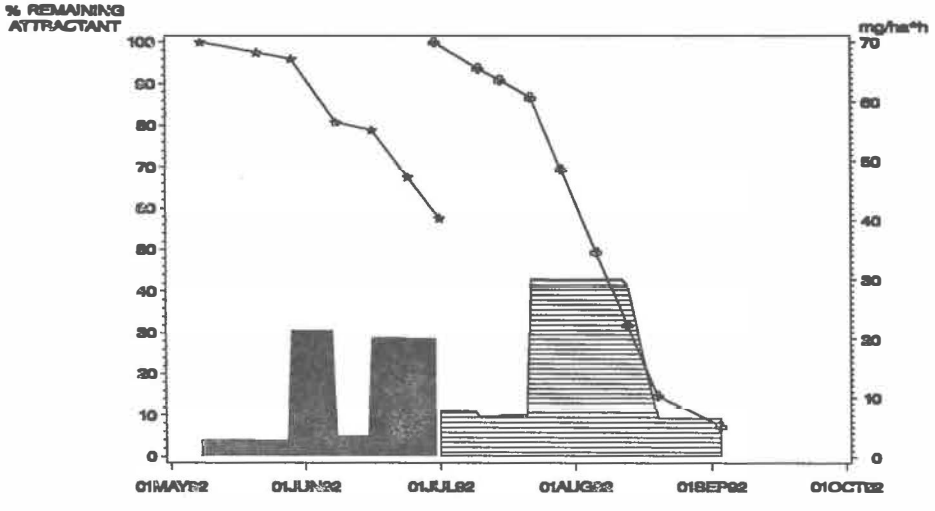
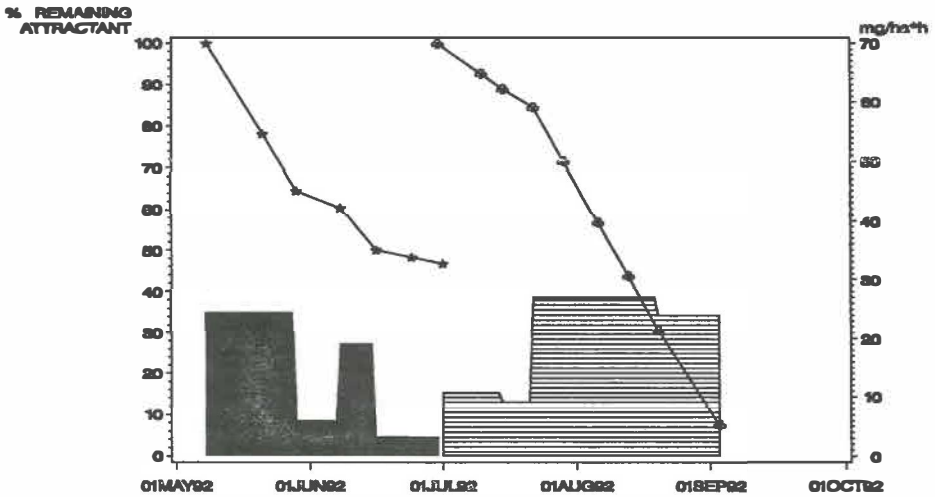


Figure 6 - Diffusion rate of pheromone from Checkmate CM 'dual release area dispenser' assessed by GC-analyses in 1992.



References

ARN, H., 1992. Mating disruption on its way to perfection: some thoughts. IOBC/WPRS Bulletin **XV/5**: 3-5.

AUDEMARD, H., 1986. Confusion sexuelle avec des phéromones en Europe de l'Ouest. Agric. Ecosyst. & Environ. **21**: 101-110.

BARTELL, R.J., 1982. Mechanism of communication disruption by pheromone in the control of Lepidoptera: a review. Physiol. Entomol. **7**: 353-364.

CHARMILLOT, J.P., 1987. La technique de la confusion sexuelle: un moyen spécifique de lutte contre le carpocapse *Cydia pomonella* L. Revue suisse Vitic. Arboric. Hortic. **19** (2): 129-138.

THE SYNTHETIC SEX PHEROMONE FOR STUDYING DISTRIBUTION, FLUCTUATION AND MONITORING THE ARMY WORM *SPODOPTERA LITURA* F. (LEPIDOPTERA: NOCTUIDAE) ON SOYBEAN

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Abstract Water trap with a synthetic sex pheromone as an attractant to the male moth of the army worm *Spodoptera litura* F. (AW) was used to study distribution, fluctuation of population and AW infestation on soybean. The synthetic sex pheromone was a mixture of cis-9, trans-11-TDDA and cis-9, trans-12-TDDA (Takeda Chemical Industries).

Observations from 1975 through 1979 revealed that AW distributed throughout Java island throughout the year. Population of the Bandung area (high elevation planted with mixed vegetation) was higher than that in four lowland areas with rice as the main crop. High population was observed during period June through August. Climatic factors did not influence fluctuation of the insect. With the increasing acreage of soybean in the recent years, AW becomes one among the major pests of soybean in Indonesia. Results from experiment done after 1989 revealed that area with a mixed vegetation such as in Kuningan (West Java), the number of trapped moths did not indicate population of the insect on soybean in the field. While at Sukamandi, there was a relationship between the number of trapped moth obtained from trap located on the ground with number of egg clusters observed in the field. A high number of moth catches indicated a high number of egg clusters at the same period. Even though the number of moths obtained from traps located at one or two meters above the ground was high, but it did not indicate population of the army worm in soybean crop the field. In area having rice-rice-soybean cropping pattern such area as at Sukamandi, controlling AW was mainly rely on insecticide. In such areas, the synthetic sex pheromone would be useful as a tool in monitoring the population of the AW.

Introduction

Army worm *Spodoptera litura* F. (AW) is a cosmopolitan insect and has a wide range of host plants. In Indonesia this insect is one of the insect pests of soybean. The acreage of soybean crop in increased in the recent years, the army worm became a major insect pest in soybean. As controlling AW is heavily rely upon insecticide application, a reliable monitoring method will be very useful in reducing insecticide application.

The occurrence and fluctuation of the AW in Java Island, was conducted by monitoring AW

by trapping male moth by. Male moth was trapped by a water trap with a synthetic sex pheromone as an attractant. Water trap, with 26 cm diameter and 26 cm height and sufficient hole in its side was used and placed on the ground. A small rubber dispenser containing 1 mg of a mixture of cis-9, trans-11-tetradecadien-1-01 acetate (= cis-9, trans-11- TDDA) and cis-9, trans-12-tetradicadien-1-01 acetate (= cis-9, trans 12-TDDA) (Takeda Chemicals Industries), was used as the synthetic AW female sex pheromone. The dispenser was kept about 15 cm above the surface of water of the trap. The bottom of the trap was filled with a solution of detergent. This paper summarised synthetic sex pheromone of AW studies at Sukamandi Rice Research Institute for Food Crops.

Distribution of the army worm

To study distribution and occurrence of AW in Java island two traps were kept in each of four locations, Banyuwangi (East Java), Kendal (Central Java), Bandung (Horticultural Research Institute, West Java), and Lebak (West Java). Four traps were kept in the farm of Sukamandi (West Java). The observation was done from August 1975 to March 1979, by counting male moths collected by traps every day.

AW population was high at Margahayu in Bandung Region where is a vegetable growing area, about 1200 m above the sea level, whereas the populations were low at four locations where are in lowland rice growing areas in Java (Figure 1). Though the number of moths collected fluctuated annually, locally, and seasonally, the populations in June to September were frequently higher than in other months. These studies showed that AW occurred even in the lowland rice growing areas throughout the year. Then, the AW commonly occurs at many locations in Java throughout the year, or migrate. Judging from a great number of eggs produced by a female, AW had a possibility to occur sporadically in large scales.

From data collected from those four years, the rainfall did not control fluctuation in populations of AW. However, at four locations other than Margahayu, the number of male moths collected was less then 50/10 days when rainfall was more than 100 mm/10 days.

To confirm that finding, experiment in monitoring AW population was done again in July 1983 up to July 1984 at Sukamandi and climatic factors was also recorded.

The fluctuation of AW population did not correlate with climatic factors such as temperature, humidity and rainfall (Figure 2). High catches of moths in August and September 1984 may possibly come from population surrounding the trap, since secondary crops such as soybean was planted in June to August. In October and November the field was fallow and low catches of moths. From May to June 1984 no catches of male moth even- though some soybean was planted the vicinity of the traps. There was also no record of damaged plant by AW during that period.

The effect of the height trap placement on male moth catches

Six pheromone traps were placed at varying heights namely 0, 1, 2, 3, 4, and 5 meters(s) above the ground. A set of the six traps was placed at Jatisari , Sukamandi and Pusakanegara. These places represented a typical lowland rice growing area under Jatiluhur irrigation scheme and distance among places were 15 to 40 km. The experiment was conducted in RCBD with the height at which the height of trap placement as treatment and locations as replication. Observation were done every five days on the number of trapped male moth.

Seven observations showed that traps placed on the ground caught the least number of male moths. The traps placed at 1,2,3,4, and 5 meter (s) above the ground caught more male moths.

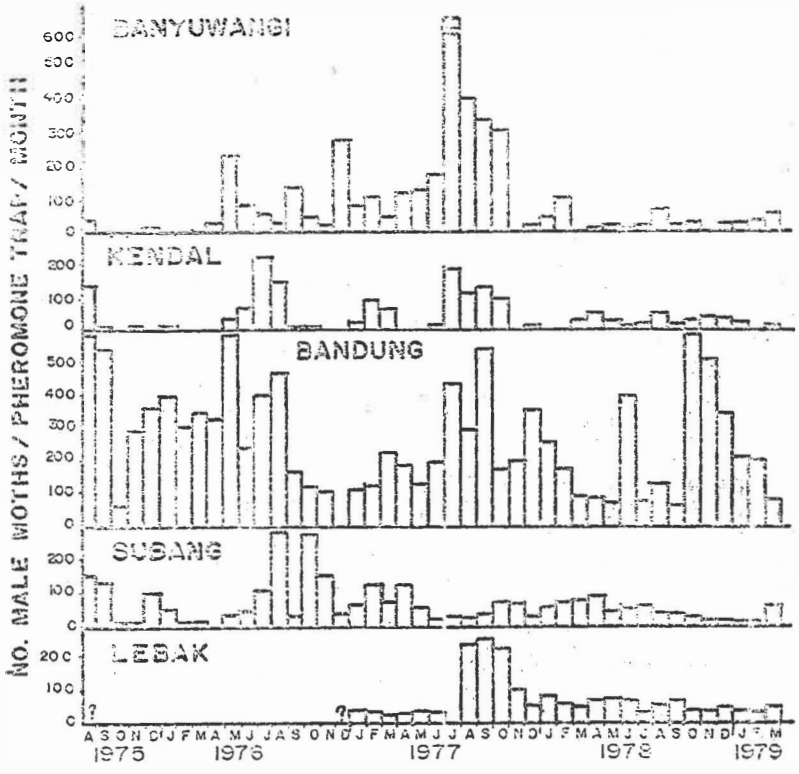

Fig. 1. Pheromone trap catches of S. litura male moths at five location in Java 1975 to 1979. Question mark indicates no data. (Source: Mochida et al 1979.

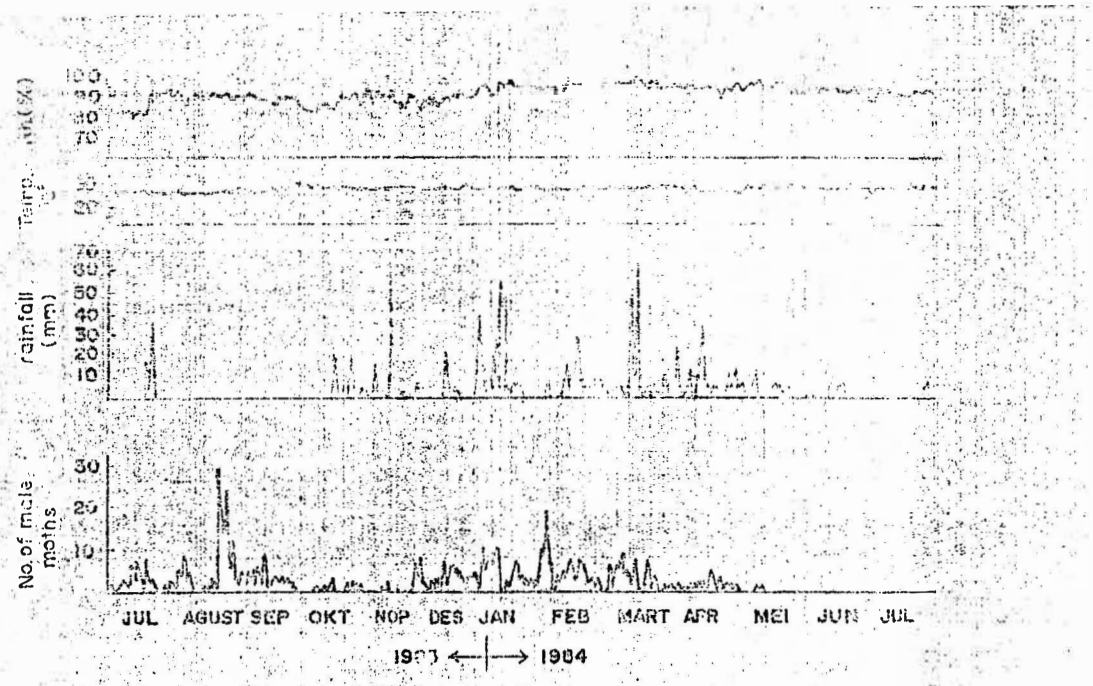

Fig.2. Number of male moth of S. litura, relative humidity, temperature and rainfall at Sukamandi from July 1983 to July 1984. (Source: Hendrisil et al 1988)

Traps placed on the ground may only catch the moths from nearby places. The highest number of catch were by traps placed two meters above the ground (Table 1). The number of male moths caught indicated both the populations of army worm in the field and also the migrant moths. It is likely that the male moths come from the field surrounding the traps.

The number of trapped moths at these three places did not differ significantly for each height of trap placement. Tbis indicated that those three places had similar cropping pattern and vegetation.

Table 1. Number of male moths caught by traps at six different heights from the ground, West Java, 1984

Height of trap above the ground	Average of the number of male moths/observation						
	I	II	III	IV	V	VI	VII
0m	1.00 b	1.25 a	1.25 b	0.50 b	0.25 c	0.50 d	5.50 bc
1m	4.00 ab	8.25 a	8.25 ab	2.55 ab	2.50 b	2.75 ab	13.0 0a
2m	12.2 5a	12.5 0a	17.5 0a	7.25 a	7.25 a	4.25 a	12.7 5a
3m	6.75 a	8.75 a	8.75 ab	3.25 ab	3.25 b	2.00 b	8.00 b
4m	2.30 ab	3.50 a	5.50 ab	0.75 ab	0.75 c	1.75 bc	4.00 cd
5m	4.00 ab	5.75 a	5.55 ab	3.00 ab	0.50 c	0.75 cd	2.25 d

[a] An observation was done within 5 days; Data was analysed after transformation V x +0.5; number followed with the same letter in a column indicates no significant difference at DMRT 0.05

The fluctuation of male moth catches and AW infestation in the soybean field.

To evaluate the sex pheromone as monitoring tool for AW population on soybean, experiment was done in Kuningan Experiment Station (Elevation 700 m, mixed vegetation) in year 1989. Similar experiment was done in consecutive dry seasons 1991 and 1992 on soybean field that planted after rice at Sukamandi (rice-rice- soybean cropping pattern). At each location AW male moth was trap by traps located at three different height from the ground, namely, 0, 1 and 2 m above the ground.

At Kuningan moth catches did not have any relationship with the occurrence of AW in the field. At similar experiment in the dry season 1991 at Sukamandi, AW infestation was high causing 40% leaf damage. Traps placed at 1 and 2 m above ground steadily caught many of moths (Figure 3). The traps placed on the ground caught a fluctuated number of moths. There was no egg on plant older than 6 weeks. This indicated that the early infestation of AW was from migrating moths and chose young plant and they did not produce second generation at the same cropping. High moth catch coincided with a higher number of egg clusters in the field. In dry season 1992, the AW

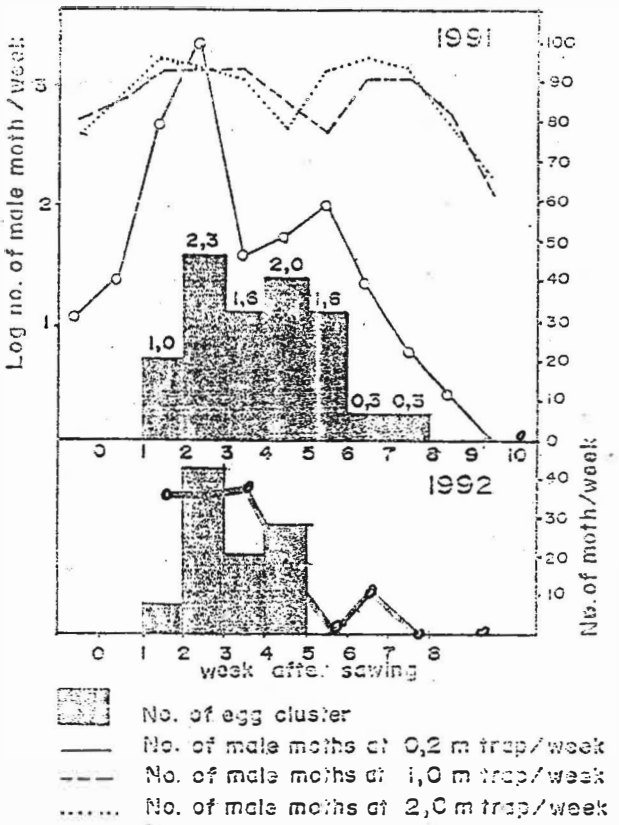

Figure 3. The relationship between number of trapped male moths and number of egg clusters of army worm *Spodoptera litura* F. on soybean, Sukamandi 1992. (Source: Hardaniel 1992).

infestation was low and leaf damage was less then 5%. Moth catches did not show any relationship with number of egg.

The AW infestation on soybean was influenced by many factors especially by cropping pattern, vegetation and age of plant.

Calculating from a regression equation, if a trap caught 65 moths/week at crop stage less then 6 week old plant, the number of egg clusters in the field reaching economic injury level. While the capture was less than 38 moths/weeks no serious infestation in the field.

Conclusion

Studies showed that the synthetic female sex pheromone for the army worm *Spodoptera litura* F, a of mixture cis-9, trans-11- TDDA and cis-9, trans-12-TDDA was very effective in attracting male moth. Using water traps with this lure as an attractant in monitoring AW resulted in:

1. AW was distributed though out Java including rice growing area, the population in mixed vegetation area was higher than that rice growing area.
2. Climatic factors did not influence fluctuation of AW, however, the cropping pattern was more significant in influencing AW fluctuation.
3. Traps which were placed on the ground caught the least number of male moths, as the height placement of trap increased the number of male moth captured increased reaching the highest number at 2 m above the ground. Number of captured moth decreased at those traps located higher than 2 m.
4. No correlation between moth catch by trap placed at 1 and 2 m above the ground with AW occurrence in the soybean field. Cluster of egg of AW was found as early as two week old soybean plant and the peak of number of egg cluster was on 5 week old plant. Traps placed on the ground showed a relationship with number of egg clusters up to 5 week old plant. This relationship only occurred on soybean in a district rice- rice-soybean cropping pattern, while in mixed vegetation area did not.

References

HENDARSIH SUHARTO, INDAH N.P AND ABDULLAH, 1988. The Evaluation of the Synthetic Sex Pheromone of *Spodoptera litura* F. For Monitoring the Army Worm Population. In Proc. of the Symposium on Pest Ecology and Pest Management, Bogor Indonesia. 9-12 April 1985. Biotrop Special Publication no 32. SEAMEO. BIOTROP.

HENDARSIH SUHARTO AND INDAH N.P., (1990). The Use of Sex Pheromone Trap in Predicting Army Worm Infestation. (*In* Indonesian). Seminar in 1989/1990 SURIF Research Report, Sukamandi 3-6 September 1990, 7pp.

HENDARSIH SUHARTO, 1992. The Synthetic Sex Pheromone for Monitoring the Army Worm *Spodoptera litura* F on Soybean in Rice Soybean Cropping Pattern.(in Indonesian, unpublished)

INDAH N.P, A. RAHAYU AND O. MOCHIDA. 1979. A Preliminary Report on the Bionomic, Occurrence and Soybean Yield Reduction Analysis in *Spodoptera litura* (Fabricivs. (Lep; Noctuidae) an Insect Pest on Legumes in Java. Paper Presented at the Seminar at CRIA - IRRI. December 19, 1979. mimeograph 25 pp

USE OF INSECT SEX PHEROMONES IN CHINA

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Abstract. In China systematic research on insect pheromones began in the early 1970's. Up to now, more than 20 insect sex pheromones of important species have been studied. Major research interests are focused on the structural elucidation, synthesis and application in pest management programs.

Insect sex pheromones have been used extensively in pest monitoring throughout the country and great benefits have been gained. Experiments by using sex pheromone for direct control of some fruit pests, sugarcane borers, some cotton and forest pests, have been conducted with success.

Sex pheromones of the following pests, such as *Ostrinia furnacalis* Guenee, *Ancylis sativa* Liu, *Parathrene tabaniformis* Rotenberg, *Chilo infuscatallus* Snellen, *Pectinophora gossypiella* Saunders, *Scirpophaga incertulas, Chilo suppressalis, Chilo venosatus, Dichocrocis punctiferalis* Guenee, *Carposina niponensis, Grapolitha molesta, Heliothis armigera, Heliothis asaulta, and Plutella xylostella*, have been used more popularly in pest management programs.

Monitoring Application

In China, several research institutes from the Chinese Academy of Sciences, the government institutions and universities have set to deal with structural identification, synthesis and trapping tests of the sex pheromones of the major pests since the early 1970's, and put into field trapping tests throughout the country. There were all together more than 20 sex pheromones of important species have been synthesized, most of them are used as monitoring tools in pest management program. High activity, high specificity, harmless, simplicity in use and comparatively low costs are all the advantages offered by pheromone monitoring systems. It has been extended to large areas, and great benefits have been resulted. Especially, the pheromone monitoring systems in fruit tree pests have been accepted by the fruit growers. Fruit orchards have their own forecasting systems, including to use pheromone traps as the major tools. Chemical pesticide usage can be cut down about 50%, due to grasping the correct spraying time by using the pheromone traps as monitoring devices. Thus the efficiency of chemical control can be improved and environmental pollution greatly decreased (Liu, 1986, 1989).

Control Application

In China, use of insect sex pheromones for direct control of pests is still in the stage of experimental tests. However, there are some success in a few species.

Forest Pests

Sex pheromones of forest pests, such as *Dendolimus punctatus, Lymantria dispar* and *dioryctria rubella* have been developed. In control application, the unique example is the sex pheromone of popular clearwing moth, *Parathrene tabaniformis,* which has been used successfully as controlling measure in artificial regeneration forest by mass trapping in wide areas in North China. After treating with mass trapping technique for 3 consecutive years in large areas, this pest can be controlled by reducing its density to 1%(65-80% reduction). The popular clearwing moth has one generation per year, its sex ratio of females to males is 0.92-1.0, the males mate only once in their life time. The population densities were relatively low in the experimental areas. Under this circumstances mass trapping seems to be very effective. More than 100 thousand hectares of forest has been treated per year (Du et al., 1984, Miao et al., 1985).

Fruit Tree Pests

Fruit borers and leafrollers are serious pests in North China, attacking on apples, pears, chestnut. 6-8 times of pesticide sprays should be applied per year. For better qualities of fruit production it is needed to use sex pheromones instead of conventional insecticides for controlling these species. Experiments on mass trapping for controlling *Grapholitha molesta* (Meng et al., 1981, 1985), *Carposina niponensis* (Liu et al., 1988a), *Dichocrocis punctiferalis* (Liu et al., 1988b), *Phyllonorecter ringoniella* (Su et al., 1992) and *Acylis Sativa* (Li, L.-C. et al., 1988) in pear, apple, jujube and chestnut orchards have been conducted for many years. Usually 45-60 traps (water bowl traps) were put per hectare. Under low population conditions, pesticide sprays can be saved. If the population was high it is better to use the combination of pheromone traps and one or two pesticide treatment. Notable results were achieved on oriental fruit moth in an area of 10,000 hectares. Mating disruption was also successful in controlling *Grapholitha molesta* (Meng et al., 1988) and *Carposina niponenesis* (Liu et al., 1990a) by using pheromone dispensers.

Sugarcane Borers

Sugarcane stem borers cause great damage in sugarcane production in South China. Sex pheromones of *Chilo infuscatellus* (Wu et al., 1984, Wu, 1988), *Chilo vensatus* (Wu, 1982) and *Scirpophaga excerpalis* (Wu et al., 1990, Liu et al., 1992) have been developed. Mating disruption techniques have been developed for controlling of *C. venosatus* in sugarcane fields in Guangdong Province and were used as the major controlling measure for many years. When 7500 polyethylene pheromone tubes were applied per hectare (3.75-4.50 g of active ingredients per hectare), the mating rate was reduced by 70-85% and the damage could be reduced by 70-80%. The cost of mating disruption treatment was compatible with that in conventional chemical treatment areas (Li, 1991).

Cotton Pests

There are two major cotton pests in China: cotton pink bollworm, *Pecyinophoroa goosypiella,* in East and Central China, and oriental cotton bollworm, *Heliothis armigera,* in North China. Tests for controlling cotton pink bollworm have been conducted for many years either by mass trapping or by mating disruption with domestic or foreign pheromone preparations (Cao et al.,

1988, Li et al, 1982, Xie, J.-L. et al., 1993). Results were positive, but until now practical application remained to be solved partially because of small farming and varieties of pest species. Recently, Oriental cotton bollworm became a serious pests and pesticide resistant increased rapidly in North China. Scientists are trying to control this species by the integration of sex pheromone mass trapping and chemical or biological agents(Xie, B.-Y., 1993)

Vegetable and other Pests

Diamondback moth, *Plutella xylostella,* is the most widespread vegetable pest in China, especially in the South, where 20-30 generations were occurred per year. Heavy insecticide spraying caused negative effect on qualities of products and the environment. Sex pheromone has attracted attention in this species as an alternative of conventional pesticides(Liu, X., 1987, Chen, X., 1990). *Heliothis assulta* is another vegetable pest in China. It attacks green pepper as well as tobacco. Field tests on sex pheromone of this species have been conducted recently (Cai et al., 1992). Field experiments on sex pheromones of *Ostrinia furncalis*(Li, W.D. et al., 1988), *Mythimna separata* (Liu et al., 1985) and *Chilo, suppressalis* (Kong et al., 1990) were reported.

Conclusion

1. Pheromone traps are good tools for monitoring pest population and indicating for other control measures. It has been acknowledged by farmers and growers.
2. Most of the pheromone preparations used in controlling insects are still in experimental stages. The complicity in evaluation of the efficiency and the high cost of preparations are the main factors hindering their development.
3. We should pay more attention to the integration of the pheromone preparations with other measures.
4. The demand for green food (ecological or organic farming) and the request for reducing the amount of pesticides certainly could accelerate the development of the pheromone technology.

References

CAI, J.-P. and LIU, M.-Y., 1992. Field trapping tests of sex pheromone of oriental tobacco budworm, *Heliothis assulta* Guenee, Sinozoologia, **9**:11-15.

CAO, C.-Y. and SHU, C.-E., 1988. The strategy of applying Gossyplure in controlling pink bollworm, *Pectinphora gosspiella* Saunders, on cotton. China-Japan Seminar on Insect Semiochemicals (Abstract) 1988, Beijing, P56-61.

CHEN, X.-G., LI, Z.-B., LIU, X., KONG, J., ZHANG, Z.-N., & LOU, Z.-X., 1990. Field application of synthetic sex pheromone of the diamondback moth *Plutella xylostella*, in monitoring, mass trapping and mating disruption. Sinozoologia, **7**:17-23.

DU, J.-W., XU, S.-F., DAI, X.-J. & ZHANG, X., 1984. Field test on controlling popular clearwing moth, *Paranthrene tabaniformis* Root, by mass trapping. Contr. Shanghai Inst. Entomol. **4**:53-58.

KONG, J., LOU, Z.-X., LIU, X., PAN, Y.-C., MENG, X.-Y., LI, Z.-P. & HU, G.-W., 1990. Field attraction of the female sex pheromone to rice stem borer, Chilo suppressalis, Sinozoologia **7**:31-34.

LI, J.-L., 1991. Control of striped sugarcane stem borer, *Chilo venosatus* Walker, by mating disruption with sex pheromone. Presented at the First Meeting on the Chinese Society of Chemical Ecology. Shanghai, China.

LI, L.-C., LI, L.-Z., Duan, H.-Y., WU, S.-N. & ZHUO, M.-J., 1988. Study on the application of the sex pheromone of *Acylis sativa* Liu. China-Japan Seminar on Insect Semiochemicals (abstracts) 1988, Beijing, P75-78.

LI, W.-D., LI, W.-G., CHEN, S.-S., ZHU, P.-Q., YU, G.-X. & ZHANG, S.-L., 1988. Mating disruption with synthetic sex pheromone for controlling the Asia corn borer, *Ostrania furnacalis* Guenee. China-Japan Seminar on Insect Semiochemicals(abstracts), 1988, Beijing, P146-151.

LI, W.-G., LUO, Z.-Y., JENKINS, J. W. & DOANE, C. C., 1982. Demonstration of disrupting mating communication for controlling *Pectinophora gossypiella* Saunders with Nomate PBW. Contr. Shanghai Inst. Entomol., **3**: 1-8.

LIU, M.-Y., PAN, H., WU, D.-M., SU, R.-H. & MENG, X.-Z, 1985. Attractiveness of synthetic sex attractant to male moth of armyworm, *Mythimna separata*. Chinese Journ. Biol. Control, **2**:1-5.

LIU, M.-Y., 1986. Insect pheromones and their applications in integrated pest management. Selections from the Bulletin of the Chinese Academy of Sciences, 282-287.

LIU, M-Y., MENG, X.-Z., YAN, Z.-C., WANG, Y.-H., YE, M.-X. & CUI, D.-N., 1988a. Field trials of controlling peach fruit moth, *Carposina niponensis*, by mass trapping. Ecomones. No. **1**: 30-31.

LIU, M.-Y., MENG, X.-Z., YANG, Z.-Y., SONG Q.-X. & LU, J.-W., 1988b. Field trials of controlling yellow peach moth, *Dichcrocis punctiferalis*, by mass trapping. Ecomones, No.**1**:32-33.

LIU, M.-Y., 1989. Insect pheromone research and its application in China. Proceeding I, The First Asia-Pacific Conference of Entomology (APCE), November 8-13, 1989, Chiang Mai, Tailand, P142-150.

LIU, M.-Y., SU, X.-P., YAN, Z.-C. & YAO, Y. 1990a. Control of peach fruit moth, *Carposina niponensis* (Lep.: Carposinidae) by mating disruption with sex pheromone. Chinese Journ. Biol. Control. No. **2**: 184.

LIU, M.-Y., MENG, X.-Z., MENG, Q.-X. & LI, S.-X, 1990b. Field trials of the sex pheromone of yellow peach moth, Dichocrocis punctiferalis. Sinozoologia, **7**:1-5.

LIU, M.-Y., YAN, Y.-H., CAI L.-M., CHEN, Y. & YANG, C., 1992. Field trapping tests on synthetic sex pheromone of white sugarcane stem borer, *Scirpophaga rxcerpalis*. Chinese Journ. Biol. Control. **8**:68-61.

LIU, X., KONG, J., ZHANG Z.-N., PICKETT, J. A., PAN, Y. C. & MENG, X.-Y., 1987. Field attraction of the photosensitive propheromone to diamondback moth, *Plutella xylostella*. Acta Ecol. Sinica, **5**: 15-9.

MENG, X.-Z., HU, J.-H., LI, P.-S., GUO, R.-H. & LI, Z.-S., 1981. The influence of different dispensers on the attraction efficiency and duration of sex pheromone traps of the Oriental fruit moth. Acta Entom. Sinica, **24**:332-335.

MENG, X.-Z., WANG, Y.-H. & YE, M.-X., 1985. Field trials on mass trapping for the control of the Oriental fruit moth with synthetic sex pheromone. Acta Entom. Sinica, **28**:142-147.

MENG, X.-Z, WANG, Y.-H. & CUI D.-N., 1988. Mating disruption trials with synthetic sex pheromone for control of Oriental fruit moth, *Gropholitha molesta* Busck. Sinozoologia, **6**:19-23.

MIAO, J.-C., JIANG, R.-H., XU, B.-R., HAN, W.-X., SU, G.-Q., LI, W.-H. & DENG, L.-W., 1985. Experiments on sex pheromone of popular clearwing moth, *Paranthrene tabaniformis*, and technique of application. Journ. of North-East Forestry University, **13**:123-127.

SU, R.-H., LIU, M.-Y., QIU, T.-D. & CHEN, H.-J., 1992. Synthesis of the sex pheromone components and analogs of apple leafminer moth, *Phyllonorycter ringoniella*, and field trials. Sinozoologia, **9**:1-10.

WU, D.-M., & LI, J.-L., 1982. EAG analysis and field attractant effect of the sex pheromone of the striped sugarcane borer. Act Entom. Sinica, **25**:358-362.

WU, D.-M. , YAN, Y.-X., Wang, B.-H. & Huang, H.-L., 1984. Structure and field attraction effect of the sex pheromone of sugarcane stem borer, *Chilo infuscatellus* Snellen. Acta Entomological Sinica, **27**: 368-374.

WU, D.-M., 1988. The reliability in forecasting the sugarcane stem borer, *Chilo infuscatellus* by using its sex pheromone. Sinozoologia, **6**:13-18.

WU, D.-M., YAN, Y.-X., CUI, J.-R., LIU, M.-Y., REN D.-F., CHEN, Y., & ZHANG, Q., 1990. Sex pheromone of sugarcane tip borer *Scirpophaga excerpalis* Walker. Chinese Scien. Bull.. **35**:1469-1473.

XIE, B.-Y., 1993. Personal communication.

XIE, J.-L., CHEN, Y.-G., ZHANG L. & TANG, X.-H., 1993. The research on the forecasting models of pink bollworm with the sex pheromone traps. Chemical Ecology I (Proceedings of First Meeting of the Chinese Society of Chemical Ecology). edited by Chen Yuan-guang et al. Shanghai Science and Technology Publishing House. P1-4.

CHEMICAL MEDIATION OF COMMUNAL OVIPOSITION IN THE RIVER BLINDNESS VECTOR *SIMULIUM DAMNOSUM* S.L. IN SIERRA LEONE

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Abstract The phenomenon of aggregated oviposition in blackflies (Diptera:Simuliidae) was investigated under controlled laboratory conditions within an endemic onchocerciasis area in Sierra Leone, using wild populations of the local vector species, *Simulium damnosum* s.l.. A bioassay was developed and in a series of two-choice tests showed that significantly more gravid flies chose oviposition substrates already containing eggs over control substrates (91% of ovipositing flies chose the substrates with eggs already present; $p = 0.004$). The time from introduction of gravid flies into the oviposition system to the onset of egg-laying was significantly reduced when eggs were already present ($p = 0.049$). Furthermore, the relationship between egg-batch number and the time of this response was curvilinear, with flies laying eggs sooner when more eggs were present ($p = 0.012$). The volatile compounds from the eggs have been trapped using a closed volatile collection system and when assayed, this was also found to be significantly more attractive to ovipositing flies than the relevant control ($p = 0.036$), indicating that aggregation during oviposition in blackflies is at least in part, pheromonally mediated.

Introduction

Blackflies (Diptera: Simuliidae) are the vectors of onchocerciasis or river blindness, a chronic disease caused by the filarial nematode *Onchocerca volvulus*, which is found in Africa, Central and South America and in the Yemen, and which infects about 18 million people, mostly in Africa where its major vectors are blackflies within the *Simulium damnosum* s.l. species complex. Control of the disease is effected either by vector control (involving insecticide spraying of the rivers where the aquatic filter-feeding larvae occur) or more recently by mass treatment of the human population with the drug ivermectin, which will limit the progress of the infection. Monitoring of wild fly populations for estimates of disease transmission levels still relies on the collection of flies from human bait, a procedure which is often unreliable and which can expose the catcher to infection, but for which a more reliable alternative has not yet been found. For this reason we became interested in the phenomenon of aggregated oviposition in blackflies, since it represented a stage in their life-cycle which might render them vulnerable to mass trapping. Crosskey (1990) has reviewed all aspects of oviposition in these flies. In those species which aggregate, the gravid females assemble in large "swarms" at dusk, close to a favourable site (usually near some trailing vegetation or partially submerged stones in a region of turbulent river water), before landing and laying their eggs

simultaneously on a limited number of substrates, producing egg masses of up to 1cm thick and consisting of up to half a million eggs on one substrate. Walsh (1984) showed that this behaviour was not the result of a limitation of suitable sites but an attraction to the eggs themselves. Here we report on a series of controlled laboratory based studies on the responses of wild gravid flies to conspecific eggs and to egg volatiles.

Materials And Methods

Flies:

The experiments reported here were carried out at the British Medical Research Council Laboratory at Bo, Sierra Leone, during November and December, 1992. Wild flies were collected in aspirators from human bait at the riverside, and allowed to engorge on domestic pigs. The flies were then maintained in the dark at 23-30^{0}C and 75-100%RH, and fed on 20% sucrose supplemented with antibiotics (penicillin, streptomycin @ 200 units/ml, nystatin @ 100 units/ml), for 3 days until used in the bioassays. Of the 5 cytospecies of *S. damnosum* s.l. found within this area at this time of year, over 90% are *S. soubrense* 'B', and the remainder are *S. squamosum* and *S. yahense* (Davies *et al.*, 1988).

Bioassays:

Bioassays were carried out by allowing the gravid flies to choose between 2 substrates set 6cm apart (one with the test attractant, and the other the appropriate control), in a net cage with a polystyrene floor and top, 16 x 16 x 10cm. As a crude simulation of the natural conditions preferred by blackflies for oviposition, each substrate consisted of a nylon netting disc (4 cm diam.) stretched taut over a glass funnel and set flush into the floor, with water dripping onto each disc (20ml/min). Each substrate had a separate water system and the whole setup was lit only from beneath through the substrate netting. Flies laid eggs directly onto the substrate. In the bioassay procedure, 20 flies were introduced into the chamber and allowed 20 minutes to choose a substrate and lay their eggs. They were then removed, the relative positions of the substrates reversed, and 20 more flies introduced for a further 20 minutes. A choice was recorded only when oviposition began on either substrate. In addition, the time elapsed from introduction of the flies into the bioassay chamber to the first oviposition, and the number of egg batches present when each group of flies was introduced was recorded for every group of 20 flies.

Volatile Collections and Analyses:

Volatile compounds were collected using a system based on that described by Turlings *et al.* (1991). Air was purified with an in-line activated charcoal filter, before flowing through a glass sinter over the test material within a closed Pyrex glass container. The air exited the system through a collection trap, consisting of a 4cm length of glass tube (6mm OD), packed with 25mg Super Q adsorbent (80-100 mesh; Alltech, Deerfield, Illinois). The components of the system in contact with the test material consisted solely of the glass containers, teflon tubing and brass fittings, to facilitate cleaning. Collection traps were rinsed with 200µl dichloromethane and a fresh aliquot of 50µl (equivalent to 3-5 egg-batch volatiles/ hour) used as attractant at each bioassay stage.

Results

Responses to Conspecific Eggs:

Of the total of 55 ovipositions (22.9% of 240 flies tested) obtained in 6 assays (Table 1), 50 were on the substrates already with eggs, a result which was highly significant (p=0.004, Wilcoxon signed-rank test). In addition, the elapsed time after entry to the chamber before oviposition was significantly shorter (p= 0.049, Mann-Whitney test), if eggs were already present on substrates (mean time to first oviposition= 4.95 mins, SD= 5.54) than if no eggs were present (mean time= 22.8 mins, SD= 20.8). As Figure 1 shows, the relationship between egg-batch number and the time of this response was curvilinear (p= 0.012), with flies laying eggs earlier when more eggs were present.

Responses to Egg Volatiles:

As shown in Table 2, the volatile collections from the eggs also attracted significantly more ovipositions than a control substrate (16 *v.* 3; P= 0.036), using the same bioassay procedure, though the overall responses were lower than with whole fresh eggs, with a mean of 15.8% of flies ovipositing in the three tests. It was noted that the other gravid flies would land on the substrate baited with egg volatiles but not lay, indicating that a further oviposition cue may have been absent (McCall, unpublished).

Table 1. The responses of gravid flies to a choice of substrates with or without fresh eggs already present, showing the numbers of flies ovipositing on either substrate, over 6 assay repeats, and the proportion of flies ovipositing in each assay.

Assay no.	eggs present	eggs absent	% ovipositing
1	8	3	27.5
2	10	0	25
3	6	1	17.5
4	11	1	30
5	4	0	10
6	11	0	27.5

Figure 1. The effect of the number of initial egg batches on the speed of response of gravid flies (time to oviposit = time from introduction into the chamber until the first oviposition (y= 1.04-0.117x; p= 0.012, 16df).

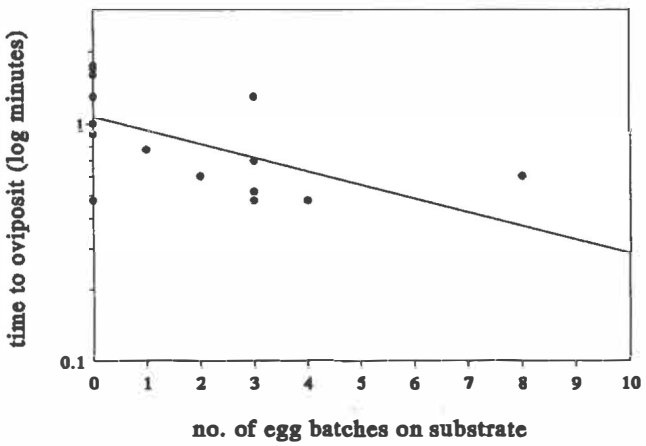

Table 2. The responses of gravid flies to a choice of substrates baited with egg volatiles or volatile control, showing the numbers of flies ovipositing on either substrate, over 3 assay repeats, and the proportion of flies ovipositing in each assay.

Assay no.	egg volatiles	control	% ovipositing
1	5	1	15
2	7	2	22.5
3	4	0	10

Discussion

These results clearly show that *S. damnosum* s.l. preferentially select substrates where eggs have already been laid. The ability of the volatile compounds from the eggs to also attract gravid flies and to partially reproduce this behaviour suggests that the behaviour is at least in part controlled by a pheromone derived from the eggs, though its reduced activity in comparison to intact eggs suggests that other cues, possibly visual, tactile or non-volatile chemical cues, may also be involved.

Initial gas chromatographic analyses show a limited number of compounds present in the egg volatile collections, and studies are underway to determine the specific role of these compounds in mediating communal oviposition. Clearly the identification of any attractants, especially those involved in the mediation of a susceptible behavioural strategy, would be of great use in the development of an effective trap for this important vector.

References

CROSSKEY, R.W. 1990. The Natural History of Blackflies. pp. 711, John Wiley & Sons.

DAVIES, J.B., P.A. BEECH-GARWOOD, M.C. THOMSON & J.E. MCMAHON. 1988. Onchocerciasis transmission levels and *Simulium damnosum* complex biting activity at riverside and rice field sites in Sierra Leone. Med. Vet. Entomol. **2**: 357-369.

TURLINGS, T.C.J., J.H. TUMLINSON, R.R. HEATH, A.T. PROVEAUX & R.E. DOOLITTLE. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. J. Chem. Ecol. **17**: 2235-2251.

WALSH, J.F. 1984. Aspects of the biology and control of *Simulium damnosum* s.l. (Diptera: Simuliidae) in West Africa. Ph.D. Thesis, Salford University.

MEASUREMENTS OF THE PHEROMONE DENSITY IN COTTON FIELDS AND VINEYARDS BY EAG-METHOD.

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Abstract When experimenting with mating disruption, it is important to know about the average density and distribution of the pheromone generated by the dispenser system in use. In addition, the interaction between plant surfaces and pheromone may modify the total pheromone density and distribution considerably (Karg et al. 1990). Standard chemical methods to measure airborne densities of the order of ng/cubic meter need a long sampling time and do not permit to follow the change in climatic conditions occurring continuously.

We have used a measurement system based on the quantitative evaluation of Electroantennogram signals (Sauer et al. 1992) to measure the densities of the pheromone of the Wine Grape Moth (*Lobesia botrana*) in vineyards and of the Pink Bollworm (*Pectinophora gossypiella*) in cotton fields.

The measurement system consists basically of an antenna mounted in a special holder, contained in an enclosure which is constantly flushed with ambient air moved by a suction pump. For calibration, the incoming air is filtered using a charcoal filter, and pulses of pheromone with known density are applied, generating calibration EAG responses. When the filter is opened, the pheromone content of the air produces EAG responses which after suitable evaluation yield pheromone density values. At present, density results can be given in values relative to a reproducible standard generated by mixtures of pheromone in silicon oil.

In this contribution, two sample measurements are shown: Measurements in vineyards show the dependence of pheromone density on the height of the measurement probe: In positions higher than the leave's canopy (2m), the pheromone density decreases gradually, but in a height of 4m, there is still 10% of the value measured at 1m.

The measurements in cotton fields were taken in a series taken over a large part of the total cotton season. The individual points show almost constant pheromone densities and height profiles, indicating long-lasting and stable pheromone diffusion by the dispensers. In one set of measurements however, the recorded pheromone density was about 6 times below its neighbouring values. The reason for this was strong winds (>5m/s) which did not permit the formation of a high density pheromone atmosphere.

This system can be easily adapted to measure pheromones of other species, provided that a suitable holder for the corresponding antenna can be made.

References

KARG, G., SAUER, A.E. and KOCH, U.T. 1990 The influence of plants on the development of pheromone atmospheres measured by EAG-method. In: N. Elsner (ed.) Proceedings of the 18th Göttingen Neurobiology Conference, Thieme Verlag, Stuttgart, New York, p. 301.

SAUER, A.E., KOCH, U.T., DE KRAMER, J.J. and MILLI, R. 1992 A portable EAG system for the measurement of pheromone concentration in the field. Chemical Senses **17**(5): 543-553.

'isolated-traps' ratio at the distance which approximates to the active-range of the upwind lure. At this point the plume from the upwind trap no longer overlaps the downwind trap, at greater distances the value of D/U should remain constant. Observation of the variation of the D/U ratio with inter-trap distance should thus enable estimation of the active-range of the *upwind* lure. In practice data interpretation should be easier if a relatively weak upwind lure is used, as the isolated-traps ratio then exceeds unity and deviations from it are more clear-cut.

The isolated-lures ratio for doses used in the experiment is required. Accordingly, experiments to compare the catches using lures of varying initial loadings (doses) were conducted. Video observations in the field and in a wind-tunnel have previously shown that overshooting is a real phenomenon with *S. littoralis* (Downham, unpub. data).

Materials And Methods

Polythene vials (20 x 9 x 1.5 mm thick) were loaded with a 99:1 mixture of Z,E-9,11-tetradecadienyl acetate (Z,E-9,11-14:Ac) and Z,E-9,12-14:Ac respectively. Yellow, plastic funnel traps (Critchley and El-Dieb, 1981) were placed in cotton fields in Sinnuris District of Fayoum governorate, Egypt. Counting and replacing of lures was carried out on a daily basis.

In a series of dose-response experiments 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg doses were compared in traps with a minimum separation of 200 m. Data were transformed to $\log_{10}(x+1)$ values before analysis of variance. Using the data for doses common to different experiments the results were normalized to generate a set of comparable means.

For the active-range experiment pairs of traps were aligned with the mean wind-line (from 22.5°). Inter-trap spacings were 15, 30, 60, 100 and 150 m (4 or 5 replicates per spacing). Trap-pairs were separated by a minimum of 100 m across-wind or 300 m along the wind-line. Based on the results of the dose comparisons 0.003 and 0.01 mg lures were placed in the upwind and downwind traps respectively.

Only trap-catch data from nights in which, during the peak catching period (midnight to 04.00), wind-speed did not fall below 1.5 m/s and the four hourly estimates of mean wind-direction did not depart from the ideal bearing of 22.5° by more than 25° were considered admissible. A Lambrecht wind-recorder was used to provide continuous wind-speed and -direction data. For analysis of trap data the D/U ratios were calculated for each trap-pair, and these transformed to $\log_{10}$ values before analysis of variance.

Results

Details of the normalized means from the dose-response comparisons are given in Table 1.

Table 1: Mean transformed catches from the dose-response comparisons normalized for comparability

Dose (mg) per lure	Transformed catch (normalized)
0.001	0.411
0.003	0.990
0.01	1.429
0.03	1.584
0.1	1.791
0.3	1.942
1.0	1.878

The slope of the dose-response curve at the higher doses was shallower than at the lower doses. This implied either a direct inhibition of catch or that a large part of the pheromone plumes from the higher dose lures lay outside crop areas - where no moths were present. 0.001 mg was too weak to provide reliably adequate catches, hence the choice of 0.003 and 0.01 mg doses for the active-range experiment. The difference in transformed catch was 0.439 - the isolated lures ratio for these doses. This equated to an actual catch ratio of 2.75.

In the active-range experiment 3 nights satisfied the criteria for wind-direction and wind-speed, 3 were unacceptable and 2 close to acceptable. Analysis of variance showed that there was no significant effect of spacing on the D/U ratio, for the combined data of the three acceptable nights (P=0.33), or for the three unacceptable nights combined (P=0.31). However the trends differed somewhat. Figure 1 shows that for the set of acceptable data the mean D/U ratios rose steadily from 15 m up to 100 m with a slight dip at 150 m. The $\log_{10}$ ratios 0.183, 0.329, 0.382, 0.487 and 0.437 equated to actual ratios of 1.53, 2.13, 2.41, 3.07 and 2.70 at 15 - 150 m respectively. The data for the three unacceptable nights (Figure 2) lacked any interpretable trend. Mean catch ratios were 2.61, 1.50, 1.83, 2.88 and 3.19 at 15 - 150 m respectively.

Discussion

The data for the unacceptable nights can be viewed as a check for the assumed model of active-range and pheromone response. Wind 90° to the axis of the trap-pair (i.e. "perfectly unacceptable") would produce negligible overlap of pheromone plumes, and so probably result in values close to the isolated lures ratio at all spacings. Erratic or little wind should also produce no clear trend. In fact, whereas the acceptable nights' data produced a rising trend in mean D/U ratios, no such trend was observed for unacceptable nights. This supports this method of estimation of active-range, though confidence in the result would be improved if the effect of spacing in the analysis of variance of acceptable nights' data had been significant.

The isolated lures ratio of 2.75 was intermediate between the D/U ratios for 60 and 100 m from the acceptable nights indicating that the active-range of the 0.003 mg vial lay between these distances. Assuming the figure to be 80 m, then it is possible to estimate the active-range of higher

FIGURE 1: ACTIVE-RANGE EXPERIMENT - LOG10 RATIO OF DOWNWIND/UPWIND TRAP-CATCH vs. TRAP-SPACING FOR 3 NIGHTS OF ACCEPTABLE WIND

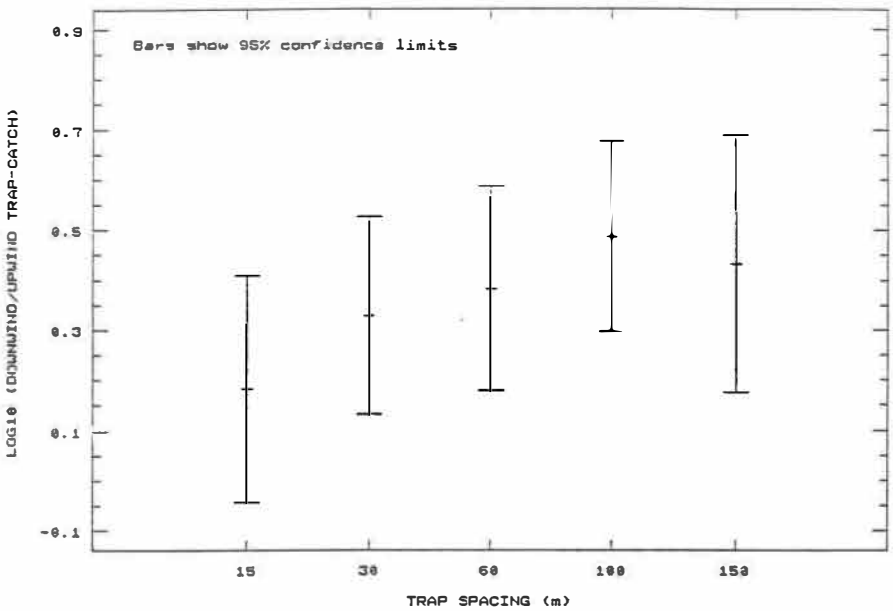
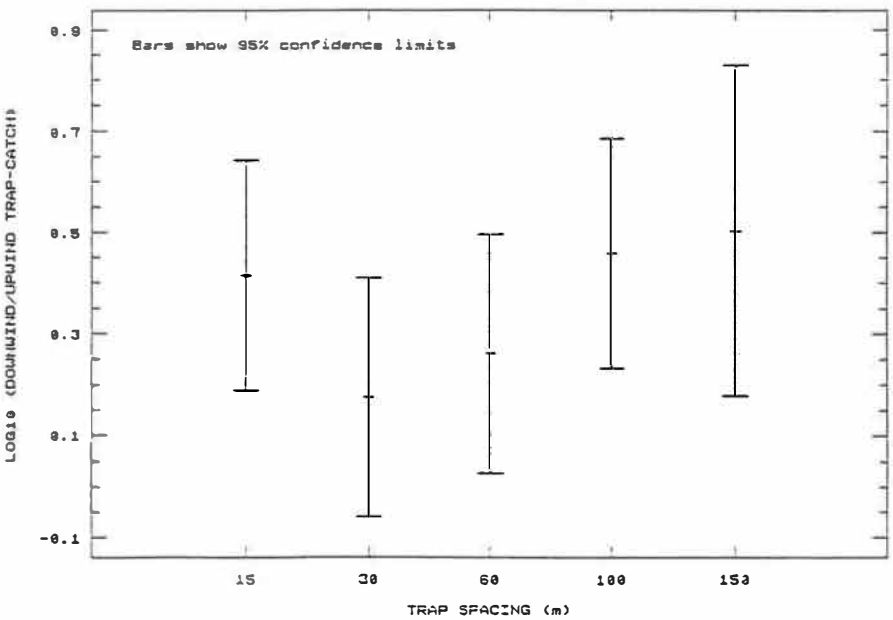


FIGURE 2: ACTIVE-RANGE EXPERIMENT - LOG10 RATIO OF DOWNWIND/UPWIND TRAP-CATCH vs. TRAP-SPACING FOR 3 NIGHTS OF UNACCEPTABLE WIND



dose lures, e.g. 0.01 mg, from the appropriate isolated lures ratio. From Stanley *et al.* (1985) if the *length* of the pheromone plume detectable by the moths (the active-range) totally governs catch then:

$$\text{length}(0.01) = \text{catch ratio} \times \text{length}(0.003)$$

$$\text{i.e. active-range 0.01 mg vial} = 2.75 \times 80 \text{ m} = \underline{220 \text{ metres}}$$

If the *area* of the pheromone plume detectable by the moths (the active-area) totally governs catch then:

$$\text{length}(0.01) = \{\text{catch ratio} \times [\text{length}(0.003)]^2\}^{0.5}$$

$$\text{i.e. active-range 0.01 mg vial} = \{2.75 \times 6400\}^{0.5} = \underline{132.7 \text{ metres}}$$

Probably the true value lies between these values. These lures were much weaker than those used in any mating-disruption treatment, the latter would have much greater active-ranges (and active-areas). Superficially, this indicates that in typical source densities of 500-1000/ha the plumes would overlap greatly and that some scope for reducing density was available. However, the estimate made here was for free-moving air above the crop. The corresponding within-crop figure would be relatively much reduced. Some estimate of the extent of this reduction would be very useful. The results demonstrate that lures in traps may mutually interfere over considerable distances, depending on the lure release-rate.

References

BRADSHAW, J.W.S., ELLIS, N.W., HAND, S.C. AND STOAKLEY, J.T. (1989). Interactions between pheromone traps with different dose lures for the pine beauty moth, *Panolis flammea*. J. Chem. Ecol. **15**(10): 2485-2494.

CRITCHLEY, B.R. AND EL-DIEB, Y.A. (1981). An improved pheromone trap for the mass-trapping of cotton leafworm moths in Egypt. Appropriate Technology for Egyptian Agriculture **4**: 6-10

DE SOUZA, K.R., MCVEIGH, L.J., DOWNHAM, SMITH , J., AND MOAWAD, G.M. (1993). Mating-disruption of *Spodoptera littoralis* in Egypt. This volume.

STANLEY, B.H., HUMMEL, H.E., RUESINK, W.G. (1985). Estimating Maximum Horizontal Area of Pheromone Plumes. J. Chem. Ecol. **11**(8): 1129-1146.

WALL, C. AND PERRY, J.N. (1980). Effects of spacing and trap number on interactions between pea moth pheromone traps. Ent. exp. & appl. **28**: 313-321.

WALL, C. AND PERRY, J.N. (1987). Range of action of moth sex attractant sources. Ent. exp. & appl. 44: 5-14.

ELECTROPHYSIOLOGICAL RESPONSES OF MALE CODLING MOTHS (*CYDIA POMONELLA*) TO HALOGENATED ANALOGUES OF CODLEMONE

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Abstract The biological activity of halogenated analogues of codlemone, the main pheromone component of the codling moth, was measured on the pheromone receptor system of males. In EAG screening, codlemone was the most active compound, but F10,11-codlemone and C1-codlemone elicited significant responses. EAG cross adaptation experiments and single sensillum recordings revealed that these compounds were detected by the same receptor neurone type as codlemone. No competitive inhibition with codlemone was observed from non-active analogues. In field trapping, F10,11-codlemone and C1-codlemone were more attractive to males than codlemone itself. The strong activity of the F10,11-codlemone and C12-codlemone may be due to different behaviours of these compounds in the field due to their physical properties.

Introduction

Chemists of our group synthesised flourinated and chlorinated analogues of codlemone (8E,10E-dodecadienyl-1-ol) the main pheromone component of *Cydia pomonella* (Lepidoptera: Tortricidae) (Hammoud, 1988; Tellier *et al.*, 1989; Tellier and Sauvêtre, 1992; Tellier *et al.*, 1993). These analogues consisted in isosteric replacements of hydrogens by flourine atoms in the terminal CH_3 group or in the diene system of codlemone or in the replacement of the terminal CH_3 group by a chlorine atom (Table 1).

Traps baited with C1-codlemone and F10,11-codlemone caught respectively 73 % and 43 % more males in the field than codlemone (Audemard, unpublished data). Thus, electrophysiological experiments were conducted to test if the stronger field activity of these two compounds when compared to codlemone was due to their higher activity on the pheromone receptor cells. The mechanisms of detection of F10,11-codlemone, C1-codlemone and of a series of 9 other halogenated analogues of codlemone by the pheromone receptor system of the males were compared to those of

TABLE 1: List of compounds tested. Underlined short names designe codlemone analogues in the text and figures.

(E,E)-8,10-dodecadienol: codlemone (= E8,E10-12:OH)

FLUORINATED COMPOUNDS:
(E,E)-8,9-difluoro-8,10-dodecadienol: F8,9-codlemone
(E,E)-10,11-difluoro-8,10-dodecadienol: F10,11-codlemone
(E,E)-8,9,10,11-tetrafluoro-8,10-dodecadienol: F8,9,10,11-codlemone
(E,E)-12,12,12-trifluoro-8,10-dodecadienol: F12,12,12-codlemone

CHLORINATED COMPOUNDS:
(E,E)-11-chloro-8,10-undecadienol: Cl-codlemone (= Cl EE OH)
(E,E)-11-chloro-8,10-undecadienyl acetate: Cl EE Ac
(Z,E)-11-chloro-8,10-undecadienol: Cl ZE OH
(Z,E)-11-chloro-8,10-undecadienyl acetate: Cl ZE Ac
(E)-11-chloro-8-undecyn-10-enol: Cl Yne Z OH
(Z)-11-chloro-8-undecyn-10-enyl acetate: Cl Yne Z Ac
(E)-11-chloro-8-undecyn-10-enyl acetate: Cl Yne E Ac

FIGURE 1: EAG response profile of males *C. pomonella* to codlemone and to a series of 11 halogenated analogues tested at the dose of 1 μg (n = 13). Means associated to a same letter are not significantly different at $P<0.05$ (one-way ANOVA, Newman-Keuls test).

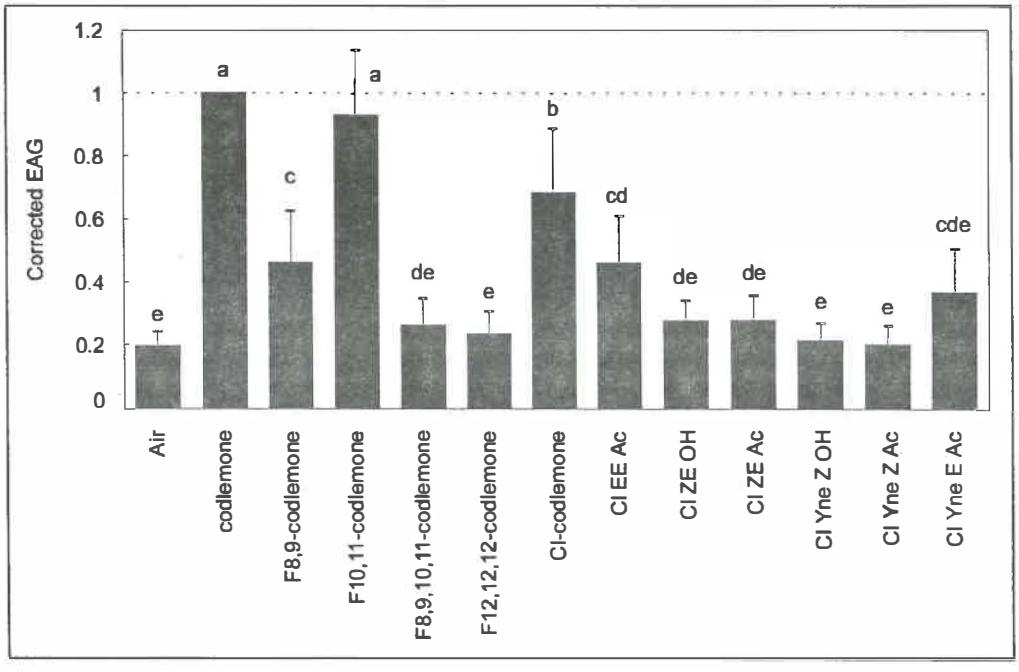


FIGURE 2: EAG responses to codlemone alone or in mixture with increasing amounts of F12,12,12-codlemone (n = 7) or F8,9,10,11-codlemone (n = 8). EAG responses to these blends were compared to responses to pure codlemone obtained on each male before and after testing the blends. Mean responses did not differ significantly at $P<0.05$ (one-way ANOVA, Newman-Keuls test).

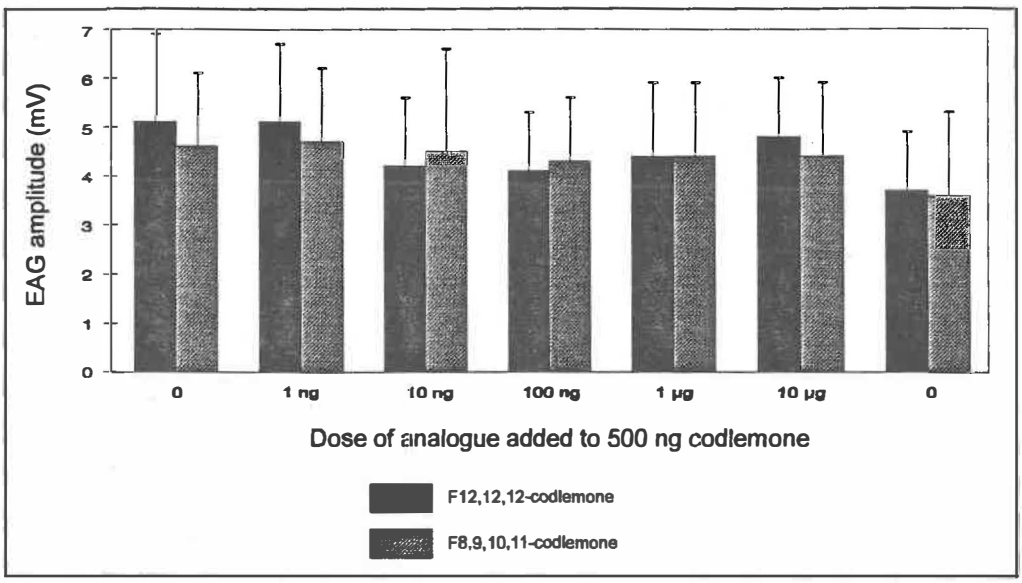
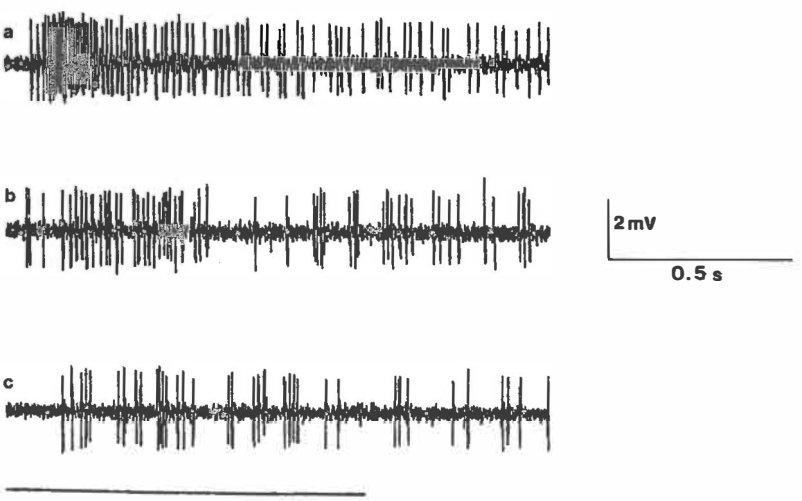


FIGURE 3: Samples of responses of a sensilla trichodea to 1 μg codlemone (a), 1 μg F10,11-codlemone (b) and 1 μg Cl-codlemone (c). The bar below the recording indicates the duration of the olfactory stimulation (1 s).



codlemone. In particular, using EAG and single sensillar recordings, we tested if F10,11-codlemone and C1-codlemone excite the same receptor neurones as codlemone and if non-active compounds have the ability to bind the dendritic receptor sites and thus to compete with codlemone at this level.

Results and Discussion

In EAG screening, codlemone was the most active compound but F10,11-codlemone, F8,9-codlemone, C1-codlemone and C1 EE Ac elicited significant responses (Fig. 1). Differences between the responses to codlemone and F10,11-codlemone were not significant whatever the dose. Hence, flourination in the diene system maintained some biological activity. Triflourination of the terminal CH_3 group abolished the activity of the compound while its replacement by a chlorine atom gave only a 10-fold reduction in activity. The CF_3 being amongst the most lipophilic of all substituent, this illustrates the importance of the terminal CH_3 group, in particular its lipophily, in pheromone-receptor binding.

Competition experiments were conducted with 2 flourinated analogues without electrophysiological activity. Increasing amounts of these 2 analogues in mixture with codlemone had no effect on responses demonstrating that they do not compete with codlemone (Fig. 2). Thus, the absence of activity of these 2 compounds is likely due to their low affinity for dendritic receptor site and/or for pheromone binding proteins present in the sensillar lymph.

After repetitive stimulations by 20 µg of codlemone, F10,11-codlemone or C1-codlemone (adaptation stimulus) responses to 1 µg of these 3 compounds were reduced to 30 to 69 % of their pre-adaptation values. The level of adaptation was found to be correlated with the EAG activity of the adaptation compounds. The cross adaptation between codlemone F10,11-codlemone and C1-codlemone was in favour of their detection by the same cell type but it had to be confirmed by single sensillar recordings.

Codlemone, F10,11-codlemone and C1-codlemone were screened on 5 different sensilla belonging to 3 different males. All sensilla were very responsive to codlemone, F10,11-codlemone and to a lesser extent to C1-codlemone (Fig. 3). Action potentials of the same class of amplitude were emitted in response to these 3 compounds, indicating that they are probably detected by the same receptor cells.

Conclusion

Isosteric replacements of hydrogens by flourine atoms in the diene system of codlemone or replacement of the terminal methyl group by a chlorine atom produced halogenated analogues with electrophysiological activity. F10,11-codlemone and C1-codlemone interacted with the pheromone receptor system of the males as agonists of codlemone, C1-codlemone having a weaker activity. No competitive inhibition was observed with inactive analogues.

The higher level of trap catches obtained with C1-codlemone or F10,11-codlemone than with codlemone could not be expected on the basis of their relative physiological activity. The field activity of a pheromone compound results both from its activity on the sensory receptors and from its availability at this level. Thus, the higher field activity of C1-codlemone and F10,11-codlemone is likely due to their

physical properties modifying their behaviour in the field (chemical stability, volatility...) which compensate for their reduced electrophysiological activity when compared to codlemone

One of the major problems concerning the use of synthetic pheromones is their chemical degradation and/or isomerisation in the field. In particular this is true for molecules bearing a conjugated diene system such as codlemone. The use of pheromone analogues having a stronger stability in the field than the parent molecules is of considerable interest. This constitutes an attractive approach towards the development of new pest-control strategies.

References

HAMMOUD, A. 1988. Synthèse de phéromones et de molécules isostères de phéromones d'insectes pour préciser le mécanismes moléculaires de la reconnaissance des signaux chimiques au niveau des récepteurs antennaires. Thèse de doctorat d'état, Paris VI, 200 pp.

TELLIER, F., SAUVETRE, R. and NORMANT, J.-F. 1989. Synthèse de flourocodlémones. J. Organomet. Chem. **364**: 17-28.

TELLIER, F. and SAUVETRE, R. 1992. Synthesis of a new flourinated analog of (E,E)-8,10-Dodecadienol (Codlemone). Tetrahedron Lett. **33**: 3643-3644.

TELLIER, F., HAMMOUD, A., RATOVELOMANANA, V., LINSTRUMELLE., G. and DESCOINS, C. 1993. Synthesis of a chlorinated analog of (E,E) 8,10-dodecadienol (Codlemone). Bull. Soc. Chim. France. In press.

LUCAS, P., RENOU, M., TELLIER, F., HAMMOUD, A., AUDEMARD, H. and DESCOINS, C. 1993. Electrophysiological and field activity of halogenated analogues of (8E,10E)-dodecadienyl-1-ol, the main pheromone component of the codling moth. Submitted to J. Chem. Ecol.

RESPONSE OF *ORYZAEPHILUS SURINAMENSIS* TO CAROB EXTRACTS

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Abstract A computerised tracking system has been used to investigate the behaviour of *Oryzaephilus surinamensis* to different patterns of delivery of carob extract. A number of different parameters have been used to investigate insect response. Each parameter suggest that pulsed delivery may provide a better response than continuous delivery.

Introduction

During storage, cereal crops are vulnerable to infestation by numerous species of insects, especially *Oryzaephilus surinamensis*, the saw-toothed grain beetle. O'Donnell et al., (1983) and Stubbs et al., (1985) have described how the extracts of carob may be used to attract O. *surinamensis* to a wick or a pitfall trap, where the beetles may be caught. This poster described experiments undertaken to improve the understanding of the detailed behaviour of the insects in the presence of carob extract vapour and to determine the optimum delivery conditions.

All experimental work was undertaken using the tracking system recently developed at The University of Glamorgan. Details of the system are the subject of a paper at this Conference.

Experimental Procedure

Beetles were placed in a 100 mm diameter arena bounded by a 10 mm high fluon coated ring, and allowed to move on a white filter paper. A computer controlled micro-syringe delivery unit was used to delivery specific amounts of carob extract, dissolved in a mixture of pentane and heptane, to a small area at the centre of the filter paper. In order to ensure that carob vapour was not released prior to delivery period, the tip of the syringe needle was embedded inside a septum until delivery was initiated. The beetles used were aged between 8 weeks and 10 weeks. Twenty replicate runs were carried out for each series, the routine for each run being:

1. Cleaning of arena.
2. Filling micro-syringe and adjusting tip to be inside septum.
3. Introduction of beetle to arena followed by a settling period.
4. Tracking for 500 sec (pre-treatment period).
5. Needle forced through septum and delivery initiated.
6. Tracking for 500 sec (post-treatment period).

During pre- and post-treatment periods the operator observed the beetles for antennae response (where the beetles stopped and appeared to be raising their antennae to sense the air). Each response was recorded on the computer as an event.

The carob extract dissolved in pentane was prepared by CSL and then diluted by x 100 with heptane. Heptane was used instead of pentane in order to lower its volatility in the micro-syringe. Delivery rates are however quoted in terms of volume per second of extract as supplied by CSL.

Results

The analysis software provided with the tracking system enables many parameters to be investigated. For this study the plot of distance between insect and the arena centre with time, and recorded antennae events, were extensively used. Whole and part of tracks, as well as plots of insect speed and direction of movement were also studied. One of the main objectives of this work was to investigate the effect of pulsing carob rather than continuous delivery. Consequently three sets of replicates were carried out where the total amount of delivered carob was the same, but each set had a different delivery profile. Table 1 shows the conditions for the sets of experiments described.

Table 1 Details of Experimental Conditions

Insect - *Oryzaephilus Surinamensis*, LS strain held at CSL
Insect age - between 8 and 10 weeks
Storage and test temperature - between 23ºC and 25ºC
Insect settling period - 10 min
Pre-treatment tracking period - 500 sec
Post-treatment tracking period - 500 sec
Sampling interval - 1.0 sec
Attractant - Carob extract in pentane (CSL) diluted x 100 with heptane
Number of replicates - 20

Delivery conditions:-

Series	Post-treatment delivery
A	Constant delivery at 0.0026 nls^{-1}
B	Pulsed delivery with a single pulse of 13 nl during the first 10 s of tracking.
C	Pulsed delivery with two equal pulses at times 0 to 10 sec and 250 to 260 sec.

Note that the total carob delivered was the same for each run.

Three main parameters have been used so far to assess beetle behaviour.

1. Frequently of visits to the centre, where the centre was defined to be a circle of diameter 5 mm, 10 mm or 20 mm.

Table 2 shows the ratio of post-treatment frequency of visits to pre-treatment frequency of visits for different centre diameters and for different time intervals since the start of either pre- or post-treatment periods. The table demonstrated how small centre diameters give greater divergence between pre- and post-treatment results. Fig. 1 provides the variation of frequency of visits with time. Both figure and table show that the pulsed delivery provides greater response during the tracked period of 500 s.

Table 2: Results showing number and duration of visits to the centre

Centre diameter	Time allowed	Post-treatment visits / Pre-treatment visits			% of post-treatment visits of duration > T sec		
		A	B	C	A T = 2 sec	B T = 4 sec	C T = 8 sec
5	150	1.2	4.8	2.3	50	77	78
5	250	1.3	3.7	2.6	44	75	79
5	500	2.4	4.1	2.1	47	68	74
10	150	1.2	2.5	1.9	40	60	67
10	250	1.1	1.5	2.3	48	54	72
10	500	1.4	1.7	1.6	49	52	73
20	150	0.8	2.4	3.1	58	32	36
20	250	0.9	1.6	2.3	48	30	47
20	500	1.2	1.4	1.6	53	30	51

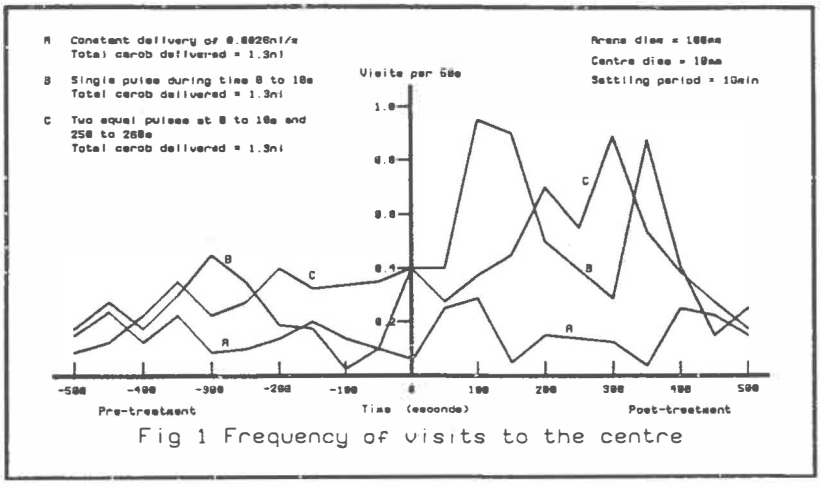

Fig 1 Frequency of visits to the centre

2. Duration of stay within the specified centre circle.

Table 2 gives the % of post-treatment visits which are of duration greater than approximately 1.5 times the time that a beetle would take to cross the centre without stopping. The value may then be considered to be a measure of effectiveness of the attractancy of the carob. A value of 0% would result if there were no visits to the centre of duration >T, while 100% means that all the beetles remain at the centre for a reasonable time. Again higher values are found in the case of 5 mm diameter centre, demonstrating retention response of the insects to carob. Figure 2 gives a plot of length of stay at the centre with time. Plot shows a marked response within 50 sec of start of delivery.

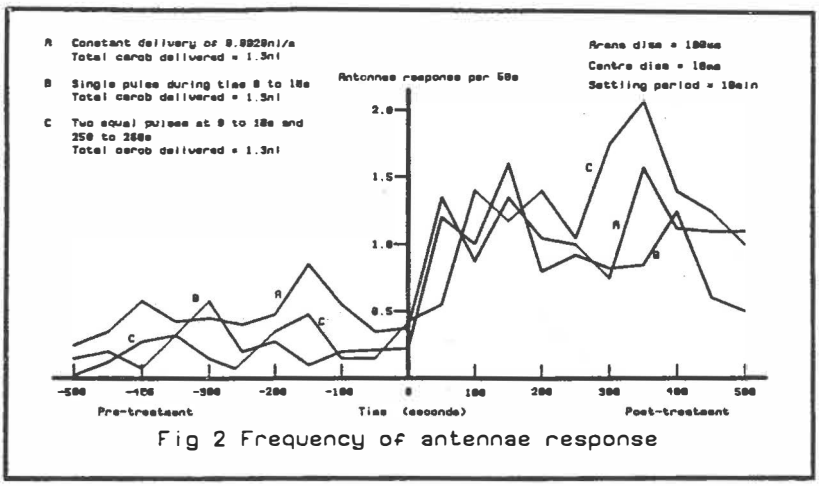

Fig 2 Frequency of antennae response

3. Number and total time of antennae responses (events).

Table 3 shows the ratio of number of antennae responses, and the ratio of total response time, during post-treatment period to that during pre-treatment period. Fig. 3 gives a plot of antennae response frequency with time. It is noted that this parameter shows a marked increase within 50 sec of delivery in the case of experiment A. This was not observed for frequency of visits. The insects are therefore sensing the presence of the carob but appears to be unable to determine its source. This may be due to the fact that either the actual concentration, or the gradient in concentration or the rate of change in concentration with time is too low.

Table 3 Data on antennae responses

Experiment	Ratio of response parameter Post-treatment/Pre-treatment		% visits to 10 mm centre in 500 sec with response within 20 sec prior to visit	
	Number of responses	Total response time	Pre-treatment	Post-treatment
A	2.5	2.8	33	23
B	3.5	3.8	22	51
C	5.1	6.3	20	52

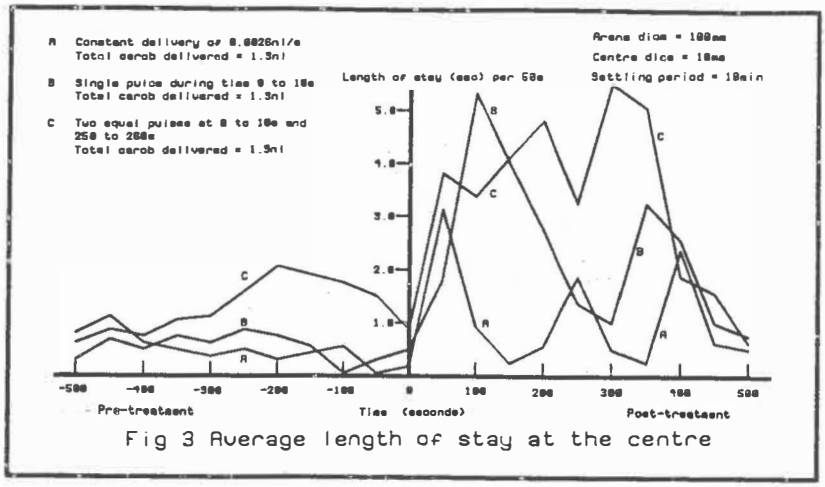

Fig 3 Average length of stay at the centre

Main Conclusions

1. The tracking system provides extensive data on insect behaviour. The interpretation of the data required further research.

2. Small diameter centres provide improved data on response to an attractant.

3. Beetle response as measured by duration of stay at the centre and antennae response appears to be more sensitive to low delivery rates than frequency of visits to the centre.

4. Initial studies suggest that a pulsed delivery provides better response in those cases where the total amount of attractant available for use is limited.

References

1.STUBBS, M.R., CHAMBERS, J., SCHOFIELD, S.B. and WILKINS, J.P.G. Attractancy to *Oryzaephilus surinamensis* (L.) of volatile materials isolated from vacuum distillate of heat-treated carobs. J. Chem. Ecol. Vol. 11, No. **5**, 565-581, 1985.

2.O'DONNELL, M.J., CHAMBERS, J., and MCFARLAND, S.M. 1983. Attractancy to *Oryzaephilus surinamensis* (L.), saw-toothed grain beetle of extracts of carobs, some triglycerides, and related compounds. J. Chem. Ecol. **9**:357-374.

BEHAVIOURAL EFFECTS PRODUCED BY THE SEX PHEROMONE COMPONENTS OF THE EGYPTIAN ARMYWORM *Spodoptera littoralis*

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ABSTRACT

The behavioural effects induced on males by the sex pheromone components, (*Z,E*)-9,11-tetradecadienyl acetate (**I**), (*Z*)-9-tetradecenyl acetate (**II**), (*E*)-11-tetradecenyl acetate (**III**) and tetradecyl acetate (**IV**), of our strain of the Egyptian armyworm *Spodoptera littoralis* in wind tunnel has been studied. The parameters considered comprise the air speed, moth age and amount of chemicals. The males were scored according to their behaviour performed in the sequence: taking flight, upwind flight to the middle of the tunnel, close approach and contact with the source. Best results were obtained with males and females of 30-34 h after emergence, regardless the air speed considered. Compound **I** peaked its activity (60% of males contacting with the source) at 5000 μg dose with a wind speed of 40 cm/s. Among the minor components, only compound **III** showed some activity alone (25% of males attempting copulation) at 100-1000 μg loadings. The diene acetate (*Z,E*)-9,12-tetradecadienyl acetate (**V**), which has also been detected in other strains, did not induce male landing at any of the concentrations tested.

INTRODUCTION

The Egyptian armyworm *Spodoptera littoralis* is an important pest of cotton and vegetable crops in Europe, Asia and Africa. Composition of the sex pheromone was initially reported by Nesbitt et al. (1973) as a mixture of (Z,E)-9,11-tetradecadienyl acetate (**I**), (Z)-9-tetradecenyl acetate (**II**), (E)-11-tetradecenyl acetate (**III**) and tetradecyl acetate (**IV**). Later, Tamaki and Yushima (1974) found in insects from Nairobi (Kenya) that (Z,E)-9,12-tetradecadienyl acetate (**V**) was also a major component of the sex pheromone, which synergized the activity of the major component **I** in laboratory bioassays. Campion et al. (1980) analysed virgin females from several origins (Crete, Israel and Egypt) and found that insects from Crete produced only compounds **I** and **II**, whereas those from Israel and Egypt contained, respectively, compounds **I**, **IV** and **V** and **I**, **II**, **IV**, **V** and/or (E)-11-tetradecenyl acetate. The authors finally suggested that insects from different areas may respond in a different manner to the minor components found in the sex pheromone glands. Dunkelblum et al. (1982) confirmed the presence of compound **V** in the extracts although in very scarce amounts (0.5-1%). The presence of the unconjugated diene seemed to be essential for an optimum trap catch in the field.

Although most of the formulations have been tested in laboratory bioassays and in the field, only one report about the effects of the minor components on the behaviour of males in wind tunnel has been reported (Haines, 1983). Our studies brought about on our own strain show that the pheromone gland contains acetates **I**, **II**, **III** and **IV** in 66:12:10:11 ratio. The estimated amounts of these compounds were ca. 40, 7, 6 and 7 ng, respectively per female. We report herein our results on the behavioural effects induced by the pheromone components **I-IV** as well as **V**, on *S.*

littoralis males in a wind tunnel.

MATERIALS AND METHODS

Insects.

Insects were reared on an artificial diet and kept on a 16:8 light:dark regime at 25±2°C with a relative humidity of 60% Pupae were sexed in groups of 20-25 and placed in 20 x 20 cm plastic boxes until emergence. Adults were provided with 10% sucrose solution. Activity of females was tested by placing 3-4 individuals in aluminum cages and hanged at 25 cm from the floor. Males, in groups of 5, were placed on a filter paper standing on a stainless-steel jack, at the far end of the tunnel and at similar height than the source. Twenty five males were used per each experiment.

Wind tunnel.

The bioassays were performed in a rectangular wind tunnel, made of glass, of 200 cm long, 50 cm wide and 50 cm high. The air is pushed through with a fan and pulled out of the building with the aid of an exhaust blower. The fan and the blower function simultaneously, and their speed were carefully regulated in order to avoid undesired turbulence in the moving air. The air from the observation room is purified through a thick glass wool bed and conducted through a nylon screen to smooth the airflow. The experiments were carried out at an ambient temperature of 22±1°C and with a relative humidity of 60%. The air speed varied between 40 and 80 cm/s. At the top of the tunnel two infrared lamps provided the required illumination (10 lux).

Compounds.

The chemicals were commercially available from Fluka. The required amount of the compounds, previously dissolved in hexane, was dispensed in a cotton wick and hanged at a height of 25 cm.

RESULTS AND DISCUSSION

Males behaviour was scored according to the sequence: taking flight, upwind flight to the middle of the tunnel, close approach to the source (ca. 10 cm) and contact with the lure, and the effects produced by three key parameters, air speed, age of insects and amount of whereas chemicals, were considered. In preliminary experiments we noticed that 1-3 day-old males were the most active 1-2 day-old females displayed the highest attractant activity. Therefore, only virgin females of 10-34 h and males of 10-54 h were considered in this study.

Activity of the virgin females was found to be optimal at 30-34 h after emergence, which corresponds to the maximum sex pheromone titer found in the pheromone gland (Martínez and Camps, 1988). Almost 80% of males (30-34 h of age) closely approached to the source at any air speed considered, but only at 50 and 55 cm/s over 60% of males attempted copulation with the females (Fig. 1). Younger males, of less than 30 h after emergence, required the same speed to behave in a similar manner, but the number of insects flying upwind following the plume was reduced to 80%.

The major component I was tested at several doses (100-5000 μg), under different air speed (40-80 cm/s) and on males of several ages (10-30 h, 30-34 h and 34-54 h). Although there was great variability in the average response of moths of 10-30 h, the males were highly activated only at high speed (80 cm/s) and at all doses, but their ability to land was greatly impaired. Again, high proportion of males of 30-34 h approached to the source at low speed regardless the loading of the compound used. Low dose (100 μg) elicited less attraction to the cotton wick but the males showed

relatively high capacity to land. The highest response was shown by males (30-34 h) at 5000 μg and a wind speed of 40 cm/s (Fig. 2). At low speed, older males (34-54 h) were also highly activated, flying upwind to the source, but were relatively unable to find the source.

The minor components present in our strain **II-IV**, as well as the unconjugated diene **V**, were also tested for activity under the same conditions. However, neither of them provoked a remarkable effect on males (10-34 h) at any dose or air speed. Only compound **II** at 100 μg and 45 cm/s induced some response on 10-34 h males, taking flight ca. 75% and 20% atempting copulation (Fig. 3). Slightly less pronounced effect was exerted by compound **III** at 500-1000 μg dose at the same air speed (Fig. 4). The effect induced by compounds **IV** and **V** was practically negligible at all concentrations tested.

Although more work has to be done, particularly with binary mixtures of the major component **I** with the secondary components **II-IV**, our results show that in wind tunnel and under certain conditions, acetate **I** can elicit a remarkable, full response on 60% males of *S. littoralis*, which is almost comparable to the activity displayed by virgin females.

ACKNOWLEDGEMENTS

We gratefully acknowledge CICYT (PB90-0089) for financial support and KenoGard, S.A. for a predoctoral fellowship to C. Quero.

Figure 1. Percentage of S. littoralis males (30-34 h) attracted to virgin females (30-34 h).

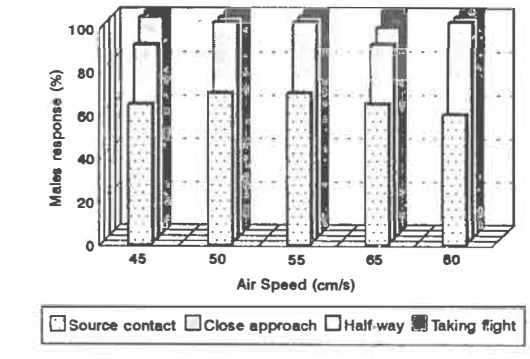


Figure 2. Percentage of S. littoralis males (30-34 h) attracted to (Z,E)-9,11-14:OAc (speed 40 cm/s)

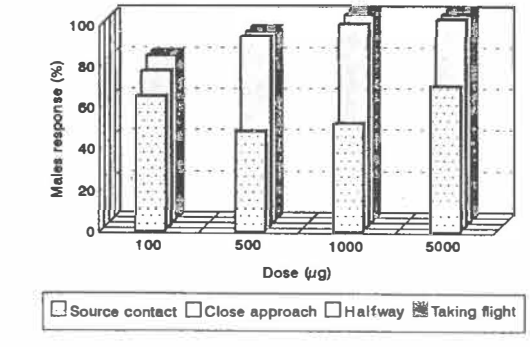


Figure 3. Percentages of S. littoralis males (10-34 h) attracted to Z9-14:OAc (speed 45 cm/s)

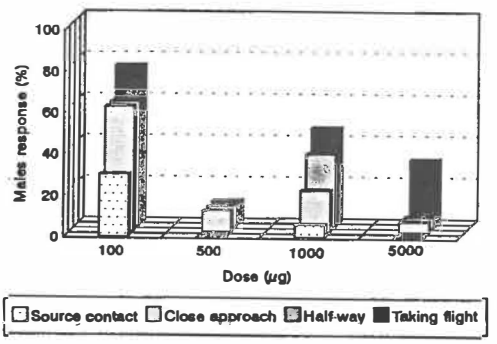
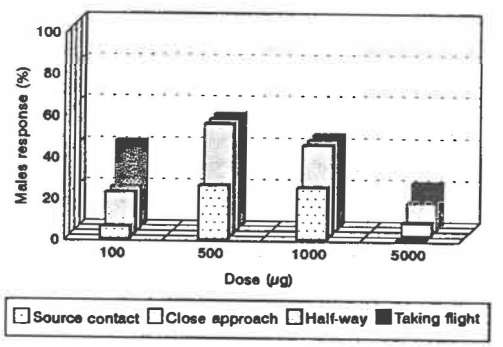


Figure 4. Percentage of S. littoralis males (10-34 h) attracted to E11-14:OAc (speed 45 cm/s).



REFERENCES

CAMPION, D.G., HUNTER-JONES, P., McVEIGH L.J., HALL, D.R., LESTER, R. & NESBITT, B.F. 1980. Modification of the attractiveness of the primary component of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), by secondary pheromone components and related chemicals. Bull. Entomol. Res. **70**:417-434.

DUNKELBLUM, E., KEHAT, M., GOTHILF, S., GREENBERG, S. & SKLARZ, B. 1982. Optimized mixture of sex pheromonal components for trapping of male *Spodoptera littoralis* in Israel. Phytoparasitica **10**:21-26.

HAINES, L.C. 1983. Wind tunnel studies on the effects of secondary sex pheromone components on the behaviour of male Egyptian cotton leafworm moths, *Spodoptera littoralis*. Physiol. Entomol. **8**:29-40.

NESBITT, B.F., BEEVOR, P.S., HALL, D.R., LESTER, R. & POPPI, R.G. 1973. Sex pheromones of two noctuid moths. Nature: New Biology **244**:208-209.

TAMAKI, Y. & YUSHIMA, T. 1974. Sex pheromone of the cotton leafworm *Spodoptera littoralis*. J. Insect Physiol. **20**:1005-1014.

MARTINEZ, T. & CAMPS, F. 1988. Stimulation of sex pheromone production by head extract in *Spodoptera littoralis* at different times of the photoperiod. Arch. Insect Biochem. Physiol. 211-220.

INHIBITION ACTIVITY OF ANTENNAL ESTERASES OF THE EGYPTIAN ARMYWORM *Spodoptera littoralis*

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ABSTRACT

A series of aliphatic and aromatic trifluoromethyl ketones has been tested as inhibitors of the antennal esterases in the Egyptian armyworm *Spodoptera littoralis*, by evaluation of the extent of hydrolysis of tritium-labeled (*Z,E*)-9,11-tetradecadienyl acetate, the major component of the sex pheromone. Most of the compounds tested were slow-binding inhibitors, being the most potent, among the aliphatic compounds, 3-octylthio-1,1,1-trifluoropropan-2-one (**2**) (I_{50}=0.55 μM) and 1,1,1-trifluotetradecan-2-one (**6**) (I_{50}=1.16 μM). The aromatic structures were less potent inhibitors, being β-naphthyl trifluoromethyl ketone (**3**) the one which displayed the most favourable results (I_{50}=11.0 μM). Compounds **2**, **3** and **6** exhibit a competitive inhibition with inhibition constants (K_i) of 2.51×10^{-5} M, 2.49×10^{-4} M and 2.98×10^{-5} M, respectively.

INTRODUCTION

Degradation of sex pheromones in insects has been shown to occur preferentially in the antennal receptors. Two possible inactivation mechanisms have been postulated, either by binding of the stimulus molecules to the pheromone binding proteins located in the sensillum limph or by enzymatic catabolic activity of the pheromone molecules associated with the hairs. In any case, it is generally believed that the transductory process of olfaction modulates the behavioral response of the insect.

Trifluoromethyl ketones have been proven to inhibit the action of a variety of serine esterases (Gelb et al. 1985), juvenile hormone esterase (Abdel-Aal and Hammock, 1985), or mammalian carboxylesterases (Ashour and Hammock, 1987). Some of them have been tested in a limited number of insects as inhibitors of the antennal esterases (Prestwich, 1987) and their activity reasoned in terms of the tetrahedral geometry of the geminal diol produced by hydration of the ketones in aqueous solution. In the presence of esterases, trifluoromethyl ketones may form hemiketals with the serine residue present at the active site of the enzyme, acting, therefore, as a reversible inhibitor. We have prepared crude antennal preparations of the Egyptian armyworm *Spodoptera littoralis*, and tested *in vitro* a variety of aliphatic and aromatic trifluoromethyl ketones **2**-**13** (Scheme 1) as inhibitors of the antennal esterases, by evaluating the extent of hydrolysis of the major component of the sex pheromone, (*Z,E*)-9,11-tetradecadienyl acetate, labeled with tritium at C-1.

MATERIALS AND METHODS

Insects.

Insects were reared on an artificial diet and kept on a 16:8 light:dark regime at 25±2°C with a relative humidity of 60%. Pupae were sexed in groups of 20-25 and placed in 20 x 20 cm plastic

boxes until emergence. Adults were provided with 10% sucrose solution.

Synthesis.
Tritium-labeled pheromone **1** was prepared from the corresponding enyne aldehyde by BT_4Na (469 mCi/mmol) reduction followed by acetylation (acetic anhydride/pyridine). The resulting stock solution was $5.64x10^{-2}$ M with a specific activity of 137 mCi/mmol (radiochemical yield 77.6%). 3-Octylthio-1,1,1-trifluoropropan-2-one (OTFP) was a gift of Prof. G.D. Prestwich. Trifluoroacetophenone was commercially available from Fluka. The remainder trifluoromethyl ketones were prepared previously in our laboratory (Parrilla, 1993).

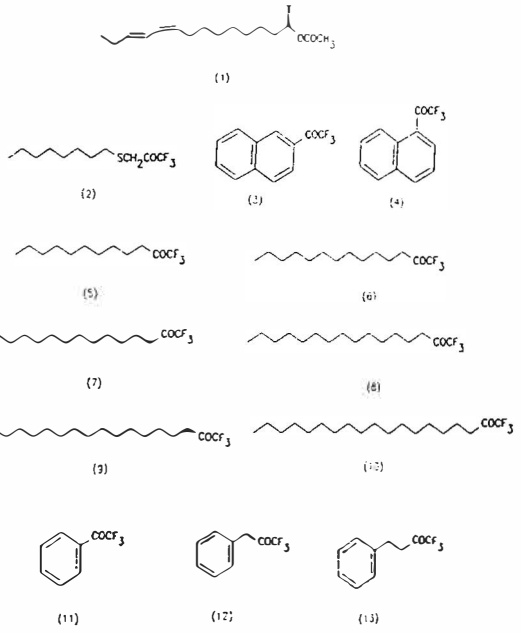
Enzyme preparation.
The enzyme preparation used was the crude homogenate of freshly emerged male antennae, since trials to separate pheromone-specific sensory hairs from the antennal branches were unsuccessful. The crude antennal homogenate was prepared as follows. Antennae from 1-2 day old anesthesized males were removed and immediately frozen at -80°C. When required, the antennae were homogenized in an ice bath with the aid of a mechanical stirrer at 680 rpm, in the presence of 10 mM Tris-HCl buffer (pH 7.2) and centrifuged at 12000 g for 2 min and 4°C, to remove the cuticular debris. Dilutions of the stock antennal solutions were made just before the inhibition assays.

Inhibition assays.
Unlabeled solutions of the synthetic pheromone and alcohol were spotted on analytical 10 x 5 cm TLC plates (DC Plastikfolien Kiesegel $60F_{254}$ Merck). In glass tubes, previously cooled on a ice bath, several concentrations of the inhibitor (0.6, 6 and 60 μM) and Tris-HCl buffer to get a total volume of 100 μl were added. The tubes were vortexed for 30 sec; an aliquot of the esterase extract corresponding to 2 antennae was added to the tubes, vortexed again for 5 sec and the tritiated substrate added. The mixture was immediately incubated on a thermostatized water bath at 25°C for 30 min. The tubes were cooled in an ice bath and treated with 100 μl of ethyl acetate. The two phases were clearly separated by vortexing for 1 min and 5 μl aliquots of the organic solution, taken with a calibrated disposable glass micropipet, were spotted on the TLC plates. The plates were eluted with hexane:ethyl acetate 5:1 and the spots visualized with iodine (r_f 0.46 for the substrate, r_f 0.1 for the alcohol). The spots were scraped into poliethylene scintillation vials to which 4 ml of Unisolve scintillation liquid had been added. The vials were counted for 5 min in a Kontron Betamatic scintillation counter. The extent of hydrolysis was calculated as the ratio of dpm of the tritiated resulting alcohol to the total amount of dpm of the alcohol plus the unreacted labeled acetate. Three replicates of each inhibitor concentration were used.

RESULTS AND DISCUSSION

In previous experiments we have noticed that fresh preparations of 5, 7 and 10 antennae did not lose appreciable activity when they were immediately frozen at -80°C. Therefore, most of our inhibition studies have been carried out with frozen extracts. The K_m and V_{max} values of the enzyme, 13.9 μM and $4.66x10^{9}$ M/s, were calculated according to Lineweaver and Burk (1934), using homogenates of 2 antennae and several concentrations of substrate (0.56, 1.86, 5.64, 11.3, 22.6 and 45.1 μM).

Studies of the inhibition activity of aliphatic and aromatic trifluoromethyl ketones **2-13** were carried out with and without preincubation to determine the mode of binding to the active site. 3-Octylthio-1,1,1-trifluoropropan-2-one (OTFP, **2**) was also included in our study for comparison purposes, since it has been demonstrated to be a potent, slow-tight binding inhibitor of juvenile



Scheme 1

Table 1. Inhibition of antennal esterase extract of *Spodoptera littoralis* by TFMKS **2-13**.

Compound	Inhibitor Concentration			I_{50} values (μM)	
	60 μM	6 μM	0.6 μM	With preincub.	Without preincub.
2	83.4	66.2	24	0.55	3.7
3	77	51	30	10.4	7.9
4	38	16	0	--	(38%)[a]
5	--	--	--	19.3	--
6	75.5	57	34.5	1.16	10.6
7	--	--	--	4.4	11.0
8	--	--	--	18.4	--
9	50	30.3	0	--	(50%)[a]
10	44	0	0	--	(44%)[a]
11	44.4	32.2	22	40.7	(31%)[a]
12	36.3	25.7	24	--	(36%)[a]
13	43.5	23	12.8	--	(43%)[a]

[a]Values between parenthesis represent the inhibition values at 63 μM inhibitor concentration.

hormone in the tobacco hornworm *Manduca sexta* (Abdel-Aal and Hammock, 1985). Initial experiments on the effect of a preincubation period of **2** (0.6 μM), ranging from 0-15 min, on the time course of hydrolysis of labeled **1** (11.3 μM) showed higher inhibition effect at preincubation times shorter than 5 min. When we applied the protocol cited above (see Materials and Methods), we found that the best inhibitor found was compound **2** (I_{50} 0.55 μM, with preincubation), confirming that compounds with a β-thiotrifluoropropanone group are good inhibitors of antennal esterases, although the role of the thioether in enhancing inhibitor potency is not general (Ashour and Hammock, 1987). Other aliphatic compounds were also good inhibitors, in particular 1,1,1-trifluorotetradecan-2-one (**6**) (I_{50} 1.16 μM, with preincubation), which agrees with a previous report (Hammock et al., 1982) in which compound **6** is thought to be a transition-state inhibitor, binding to the active site of the enzyme almost irreversibly although non-covalently. Trifluoromethyl ketones with longer or shorter aliphatic chain length led to lower inhibitory potency. The aromatic compounds were much less potent inhibitors, except β-naphthyltrifluoromethyl ketone (**3**), which showed I_{50} values of 10.4 and 7.9 μM with and without preincubation, respectively. On the other hand, compound **3** behaved as the only non slow-binding inhibitor found among the other most potent inhibitors found **2, 3, 6** and **7**, which were also tested under non-preincubation conditions.

The inhibition constants for compounds **2, 3** and **6** were determined by plotting 1/V vs 1/S for several concentrations of inhibitor, ranging from 0 to 1.2×10^{-4} M, and substrate, which concentration varied from 1.15 to 45 μM. The compounds had a K_i of 2.51×10^{-5} M for **2**, 2.49×10^{-4} M for **3** and 2.98×10^{-5} M for **6**, and all behaved as competitive inhibitors according to Cornish-Bowden's plot of [S]/V vs [I].

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REFERENCES

ABDEL-AAL, Y.A.I. & HAMMOCK, B.D. 1985. Apparent multiple catalytic sites involved in the ester hydrolysis of Juvenile Hormones by the Hemolymph and by an Affinity Purified Esterase from *Manduca sexta* J. (Lepidoptera, Sphingidae). Arch. Biochem. Biophys. **243**:206-219.

ASHOUR, M.B.A., & HAMMOCK, B.D. 1987. Substituted trifluoroketones as potent selective inhibitors of mammalian carboxylesterases. Biochem. Pharm. **36**:1869-1879.

GELB, M.H., SVAREN, J.P. & ABELES, R.H. 1985. Fluoroketone inhibitors of hydrolytic enzymes. Biochemistry **24**:1813-1817.

HAMMOCK, B.D., WING, K.D., McLAUGHLIN, J. LOWELL, V.M. & SPARKS, T.C. 1982. Trifluoromethyl ketones as possible transition state analog inhibitors of juvenile hormone esterase. Pestic. Biochem. Physiol. **17**:76-88.

PRESTWICH, G.D. (1987) in Pheromone Biochemistry, G.D. Prestwich and G.J. Blomquist eds. Academic Press, N.Y.

LINEWEAVER, H. & BURK, D. (1934). The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56**:658-666.

PARRILLA, A. 1993. Ph.D. Dissertation, University of Barcelona.

CORNISH-BOWDEN, A.J. (1973). A simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. J. Biochem. **137**:143-144.

HALOACETATE ANALOGUES AS INHIBITORS OF THE SEX PHEROMONE OF *SESAMIA NONAGRIOIDES* LEF.

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Abstract A series of haloacetate analogues of (Z)-11-hexadecenyl acetate, the major component of the sex pheromone of the corn stalk borer *Sesamia nonagrioides*, as well as (Z)-1,1,1-trifluoro-14-nonadecen-2-one, have been prepared and tested as inhibitors of the pheromone action in EAG bioassays and in the field. In the laboratory, while the compounds *per se* were poor to moderate EAG active, most of them displayed significant inhibitory activity at concentrations 1, 100 and 1000 µg. In the field, whereas the monofluoroacetate analogue displayed the highest disruptant activity, the trifluoromethyl ketone, unexpectedly, behaved as a synergist, thereby enhancing the trap catches when mixed with the pheromone blend in 1:10 ratio. The effect of the chemicals on catches of *Mythimna unipuncta* and *Discestra trifolii* males, two related species usually caught in *S. nonagrioides* pheromone traps, is also reported.

Introduction

Disruption of the antennal perception process in insects by pheromone analogues represents a potential approach towards the development of new pest-control strategies. Among them, haloacetate analogues which proceed from replacement of the acetate hydrogens of the parent molecule by halogens, have been found to be good inhibitors of the perception process in the processionary moth *Thaumetopoea pityocampa* (Camps et al., 1990). Some of the compounds are also potent competitive inhibitors of the hydrolysis of the tritium-labeled pheromone by the antennal esterases in *Plutella xylostella* (Prestwich & Streinz, 1988), as well as inhibitors of the upwind flight response of *Ostrinia nubilalis* males (Schwartz et al., 1990).

The corn stalk borer *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae) is one of the most dangerous pests of corn, particularly in the Mediterranean region. The sex pheromone of the insect has been described as a blend of 4 components: Z11-16:Ac (**II**), Z11-16:OH (**I**), Z11-16:Ald (**III**) and 12:Ac in the ratio 69:8:8:15 (Mazomenos, 1989).

Continuing our efforts on the development of inhibitors of the pheromone action of important pests, we have turned out our attention to *S. nonagrioides* wherein no such study had been undertaken. In this paper we report on the electrophysiological activity of haloacetate analogues **IV-X** and trifluoromethyl ketone **XI** (**Fig. 1**) on EAG and in the field, and at the same time, the effects

produced by several formulations of the potential inhibitors with the pheromone complex on two other noctuids *Mythimna unipuncta* and *Discestra trifolii*, very often caught in *S. nonagrioides* traps.

Materials And Methods

Fig. 1. Structures of the analogues tested.

IV: R=O-CO-CH_2F **V**: R=O-CO-CHF_2
VI: R=O-CO-CF_3 **VII**: R=O-CO-CH_2Cl
VIII: R=O-CO-$CHCl_2$ **IX**: R=O-CO-CCl_3
X: R=O-CO-CH_2Br **XI**: R=CH_2-CO-CF_3

EAG bioassays.

Inhibition experiments were carried out by placing one to two day old males in 10 x 2 cm Petri dishes containing a piece of filter paper (2 x 2 cm), to which different doses of test compounds (1, 10, 100 and 1000 µg in 100 µl of hexane), had been applied. Insects were exposed to the vapors of the compounds for 2 hours in the dark, taken out and their antennae removed. Ten "puffs" with 10 µg of synthetic pheromone were insufflated over the antenna and the EAG responses recorded at 40 s intervals. Eight replicates were used for each test, and inhibition values were determinated as percent of the relative decrease of the EAG response in relation to the values exhibited by control insects.

Field tests.

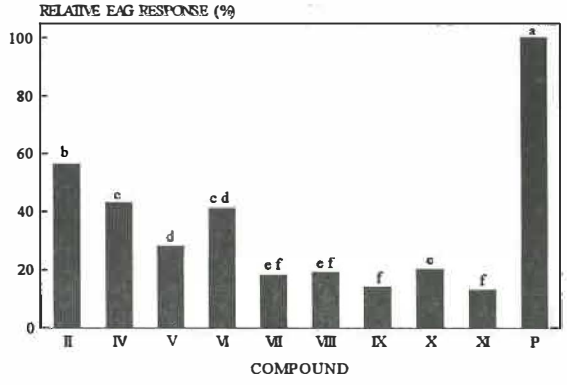
Experiments were conducted in 1992 from July 27th to October 27th in a maize field of Lleida province (Spain). Catches were evaluated using water traps and polyethylene vials (3 x 1.1 cm i.d.), baited with 0.2 mg of pheromone and 2 mg of the test compounds as dispensers. Traps, spaced 30 m apart, were hung at a height of ca. 1 m around the field in a linear randomized complete block design. Three traps were used for each formulation and the number of catches of *S. nonagrioides* recorded weekly, as well as those of the two related species *M. unipuncta* and *D. trifolii*.

Results And Discussion

Among the compounds tested, acetate **II** was the most potent (56% activity in comparison with **P**), followed by mono- and trifluoroacetate (43% and 41% respectively). As anticipated, the fluoroanalogues clearly elicited higher responses than the corresponding chloroderivatives, which were only slightly active. Bromoanalogue **X** displayed analogous activity than mono- and dichloroacetate **VII-VIII**, whereas trifluoromethyl ketone **XI** behaved similarly to trichloroacetate **IX** (**Fig. 2**). The results agree with those previously reported (Prestwich & Streinz, 1988; Camps et al., 1990) and it is worth of note the high activity shown by mono- and trifluoroacetate **IV-VI** in comparison to the major component **II**. This reflects once more that the steric size of the acetate

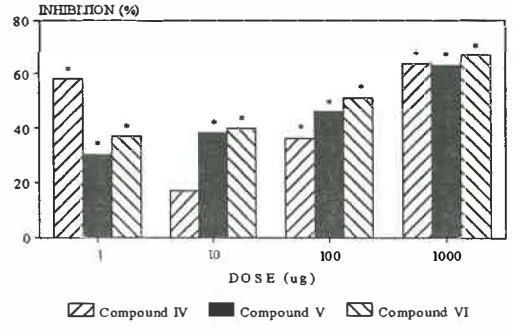
function predominates over the dipole moment in eliciting good EAG responses, and that the replacement of hydrogen by fluorine, gives rise to analogues with high electrophysiological activity.

Fig. 2. Relative intrinsic EAG activity showed by 10 µg of compounds **II-XI** compared to the natural pheromone blend (**P**). Bars represent the mean responses of 5 separate trials, and those with different letters are significantly different at $p<0.05$ (LSD procedure)



In the inhibition tests, the fluorinated analogues **IV-VI**, except compound **IV** at 1 µg, displayed a linear dose-effect relationship, the inhibition activity being higher at increasing doses (**Fig. 3**). In general, the inhibition values were moderate to good, ranging from 20% to 70% being

Fig. 3. Inhibition of the natural pheromone EAG response promoted by monofluoroacetate **IV**, difluoroacetate **V** and trifluoroacetate **VI**. Inhibition values correspond to the mean response of eight experiments. (*): Significantly different from control insects at $p<0.05$ (Student's *t*-test).



bromoacetate **X** was tested, again a moderate inhibition (35-45%), but higher than the corresponding monochlorinated **VII** at 1 and 10 µg doses, was obtained (**Fig. 5**).

Fig. 4. Inhibition of the natural pheromone EAG response promoted by monochloroacetate **VII**, dichloroacetate **VII** and trichloroacetate **IX**. Inhibition values correspond to the mean response of eight experiments. (*): Significantly different from control insects at $p<0.05$ (Student's *t*-test).

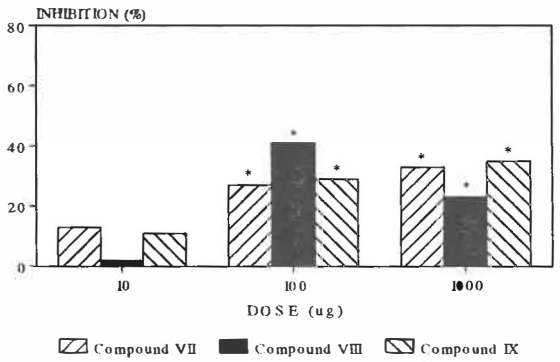
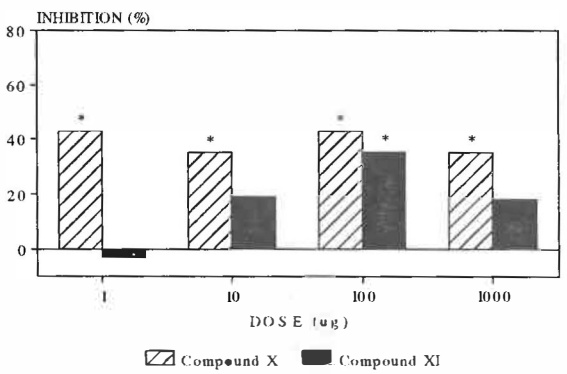


Fig. 5. Inhibition of the natural pheromone EAG response promoted by monobromoacetate **X** and trifluoromethyl ketone **XI**. Inhibition values correspond to the mean response of eight experiments. (*): Significantly different from control insects at $p<0.05$ (Student's *t*-test).

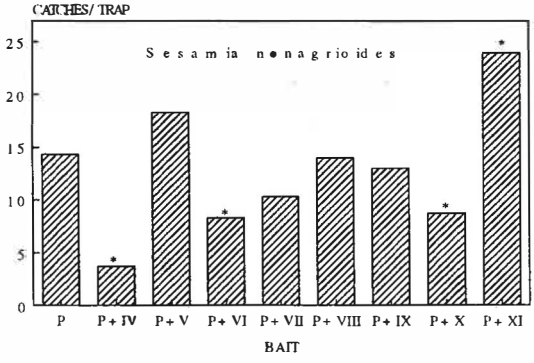


Apparently, the concentration had no effect on the activity of the compound. Trifluoropropanone **XI** was a poor inhibitor of the pheromone perception of the insect. Overall, the results are consistent with the relative molecular size of the analogue although the relatively high capacity of dichloro-,

trichloro and bromoderivatives **VIII**, **IX** and **X** to block the antennal receptors at certain concentrations is somewhat surprising.

In the field tests, fluoroacetates **IV** and **VI**, and monobromoacetate **X** appeared to be the best antagonists of the pheromone action (**Fig. 6**), while a synergistic effect was obtained when trifluoromethyl ketone **XI** was mixed with the pheromone. The other compounds didn't show any significant effect.

Fig. 6. Catches per trap of *S. nonagrioides* with baits of the sex pheromone complex **P** mixed with the potential inhibitors **IV-XI** in 1:10 ratio. Bars marked with (*) show the formulations with a synergistic or inhibitory effect compared to pheromone alone (**P**) at $p<0.05$ (Student's *t*-test).



For the other species, the results markedly differ. Thus, in *M. unipuncta* (**Fig. 7**), the antagonist compounds were difluoroacetate **V**, trichloroacetate **IX** and trifluoromethyl ketone **XI**, whereas trifluoroacetate **VI** showed a clear synergistic effect. In *D. trifolii*, all the compounds showed a significant inhibitory effect on male catches, except monofluoroacetate **IV** that exhibited a strong synergistic effect (**Fig. 8**).

Fig. 7. Catches per trap of *M. unipuncta* with baits of the sex pheromone complex **P** mixed with the potential inhibitors **IV-XI** in 1:10 ratio. Bars marked with (*) show the formulations with a synergistic or inhibitory effect compared to pheromone alone (**P**) at $p<0.05$ (Student's *t*-test).

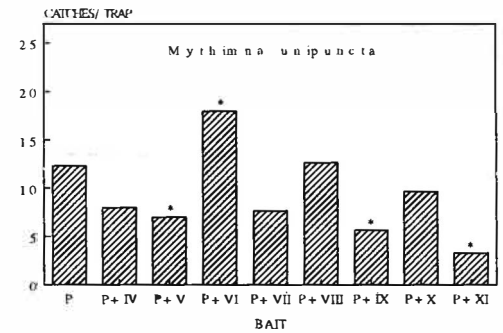
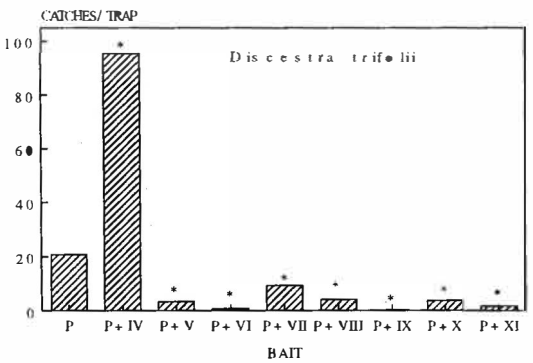


Fig. 8. Catches per trap of *D. trifolii* with baits of the sex pheromone complex **P** mixed with the potential inhibitors **IV-XI** in 1:10 ratio. Bars marked with (*) show the formulations with a synergistic or inhibitory effect compared to pheromone alone (**P**) at $p<0.05$ (Student's *t*-test).



References

CAMPS, F., GASOL, V., GUERRERO, A., HERNANDEZ, R. & MONTOYA, R., 1990. Inhibition of the processionary moth sex pheromone by some haloacetate analogues. Pestic. Sci. **29**: 123-134.

MAZOMENOS, B.E., 1989. Sex pheromone components of corn stalk borer *Sesamia nonagrioides* (Lef.). Isolation, identification, and field tests. J. Chem. Ecol. **15**: 1241-1247.

PRESTWICH, G.D. & STREINZ, L., 1988. Haloacetates analogs of pheromones: Effects on catabolism and electrophysiology in *Plutella xylostella*. J. Chem. Ecol. **14**: 1003-1021.

SCHWARTZ, M., KLUN, J.A. & UEBEL, E.C., 1990. European corn borer sex pheromone. Inhibition and elicitation of behavioral response by analogs. J. Chem. Ecol. **16**: 1590-1604.

OVIPOSITION ATTRACTANTS FOR *CULEX QUINQUEFASCIATUS*

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Abstract A polluted water sample was prepared to simulate the natural breeding waters of *Culex quinquefasciatus*. The water sample (0.01 - 80%) as well as the oviposition pheromone (0.01 - 80 µg) increased oviposition by gravid females in a choice bioassay. When 0.05 µg pheromone was added to the polluted water dilution series an additive effect resulted. An additive effect was also seen in an electrophysiological assay, recording electroantennograms (EAGs) from insect antennae.

Introduction

The vector status of *Culex quinquefasciatus* Say for a variety of arboviruses is well established. It is an important vector of filiariasis in the tropics (Reiter, 1985) and in the USA it transmits a variety of arboviruses, including St Louis encephalitis (Reeves and Milby, 1990). Pathogen acquisition and subsequent disease transmission requires two blood meals to be taken and one oviposition cycle to be completed. Hence, the capture of gravid females would enable estimates of the infective population to be made, as well as effecting a degree of control. Successful "ovitraps" to capture gravid females are presently being researched.

C. quinquefasciatus females lay their eggs in water polluted with sewage and other organic matter (Reiter *et al.*, 1991; Millar *et al.*, 1992) and are also attracted to a volatile pheromone (*erythro*-6-acetoxy-5-hexadecanolide) released from the apical droplets of egg rafts (Laurence *et al.*, 1985; Dawson *et al.*, 1989).

We have investigated the attractancy resulting from a combination of the *Culex* oviposition pheromone and a polluted water sample, using both behavioural and electrophysiological techniques.

Materials and Methods

Bioassays

Groups of twenty gravid *Culex quinquefasciatus* females were offered the choice between two glass bowls for oviposition: one test and one control. Test bowls contained either 100 ml distilled water plus 100 µl of the test material (oviposition pheromone, extraction fractions of polluted water or 3-methylindole), or 100 ml polluted water (100% = 25g rabbit dropping in 500 ml distilled water, fermented for three days). Control bowls contained 100 ml distilled water plus 100 µl solvent where appropriate. Bowl positions were alternated between replicates. The trials were run overnight T 27 ± 2°C and assessed the following morning by counting the number of egg rafts in each bowl. Results are expressed as the percentage of egg rafts in the test bowl compared with the total number of egg rafts in the cage.

Electrophysiology

Electroantennograms (EAGs) were recorded with Ag-AgCl electrodes from excised heads of gravid female mosquitoes, using methods described previously (Mordue (Luntz) *et al.*, 1992). Test compounds were applied in 10 µl solvent to filter papers inserted into glass Pasteur pipettes. Stimulus molecules were introduced into a filtered, humidified airstream directed onto the antenna.

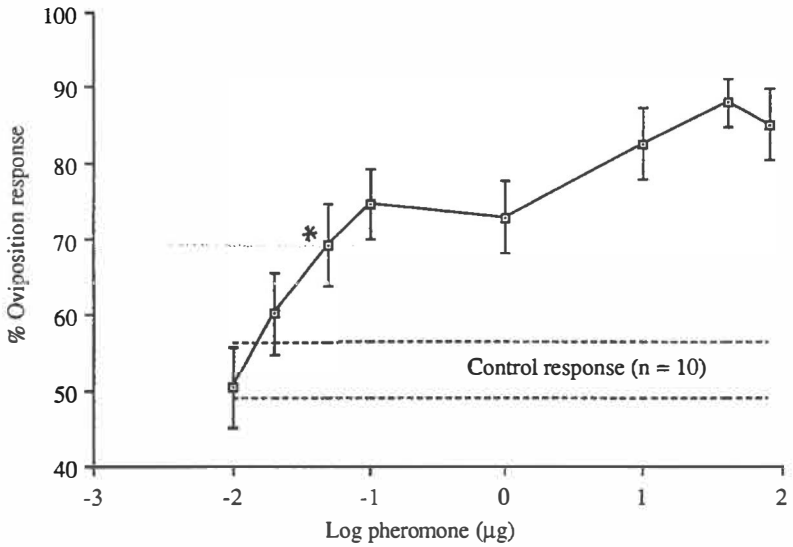
Results and Discussion

The synthetic oviposition pheromone increases egg laying by *C. quinquefasciatus* females in a dose-dependant manner within the dose range tested (0.01 - 80 µg), (Fig. 1A). The threshold concentration increasing oviposition significantly compared with the control was 0.05 µg (69.3 ± 5.4%, n = 7). The pheromone was also electrophysiologically active (Fig. 1B): the threshold concentration of 49.05 µg (0.18 ± 0.05 mV) was three orders of magnitude greater than the behavioural threshold.

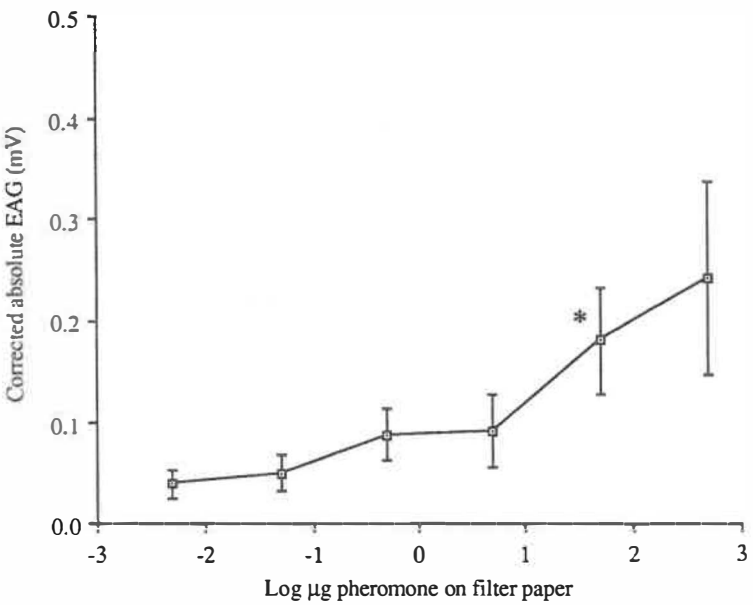
Three-day polluted water was strongly attractive to gravid females, with a 2% solution significantly increasing oviposition (91.4 ± 3.8%, N = 8), compared with the control value (47.4 ± 6.2%, n = 9 (Fig. 2A) An additive effect resulted from combining 0.05 µg oviposition pheromone with the polluted water, reducing the polluted water threshold to 0.2% (85.4 ± 2.9%). An additive effect was also seen with EAG assays, with the addition of pheromone reducing the threshold for the polluted water from 100% (0.26 ± 0.05 mV to 10% 0.23 ± 0.06 mV), (Fig. 2B).

Six day fermented water was extracted in ether and the resulting fractions assayed for activity (Table 1). A 2% solution of the unextracted water was unattractive to the mosquitoes. After extraction, both the extracted water and the ether component showed activity. Vacuum distillation of the ether extract did not alter the activity of the sample. The extraction fractions were also electrophysiologically active, with threshold of 1% (vacuum-distilled ether extract), 10% (2% polluted water after extraction) and 100% (unextracted polluted water (2%) and ether extract of 10% polluted water).

Fig. 1 Responses of female C. quinquefasciatus to the synthetic oviposition pheromone, erythro-6-acetoxy-5-hexadecanolide. Vertical lines = SEM; * = threshold concentrations (see text).

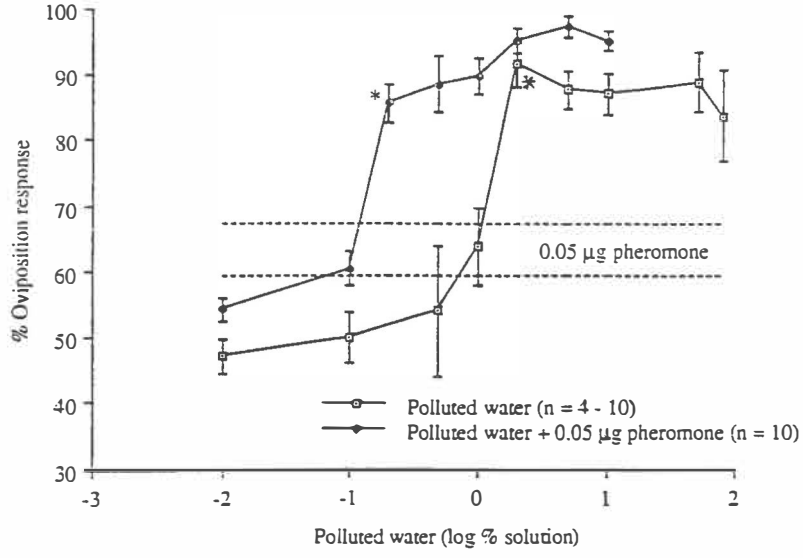


A. Behavioural response (n = 3 - 7 per concentration). The broken lines represent the upper and lower values of the control mean.

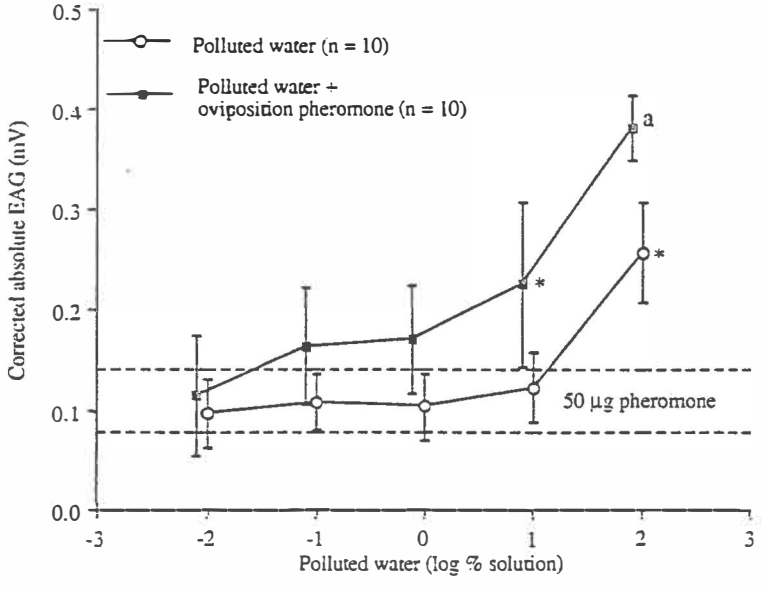


B. EAG response (n = 10).

Fig. 2 Responses of gravid female C. quinquefasciatus to three-day polluted water and three day polluted water plus oviposition pheromone. Vertical lines = SEM, * = threshold concentrations (see text).



A. Behavioural response. The broken lines represent the response to 0.05 µg pheromone alone.



B. EAG response. The broken lines represent the response to 50 µg pheromone alone.
a: polluted water + pheromone > polluted water alone ($P < 0.05$).

Table 1. Oviposition behaviour of gravid *C. quinquefasciatus* females in the presence of six-day fermented polluted water and its extraction products. *: $p < 0.001$ compared with the control mean (Student's t-test).

Test solution	Mean % egg rafts (of total) laid in test bowl ± S.E. (n)
Ether control	47.2 ± 2.7% (8)
2% polluted water	29.8 ± 10.6% (3)
2% polluted water after extraction	76.3 ± 3.2%* (3)
Ether extract of 10% polluted water	70.9 ± 4.4%* (8)
Undiluted ether extract of polluted water following vacuum distillation	92.7 ± 3.1%* (8)

One possible constituent of the polluted water was 3-methylindole (skatole), a common natural product in animal excreta (Millar *et al.*, 1992). However, although 3-methylindole was strongly stimulatory in both the behavioural and electrophysiological assays, it was not detected in GC and GC-MS analysis of a vacuum-distilled ether extract of the polluted water. Work is currently in progress to determine the active constituents of the polluted water, which together with the oviposition pheromone, have the potential to form an efficient and convenient synthetic oviposition attractant for use in control and monitoring programmes.

References

DAWSON, G.W., LAURENCE, B.R., PICKETT, J.A., PILE, M.M. and WADHAMS, L.J. 1989. A note on the mosquito oviposition pheromone Pest. Sci **27**: 277-280.

LAURENCE, B.R., MORI, K., OTSUKA, T., PICKETT, J.A. and WADHAMS, L.J. 1985. Absolute configuration of mosquito attractant pheromone, 6-acetoxy-5-hexadecanolide. J. Chem. Ecol. **11**: 643-648.

MILLAR, G.J., CHANEY, J.D. and MULLA, M.S. 1992. Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. J. Am. Cont. Assoc. **8**: 11-17.

MORDUE (Luntz), A.J., BLACKWELL, A., HANSSON, B.S., WADHAMS, L.J., and PICKETT, J.A. 1992. Behavioural and electrophysiological evaluation of oviposition attractants for *Culex quinquefasciatus* Say (Diptera: Culicidae). Experientia **48**:1109-1111.

REEVES, W.C. and MILBY, M.M. 1990. Natural infection in arthropod vectors. In: Epidemiology and control of mosquito-borne Arboviruses in California, 1943-1987. Calif. Mosq. Control Assoc., Sacramento, CA (ed by W.C. Reeves), pp. 128-129.

REITER, P. 1985. A field trial of expanded polystyrene balls for the control of *Culex* mosquitoes breeding in pit latrines. J. Am. Mosq. Cont. Assoc. **1**: 519-521

REITER, P., AMADOR, M.A. and COLON, N. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. J. Am. Mosq. Cont. Assoc. **7**: 52-55.

NOVEL SYSTEMS FOR PHEROMONE RELEASE, USING *CULEX QUINQUEFASCIATUS* OVIPOSITION PHEROMONE

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Abstract Mixed carboxylate glasses have been shown to successfully dissolve *Culex quinquefasciatus* oviposition pheromone. These matrices degrade in a humid environment and release the pheromone in a controlled fashion. Pheromone encapsulation of the glasses was >70% and successful aerial release of the pheromone was demonstrated in an oviposition choice bioassay, with dose-response relationships with both time and dose. The glasses alone did not affect the behavioural responses of the mosquitoes.

Introduction

The composition of glass can be adapted to suit a variety of applications, including those which depend on its solubility and hence rate of dissolution. Thus, glass has great potential for controlled release applications, although to date only for materials capable of withstanding the high temperatures involved in the preparation of phosphate glasses. It is possible, however, to produce glasses from mixtures of metal carboxylates which are liquid at much lower temperatures and are excellent solvents. This allows the incorporation of thermally unstable biological molecules into the metal carboxylate glasses, with solubility usually ca. 5% by weight of solute. The glasses require humidity for breakdown and release but can be stored indefinitely in an anhydrous environment without affecting the incorporated compound. The breakdown products of the glass are harmless in the environment. The potential for such glasses would relate for example to the slow release of insecticides or nutrients into the soil or the release of pheromones into the air. In this paper selected water-soluble, low softening-temperature carboxylate glasses have been investigated with respect to the incorporation and controlled release of *C. quinquefasciatus* oviposition pheromone (*erythro*-6-acetoxy-5-hexadecanolide) which has demonstrated uses in mosquito control (Otieno *et al.*, 1988).

Materials and Methods

Controlled release matrices

Glasses were prepared by grinding together the component carboxylated salts followed by melting and quenching (Blair *et al.*, 1992). *C. quinquefasciatus* oviposition pheromone was introduced into melted glass by injection below the surface. After stirring, the melt was quickly quenched in bronze moulds to form discs of glass (3mm x 10mm and 0.8g weight). Such tablets were stored in sealed, dry flasks until required. Pheromone content of the glass was analysed by GC using a Perkin-Elmer 8320 capillary gas chromatograph and 25 m S.G.E. 0.25μ bonded phase fused silica BP5 column (50°C, 2 min; 20°C, min^{-1}, 230°C, 15 min) with N_2 at 15 psi. The pheromone was soxhlet extracted in dichloromethane.

Bioassays

Groups of gravid *C. quinquefasciatus* females were offered the choice between two glass bowls for oviposition: one test and one control. Test bowls contained either 200 ml distilled water plus pheromone alone, applied onto a glass coverslip, floated on a plastic vial cap, or pheromone encapsulated into a carboxylated glass, also on a plastic vial cap. Control bowls contained 200 ml distilled water plus a glass coverslip with solvent with solvent alone, or the relevant undoped glass. Control experiments of glass versus no glass were also run. The bowls were placed in diagonally opposite corners of the cage some 30 cm apart. Neither the relative position of the test and control bowls not the density of gravid females (20-200 per test) significantly egg laying behaviour (Blair, 1992). The trials were run overnight at 27 ± 2°C and assessed the following morning by counting the number of egg rafts in each bowl and expressing the results as percentage in the test bowl compared with the total number in both tests and control bowls.

Results and Discussion

The glasses did not adversely affect the oviposition behaviour of the mosquitoes and recovery of the pheromone from the glasses showed it to be unaltered by the encapsulation procedure (Table 1).

The release of pheromone in time is expressed as mean % retention for each of the glass matrices (Fig. 1). It is clear that the more hygroscopic glasses release pheromone at a faster rate. The acetate glass broke down completely in 70-80% humidity releasing most of the encapsulated pheromone within one month, whereas the more resistant glass exhibited a slower or greatly reduced release rate (Fig. 1).

As with the work of Laurence and Pickett (1985) and Mordue (Luntz) *et al.* (1992), a maximal behavioural response to pheromone was achieved at 0.1 μg with the threshold response t 0.05 μg (Fig. 2). When incorporated into carboxylate glasses the pheromone also brought about a positive response (Fig. 2). The threshold response for acetate glass was 25 μg pheromone/glass disc ($p < 0.05$) and for propanoate glass, was 1000 μg pheromone/glass disc (Fig. 2). The mixed-anion matrix, even at an initial loading of 4.2 mg/glass disc, showed no preferential response until the overall surface area was increased by powdering the tablet (response for 250 μg pheromone in powdered tablet was 66.4 ± 2.6%; $p < 0.01$, $n = 8$) (Blair, 1992).

Further work has shown that there is potential for regulating both the initial loading of the glass

Table 1 Properties of selected carboxylate glasses as controlled release media

Glass Composition	Ratio	Encapsulation temperature /°C	Solubility[1]	Density g cm^{-3}	Mean % encapsulation of pheromone	Ovipositional response of *C. quinquefasciatus* to glass alone[2]
1 Sodium acetate Potassium acetate Calcium acetate	1:1:1	140	2 hours	1.478	84.1±3.76(5)	52.6±3.70(10)
2 Sodium propanoate Calcium propanoate	1:1	180	8 hours	1.322	74.0±5.18(3)	51.9±3.02(9)
3 Sodium butanoate Calcium propanoate Sodium octanoate	1:2:2	160	2 weeks	1.185	72.4±1.94(5)	49.5±3.46(5)

1. Time for 1 g tablet to lose half its weight in still water.
2. Expressed as mean % egg rafts laid in test bowls vs control. No significant difference between the glass control and the blank control values, 50.5±5.29(3).

Figures in brackets refer to number of replicates.

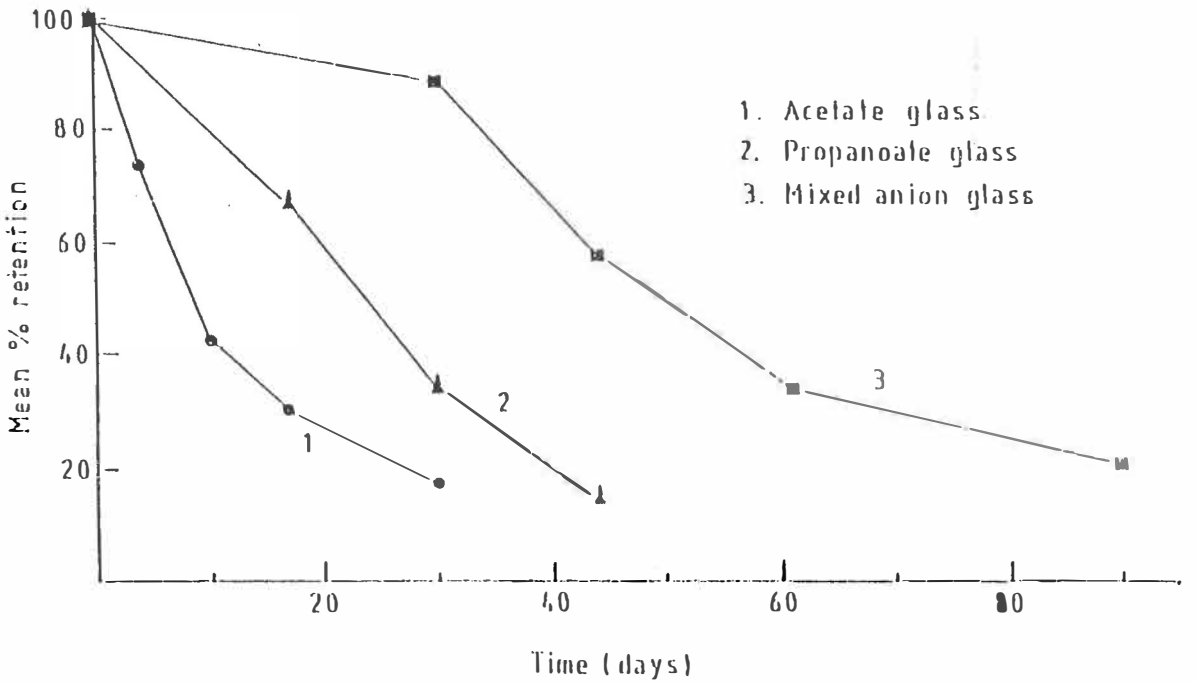

Figure 1 The release of *C. quinquefasciatus* oviposition pheromone with time from carboxylate glasses, in a humid environment, when held over water, as measured by the residual pheromone in the glass (n=3). Loading of the glasses was 8mg g^{-1}.

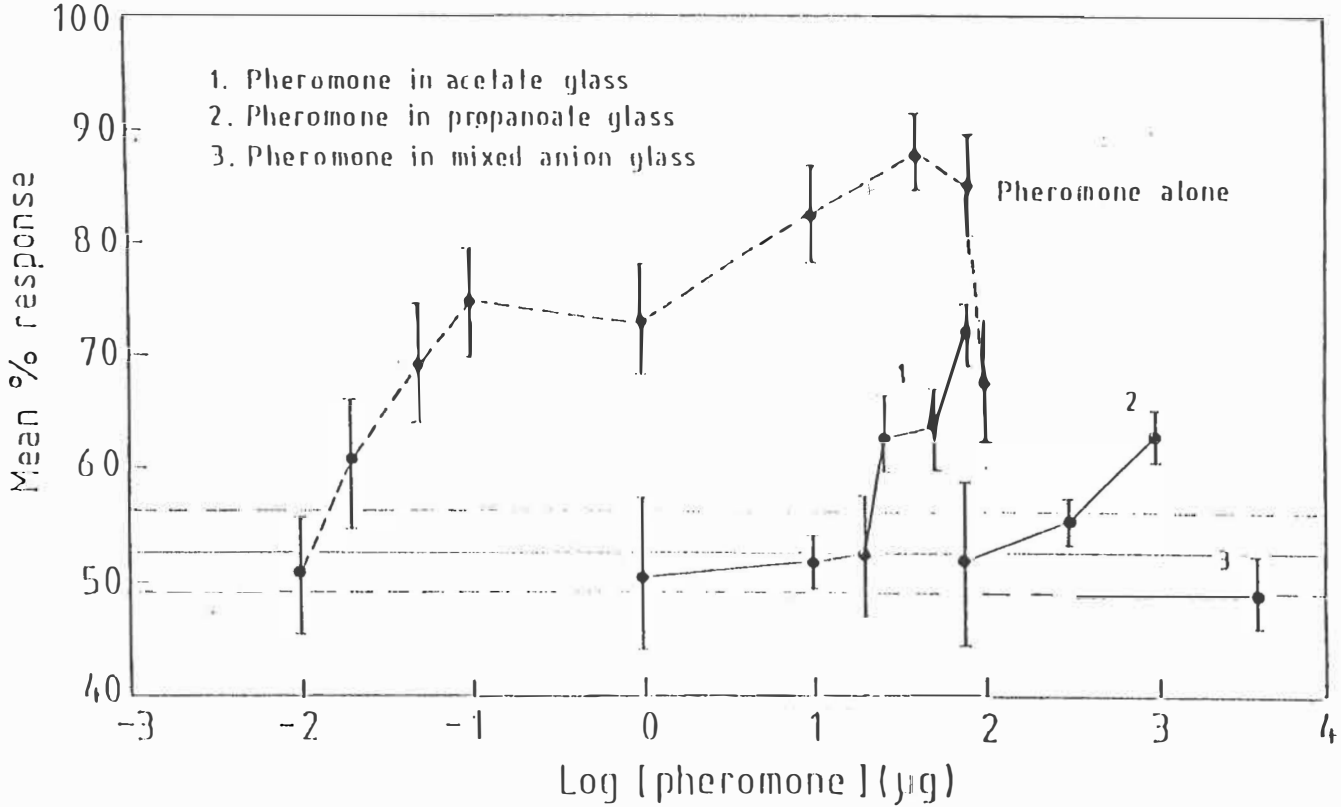

Figure 2. Response of *C. quinquefasciatus* to oviposition pheromone either alone or incorporated into different carboxylate glasses. Pheromone loading is expressed as log[pheromone] per glass disc. Response is measured as the number of egg rafts in the test bowl against total number of egg rafts laid (n=5-11). Control response (±SEM) in the absence of pheromone is shown by continuous and dashed lines.

and the type of glass used to produce glasses with the capacity for holding high concentration of pheromone for long term controlled release (Blair, 1992).

References

BLAIR, J.A. 1992. Development and application of metal carboxylate glass matrices. PhD thesis, University of Aberdeen, 1992.

BLAIR, J.A., DUFFY, J.A. and WARDELL, J.L. 1992. A new glass for hosting organic compounds with optical, electronic and biological applications. Phys. Chem. Glasses **33**: 191-198.

LAURENCE B.R and PICKET, J.A. 1985. An oviposition attractant in *Culex quinquefasciatus* Say (Diptera: Culicidae). Bull. ent. Res. **75**: 283-290.

MORDUE (LUNTZ), A.J., BLACKWELL, A., HANSSON, B.S., WADHAMS, L.J. and PICKETT, J.A. 1992. Behavioural and electrophysiological evaluation of oviposition attractants for *Culex quinquefasciatus* Say (Diptera: Culicidae). Experienta **48**: 1109-1111.

OTIENO, W.A., ONYANGO, T.O., PILE, M.M., LAURENCE, B.R., DAWSON, G.W., WADHAMS, L.J. and PICKETT, J.A. 1988. A field trial of the synthetic oviposition pheromone of *Culex quinquefasciatus* Say (Diptera: Culicidae) in Kenya. Bull. ent. Res. **78**: 463-478.

DEVELOPMENT OF A MONITORING TRAP FOR THE PEA AND BEAN WEEVIL, USING THE AGGREGATION PHEROMONE

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Abstract The effect of trap type, height and colour on the capture of the pea and bean weevil, *Sitona lineatus* L., was studied in a series of field experiments. All traps were baited with the aggregation pheromone, 4-methyl-3,5-heptanedione. A small cone trap placed on the ground was found to be the most effective and practical design. The colour of the trap did not have a significant effect on the number of weevils captured.

Introduction

The pea and bean weevil, *Sitona lineatus* L., occurs widely throughout Europe and the Middle East and is considered to be a serious pest of leguminous crops (Bardner et al., 1983; Oschmann 1984; Nielsen, 1990). Work in the UK has shown that effective control produces yield increases for peas and beans of 7-31% (King, 1981; Bardner et al., 1983; Baughan et al., 1985).

A male-produced pheromone, 4-methyl-3,5-heptanedione, has been identified and shown to be attractive to both sexes in the field (Blight et al., 1984). Peak pheromone production occurs in the spring and facilitates aggregation of overwintered adults on the host crop, where feeding produces characteristic leaf notching. Although the aggregation pheromone is not produced by newly-emerged males in the autumn, both sexes respond to synthetic pheromone during the autumn dispersive phase (Blight et al., 1991).

At present, control is prophylactic or by pesticide application in response to the appearance of leaf notching. However, an effective monitoring system would give earlier warning of movement into the host crop and should lead to more accurately timed application, and reduced use, of pesticides.

The aim of the work presented here was to determine an optimal design of trap, baited with aggregation pheromone, to form the basis of a monitoring system for this insect.

Methods

The experiments were carried out on Rothamsted Farm between early April and June 1992. Traps were compared using a randomised block (Latin square) design with periodic rerandomisation within the blocks. In all experiments, traps were baited with lures which released 4-methyl-3,5-heptanedione.

Data were analysed using ANOVA. Where appropriate the Least Significant Difference (LSD) test was applied.

I. Comparison of trap type

The following types of trap were compared:

a) Modified cotton boll weevil `Scout' cone traps (Forey and Quisumbing, 1987). Four semicircular holes were cut at the lower edge of the base of the trap and the top of the base was removed to allow the weevils to pass from the base into the cone.
b) Cotton boll weevil `Hardee' cone traps (Mitchell and Hardee, 1974), modified as for (a).
c) Large yellow bucket traps (Blight and Wadhams, 1987).
d) Box trap, with four yellow sticky cards attached. (Smart et al., 1989).
e) Oecos Delta trap no. 1 painted yellow, with sticky card insert.

All traps were placed at ground level, on sites where *S. lineatus* had overwintered.

II. Comparison of trap height

The modified boll weevil `Scout' cone trap was compared with a sticky trap, consisting of two boards coated with Oecos `Master trap' attached to a wooden stake. The boards were oriented so that one faced north/south and the other east/west.

Both types of trap were placed at ground level and at a height of 1m on the edge of fields of spring sown field beans.

III. Comparison of different coloured modified `Scout' traps

Unpainted (fluorescent green) cones were compared with cones painted yellow, white, black and dark green. (These were British Standard colours). The experimental blocks were placed on sites where *S. lineatus* had overwintered.

Results

Experiment I.

The three plastic cone traps caught significantly more *S. lineatus* than the sticky delta and box traps ($p<0.001$, LSD test). However, the small, boll weevil `Scout' and `Hardee' cone traps were more attractive than the larger bucket trap, ($p = 0.05$, LSD test) and were also more practical to use.

Experiment II.

Modified `Scout' cone traps, at ground level, caught significantly more weevils than `Scout' traps at 1m or sticky boards at either height ($p<0.001$, LSD test). Additionally, the sticky boards proved inefficient at retaining trapped weevils.

These results support the observation that overwintered weevils fly to the vicinity of host crops and then walk to the point source of attractant.

Experiment III.

Although yellow traps caught more weevils than any other colour there were no significant differences between the numbers caught by the five colours.

Discussion and Conclusions

This work demonstrated that a small cone trap, placed at ground level and baited with the aggregation pheromone is an effective and practical design for capturing the pea and bean weevil. Trap colour does not appear to be important. This was rather surprising since many phytophagous insects appear to have a colour preference, e.g. the cotton boll weevil is strongly attracted to yellow and fluorescent green pigments (Cross et al., 1976; Leggett and Cross, 1978).

The baited cone trap is highly specific. It is unattractive to other *Sitona* species and >98% of the insects trapped are *S. lineatus*. A monitoring system based on the use of this trap should facilitate more efficient timing of pesticide application and lead to better pest control with lower pesticide application.

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References

BARDNER, R., FLETCHER, K.E. & GRIFFITHS, D.C., 1983. Chemical control of the pea and bean weevil, *Sitona lineatus L.*, and subsequent effects on the yield of field beans *Vicia faba L.* J. Agric. Sci. (Camb.) **101**: 71-80.

BAUGHAN, P.J., BIDDLE, A.J., BLACKETT, J.A. & TOMS, A.M., 1985. Using the seed as a chemical carrier. pp. 97-106. *In* E.S.E. Southcombe (ed). Symposium on application and biology. British Crop Protection Council Monogram No. 28.

BLIGHT, M.M., DAWSON, G.W., PICKETT, J.A. AND WADHAMS, L.J., 1991. The identification and biological activity of the aggregation pheromone of *Sitona lineatus*. Aspects of Applied Biology **27**. Production and Protection of Legumes: 137-142.

BLIGHT, M.M., PICKETT, J.A., SMITH, M.C. & WADHAMS, L.J., 1984. An aggregation pheromone of *Sitona lineatus*. Naturwissenschaften **71:** 480-481.

BLIGHT, M.M., & WADHAMS, L.J., 1987. Male produced aggregation pheromone in the pea and bean weevil, *Sitona lineatus* (L.). J. Chem. Ecol. **13**: 733-739.

CROSS, W.H., MITCHELL, H.C. & HARDEE, D.D., 1976. Boll weevils: Response to light sources and colours on traps. Environ. Entomol. **5** : 565-571.

FOREY, D.E. & QUISUMBING, A.R., 1987. Newly designed boll weevil SCOUT trap. pp. 139-141, *In* Proceedings, Beltwide Cotton Production Research Conferences, Jan. 4-8, Dallas. National Cotton Council of America, Memphis.

KING, J.M., 1981. Experiments for the control of pea and bean weevil (*Sitona lineatus*) in peas, using granular and liquid insecticides. pp. 327-331. *In* Proc. British Crop Protection Council.

LEGGETT, J.E. & CROSS, W.H., 1978. Boll weevils: The relative importance of color and pheromone in orientation and attraction to traps. Environ. Entomol. **7**: 4-6.

MITCHELL, E.B. & HARDEE, D.D., 1974. In-field traps: A new concept in survey and suppression of low populations of boll weevils. J. Econ. Entomol. **67**: 506-508.

NIELSEN, B.S., 1990. Yield responses of *Vicia faba* in relation to infestation levels of *Sitona lineatus* L. (Col.: Curculionidae). J. Appl. Entomol. **110**: 398-407.

OSCHMANN, M., 1984. Untersuchungen zur Ertragsbeeinflussung der Ackerbohne (*Vicia faba* L.) durch den Liniierten Blattrandkäfer (*Sitona lineatus* L., Coleoptera, Curculionidae). Arch. Phytopathol. Pflanzenschutz **20**: 371-381.

SMART, L.E., STEVENSON, J.H. & WALTERS, J.H.H. 1989. Development of field trial methodology to assess short-term effects of pesticides on beneficial arthropods in arable crops. Crop Protection **8**: 169-180.

DEVELOPMENT OF A MONITORING SYSTEM FOR THE CABBAGE SEED WEEVIL AND THE POLLEN BEETLE

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Abstract The effect of trap type and an isothiocyanate lure on the capture of the seed weevil, *Ceutorhynchus assimilis*, and pollen beetles, *Meligethes* spp., was studied in a series of field experiments. Yellow sticky traps and water traps were equally effective, but traps baited with the isothiocyanate lure caught significantly more insects than unbaited traps. A baited yellow sticky card trap angled at 45° to the vertical, appears to be suitable for the simultaneous monitoring of the two insect species.

Introduction

The seed weevil, *Ceutorhynchus assimilis* Payk., and the pollen beetles, *Meligethes* spp. Fab., are important pests of oilseed rape, *Brassica napus*, in the UK and Europe (Nolte & Fritzsche, 1954; Bonnemaison, 1957; Jourdheuil, 1962; Nilsson, 1987; Alford et al., 1991). Larvae of *C. assimilis* can reduce yields of both winter and spring sown rape by feeding on the seeds. Additional crop loss occurs when adult feeding and egg laying punctures in rape pods provide access for another major inflorescence pest, the Brassica pod midge, *Dasineura brassicae*.

In contrast to the weevil, pollen beetles are major pests of spring rape in the UK, but only cause loss of winter rape when growth is poor. Damage is caused by adult feeding on the pollen of both buds and flowers, but the larvae are not injurious (Free & Williams, 1978).

Effective monitoring of both pests would reduce unnecessary use of pesticides because control measures are not justified on every site in every year (Alford et al., 1991).

Both visual and olfactory cues appear to be involved in the orientation of seed weevils and pollen beetles to Cruciferous plants (Moericke, 1953; Nolte & Fritzsche, 1954; Free & Williams, 1978). Damaged oilseed rape emits a complex mixture of volatile compounds, many of which are detected by the seed weevil antenna (Blight et al., 1992). Some of these compounds, the isothiocyanates, are characteristic of the Cruciferae. They are formed from involatile glucosinolates by the action of enzymes released as the result of tissue damage (Kjaer, 1976). There is evidence from laboratory (Bartlett et al., 1992) and field experiments (Finch, 1977; Free & Williams, 1978; Lerin, 1984) that isothiocyanates may be attractive to seed weevils and pollen beetles.

The effects of an isothiocyanate lure on the capture of these insects by water and sticky traps was investigated in a series of field experiments. It is possible that a baited trap could be used to monitor both pests.

Methods

The experiments were carried out during the late summer, 1991, on Rothamsted Farm on a site where winter rape had recently been harvested. Randomised block (Latin Square) designs were used and the traps were set up, 10 m apart, alongside hedgerows at the edges of the field.

Comparison of trap type

Three types of yellow trap were compared:

a) Box trap, with four sticky cards attached (Smart et al., 1989);
b) Water trap, constructed from a petri dish, diameter 14 cm x 1.7 cm deep;
c) Water bowl trap, diameter 21 cm x 9 cm deep.

All traps were mounted on poles at a height of 1 m. They were either unbaited, or baited with a lure which released allyl isothiocyanate at 60 mg per day and the 3-butenyl, 4-pentenyl and phenylethyl isothiocyanates at 6 mg per day per compound.

Effect of angle of inclination of a sticky card trap on insect capture

Yellow sticky cards (20 x 10 cm) were set up vertically, horizontally, or at a 45^{o} angle, at a height of 1 m above ground. The traps were unbaited, or baited with the isothiocyanate lure used in Experiment I.

Results

Experiment I

All three trap types were equally effective in capturing both insects (Factorial ANOVA, $P=0.05$) but baited traps were significantly more attractive than unbaited to both pests (Factorial ANOVA, $P<0.001$). Five to seven times as many seed weevils were caught by the baited, compared with the unbaited traps, and pollen beetle capture was doubled when the lure was used.

Experiment II.

As in the previous experiment, baited traps were more effective than unbaited (Factorial ANOVA, $P<0.001$). Use of the lure doubled the capture of pollen beetles and doubled or quadrupled the capture of seed weevils.

Baited vertical traps were significantly more attractive to the seed weevil than all other traps, but baited vertical and 45^{o} angled traps were equally effective for pollen beetle capture. Horizontal traps of both types caught significantly fewer numbers of both pests (Duncan's multiple range test, $P=0.05$).

Conclusions

These experiments demonstrated that baited water and sticky traps are equally effective in capturing both seed weevils and pollen beetles. Both trap types could therefore be used for monitoring these insects, but sticky traps may be more convenient for use by the grower. They have

been used successfully by the non-specialist to monitor other species of insects (Muirhead-Thomson, 1991).

The small sticky card trap was simple to use and was effective. In these experiments, conducted during the autumn dispersal phase, the vertical trap was the most efficient, but these and additional results (Blight and Smart, unpublished) suggest that a 45^{o} orientation may be the best compromise for trapping both species throughout the year.

The isothiocyanate lure was attractive to both species, but at least twenty-two additional host plant volatiles are detected by the seed weevil antenna (Blight et al., 1992) and many of these may be attractive to both insects. The use of these volatiles in a lure for monitoring both pests is being investigated.

References

ALFORD, D.V., COOPER, D.A, & WILLIAMS, I.H., 1991. Insect Pests of Oilseed Rape. HGCA Oilseeds Research Review no. OS1. Home Grown Cereals Authority, London. 130 pp.

BARTLET, E., BLIGHT, M.M., HICK, A.J., & WILLIAMS, I.H., 1993. The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape (*Brassica napus*) and to some volatile isothiocyanates. Entomol. Exp. Appl. In press.

BLIGHT, M.M., HICK, A.J., PICKETT, J.A., SMART, L.E., WADHAMS, L.J. & WOODCOCK, C.M., 1992. Volatile plant metabolites involved in host plant recognition by the cabbage seed weevil, *Ceutorhynchus assimilis*. pp. 105-106. *In* S.B.J. Menkin, J.H. Visser & P. Harrewijn (eds). Proc. 8th Int. Symp. Insect-Plant Relationships. Kluwer Acad. Publ. Dordrecht

BONNEMAISON, L., 1957. Le charancon des siliques (*Ceutorhynchus assimilis* Payk.). Biologie et méthodes de lutte. Ann. Épiphyt. **8:** 387-543.

FINCH, S., 1977. Effect of secondary plant substances on host-plant selection by the cabbage root fly. Colloques Internationaux du CNRS no. 265, 251-267.

FREE, J.B., & WILLIAMS, I.H., 1978. The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceuthorhynchus assimilis*, to oilseed rape, *Brassica napus*, and other plants. J. Appl. Ecol. **15:** 761-774.

JOURDHEUIL, P., 1962. Famille des Nitidulidae. pp. 318-330. *In* A.S. Balachowsky (ed). Entomologie appliquée à l'agriculture. I. Coléoptères. Masson et Cie. Paris.

KJAER, A., 1976. Glucosinolates in the Cruciferae. pp. 207-219. *In* J.G. Vaughan, A.J. MacLeod & B.M.G. Jones (eds). The biology and chemistry of the Cruciferae. Academic Press, London.

LERIN, J., 1984. Effet de deux isothiocyanates sur les niveaux de capture en cuvettes jaunes d'insectes ravageurs du colza. Acta oecologia; Oecol. Applic. **5:** 61-70.

MOERICKE, V., 1953. Wie finden geflügelte Blattläuse ihre Wirtspflanzen? Mitt. Biol. Zentralanst. **75:** 90-97.

MUIRHEAD-THOMSON, R.C., 1991. Plant pest responses to visual and olfactory `sticky' traps. pp. 180-196 *In* Trap Responses of Flying Insects - The Influence of Trap Design on Capture Efficiency. Academic Press.

NILSSON, C., 1987. Yield losses in summer rape caused by pollen beetles (*Meligethes spp.*) Swed. J. Agric. Res. **17:** 105-111.

NOLTE, H.-W. & FRITZSCHE, R., 1954. Untersuchungen zur Bekämpfung der Rapsschädlinge. III. Zur Biologie und Bekämpfung des Kohlschotenrüsslers (*Ceuthorrhynchus assimilis* Payk.) und der Kohlschotengallmücke (*Dasyneura brassicae* Winn.) NachrBl.dt. PflSchutzdienst, n.F. **8:** 128-135.

SMART, L.E., STEVENSON, J.H. & WALTERS, J.H.H., 1989. Development of field trial methodology to assess short-term effects of pesticides on beneficial arthropods in arable crops. Crop Protection **8:** 169-180.

CONTROLLING THE VINE BERRY MOTH, *LOBESIA BOTRANA* DEN & SCHIFF., WITH MATING DISRUPTION TECHNIQUE BY PHEROMONES IN GREECE

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Abstract The mating disruption technique by pheromones was used to control the vine berry moth, Lobesia botrana, in part of an isolated vineyard in Tsaritsani, Thessalia, Greece. The total area covered by pheromone dispensers was 16ha. In the central 4ha only pheromones were applied while in the surrounding buffer zone of 12ha insecticides were also used. About 500 BASF (RAK 2) dispensers were used per ha.

The results showed effective control, comparable to that with insecticides, against the pest.

ANNEX

ROLE OF PHEROMONES IN INTEGRATED PEST MANAGEMENT (SURVEY CONDUCTED IN JULY - NOVEMBER 1990)

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Abstract The extensive application of pesticides has not only upset the delicate biological equilibrium of nature, but it also poses a danger to human health and causes problems of resistance. As a result, the emerging "alternative agriculture" and a worldwide shift towards integrated pest management (IPM) are bringing behavior-modifying chemicals (BMC), particularly pheromones, to the forefront of pest control. High expectations have been associated with BMC as ecologically friendly, chemically safe, and efficient tools in pest, especially insect, control. Monitoring by means of pheromones is a technique that has found considerable success in IPM. However, real control by mass trapping and disruption of communication ("confusion") is still not applicable on a large scale, except for a few isolated examples.

To evaluate the future role of pheromones in IPM, a questionnaire was sent to 142 researchers, field workers, companies, agencies, and other bodies around the world. The questions dealt mainly with real applications at present and predictions for 1, 5 and 10 years ahead. The information gathered, albeit not complete, shows that worldwide some 1,300,000 ha of a variety of crops are treated with pheromones by means of all the techniques available. Pheromones find their widest application in the "confusion" of the pink bollworm in cotton and in the mass trapping of the pine processionary moth in forests. Fruit moths also constitute a group of pests that are being controlled with pheromones. Expectations are that pheromones will capture about 15-40% of the insecticide market within 10 years. The current approach, which consists of applying small quantities at high cost, will probably shift to treatment with larger quantities of cheap pheromones. To this we should add that ecological considerations will play an ever increasing role in the behavior of customers who will probably be ready to pay more for "clean" agricultural products, and government regulations than will ban more pesticides for wide-spread application.

Introduction

The classical pesticides, even the latest generation of pyrethroids, cannot eliminate or even significantly reduce the damage caused by pests in the field. Despite the application of 225,000 tons of synthetic pesticides annually on crops in the USA (a 33-fold increase since 1945), crop losses

from insects, diseases and weeds has increased from 31% in 1945 to 37% in 1990 (Hilman, 1990). In parallel, about 440 insect and mite species and 70 fungus species have become resistant to some pesticides, as have 80 out of more than 500 weed species to herbicides. Resistance has even developed to the relatively new group of pyrethroids within a few years of application. Another drawback of the application of pesticides is their penetration into ground water and into the food chain. Almost 50 types of pesticide have been detected in ground water in many states in the USA (Hilman, 1990). In Israel, several pesticides have been found in underground water near chemical factories and storage facilities (L. Muszkat, personal communication). In addition, beneficial insects such as honey bees which are responsible for pollinating tens of billions of dollars worth of fruit, vegetable and forage crops are being destroyed by pesticides. A new group of insect growth regulators (IGRs) is now being introduced into pest control, but no-one knows how nature will react to this pressure.

The prevailing attitude is integrated pest management (IPM), which is an ecological approach to reducing pest damage using all the available techniques to maintain pests below damaging levels (pestistasis). This strategy is based on the determination of the point at which a pest population approaches the level at which control is necessary to prevent a decline in net returns.

Fortunately, we are already witnessing a change in attitudes to pesticides, and their use has declined slightly since the early 1980s (Hilman, 1990). The EPA is in the midst of a re-registration program for all pesticides registered prior to November 1984. One of the results of this exercise will be the drastic reduction (estimated to be as much as 80%) in crop protection agents available to the farmer (Weatherston, 1990).

At present "alternative agriculture" is gaining momentum, and sales of "organic" produce have doubled to $1 billion in 1988 from 1983 levels (Hilman, 1990). It should be emphasized here that pheromones are by no means pesticides, since they do not actually kill the pests. Moreover, they are essential for survival, playing a role in reproduction, defense, food location, and other important aspects of normal animal behavior. Behavior-modifying chemicals (BMC) have started to play a more significant role in IPM, and pheromones in particular will come to the forefront of the endless battle for food between mankind and pests (Shani, 1980). Possible modes of pheromone application in pest control are described in Scheme 1. Two recent books (Ridgway ***et al***, 1980, Jutsum and Gordon, 1989) describe the applications of pheromones as well as problems encountered in the wide range of implementations. Short critiques of the status and applications of pheromones are given by Arn (1990) and Silverstein (1990). Despite all the efforts described above, the entry of pheromones into conventional agriculture is slow.

Results

To evaluate the future role of pheromones in pest control I conducted a survey during the second half of 1990. A questionnaire (Appendix A) was sent to 142 people in 25 countries, 72 are involved in research at universities and research institutes, and 70 are employed in industry (managers and employees of companies, agents, etc). Eighty-four people (59%) replied, 48 from universities and research institutes and 36 from the commercial sector (in some cases more than one person from the same company). Fifty six out of 84 (67%) are personally involved in application of pheromones in pest control (question no. 1 of the questionnaire), while most (23) of the 28 who are not directly involved work at institutes which are engaged in pheromone applications.

The types of activities of the respondents and the application techniques used by them are summarized in Tables 1 and 2 (questions 2 and 3).

Table 1. Type of activities of the respondents

Activity	No. of respondents[a]
Basic research	61
Applied research	66
Industrial synthesis of pheromones	25[b]
Dispenser production	30
Trap production	25
Controlled-release formulations	29
Distributor, agent	19
Others:	
Service to food warehouses	1
Consulting	1
Advising service on risk estimation	2
Field extensionist	2
Field development	1
Regulatory	1

[a] Most of the 84 respondents are engaged in more than one type of activity.

[b] One claimed "on small scale."

Table 2. Techniques used in pheromone application[a]

Technique	No. of respondents
Monitoring	58
Mass trapping	38
Disruption of communication ("confusion")	44
Attract & kill	21
Others:	
Resistance monitoring	1
Dispersion (alarm pheromone)	1
Toxic baits	1
Bioirritants	1
Detection mapping	1

[a]Most of the 84 respondents apply more than one technique.

The information about acreage treated and sales (questions 5 and 6) is not complete (see Discussion), but is still very impressive in terms of area treated, as summarized in Scheme 2. In this Scheme, the data are classified according to pest, type of pheromone, technique of application, crop and geographical zones. The figures might not be complete, but the total area is about 1,300,000 ha worldwide, which is less than 0.1% of cultivated land on the globe.

Scheme 2.

Subgrouping Of Total Area (1,313,000 Ha) Treated Worldwide With Pheromones

PEST		CROP	
Moths	1250	Cotton	550
Beetles	45	Deciduous fruit	322
Flies	17	Forests	295
Scales, aphids, mites	1	Grapevines	108
		Olives	17
		Cocoa	10
		Vegetables	9
		Citrus	1.6
TYPE OF PHEROMONE		Rice	0.1
Sex	1280	Kiwi	0.1
Aggregation	32	Stored products	na*
Alarm	<1	Roses	na*
		GEOGRAPHICAL ZONE	
		Europe	690
TECHNIQUE		Northern America	300
Monitoring	421	Oceania	250
Masstrapping	306	C.& S. America	53
Confusion	556	Africa &	20
Attract&kill	30	Middle East	

*na = not available

Values in the chart are ha x 10^3 of land in which pheromones are applied

The sales of pheromones and devices as reported by only part of the respondents are close to $6 million. It is much likely that worldwide this figure is much higher, and if we compare it to $7 billion sales of insecticides annually* , we may envisage a small niche of 0.05-0.5% for pheromones in the pesticide market. This figure fits with the estimation of experts about future sales in 1, 5 and 10 years (questions 7-9), as shown in Tables 3 and 4.

*A recent figure quotes a $20 billion pesticide market worldwide with insecticides estimated to capture as much as $7 billion, Thayer AM, Chem. Ind. News Aug. 6, 1990, pp. 15-17.

Table 3. Forecast for worldwide sales (in 10^6) of pheromones as chemicals and of devices for pheromone application in 1, 5, 10 years from 1990

Predicted sales in $ x10^6 for the year:		
1991	1995	2000
1-50	5-250(500)[a]	10-400(1,000)[a]

[a]An optimistic view of one respondent.

Table 4. Pheromones in IPM - percentage of insecticide market in 1, 5, 10 years from 1990

Technique of pheromone application	Predicted % of insecticide market for the year:		
	1991	1995	2000
Monitoring	0.01-5.0	0.4-7.0	1.0-10.0
Mass trapping	0.01-1.0	0.1-2.0	1.0-10.0
Disruption of communication ("confusion")	0.01-1.0	3.0-10.0	10.0-20.0
Attract & kill	-	-	0.5-1.0
Total	0.03-7.0	3.5-19.0	12.5-41.0

Thus, the forecast for 1991 matches the range of sales reported (and unreported because of commercial confidentiality). The percentage for each technique (Table 4) shows that in the near future monitoring will dominate the pheromone market (0.01-5% of total insecticide market), while in the long run, disruption of communication will prevail.

Question 10 related to the specific insect pest target for pheromone application. The results are summarized by groups of insects in Table 5, sex pheromones of moths being the most prevalent group.

Table 5. Insect pest targets for pheromone application

Pest and crop	Type and mode of application of pheromone									
	Sex				Aggregation		Alarm	Oviposition deterrent	Queen	Trail
	A	B	C	D	A	B	E			
Moths										
Cotton	x		x	x						
Field crops	x		x	x						
Fruit	x	x	x							
Vegetable	x	x	x							
Forest	x	x	x							
Stored products	x	x	x							
Beetles										
Forest					x	x				
Stored products				x	x					
Gardens		x								
Aphids							x			
Flies										
Fruit		x		x						
House fly				x						
Mosquitos								x		
Fire ants									x	
Cockroaches		x		x		x				
Termites										x

A = monitoring; B = mass trapping; C = "confusion"; D = attract & kill; E = dispersion.

The vast majority of the 48 respondents to question 11 envisaged an important role, "essential," "versatile" or "indispensible", for phermones in future pest control. Twenty-one responded in general terms, 13 emphasized monitoring as the major mode, seven favored mass trapping and three others emphasized disruption of communication. Three respondents had some reservations and saw the need for combination with other approaches, and one envisaged the application of pheromones only in the distant future. Many respondents emphasized the need for more basic research before embarking on real application.

The last question (no. 12) dealt with possible application of other "innocent chemicals," which usually do not kill or damage the pest, but affect its behavior, inhibit, deter, or repel it. Out of 50 replies, 38 saw an active role of these chemicals in IPM, five envisaged only a limited role, two doubted whether they would be applied and five did not see any role (one of them "not for the time

being"). Table 6 summarizes the different groups of chemicals and approaches mentioned in the replies.

Table 6.
Type of natural or synthetic chemicals (which are not classified as hard poisonous chemicals) and approaches other than pheromones which are proposed for IPM

Type of chemical/approach	No. of replies [a]
Control of oviposition and hatching	9
Isomers of pheromones, analogs	8
Plant volatiles	7
Antifeedants	7
Insect growth regulators	7
Kairomones	5
Pheromone inhibitors	4
Repellents	3
Control of PBAN[b] release	3
Predators, parasites, microorganisms	7
Attract & kill	2
Agrochemical approaches	1
Education of farmers	1
Public opinion	1

[a]Total number exceeds number of replies.

[b]Pheromone biosynthesis activating neuropeptide.

Several companies added brochures and lists of pheromones and devices including prices (which should be taken only as indicative). Large quantities (³1 kg) of long-chain monoenes, alcohols and acetates, sell at \$1-3/g,* whereas 1-10 g quantities sell at \$20/g. Conjugated dienes are more expensive. Gossyplure, the pheromone of the pink bollworm, sells at \$0.5-0.7/g. Dispensers sell at \$1-3 each, but in large numbers they are cheaper. The same holds for traps, which sell at \$7-10 each.

*In a recent meeting in Beijing (July 1992) representative informed that these monomers are now sold at less than \$1 per gram.

Discussion

The good response to the questionnaire (59% response) covers both research institutes and the commercial sector. I do not consider the analysis of the responses as fully comprehensive for the following reasons:

1) The results do not cover all people and bodies engaged in the application of pheromones. Inscoe and Ridgway (1990) list 64 suppliers of insect pheromones, synthetic attractants, and traps.. This review includes the responses from 25 firms.

2) Some of those who replied restricted their comments to non-confidential information, while a few others, who gave more quantitative information asked that it not be quoted. Still, the information received and checked by verbal talks, as is summarized in the Tables and in Scheme 2, is very illustrative.

Scheme 2 reveals several interesting facts:

1) Moths are almost the sole group of pests to be controlled by pheromones (Inscoe *et al*, 1990)). Among these pheromones is a unique success story, the confusion of the pink bollworm (*Pectinophora gossypiella*) in cotton fields mostly in the USA by the sex pheromone. A group of pests that will soon be controlled are fruit moths such as the codling moth (*Cydia pomonella*), the oriental fruit moth (*Grapholita molesta*), and the peach twig borer (*Anarsia lineatella*), as will another group consisting of the grape berry moth (*Eupoecilia ambiguella*) and the European grapevine moth (*Lobesia botrana*).

2) The sex pheromones dominate the market.

3) A review of the data in Scheme 2 and Table 4 shows that "confusion" already dominates the market (in 1990), although the prediction was that this domination would occur only in the long run. However, if we "remove" the figures for the pink bollworm from the analysis, "confusion" is relegated to a very small area.

4) The mass trapping of the pine processionary moth (*Thaumetopaea pityocampa*) contributes about 80% of the mass trapping application, and acreage in forests.*

5) The monitoring technique is widespread and is not limited to only one or two crops.

Change of Approach

In September 1990 a meeting on "Pheromones in Mediterranean Pest Management" took place in Granada, Spain. Some of the results presented there support the data which is presented here, and provide a more optimistic view of the future of pheromones in IPM. Disruption of communication experiments, which failed in the past when 5-20 g/ha were applied, proved to be successful when quantities of 50-200 g/ha were used. These treatments can compete effectively with conventional pesticides and bring fringe benefits such as protecting beneficial insects and predators, slowing down of the development of resistance, preventing deterioration of the biological equilibrium, and reducing pollution and ecological problems.

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*The mass trapping of the spruce bark beetle (*Ips typographus*) in Scandinavia, which was operated in the late 1970s and early 1980s is not active now, but at that time it covered 2,000,000-2,500,000 ha of forests. This area is still monitored as are forests in Germany, Poland and other Eastern European countries, but it is not included in Scheme 2. Should there be another outbreak in Scandinavia the mass trapping technique will be applied, since the use of pesticides is prohibited (Skättebøl, personal communication).

It seems that a basic change in the approach to the application of pheromones is taking place. The uniqueness of pheromones lies in the facts that they are species specific and need be used in small quantities (but at high cost). These were the slogans of the pheromone advocates in the past. The effect on both farmers and industry was mostly negative, since:

i) A species-specific agent is both good and bad: it enables the farmer to deal with one pest, without affecting other organisms, but it calls for many single treatments, which makes the pest control cumbersome and very expensive. (Results of the "cocktail" approach, namely, putting together several pheromones in one device or treatment have not always been successful (Chamberlain and Critchley, 1990, Schwalbe and Mastro, 1990), and more studies should be conducted in order to get a better understanding of the concept.)

ii) The high price of the pheromones could not compensate for the small market volume. The farmer could not afford to apply pheromones at such a cost, and the small industry hardly survived with such a small volume of sales. The chemical giants are still sitting "on the fence", watching the situation carefully. Since manpower is the major factor in the cost of the production of pheromones, manufacturing of larger amounts will reduce the costs per g (or kg) of pheromones.

The current attitude is that we have to turn our thinking through 180 degrees to larger quantities of cheap pheromones.

One aspect of the registration of pheromones might have a positive influence on their future acceptability. Since these chemicals are considered to be nonpoisonous, they are exempted from a number of toxicological tests on condition that the pheromones are applied in traps, in ropes or hollow fibers. Thus, the registration process could be made shorter and cheaper.

Push-Pull Mechanism

We are at a turning point as consumers begin to dictate the market. Psychological and environmental factors tend dominate the behavior of people (particularly in the developed countries). Customers will be ready to pay more for safe and clean agricultural products _ "organic" fruits and vegetables _ and to spend more on quality. Farmers will receive better compensation for superior products for human consumption. At the same time governments and regulatory agencies in Europe and the USA will change their attitude towards the "hard pesticides", creating a vacuum which will encourage innocent chemicals. An excellent example is the November 1990 ballot in California which, if passed by the voters, would have either totally eliminated or substantially reduced the use of pesticides in that state. The ballot did not pass, but the trend still persists. This situation may be summed up as a "push-pull" mechanism. The market will "pull" the pheromones in, while interested and active bodies will "push" them to fill the gap in the pest control arena.

With regard to field (but not food) crops, only after BMC and biological agents will have reached the limits of their effectiveness, will these crops be treated with pesticides, but with caution because of problems of resistance. This will be a case of "tea and aspirins" for a headache before going to a surgery or drastic therapeutic treatment.

Integrated pest control is not a target in itself: it is the means we require to survive without destroying the environment. We have to find the compromise between the available vs. the desirable. At the same time we have to be aware of future problems and difficulties.

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References

HILMAN, B. 1990. Alternative agriculture. Chem & Eng News, Mar. 5, pp. 26-40.

WEATHERSTON, I. 1990. Regulatory considerations - perspectives from the private sector. Presented at USDA/ARS Workshop in Semiochemicals, Atlanta, GA, May, 1990.

SHANI, A. 1980. Will pheromones be the next generation of pesticides? J Chem Educ **59**, 579.

RIDGWAY, R.L, SILVERSTEIN, R.M. and INSCOE, M.N. 1990. eds.: Behavior Modifying Chemicals for Insect Management. 761 p. + xvi, Marcel Dekker, Inc., New York.

JUTSUM, A. R. and GORDON, R. F. S. 1989. eds.: Insect Pheromones in Plant Protection. 369 p. + xvi, J. Wiley and Sons, Chichester, England.

ARN, H. 1990. Pheromones: prophecies economics and the ground swell. In: Behavior-modifying chemicals for insect management. Ridgway, RL, Silverstein RM, Inscoe MN, eds. Chap. 39, pp. 717-722, Marcel Dekker, Inc. New York.

SILVERSTEIN, R. M. 1990. Practical use of pheromones and other behavior-modifying compounds: Overview. In: Behavior-modifying chemicals for insect management. Ridgway, RL, Silverstein RM, Inscoe MN, eds. Chap. 1. pp 1-8, Marcel Dekker Inc., New York.

INSCOE, M. N. and RIDGWAY, R. L. 1990. List of Commercial Suppliers, Appendix. In: Behavior-modifying chemicals for insect management. Ridgway, RL, Silverstein RM, Inscoe MN eds. pp. 723-732, Marcel Dekker Inc., New York.

INSCOE, M. N. , LEONHARDT, B. A. and RIDGWAY, R. L. 1990. Commercial availability of insect pheromones and other attractants. In: Behavior modifying chemicals for insect management. Ridgway, RL, Silverstein, RM, Inscoe MN, eds. Chapter 38, pp. 631-715, Marcel Dekker, Inc. New York.

CHAMBERLAIN, D. J. and CRITCHLEY, B. R. 1990. Control of spiny bollworm and the pink boll worm by a single application of their combined pheromone. In: Pheromones in Mediterranean Pest Management Meeting, Granada, Spain, September, 1990.

SCHWALBE, C. P. and MASTRO, V. C. 1990. Use of pheromones and attractants by government agencies in the United States. In: Behavior modifying chemicals for insect management. Ridgway, RL, Silverstein, RM, Inscoe MN, eds. Chap. 37, pp. 619-630, Marcel Dekker, Inc., New York.

APPENDIX A

APPLICATION OF PHEROMONES IN PEST CONTROL

Questionnaire

Date: Name:
Address:

Please indicate the correct answer and add any relevant information. You may use extra pages.

1. Are you personally involved in application of pheromones (of any type) in pest control?

Yes No

If not, is the Institute/Company/Factory/Agent for which you work engaged in pheromone research and/or applications?

Yes No (Please go to question 3)

2. What type of activity related to pheromones does your Institute/Company/Factory/Agent engage in (you can mark more than one item):

Basic research
Applied research
Industrial synthesis of pheromones
Dispenser production
Trap production
Controlled release formulations
Distributor, Agent
Other (please specify)

3. If you and/or your Institute/Company/Factory/Agency are engaged in pheromone applications, what technique do you use:

Monitoring
Mass trapping
Disruption of communication ("confusion")
Attract and kill
Other (please specify)

4. What pests are controlled with your applications? (please specify)

5. What acreage is controlled by each pheromone and technique (a Table would be a good presentation):

Pheromone (sex, aggregation,etc.)	Pest	Technique	Crop (Ha)	
1	2	3	4	Tota l

6. What are your sales of pheromones in $:

As chemicals:

Devices for:
- Monitoring
- Mass trapping
- "Confusion"
- Other (please specify)

Total

7. What is your forecast for worldwide sales of pheromones as chemicals and of devices for application 1, 5, 10 years from now (in $)?

8. What is your estimation for area (acreage) treated and volume of pheromones used 1, 5, 10 years from now?

9. What portion of the worldwide insecticide market (assuming ~ $4 billion sales annually) will be captured 1, 5, 10 years from now by the following (in percents or in $)?

Monitoring
Mass trapping
"Confusion"
Other (please specify)
Total

10. Do you see any specific insect pest as a target for pheromone application? What pheromones (sex, trail, alarm, aggregation, etc.)? What technique? Please specify. (A Table could be good presentation).

11. How do you see the role of pheromones in integrated pest management?

12. Do you see any future for application of other "innocent chemicals" (Behaviour Modifying Chemicals such as inhibitors, mimics, etc.)?

Yes No

If yes, please specify.