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## Preface

*Insects use sex pheromones to communicate for mating. Pheromones elicit strong behavioural reactions at minute amounts, they are species-specific and non-toxic. By permeating the atmosphere with synthetic pheromones, olfactory communication and mate-finding can be prevented.*

The mating disruption technique had a head start. Soon after the discovery of the first lepidopteran sex pheromone in the late fifties, it was postulated that it should be possible to use synthetic pheromones for environmentally safe insect control – although virtually nothing was known about the chemistry and biology of pheromones at that time. Research techniques considered essential today, such as gas chromatography, electrophysiology or wind tunnel bioassays, were not available. The sex pheromones of economically important species and their behavioural effects had yet to be identified; synthesis, purification, and controlled release techniques had to be developed before practical applications came into reach.

In Europe, the grape berry moth was the first species to be controlled by mating disruption on a commercial scale. To mark this event, an IOBC pheromone meeting was held at Neustadt Wstr., where the field development had been made. Ten years after, the Montpellier meeting gives evidence that the mating disruption technology has reached maturity. Pheromones have become an integral part of many pest control programs and, in some cases, may even become more effective than conventional insecticides.

The Neustadt meeting of 1986 manifested the need for a better understanding of the "behaviour of moths and molecules". Since then, respectable progress has been made with the industrial synthesis of pheromones, controlled-release technology and the measurement of airborne pheromone. Growers and extension organisations have gained considerable know-how in applying the mating disruption technique. But even today, the lack of knowledge on aerial dispersal and mode of action of mating disruptant chemicals is the most serious obstacle for further developments.

The 37 years of research since the synthesis of the first insect pheromone have laid the ground for practical applications. A concerted effort of the scientific community, industry and growers must now be made to consolidate these achievements and to establish mating disruption as a reliable and cost-efficient technique. Our success will determine the public interest in further research in the field of insect olfactory communication and chemical ecology.

Alnarp, December 1996

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*Technological and commercial aspects*

## **The key to success in mating disruption**

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**Abstract** - The key factors determining the successful application of the mating disruption technique for insect control are reviewed. The composition of the disruptant blend of chemicals must be optimized in field tests. A satisfactory dispenser technology comprises sufficient release rates and dispenser life; the protection of the active ingredient; and a convenient application method. Air temperature and wind velocity determine release rates and aerial pheromone concentrations. Mating disruption works best in area-wide treatments, a large enough amount of chemicals must be applied early enough in season, before emergence of the target species. Population densities of the pest species and natural enemies, as well as economic damage thresholds play a decisive role.

**Key words** - sex pheromone, mating disruption, controlled release dispensers, integrated pest management, codling moth, *Cydia pomonella*, pink bollworm, *Pectinophora gossypiella*, Lepidoptera

### **Examples for failures of mating disruption**

There are many factors determining the effect of mating disruption. The failures experienced in field tests over the past decade may help us to determine the most critical ones. In four of nine cases, late application of the dispenser material was responsible, as farmers and researchers tend to apply pheromone dispensers at the same time as insecticide sprays. For example, the first generation of pink bollworm, *Pectinophora gossypiella*, causes no damage and sometimes dispensers are applied only against the second generation. This is not a problem at a low population density, but if the population builds up, the efficacy of the treatment against the second generation goes down considerably (Table 1). Even if the results were good for some years, there is a potential risk in treating only against the second generation.

A lack of stability of the active ingredients was responsible for failures with rice stem borer, *Chilo suppressalis*, and codling moth, *Cydia pomonella*. Low pheromone release rates during early season, problems with the dispenser system, and a low population of natural enemies were the other problems identified.

**Table 1** The effect of the time of dispenser application for mating disruption of pink bollworm, *P. gossypiella*

Application date	Boll damage (%)	
	Second half of July	First half of August
May 14	1.7	2.3
May 18	2.8	2.5
May 30	8.0	11.3
June 10	13.3	28.0

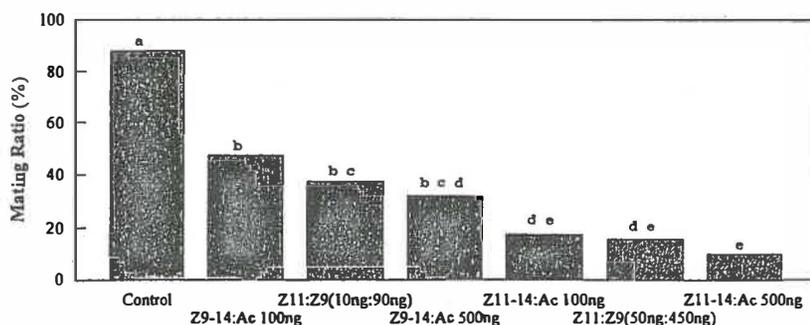
Judging from the unsuccessful cases, the conditions required to achieve a good efficacy in mating disruption are outlined below.

### Adequate controlled-release technology

Aerial pheromone concentrations of 1 to 10 ng/m<sup>3</sup> should be maintained in the crop, depending on the insect species and population density. The ideal dispenser system is required to fulfill the following properties:

#### Active ingredient

The composition of the disruptant blend of chemicals can be different from the female sex pheromone (Figure 1; Table 2). Therefore, the active ingredients used in the dispensers must be determined by field trials. Apart from a few exceptions, chemical impurities have no negative effect on mating disruption.



**Figure 1** Mating disruptant effect of (*Z*)-11-tetradecenyl acetate, (*Z*)-9-tetradecenyl acetate, and a 1:9 blend of these compounds, against summerfruit tortrix, *Adoxophyes orana*, in the laboratory. Bars with different letters are significantly different ( $P = 0.05$ ; analysis of variance)

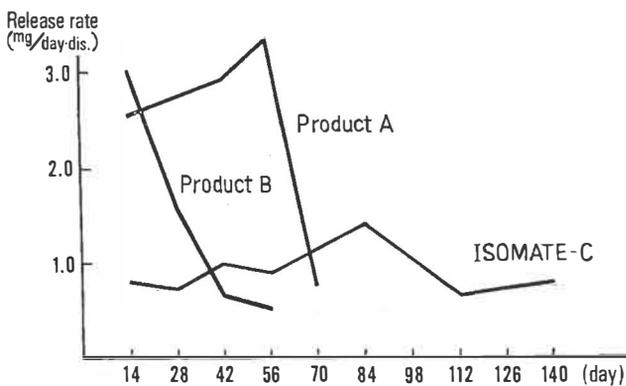
**Table 2** Active ingredients used for mating disruption in relation to female sex pheromones

Species	Compound <sup>a</sup>	Composition of		Disruptant
		Attractant (%) <sup>a</sup>	Disruptant (%)	
<i>Pectinophora gossypiella</i>	Z7, Z11-16Ac	50	50	Pheromone
	Z7, E11-16Ac	50	50	
<i>Carposina niponensis</i>	Z7-20-11Kt	95	100	Main component
	Z7-19-11Kt	5	0	
<i>Adoxophyes spec.</i>	Z9-14Ac	61		Minor component
	Z11-14Ac	31	100	
	E11-14Ac	4		
	10me-12Ac	2		
<i>Epiphyas postvittana</i>	E11-14Ac	95	66	Off-blend
	E9,E11-14Ac	5	5	
	Z11-14Ac	-	29	

<sup>a</sup> see Arn *et al.* (1992, 1996)

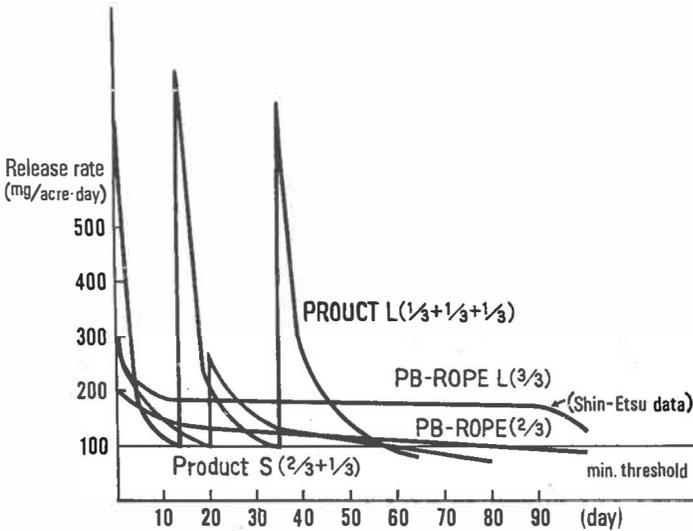
### Release rates and dispenser life

Zero order release rate is a very important characteristic of pheromone dispensers. Many dispensers have a rather high release rate in the beginning, which gradually decreases within a few weeks (Figure 2). Isomate-C dispensers contain a 60:30:1-blend of (*E,E*)-8,10-dodecadienol (*E8,E10*-12OH; codlemone), dodecanol (12OH) and tetradecanol (14OH) at 203 mg/dispenser. These dispensers still contain 75 mg after 140 days and will continue to be effective for another 30 days.



**Figure 2** Release rates of three dispensers for mating disruption of codling moth, *C. pomonella* (Gut *et al.* pers. comm.; Washington State University)

In order to allow for a too rapid decrease in release rates, a split application is recommended for pink bollworm, *P. gossypiella*, in Egypt; some dispenser types should be applied two or three times, at short intervals. However, this is not a satisfactory approach. Efforts should therefore be made to develop a zero order release system, which is almost achieved by PB-rope dispensers (Figure 3).



**Figure 3** Split application of dispensers recommended against pink bollworm, *P. gossypiella*, in Egypt

Quite obviously, a temperature-independent release system would be preferable, as currently available dispensers release high amounts of pheromone uselessly during daytime. We measured aerial concentrations in a pheromone-treated field and found that the pheromone concentrations are almost constant, regardless of different release rates at different temperatures. It seems that strong convection flows carry the pheromone upwards into the sky at high temperatures, during the daytime.

Another, most important factor is wind velocity (Tables 3, 4). An ideal dispenser should release pheromone proportional to wind velocity at low wind speeds; but should release less at strong winds, when no mating activity occurs. A dangerous situation arises from decreased release rates after periods of continuously strong winds (Figure 4).

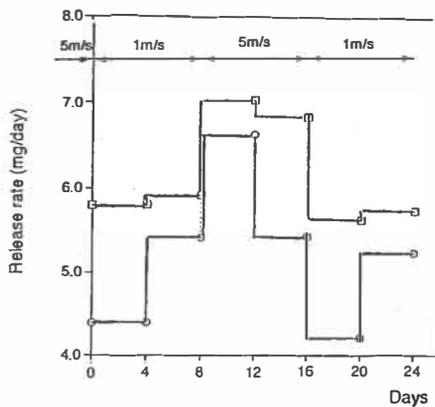
Dispenser life is obviously a most important property. Short-life dispensers cannot absorb the change of climatic conditions, they are much less reliable at hot temperatures. There is also the problem to properly time the next application.

**Table 3** Relation between wind velocity, release rates and aerial pheromone concentration

	Wind velocity (m/s)	Release rate (mg/hr/ha)	Concentration (ng/m <sup>3</sup> )
A	1.0	204	2.5
B	2.5	235	1.2
B/A	2.5	1.15	0.48

**Table 4** Dispenser life (PB-rope) in relation to wind at various locations

	Wind velocity (m/s)	Dispenser life (d)	Dosage (dispenser/ha)
Multan, Pakistan	0.5 to 1.5	70 to 90	<500
Parana, Brazil	1.0 to 1.5	70 to 80	500
Hubei, China	1.0 to 2.0	65 to 75	500
Sharkia, Egypt	1.5 to 2.5	60 to 70	750
Phoenix, USA	1.5 to 2.5	60 to 70	750
Imperial Valley, USA	3.0 to 4.0	40 to 50	1000

**Figure 4** Release rates from two dispenser types after periods of strong wind

### Stability of active ingredient

Aldehyde pheromones are easily oxidized to acids. Therefore, antioxidants and UV-adsorbers are usually added to the active ingredient, as well as to the polymers in the dispenser wall.

Pheromones also polymerize to form oligomers. Aldehydes easily form trimers. Isomerization is quite common in compounds with conjugated double bonds. Stabilizers and UV adsorbers are added to protect the compounds against these reactions.

Under certain conditions, acetates are hydrolyzed to the alcohol and acetic acid by fungi (Table 5).

**Table 5** Stability of active ingredient

Reaction	Pheromone compound	Product	Stabilizer
Oxidation	aldehyde	acid	UV absorber, antioxidant
Polymerization	aldehyde	trimer	radical absorber, PH adjustment
Isomerization	conjugated diene	oligomer	UV absorber, filler in polymer
	conjugated diene	isomer	UV absorber, filler in polymer
Hydrolysis	acetate	alcohol	fungicide

#### Others

The development of a degradable dispenser is desirable. Sprayable dispensers would be much better than existing dispensers, but it is very difficult to develop long-life sprayable dispensers.

**Table 6** Small-scale (1985) and large-scale (1990) application of mating disruption with PB-rope for control of pink bollworm, *P. gossypiella*, in cotton fields (Imperial Valley, USA)

	Number of treatments		Larvae/1000 bolls		Cost (US\$/acre)			Cotton quality
	Pheromone	Insecticide	August	Sept.	Pheromone	Insecticide	Total	
1985	0	11.4	8.5	8.8	0	114	114	bad
	6 <sup>a</sup>	10.4	9	20.8	36	104	140	bad
	1	6.6	3.2	3.9	40	66	106	fair
1990	0	9.3	4 to 5 <sup>b</sup>	-	0	93	93	fair
	1	1.8	1 to 3 <sup>b</sup>	-	40	18	58	good
	2	1	0	-	80	10	90	excellent

<sup>a</sup> Sprayable dispenser; <sup>b</sup> estimated

#### Area-wide application

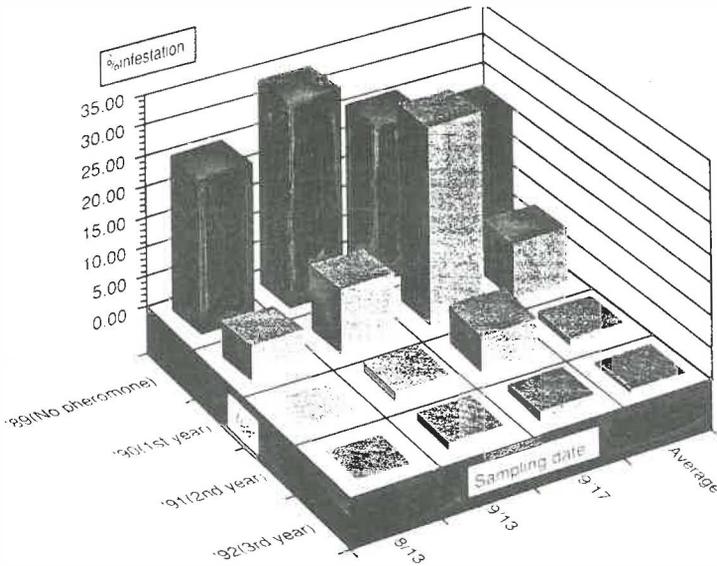
Area-wide application of mating disruption is recommended for a number of reasons. One is that a comparatively higher pheromone dose is required for small fields, and that the cost of mating disruption is therefore not competitive with insecticide treat-

ments. A rough estimation is that a ten times larger field requires only half the pheromone dosage/ha, compared to a small field. Population control of pink bollworm at late season tends to be much more efficient in larger areas, which results in a smaller first generation the next year, thus further increasing the efficacy of pheromone treatments (Tables 6, 7; Figure 5).

In larger treatments, the effect of immigrating gravid females is less critical and populations of natural enemies are expected to be higher.

**Table 7** Effect of treatment of neighbouring cotton fields on control of pink bollworm, *P. gossypiella*, by mating disruption

Surrounding pheromone-treated fields (N)	Number of fields	Male trap catch		Larval infestation of bolls (%)		
		July 1-15	July 15-30	July 1-15	July 15-30	August 1-15
≤ 1	3	0.7	1	0	2	16
2 to 4	24	0.8	3.8	0.4	2.1	4.8
5 to 8	19	0.5	2.1	0.1	0.7	0.8
8	2	0	0	0	1.5	0.5



**Figure 5** Percent boll infestation by pink bollworm, *P. gossypiella*, under Parker Valley PBW Suppression Program (1990-1992)

## Natural enemies

Mating disruption is usually designed to control one target species. Other minor pests are in part effectively controlled by their natural enemies, which benefit from the reduction of pesticide treatments. However, after insecticide sprays, the efficacy of pheromone treatment, also with respect to the target pest, is decreasing. And other species become more virulent and must be treated with additional pheromone compounds or insecticide sprays (Table 8).

**Table 8** Side effects of broad-spectrum insecticides

Insecticide	Conventional pest control	Integrated pest management
	Number of insecticide sprays/season	
Total	11.2	9.8
Pyrethroid	1 to 4	0
Imidacloprid	1 to 2	0
Pest species	Infestation (larvae/m <sup>2</sup> )	
Tea tortrix	5.5	1.2
Thrips	49.9	23.1
Scale	7.7	4.3

The use of insecticide sprays is known to induce serious secondary pests. One well-known example are gradations of mites as a result of pyrethroid sprays. Insecticides are not always efficient to control even the target species, due the induction of insecticide-resistant strains and their negative effect on beneficials (Table 9).

**Table 9** Effect of mating disruption on non-target pests

Crop	Target pest	Non-target pests	
		Increasing	Decreasing
Pome fruit	Codling moth	Leafrollers (in part)	Leafminers Mites, Aphids Psylla (pear)
Cabbage	Diamondback moth	Cabbage looper	Whitefly Mites
Cotton	Pink bollworm	Egyptian cotton leafworm	Whitefly Mites American bollworm

### **Control threshold**

The threshold of economically tolerable damage is a very important factor. In Japan, New Zealand and South Africa, the threshold of apple pests is 0.1% or lower, while it is between 1 and 2% in other countries. At extremely low population densities, the natural enemies are not blessed with enough food and their activity, therefore, remains low.

At extremely high pest population densities, on the other hand, mating disruption is often not effective and curative insecticide sprays must be applied initially.

### **Conclusion**

We have much confidence in the efficacy of the mating disruption technique, if good dispensers releasing sufficient amounts of the proper active ingredients are applied at an early stage in area-wide control systems, in the presence of natural enemies.

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## Commercial exploitation of mating disruption technology: difficulties encountered and keys to success

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**Abstract** - The use of sex pheromones for mating disruption of Lepidopteran pests of agriculture has, over the last decade, become an important component of integrated pest management packages in a number of crops. Our company has been involved in the commercialization of at least four major pest management products based on this technology, namely *Pectinophora gossypiella* in cotton, *Chilo suppressalis* in rice, *Rhyacionia buoliana* in forestry and *Keiferia lycopersicella* in tomatoes. A number of other product development projects have been started with other species but have been discontinued for a range of reasons. During the last decade we have observed several common themes which have emerged as problems during the product development and commercialization process for mating disruption products. These have included factors such as the formulations used, agronomic constraints, the pest's biology and socio-economic factors. This paper examines the problems encountered and suggests keys to how such problems can be overcome, together with indicators of how mating disruption technology may evolve in the future.

**Key words** - sex pheromone, mating disruption, integrated pest management, commercialization

### Historical perspective

Pheromones - the perfect IPM solution! This was a commonly heard statement in the 1970's and early 1980's. Pheromones are species-specific, have very low toxicity, they don't even harm the target insect and are "natural" - in the eyes of the general public, although the commercially used materials are synthetic copies of the natural product (Hodosh *et al.* 1985). At a time when insect resistance to conventional pesticides is of widespread concern and insecticides are constantly implicated as causing environmental damage, destruction of beneficial predators and explosions of secondary pests, pheromone-based pest control looks like the perfect solution.

In order to facilitate the introduction of pheromone-based technology, regulatory authorities have generally been very flexible in their data requirements given the benign nature of the technology. While demanding that control products based on pheromones be registered, such authorities usually give them an easy passage through the regulatory process compared to standard insecticides and their use for monitoring is usually exempt from any kind of regulation (Ridgway *et al.* 1992).

The scene in the mid 1980's was therefore set for success. However, it is now well over 20 years since the first mating disruption strategies were developed and shown to be effective in the field, still the market place has not experienced an explosion in the use of such technology. Under normal circumstances this should be by now an insect control technology which is approaching maturity. What therefore prevented the technology from taking hold in the market place on a large scale?

### **What have we learnt over the past 20 years?**

#### The basic principles

Mating disruption aims at preventing males from finding unmated females, thus leaving the females unfertilised and incapable of laying viable eggs.

It seems to work well when: (1) insect populations are relatively low; (2) the target insect is not very mobile or dispersive; (3) the treated area is relatively large and/or isolated from outside sources of the pest; area-wide pheromone treatment programmes have been particularly successful; (4) the target insect is a moth, although there are some cases of mating disruption that have been achieved with Hemipteran pests (Hefetz *et al.* 1988).

It seems to fail when: (1) populations are high, presumably as a result of random encounters between males and females which result in mating; (2) the target insect is mobile and capable of dispersing locally over significant distances or is migratory; (3) the areas treated are too small, have very irregular boundaries, or are poorly isolated from surrounding crops with populations of the target insect; (4) there have been errors in application - either the product was not applied evenly within the crop or it was not applied at the correct time, most commonly, the product is applied too late. (5) No apparent reason - this is probably the most concerning factor, there are occasions when it is seemingly impossible to determine the cause of a control failure given our current level of knowledge of the technology.

The net result of all these limitations is that, while there are a few products against certain pests that work very well, there are many others, either commercialised or under development, which are still questionable.

From the experience we have gathered over the last 12 years we believe the key factors for success are: (1) gaining an understanding of the principles that limit pheromone use, both technical and commercial and (2) combining this with a sound knowledge of the target pest ecology and behaviour. Since this has not always been done,

pheromone mating disruption has gained, perhaps unfairly, a reputation for not being robust enough for general use.

### The technology

A recurring problem with using pheromones for mating disruption control of insect pests is the cost of the product to the end-user. The active ingredients are difficult to produce and are often very expensive. The demands on the controlled release formulations are often very high. Formulations are expected to give a constant release and protect the active ingredient for periods of up to 6 months.

Minimising active ingredient costs is critical. This can be done by either reducing the manufacturing cost of the active ingredient and/or reducing the quantity used in the field. Development of economic synthetic routes for pheromone components is the key to reducing product costs. The pheromone is usually by far the most expensive part of the formulation and any changes in active ingredient cost will have a significant impact on the final product cost.

Being basic in the manufacture of a pheromone and having a low cost synthetic route will often determine commercial viability of a project. In the case of the pink bollworm (*Pectinophora gossypiella*), our company has been involved for a number of years in producing mating disruption products for its control. Our decision to pursue this project has to a great extent been determined by the fact that we have patented technology which produces the pheromone components in a very cost-efficient manner (Banasiak & Byers 1990).

Minimising the amount of active ingredient required for field application may be almost as critical as the cost of the active ingredient itself. This involves not only finding the minimum effective rate of pheromone use but also delivering it into the air around the crop in the most efficient manner. This is where a good controlled release formulation is vital. There is always a risk in this area that companies will, in order to achieve acceptable margins in competitive situations, cut the active ingredient rate to below a level which has a good safety margin and thereby compromise the performance of the product.

To make the most of the pheromone applied in the field, a good formulation is needed. Such formulation technology aims to trap the pheromone inside the dispenser, protect it from degradation by environmental factors and release it at a controlled rate for the required period in the field. Over the years different companies have found different solutions to achieve this. Cross-linked polymer matrixes, capsules, sandwiched polymer layers, hollow tubes, semipermeable membranes, micro-encapsulations and extruded polymers to name but a few have been used (Weatherston 1990). Broadly speaking however, they tend to fall into two categories - those that are applied by hand and have a long duration (up to 180 days) and those that are applied using spray application technology and are usually of a much shorter duration in the field (up to 30 days). The development of season-long hand-applied formulations has

undoubtedly been a significant advance in the last ten years and has led to the adoption of the technology over large areas in a number of crops. The fact that such formulations are not so demanding in terms of application skills, timing and continued vigilance while in operation, compared to the sprayable formulations, must have played a significant part in their acceptance by the end users.

Whatever form of pheromone technology that is used, it is inevitably a compromise between functionality and cost. A product based on a large amount of high quality pheromone in a hi-tech, high-priced formulation may be the best solution for a particular pest problem, but who will buy it? Investment in new product development in this field has to be directed towards economical solutions.

### **Commercial considerations**

With a few notable exceptions, for the most part the companies involved in pheromone development have either been small start-up companies or largely independent subsidiaries of larger companies. Often in these organisations there has been a youthful and enthusiastic research team keen to see this technology developed. More often than not, the focus has been very technically driven, and not enough thought has been given to basic commercial considerations which are the key to successful exploitation of any new technology.

Factors which can cause problems in commercialization of mating disruption technology: (1) Poor assessment of the economic viability of products for target pests in target crops. (2) Inaccurate and/or over-optimistic cost projections for the product when manufactured on a large scale. (3) Over-expectations of the likely acceptance of this new technology by customers. Our experience has shown that they are not willing to pay more for a system that may be more difficult to use and sometimes offers less performance or security compared to conventional technologies. (4) Poor planning of research and development strategy whereby resources are often wasted on non-productive trials.

Factors which lead to the successful commercialization of mating disruption technology: (1) Accurate assessment of the commercial potential for a product. What is the market? How will it be used? How will it be integrated with other pest control practices? What would motivate the farmers to use it? (2) Product with good efficacy and predictable reliability; sound technical support data is required to back-up the product introduction into the market. (3) Development of an economic formulation and synthesis route which is easy to produce and scale up as demand increases. While conforming to efficacy requirements, the formulation must be easy, quick and economic to produce, particularly if the product is to be produced in large volume. (4) Both public and private support for a co-ordinated implementation programme. Area-wide treatments co-ordinated by public organisations or growers' associations have consistently given good results in crops such as cotton (Jones 1994), rice (Casagrande

1993) and top fruit (Dunley 1996). (5) Well organised distribution plan and pricing policy. Knowledge of how the product is to be sold and the price structure to the end user is essential. Everyone in the supply chain has to be motivated if the technology is to be successfully introduced to the farmer.

## **Future directions**

### Continuing research

Although as an industry we have learnt a great deal about the optimal conditions for successful mating disruption over the last decade (Neumann 1992, 1993; Kirsch & Lingren 1993) there is still a great deal that we have to learn. It is a significant concern for everyone involved in this field that funding from the public purse for research in this area seems to be diminishing, especially in view of the fact that the commercial use of pheromones is still growing.

There have been a number of interesting observations which have been made recently which deserve to be researched further: (1) There is clear evidence from a number of sources that the continued use of mating disruption, where it is successfully controlling the target pest, leads to a gradual reduction in that pest over several seasons. In other words, there seems to be a "carry over" effect from one season to another as far as the population is concerned. Priority must be given to understanding how this process can be achieved in a majority of species especially if there is the concomitant possibility of reducing the amount of pheromone required for mating disruption as the population reduces. (2) Work done in the USA is clearly showing that it is possible to reduce the starting population for a multi-voltine species such as the pink bollworm by treating the over-wintering pupae with entomopathogenic nematodes. Is this a technique which can be applied to other pest species? (3) Area-wide programmes have been very successful but is this simply due to the elimination of problems related to ingress of mated females or are there more subtle reasons which deserve to be explored?

### Commercial maturity

As the pheromone industry matures, it is in danger of attracting the attention of suppliers of products of dubious quality since the barriers to entry are not as onerous as they are for conventional insecticides. Consumers of pheromone products increasingly demand of their suppliers products which conform to certain specifications and quality standards; it is incumbent on the semiochemical industry to establish high levels of product specification, quality and performance so that barriers to entry are sufficiently high to exclude the amateur, or worse still, the unscrupulous operator. The pheromone industry is coming of age and standards need to be established to protect both the consumers and the credibility of the industry. The activities of the USDA-ARS and

APHIS in establishing such standards for monitoring lures and traps have to be applauded (Leonhardt *et al.* 1990). However, it should be the industry itself that sets the standards and we have a unique opportunity to do this following the establishment of the International Biocontrol Manufacturers Association (IBMA) which has a semiochemical professional group and several other working groups to tackle areas such as registrations and specifications, and which has the potential to give the industry the level of self-regulation that it clearly needs. The IBMA will also maintain a dialogue with the regulatory authorities worldwide in order to ensure that the process of product registrations continues to strike the right balance between a fair assessment of risk and facilitating the introduction of benign technologies.

### Continuing partnership

The success that has been achieved to date has been based on partnership between the industrial semiochemical community, the publicly funded research and regulatory community, and end user groups and associations. Only through continued partnership of this nature will the technology go forward. Lindgren (1990) described the process as the "Research, Development and Application Continuum". We still have a great deal to do in order to secure the efficient operation of this process in the case of semiochemicals, especially in light of the fact that there are significant counter-currents which are acting against us. These include reduced research and development funding from the public purse; pressure in both developed and developing markets for less government intervention in pest control activities, *i.e.* privatisation of many pest management coordinating and implementation activities by government bodies; and lastly the rather feast or famine approach of financial investment communities towards the fledgling industrial companies trying to introduce pheromone technology to the field of insect pest management.

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## **Technological problems associated with use of insect pheromones in insect management**

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**Abstract** - Sex pheromone components were identified for many of the major lepidopteran pests of concern to agriculture a number of years ago. However, despite intensive efforts, implementation of large-scale, effective, and reliable insect control strategies based on pheromones remains elusive for many pest species. For example, pheromone-based mating disruption of codling moth still suffers from problems which have prevented the widespread adoption of mating disruption for control of this world-wide pest, in spite of continuous research efforts spanning 20 years. During this time, our knowledge and understanding of possible causes of unsatisfactory performance of pheromones in insect pest management has increased substantially. It is now commonly acknowledged that site characteristics such as the slope of a site and edge effects associated with adjacent cropping systems can contribute to the failure of mating disruption. However, field researchers have focused less attention on technological factors such as pheromone dispenser performance, pheromone purity, and pheromone degradation under field conditions. Both field researchers and growers are usually dependent on commercial outlets for supplies of both pheromone and dispensers, but the pressure on commercial suppliers to market product may result in products being released before adequate efficacy tests under field conditions have been conducted. In this paper, we will summarize results from several studies which examined pheromone dispenser characteristics, pheromone purity, and pheromone degradation under field conditions. The results will be discussed in terms of the performance of several pheromone products in different agricultural systems.

**Key words** - sex pheromone, mating disruption, controlled release, dispenser, *Anarsia lineatella*, *Ectomyelois ceratoniae*, *Cydia pomonella*

### **Introduction**

The first identifications of insect pheromones several decades ago generated tremendous interest and speculation in both the scientific and agricultural communities, due

to the perceived potential of insect pheromones as "magic bullets" which could be used in insect control. Indeed, insect pheromones are now used worldwide as monitoring tools, but with few exceptions, the potential for insect control with pheromones has not been realized. However, a nucleus of researchers have persisted with the development of applications for pheromones over the years, and the resulting body of empirical and experimental knowledge has resulted in the elucidation of factors which are critical to the successful use of pheromones. For example, it is now generally recognized that pheromone-based mating disruption will work best in relatively flat, isolated blocks, with low initial populations of moths. Furthermore, a relatively uniform "blanket" of pheromone above an empirically-defined critical concentration is usually provided by large numbers of point source dispensers. These guidelines have been developed more often than not as a result of control failures, and we now have a greater appreciation of the complexities of successful application of mating disruption. This complexity can be summed up simply: with mating disruption, we are attempting to modify the behavior of several generations of a population of organisms for their entire lifespans. Unlike insecticide useage, where a single contact with the active agent removes the insect from the system, behavior modification requires that pheromone coverage be continuous and complete for periods of several months.

In the following pages, we describe some of the technological problems we have encountered in the development of applications of insect pheromones, both for monitoring and mating disruption, and the solutions which have been developed.

### **Pheromones for monitoring**

#### *Peach twig borer, *Anarsia lineatella**

Peach twig borer (PTB) is a major pest of stone fruit and nut crops worldwide. The pheromone for this insect was identified by Roelofs *et al.* (1975) as a blend of *E5-10Ac* and *E5-10OH* (ca. 4:1), and it has since been used widely as a monitoring tool. However, there were two anomalies in the initial report of the pheromone. First, the blends of synthetic pheromone used in the initial studies were minimally attractive, and it was not until an entirely new batch of pheromone was used that traps began catching moths. The cause of this problem was never identified (W. Roelofs, pers. comm.). Second, there appeared to be geographical differences in the response to pheromone, with a Washington population responding best to the pure alcohol, while California populations preferred blends of the acetate with the alcohol.

We reexamined the pheromone (Millar & Rice 1992), and found several minor components in both gland extracts and effluvia, none of which had any effect on the attractiveness of baits. As part of the same study, we synthesized and screened approximately 20 analogs of the pheromone components, and found that *E6-10Ac* and *E7-10Ac* strongly antagonized the pheromone. While these studies were in progress, we received reports of failures of commercial PTB lures from a Californian company.

Careful analysis revealed that the faulty lures were contaminated with several percent of *E6-10Ac*, accounting for the poor lure performance. It should be stressed that up to this point, there had been no reason to suspect that this compound was inhibitory, and furthermore, it is not trivial to detect small amounts of *E6-10Ac* in *E5-10Ac*.

In 1993, lures from two commercial distributors again failed. Analysis followed by synthesis and screening of trace contaminants revealed a second antagonist, 5-decyn-1-yl acetate (Millar & Rice 1996). Again, this component was present in trace amounts, but it had very significant effects on lure performance. Finally, in 1996, a third failure of commercial lures occurred. Analysis determined that this pheromone, although of high chemical purity overall (>98%), was again contaminated with small amounts of *E6-10Ac*.

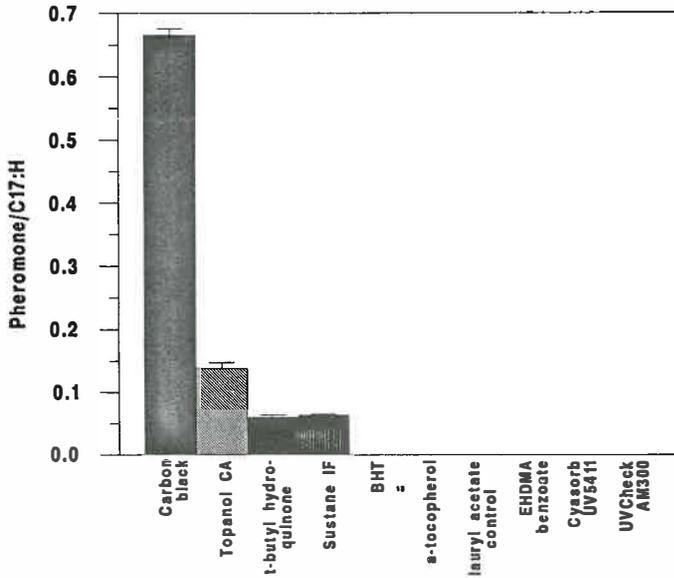
There are several points which can be drawn from this. First, if pheromone products are not reliable, their use will decline, and all the work invested in their development will be wasted. Second, synthetic pheromones used for monitoring lures, particularly for pheromones with histories of problems, should be field tested before marketing. Third, pheromone purity may be critical for efficacy, but the level of purity required will vary depending on the nature of the impurities and the insect species. Overall, researchers must be realistic with regard to the quality of pheromones which can be economically produced. However, simple precautions such as efficacy testing will be beneficial to both distributors (in terms of maintaining their markets) and to the continued development of pheromone technology.

#### Carob moth, *Ectomyelois ceratoniae*

Carob moth is the major pest of dates in California, and it is a pest of dates and almonds in other countries. The pheromone of the carob moth consists of an 8:1:1 blend of *Z9,E11,13-14Ald*, *Z9,E11-14Ald*, and *Z9-14Ald* (Baker *et al.* 1989). However, the major triene aldehyde readily degrades in light and air, making it difficult to work with, and is of questionable reliability for extended periods as a trap bait, particularly under the harsh conditions in California date gardens. The degradation problem has been addressed in two ways. First, a pheromone mimic (*Z7,E9,11-dodecatrienyl formate*) was developed, in which a formate ester replaced the aldehyde function. This compound proved to be as good or better as a trap bait than the synthetic pheromone blend (Todd *et al.* 1992).

Second, we conducted screening trials with a number of antioxidants and UV stabilizers (Figure 1) with interesting results. First, the best stabilizers of the pheromone mimic proved to be carbon black, Topanol CA® (a cocondensate of 3-methyl-6-*t*-butylphenol and crotonaldehyde; ICI Americas), and *N,N'*-diphenyl-1,4-phenylenediamine (data not shown). The latter compound had to be formulated in polyethylene glycol instead of the lauryl acetate diluent used with the other compounds because of solubility problems. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA, Sustane IF®), and  $\alpha$ -tocopherol, all of which have been used extensively

in pheromone formulations, gave intermediate or poor results. The results with carbon black were particularly surprising. This compound is thought to act by physically blocking incident radiation, but its excellent efficacy suggests that it may also have another mode of action.



**Figure 1** Carob moth pheromone remaining in plastic vials with different stabilizers after aging in sunlight for 13 days (lauryl acetate carrier)

As a result of these studies, we have increased the effective field lifetimes of our carob moth baits (0.5 mg of stabilized pheromone mimic on grey rubber septa) to at least 3-4 weeks. Our baits are currently being used extensively by the California date industry, and we have also supplied researchers in Europe, the Middle East, and Australia with baits or compounds for field trials.

Our results illustrate several points. First, in some cases, pheromone mimics may represent satisfactory alternatives to the true pheromone, at least for monitoring purposes. However, they should be used with caution, because the effects of competition from females on trap catch is unknown. Second, even very fragile molecules can be stabilized by a judicious choice of stabilizers, extending the field lifetimes of formulations to satisfactory levels. This is an area which has remained largely unexplored by academic researchers.

## Mating disruption

### Codling moth, *Cydia pomonella*

Codling moth is a major pest of apples, walnuts and pears worldwide, and despite several decades of effort, reliable and effective mating disruption of this pest has been elusive. The major component of the pheromone was identified as *E8,E10-12OH* (Roelofs *et al.* 1971), but a number of minor components have been identified from both gland extracts and effluvia (Einhorn *et al.* 1984; Arn *et al.* 1985). The effects of these components are still controversial, and commercial formulations used in mating disruption have been comprised of the major component alone or in combination with 12OH and 14OH.

One of the key factors which has slowed the development of effective mating disruption for codling moth is unsatisfactory dispenser performance: it has proven difficult to develop dispensers which release adequate quantities of high quality pheromone for periods of several months. In particular, in California, the moth has several generations per year, so that orchards need protection for periods of up to five to six months.

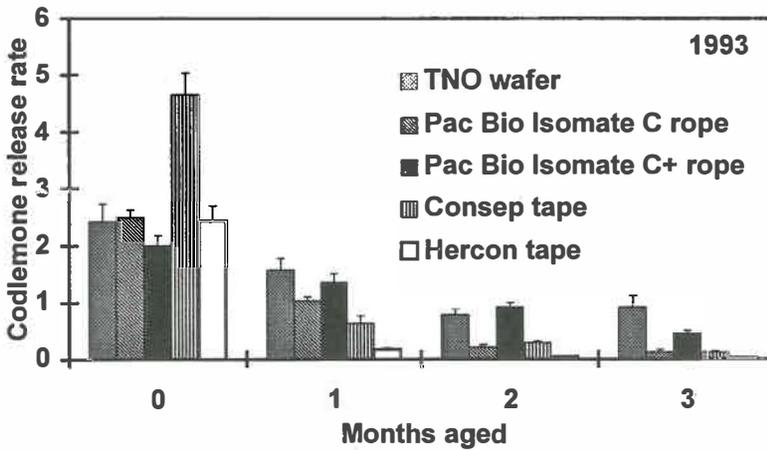
Despite the fact that codling moth mating disruption has been studied for many years, the release rates of dispensers have been poorly characterized and understood. The problem has been exacerbated by the rapid evolution of dispensers: dispensers have often been modified yearly, making it difficult if not impossible to compare results from year to year, and from one manufacturer to another. All too often, researchers have been left to speculate whether results were due to the pheromone dispensers used, or to environmental circumstances which affected moth populations. The problem was further compounded by the lack of standard protocols for conducting field trials.

Ideally a pheromone dispenser should have a constant release rate for its lifetime. In practice, release rates at a fixed temperature decrease with field aging. However, the decrease may be compensated to some extent by the increase in release rate with increasing temperatures as the season progresses.

In 1990, we began a five-year study, measuring the release rates of field-aged dispensers under standardized conditions. These measurements did not allow us to determine actual field release rates, but they did allow us to accurately compare the changes in release characteristics of dispensers over time. A representative example of the data is shown in Figure 2.

Several key points emerged. First, in many cases, release rates dropped to low levels in 60 days or less, even though projected lifetimes of the dispensers were often 90 days or more. Second, considerable degradation of the pheromone occurred with some dispensers, as demonstrated by the buildup of sticky residues on dispenser surfaces, and by the change in the ratios of components released (e.g., for one dispenser type, the ratio of *E8,E10-12OH* to dodecanol changed from 1.08 initially to 0.66 after

3 months). It must be emphasized that these linked problems of degradation and rapid decline in release rates are not unique to codling moth pheromone.



**Figure 2** Release rates of codlemone, *E8,E10*-12OH from field-aged mating disruption dispensers

However, our release rate measurements over a period of five years indicated very significant improvements in the performance of several dispensers. For the best dispensers, release rates after 12 weeks of field aging still approached 50% of the initial rates. However, other dispenser types, some of which were marketed, continued to show rapid declines in release rates with time.

In summary, information available to field researchers with regard to performance characteristics of the mating disruption dispensers that they are evaluating has often been sketchy at best. Information which has been available has often been obtained from artificially aged rather than field aged dispensers. Finally, many release rate measurements have been calculated indirectly, for example by subtracting the amount of pheromone remaining in a dispenser from the initial amount present. This fails to take into account degraded pheromone, which would not be detected or quantified by gas chromatography, resulting in actual release rates being lower than calculated. In short, adequate characterization of release rates of dispensers should become a part of the standard protocol of dispenser evaluation trials in order to introduce hard facts rather than "guesstimation" into the interpretation of field trial results.

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## **The need for standards in pheromone technology**

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**Abstract** - Industrial standards are needed to assure quality control of pheromones in pest management. Dispensers for mating disruption can be characterized by release rates of active ingredients as a function of temperature and other environmental conditions, as well as by the change of these release characteristics as a function of time. Since outdoor ageing is time-consuming and leads to highly variable results, a procedure for accelerated ageing in the laboratory should be developed. A slightly different situation exists in lures for insect monitoring and detection, where chemical composition is critical. Lures presently on the market are found to vary greatly in efficacy, mostly due to varying degree of purity of the starting materials. We propose to adopt the procedure of batch certification which identifies every batch of attractant used commercially and relates it to the published information on biological activity.

**Key words** - sex pheromone, mating disruption, dispenser, release rate, chemical purity, antagonist, batch certification, accelerated ageing

### **Introduction**

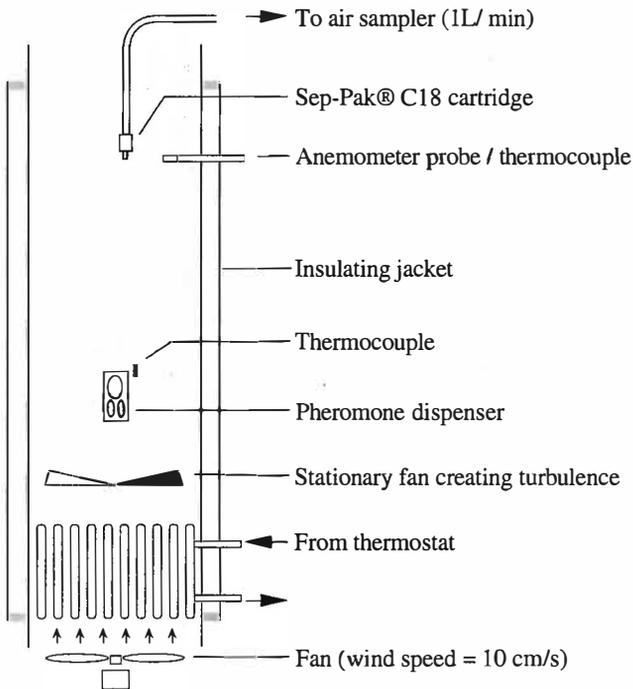
With the introduction of insect pheromones in plant protection, an old dream has come true: "Toxic" insecticides are being replaced with "harmless" natural products. This is most apparent in the technique of mating disruption which directly uses pheromone chemicals to control specific pests. But even when used to attract insects to a monitoring trap, as in hundreds of insect species worldwide, pheromones have made a significant contribution towards a reduction of pesticide use.

In spite of these achievements, progress in the application of pheromones has been rather slow, and technology is still in its infancy. While producing material for applications on a large scale, industry is still in great need to make formulations more

effective and suitable for a wide range of pests and climatic conditions. It is not only the scientific knowledge that drives this process, but to a great extent also trial and error. Users thus still find it difficult to obtain material of predictable quality for field use.

### Dispensers for mating disruption

Mating disruption can be effective as long as the proper chemical, usually a synthetic pheromone or its derivative, is present in the atmosphere in sufficient concentration to prevent male insects from locating calling females. This requires a device releasing the active ingredient over an extended period of time, usually several months.

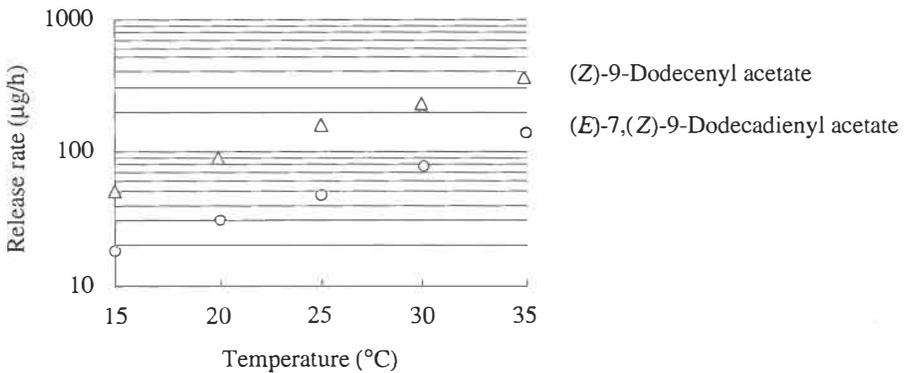


**Figure 1** Apparatus to determine release rates of mating disruption dispensers, adapted from Pop *et al.* (1993)

In most cases, the concentration of pheromone chemical required to disrupt mating is not exactly known. Approximations of minimum airborne amounts have been made from the total amount applied, based on success and failure in controlling the pest. To

improve on the existing technology, more quantitative data are urgently needed. This includes information on the amounts released by dispensers.

Measuring the release rate of dispensers is quite a difficult task. Weight loss provides reliable data only under laboratory conditions where degradation can be controlled. In the field, dispensers can even gain weight, *e.g.* with accumulation of debris, change of humidity or oxidation of the remaining product. These variations become important towards the end of the growing season when the supply of active ingredient is low and insect pressure possibly at its highest. Analysis of the remaining chemical is a more reliable, yet destructive technique, and provides no valid information about future release of the chemical. The only valid procedure is the measurement of pheromone concentration in the dispenser effluent. The techniques to accomplish this can be quite involved (Leonhardt *et al.* 1988; McDonough *et al.* 1989; Van der Kraan & Ebberts 1990).

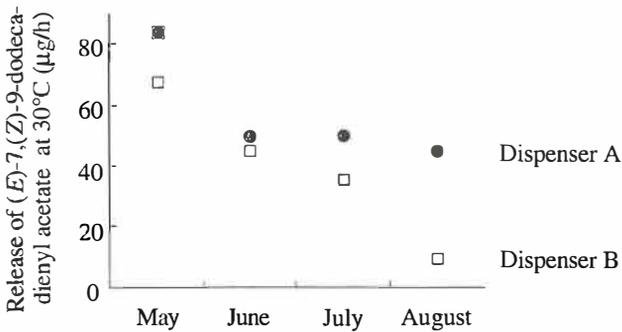


**Figure 2** Release rates of active ingredients from a RAK 1+2 (BASF) dispenser used for mating disruption in *Eupoecilia ambiguella* and *Lobesia botrana*. Since release rates vary among dispensers, the mean of multiple determinations with only one dispenser is shown.

In our laboratory we have developed and used the apparatus shown in Figure 1. To allow changing temperature, wind speed and other environmental conditions, we opted for an open system in which an aliquot of effluent is collected. This reduces errors caused by adsorption on surfaces of the apparatus. We found that degradation of dienic alcohols, *e.g.* (*E,E*)-8,10-dodecadien-1-ol, (*E,Z*)-7,9-dodecadien-1-ol or (*Z,E*)-9,12-tetradecadien-1-ol is prevented by adding  $\alpha$ -tocopherol (ca. 200 $\mu$ g) to the Sep-Pak® cartridge as an antioxidant. Internal standards are introduced prior to air collection and before gas chromatographic analysis. The apparatus is made from inexpensive parts, with a car heater used for temperature control.

The release of pheromone chemicals from an unused RAK 1+2 ampoule (BASF AG) at various temperatures is shown in Figure 2. The release rate increases by about a factor of 10 from 15 to 35°C. Similar temperature gradients were observed for dispensers for the codling moth, such as Isomate C (Shin-Etsu) or Ecopom (Isagro). Since these orchard and vineyard insects are sexually active in the evening or during night when temperatures are low - as is the case for most moths using sex pheromones - this temperature effect represents a serious waste of active material during the hot summer days.

To the user it is important to know how a dispenser will release the active material over the entire growing season. Figure 3 shows the results obtained with two dispensers of different design and lure content that were exposed facing the sun outside the laboratory. For each measurement, the dispensers were taken inside for one day and placed back outdoors again. Release rates were determined at 30°C to avoid the need for excessive refrigeration during sampling.



**Figure 3** Pheromone release from two types of dispensers for mating disruption in *L. botrana*

After 3 months of exposure, the type A dispenser still released the pheromone at ca 50% of the original rate. The release rate of type B, on the other hand, had fallen to 50% one month earlier; by mid-August the dispenser was virtually depleted. Since the second flight of *L. botrana* usually ends in mid-July, both dispensers should give satisfactory results. Dispenser B is the more economical of the two since it makes full use of the available chemical, but it contains little reserve in case of unexpected high temperatures.

#### Accelerated exposure tests

The described tests were quite useful to back up an actual field trial. They could have

given some clues in case of failure to control the specific pest. However, dispenser performance should actually be determined before their use in the field. Where the manufacturing process still evolving, a procedure is needed in which exposure to the elements, possibly under extreme conditions, is simulated in the laboratory. The Collaborative Pesticide Advisory Council (CIPAC) has adopted protocols for accelerated storage tests for pesticides. These are carried out at elevated temperatures, typically 54°C (Dobrat & Martijn 1995). Procedures to be developed for pheromone dispensers should provide for additional tortures such as UV radiation and wind.

### **Dispensers used in monitoring traps**

The chemistry of insect sex attractants is well documented. In Lepidoptera alone, pheromones and sex attractants of over 1600 species have been described (Arn *et al.* 1992, 1996). Numerous companies serve the needs of plant protection services and growers by manufacturing lures and traps. However, users of pheromone traps frequently complain about the variability of data obtained with certain commercial lures. In a letter sent to commercial suppliers in the early 90's, Günter Schruft of the Institute of Viticulture in Freiburg im Breisgau, Germany, bitterly complained that half of the lures sold to grape growers caught either nothing or the wrong species. Similar reports have been heard from various orchard and forest entomologists. This situation has led to insecurity among growers and plant protection advisors.

### **Purity requirements**

It is well known that chemical purity can be very critical to biological activity of pheromones. Insects are very sensitive to trace components present in attractant blends. Any synthetic product, crude or purified, contains some impurities. Even when close to or beyond the detection limit, these byproducts can have positive or negative effects on trap catch.

Every chemical ecologist should read the chapter by Turk & Turk (1975) on chemical purity of odorants. It begins as follows: "The idea of 'ultimate purity', usually considered to be the condition of a substance composed entirely of like molecules, has no operational meaning in the laboratory. We cannot examine each molecule in a sample, and even if we could, we might not be able to describe the purity of the sample because we would not necessarily know which differences between molecules were sufficiently permanent to persist after separation. All the chemist can do is to look for evidence of impurity. A 'pure substance' is then taken to be one for which no evidence of impurity is found. The criterion of purity is thus always conditional and temporary."

The importance of isomeric purity has been established for many species. In many cases, however, the reasons for a better performance of one batch of chemical over another have remained obscure. The detection of a positional isomer as an inhi-

bitor of the *Anarsia lineatella* pheromone (Millar *et al.*, this volume) is an example of the high quality of research needed to guarantee the efficacy of a lure. Ironically, some chemical batches are more active than others due to the presence of a synergistic impurity. This seems to be the case in the grapevine moth, *Lobesia botrana*, where highly pure (*E,Z*)-7,9-dodecadienyl acetate is less attractive than many crude synthetic products.

#### Importance of field tests

Following these considerations it is not possible by chemical analysis alone to predict whether a given batch of chemical will give a good attractant. Once chemical analysis has demonstrated the presence of essential and the absence of detrimental constituents in a synthetic product, the only demonstration of biological activity can be obtained in the field. The procedure of providing of lures suitable for distribution thus always consist of 3 steps: 1) Synthesis of ingredients, 2) chemical analysis to assure the presence of essential and absence of known antagonistic components and, if necessary and feasible, purification, and 3) field testing and comparison with standards in various habitats.

#### Batch certification

In order to assure a continuous supply of lures of comparable quality and to allow comparisons between lures of different origin, we propose to adopt the procedure of batch certification. It consists of two principles:

- 1) Any chemical or blend prepared for insect monitoring is given a batch number which is carried over to all dispensers made from it.

- 2) Each batch is field-tested by experts and the results made publicly available. Since lures for monitoring and detection are not normally subject to registration by government authorities, certification can be accomplished on a nearly informal basis within the international scientific community. Batch specifications and test results could be published on the Internet and readily updated. Some of the procedures will need to be discussed in more detail. Where the dispenser material has been shown to be important, it should be included in the procedure. A consensus should also be reached on experimental design of field tests and analysis of data.

A dilemma can be anticipated concerning the chemical information to be revealed for certification. To the educated user it is important to know the basic ingredients of each lure. Insect attractants are constantly being refined, and the user should have access to the most effective and reliable mixture. For example, racemic disparlure, which has been used for decades to monitor the nun moth, will soon be replaced by the far more attractive blend containing monachalure which was recently identified (Gries *et al.* 1996). One could even argue that analytical samples of certified batches should be made available to the scientific community and that the results of the chemical analysis be published. This could lead to an improvement of our

knowledge of effects of secondary components and lure specificity. In this context it would even be desirable to know the synthetic pathway. On the other hand, we should also respect the position that for commercial reasons the composition of a particular lure may need to remain a well-guarded secret.

Batch certification will lead to a gain of confidence in the trapping results obtained with pheromones. Attempts to relate trap catch with population density can begin to be fruitful as soon as a the batch of chemical used in different tests is the same. Certification is of critical importance for quarantine pests in which it is often impossible to confirm biological activity without going to another continent.

Batch testing has long been established for pesticides and pharmaceuticals in cases where it was necessary to track down unwanted side effects. In insect monitoring and detection, the procedure will require a closer cooperation of suppliers and scientists. This small effort is by far outweighed by the gain of confidence.

## Outlook

Pheromone technology started in the late sixties when researchers began testing their first synthetic samples in the field. Some of the home-made slow-release devices of those days, such as rubber tubes and polyethylene caps (Glass *et al.* 1970) are still in use today. Field experiments in which an attempt is made to control all the chemical and physical elements needed for biological activity are still quite scarce (Färbert *et al.* 1996; Witzgall *et al.* 1996). Such efforts are essential to guarantee the survival of pheromones as tools in pest management.

## Acknowledgement

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*Mating disruption in orchards*

## **Three years of large-scale control of codling moth by mating disruption in the South Tyrol, Italy**

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**Abstract** - In 1990 it became apparent that populations of codling moth, *Cydia pomonella*, were increasing in orchards treated with diflubenzuron. In 1992 it was shown that some codling moth strains had developed a strong resistance to chitin synthesis inhibitors (CSI); the resistance appeared after about 25 CSI applications. After having thus lost an important component of our pesticide list, there was the danger that we would not be able to maintain an integrated pest management programme that would come up to international standards. Therefore we introduced the mating disruption method on a large scale. In 1991 we started with RAK 3+4 dispensers on 110 ha. As in 1992 RAK 3+4 were not available, we made a new start with Isomate-C dispensers on 232 ha in 1993. In the following year, the area under mating disruption expanded to 2 500 ha and reached 4 500 ha in 1995, in 1996 the treated area is 3 500 ha. This paper presents the results obtained with different dispenser types (Isomate-C, Isomate-C-Plus, Isomate-C-Special, RAK 3+4, Ecopom Combi) against codling moth and leafrollers. Mating disruption was successful even in hillside orchards and smaller plots. Finally, organizational problems connected with the planning of mating disruption projects are discussed.

**Key words** - sex pheromone, mating disruption, monitoring, insecticide resistance, integrated pest management, pome fruit orchard, codling moth, *Cydia pomonella*

### **Introduction**

In 1977 the chitin synthesis inhibitor (CSI), Dimilin, was registered in Italy. Until about 1982 this pesticide had been used exclusively for leaf miners in the South Tyrolean apple growing area. Since 1983 my organization increasingly recommended it for codling moth, *Cydia pomonella* L. At the end of the 1980s some further chitin synthesis inhibitors (teflubenzuron, triflumuron, hexaflumuron) were registered in Italy.

Up to 1990, one treatment with 75 to 100 g active ingredient/ha against each codling moth generation was sufficient. In 1990 for the first time, more treatments or higher doses were no longer efficacious in a few locations. In 1991, 10 to 40% fruit

injury at harvest in spite of 3 to 5 sprays with CSIs was not unusual in these areas.

In 1992 H. Riedl (Oregon) and R. Zelger (Laimburg) proved that some codling moth strains had developed a strong resistance to diflubenzuron. Further tests by R. Zelger showed that those strains were also resistant to all other CSIs. The resistance appeared after approx. 25 CSI applications and seems to be very stable. The descendants of resistant codling moths could not be controlled by CSIs, either.

At the end of 1992 we estimated that this phenomenon appeared on 1 200 ha of our 18 000-ha apple growing area. According to our observations, these "red zones" with CSI-resistant codling moth populations have increased to 15 000 ha in the meantime.

From 1993 onwards we were forced to warn against the use of CSIs on larger and larger areas. In the so-called "red zones" the growers would have been forced to use again only organophosphates, mainly Azinphos. Their undesirable side-effects on beneficials (*e.g.* typhs, stethorus, lacewings, parasitic wasps) are already well-known. After we had stopped recommending this pesticide and others which are very noxious to typhs in 1983, we could do without acaricides in approx. 70% of the South Tyrolean orchards. Phosalone (Zolone), which worked quite well against codling moth north of the Alps, failed under our conditions. Fenitrothion (Fenitrocap) and quinalphos (Ekalux), which are about as effective as azinphos (Gusathion) but less acutely toxic, are throwbacks in comparison with CSIs. Therefore the Advisory Service tried to introduce mating disruption on a large scale in the South Tyrol.

### **Mating disruption is now used on a quarter of the South Tyrolean orchard area**

Since preliminary trials with the dual dispenser RAK 3+4 since 1988 showed promising results, we continued to test this product in 29 orchards on a total area of about 100 ha in 1991. Unfortunately, we could not carry on in 1992, because BASF decided to change the contents of the dispensers significantly.

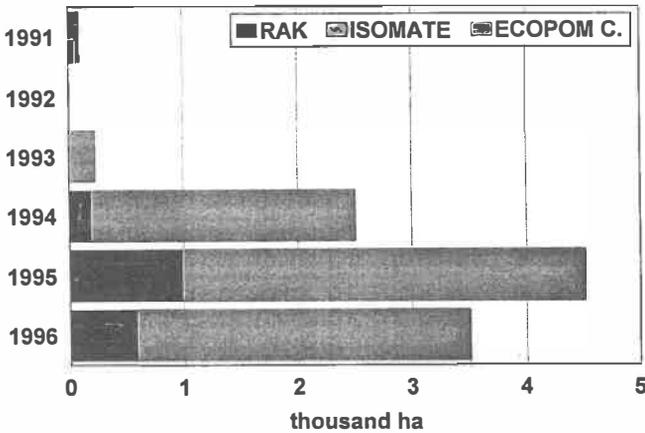
We asked Pacific Biocontrol to supply Isomate-C dispensers for a new series of tests. Based on the reports by G.J.R. Judd and M.G.T. Gardiner (Canada), as well as A. Knight (USA), we started a pilot project with 85 fruit growers in six different locations in 1993. The total area consisted of 232 ha. Per ha we recommended 1 000 dispensers at 112 mg (*E,E*)-8,10-dodecadienol (codlemone) each.

In 1994 our growers used Isomate-C-dispensers on 2 250 ha. Shin-Etsu delivered two dispenser types: Isomate-C (white) which were formulated with 112 mg codlemone and Isomate-C (brown) which contained 126 mg codlemone. RAK 3+4, which contained approximately 165 mg codlemone and 270 mg leafroller pheromone blend, were used on 200 ha. There were also two projects with other dual dispenser types: Isomate-C Special on 15 ha and Ecopom Combi on 9 ha.

In 1994 mating disruption was used in 1 600 South Tyrolean orchards on 2 500 ha altogether. We counted 131 projects. The smallest area covered by mating disrupt-

tion was 0.4 ha, the largest 135 ha.

In 1995 the total area with mating disruption expanded to 4 500 ha. This corresponds to a quarter of the whole South Tyrolean apple growing area. No other fruit growing area in the world had such a high share of mating disruption orchards. 3 500 ha were furnished with Isomate-C types, 1 000 ha with RAK 3+4 and 20 ha with Ecopom Combi. Dual dispensers were used on 2 500 ha out of the total area of 4 500 ha.



**Figure 1** Mating disruption in pome fruit orchards in the South Tyrol, Italy

In 1996 the area with mating disruption has decreased to 3 500 ha. There are two reasons: On the one hand we tried to get the growers to organize the projects themselves, on the other hand the growers ordered fewer dual dispensers. This year the South Tyrolean orchardists are using Isomate-C-Plus on 2 700 ha, RAK 3 on 400 ha, RAK 3+4 on 200 ha, Isomate-C-Special on 165 ha and Ecopom Combi on 10 ha (Figure 1). In absolute numbers, only the USA used mating disruption on a larger orchard surface in 1996.

**Planning a mating disruption project is like fitting together a jigsaw puzzle**

A typical South Tyrolean fruit farm is about 4 ha in size. The single orchards, however, are situated at different sites. It is already common knowledge that mating disruption works better in larger applications. We advise our members to assemble projects of at least 10 ha. This requires the team-work of 10 to 20 growers. As a rule, one

or a couple of growers consent to ask the owners of the neighbouring orchards if they are willing to participate in a mating disruption project. We have calculated that it takes about an hour of organizational work (map drawing and completing the order forms) per ha of mating disruption.

In some districts the packing houses have charged one of their employees with the assemblage of suitably large areas for mating disruption.

## **Barriers**

Until 1994 we recommended so-called "exterior" barriers. Dispensers were applied as far as 20 m deep in the orchards adjoining a project. Although this was very useful we had to stop doing it for two reasons: we required about 25% more dispensers on an average, which increased the costs; some "clever" growers, whose orchards were sometimes not wider than 20 m, did not participate in a project because they profited from it all the same.

Since 1995 we have been working with "interior" barriers only. If tree height exceeds 4 m, twice as much dispensers are placed on the perimeter trees than inside the orchard. If the trees have a small canopy we double the number of dispensers in the two border rows.

This constitutes an additional expenditure for dispensers and other costs of about 10% for projects over 10 ha, and of up to 30% for smaller projects. For new plantings situated within a project we recommend applying half the usual number of dispensers/ha or interior barriers.

## **Number of dispensers per ha and their placement**

With Shin-Etsu ropes we recommend in the first couple of years 1 000 dispenser/ha, without regard to tree height, size of the project and exposition. For projects over 10 ha in lower locations where mating disruption was applied in the two previous years and where the damage at harvest was below 1% without supplemental treatments we recommend in the third year only 800 dispensers/ha if tree height is below 4 m.

With RAK we recommend 750 pieces/ha for trees with big canopies, and 500/ha for trees with small canopies. Ecopom and Checkmate-CM have to be applied twice during the vegetative period. Both types are used here only on an experimental basis. No grower in our area would use a dispenser which requires two applications a season as long as there is an alternative. With large trees the application takes up to 15 hr/ha, on ground level we calculate about 4 hr/ha for smaller trees.

Where should the dispensers be placed? We recommend attaching the dispensers 0.5 to 1 m below the tree top. This seems very important to us because we have had several cases where mating disruption failed because the dispensers were positioned too low.

### Monitoring tools, orchard checks and action thresholds

L. Gut and J. Brunner (Washington) found a relation between trap catches in high-load lures and harvest damage in mating disruption orchards in their region. Under the climatic conditions in Washington each moth caught after an initial period of two weeks corresponds to an average harvest damage of 0.1%. Based on these findings we recommend at least one pheromone trap with a 10-mg lure per ha orchard area. The trap should be hung in the upper third of the canopy and the lure should be changed every 40 days. The traps are to be checked once a week.

According to our experiences a cumulative trap catch of more than eight moths is a signal that special attention is required. In this case, fruit checks are necessary after 10 to 14 days. Certainly, this monitoring method is not absolutely reliable but it helps to reduce the number of fruit checks or to determine the most convenient dates for fruit sampling.

In the preceding years we invited the growers to at least four fruit checks together with our consultants. During those checks at least 1 000 fruits/orchard were controlled for fresh entry holes. We recommend the following action thresholds in mating disruption orchards: 0.3% fruits with fresh entry holes (June); 0.5% fresh entry holes (July to mid-August); 0.8% fresh entries (from mid-August on); 1.0% wormy fruits (at harvest).

If harvest damage exceeded 1% in the previous year or is unknown as well as in small projects we recommend a supplemental treatment in any case, as soon as according to the degree day model 30% of the larvae of the first generation have emerged or when the first entry holes are detected.

### Results 1988-1996

The positive experiences with mating disruption in two orchards in the South Tyrol from 1988 to 1990 aroused the interest of the South Tyrolean orchardists in this method. In 1991, 40 fruitgrowers participated in 29 different projects with a total area of about 100 ha. On 93 ha there was less than 2% fruit injury at harvest without an additional pesticide application against codling moth. On 7 ha, that is about 7%, the fruit growers had decided to use an insecticide. On 5 ha there was a surprisingly high damage at harvest. We used 500 to 800 RAK 3+4 dispensers/ha loaded with at least 420 mg codlemone and 420 mg leafroller pheromone blend. From the beginning of May to the end of October about 100 g/ha of both pheromones evaporated. More than half remained in the dispensers.

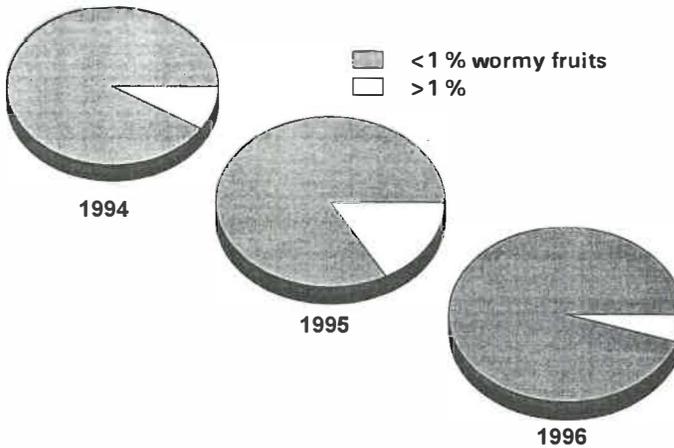
BASF did not sell this dispenser any more in 1992. In 1993 we made a new start with Isomate-C dispensers. Table 1 gives an overview of the results obtained up to now.

**Table 1** Wormy apples in mating disruption orchards, 1993 - 1996

Year	Leifers		Bozen		South Tyrol	
	Orchards <sup>a</sup>	Damage (%)	Orchards	Damage (%)	Orchards	Damage (%)
1993	80	0.9	52	1.2	-	-
1994	20	0.2	-	-	913	0.4
1995	20	0.1	-	-	984	0.7
1996	20	0.1	-	-	114	0.3

<sup>a</sup> Number of orchards checked

In 1994 the data collected in 913 disrupted orchards at harvest showed an average codling moth damage of 0.4%. In comparison with conventional orchards, the number of insecticide sprays decreased by two thirds, the average number of additional treatments was 1.4. In 1995 the average number of supplemental treatments could be reduced to 0.6. The average percentage of codling moth damage was slightly higher in 1995 with all types of dispensers. In my opinion this was mainly due to the following two reasons: In 1995 we did not recommend exterior barriers; the share of orchards with not so favourable conditions for mating disruption was higher than in 1994.



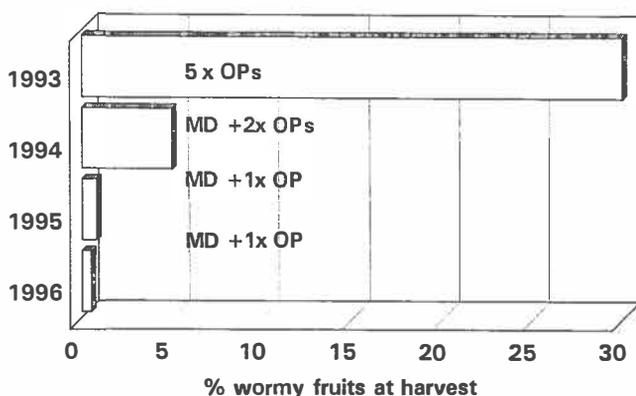
**Figure 2** Codling moth, *C. pomonella*, damage at harvest in mating disruption orchards, South Tyrol, Italy

Naturally, we have not yet obtained all the data about harvest damage in 1996. However, the first data we have collected so far indicate that harvest damage will be lower

than in 1995 (Figure 2). In the Leifers area we have recorded codling moth damage in 20 mating disruption orchards over the period from 1992-1996. The results show that there is considerably less fruit injury after four years of mating disruption (Table 1).

### Is mating disruption feasible in small orchards?

I would like to adduce as an example a small 0.5-ha orchard with partly 4 to 5 m high trees in the centre of Lana, where there are very favourable conditions for codling moth. The orchard is bordered by a barn, two houses, a street, a tall nut tree and another orchard.



**Figure 3** Mating disruption in a 0.5-ha orchard (OP: organophosphate spray; Lana, Italy)

Damage at harvest during four years is shown in Figure 3. In 1993, four treatments with phosalone (Zolone) and one with Azinphos resulted in a harvest damage of 25 to 30%. Mating disruption was employed for the first time in 1994; 1 000 Isomate-C dispensers on 0.5 ha were supplemented with two applications of Azinphos, damage at harvest was 5%. In 1995, Isomate-C Plus were used; a consultant of the Advisory Service checked the orchards weekly for fresh entry holes. The first ones were found on July 7th, and a week later 0.5% of the fruits were already damaged. On July 17th Fenitrocap at 250 ml/hl was applied. The damage at harvest was 1%. In 1996, the orchard, which was again furnished with Isomate-C dispensers, was checked once a week. After the first stings had been detected at the end of July, Fenitrocap was applied. The damage at harvest is 0.1% this year (Figure 3).

The example shows that it is very well possible to use mating disruption on small plots. It is not only of ecological importance, but it can also be economical. In

this case, three organophosphate sprays were saved in 1993, and in the last couple of years even four such treatments. Nevertheless, with small plots, the grower has to be prepared to check the fruits regularly at short intervals and to allow for one or two supplemental treatments.

### Mating disruption on slopes

The harvest damage in orchards situated on hillsides and in plains was 0.7% and 0.4%, respectively, in 1994; and 1.0% and 0.6% in 1995. The number of additional insecticide sprays in orchards situated on slopes and in plains was 1.5 and 1.4, respectively, in 1994.

In hilly country it is more difficult to compose larger areas with homogeneous and favourable conditions for mating disruption projects. Therefore we suppose that the slightly higher injury level on slopes is mainly due to the small size of the plots.

### Taller trees - more damage

The average percentage of fruit injury was considerably higher on trees taller than 4 m (0.8%) than on smaller trees (0.5%), as we found out through checks in 411 orchards on seedling and 429 orchards on dwarfing rootstocks in 1995.

### Effectiveness of the various dispenser types for codling moth

In Table 2 the results obtained with Isomate-C-Plus and Isomate-C-Special as well as RAK 3+4 dispensers in 1995 are summarized.

**Table 2** Fruit damage by codling moth, *C. pomonella*, and supplemental insecticide sprays in mating disruption orchards (South Tyrol, 1995)

	Orchards checked	Fruit damage at harvest	Supplemental sprays
Isomate-C-Plus	258	0.8 %	0.6
Isomate-C-Special	430	0.5 %	0.4
RAK 3+4	87	1.2 %	0.6

These data have to be interpreted with care. Isomate-C-Plus are mainly used in areas where codling moth is more prominent. Isomate-C-Special and RAK 3+4, on the other hand, are dual dispensers, which are more likely to be applied in areas with higher leafroller and lower codling moth density. We do not recommend dual dispensers. The effectiveness of Isomate-C-Special for codling moth control is good, where-

as the performance of RAK 3+4 is moderate, but concerning leafrollers, both types are still unreliable.

### Economic aspects

In Table 3, I have listed the costs of the various types of dispensers per ha. At present Isomate-C-Plus has the best cost/effectiveness ratio. One application of 800 dispensers/ha has the same price as three treatments with Fenitrocap (a micro-encapsulated fenitrothion). Organophosphates are noxious to typhs, among others. If mating disruption helps to do without acaricides the dispensers have already paid their way, because one acaricide application costs more than half of the dispensers/ha.

**Table 3** Cost for control of codling moth, *C. pomonella*, by mating disruption or pesticides

Pheromone dispenser	Amount (dispenser/ha)	Codlemone (g/ha) <sup>a</sup>	Cost/ha (k ITL) <sup>b</sup>
Checkmate-CM	2 x 300	83 - 108	574
Ecopom	2 x 300	138	607
Ecopom Combi	2 x 300	138	891
Isomate-C-Plus	800 - 1000	100 - 125	290 - 363
Isomate-C-Special	800 - 1000	84 - 105	344 - 430
RAK 3	500 - 750	83 - 124	378 - 568
RAK 3+4	500 - 750	83 - 124	500 - 750
Pesticide	Amount (kg/ha)		
Fenitrocap (insecticide)	3 x 3.75 - 5		235 - 300
Miro (acaricide)	1.5 - 2		150 - 200

<sup>a</sup> Codling moth pheromone (*E8,E10-12OH*); <sup>b</sup> cost in 1000 Italian Lire

The long-term advantages, *e.g.* the protection of other beneficials, the overcoming, delay or avoidance of a pesticide resistance, no reentry periods, no problems with overhead irrigation *etc.*, cannot be expressed in numbers.

### Final considerations

By now we have gathered enough facts to be able to assess the possibilities and limits of mating disruption sufficiently so as to recommend it under certain conditions as a standard measure in the future.

(1) Mating disruption will be recommended for orchards where otherwise three or more organophosphate sprays against codling moth would be needed; (2) the mating disruption area should not be smaller than 10 ha, if possible; (3) with a view to

cost reduction we will continue to recommend only interior borders, that means that the number of dispensers should be doubled in perimeter tree rows; (4) mating disruption orchards have to be furnished with monitoring traps baited with 10 mg codlemone; (5) the growers will be asked to check their orchards as soon as fresh entries are found in special orchards which will be monitored by us; (6) if there are some catches in the 10-mg traps, we will recommend fruit checks after 8-10 days; (7) in orchards with a harvest damage of more than 1% in the previous year: we recommend an additional treatment in border rows, transitional areas from small to high trees, as well as near stacks of wood, nut trees and buildings; (8) in areas with less than 1% fruit damage at harvest, after using mating disruption for two years, we will suggest 800 Shin-Etsu ropes instead of 1 000/ha.

By now the majority of the South Tyrolean fruit growing experts and fruit growers is convinced of the ecological advantages of the mating disruption technique for codling moth.

Whether the mating disruption area will further expand in the following years will largely depend also on the costs of the dispensers. The growers will base their decisions mainly on the comparison of the costs of the dispensers per ha including one eventual supplemental treatment with the expenses for three to five organophosphate sprays.

### Acknowledgements

We are indebted to many researchers who helped us to avoid mistakes when introducing this new technique. The research work by Pierre Charmillot and Heinrich Arn, Switzerland, Albert Minks, Netherlands, Gary Judd, Canada, as well as Jay Brunner, Larry Gut and Alan Knight from the USA and many others have laid the foundations for a good start. Finally I am sincerely grateful to all my collaborators who contributed by supplying data.

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## **Goading growers towards mating disruption: the South African experience with *Grapholita molesta* and *Cydia pomonella* (Lepidoptera, Tortricidae)**

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**Abstract** - *Grapholita molesta* Busck. and *Cydia pomonella* L. are key pests in South African stone and pome fruit orchards, respectively. *G. molesta* was accidentally introduced into the Western Cape in the late 1980's, where it has up to six generations per year. By 1991, despite comprehensive insecticide programmes, crop losses in canning peach orchards were as much as 60% and shoot damage as high as 80%. As a result, some growers were considering removing productive orchards. An area-wide mating disruption programme with Isomate-M was initiated during the 1991/92 season in 1 200 ha of peaches and nectarines in the Tulbagh Valley. The project was an outstanding success. By the end of the season only 69 *G. molesta* adults had been caught in pheromone traps, shoot strikes were reduced to isolated occurrences, while not a single infested fruit was recorded from the treated area. *C. pomonella* has three generations per year in South Africa and a high reproductive potential. It has recently increased in pest status due to the development of resistance to insecticides, with fruit damage in some orchards exceeding 30% after up to 13 sprays. As an exercise in resistance management, an Isomate-C and Isomate-C Plus mating disruption programme with supplementary insecticide intervention was implemented in a block of apple orchards. By the end of the third season, insecticide applications had been reduced by an average of 32%, and fruit infestation limited to an average of 0.3%. A number of factors limiting the increased use of mating disruption products in South Africa are discussed.

**Key words** - sex pheromone, mating disruption, Oriental fruit moth, *Grapholita molesta*, peaches, codling moth, *Cydia pomonella*, apple, pome fruit, resistance management

### **Introduction**

The stonefruit industry in South Africa comprises 27 000 ha of peaches, nectarines, plums and apricots. The key pest on especially canning peaches is Oriental fruit moth, *Grapholita molesta* Busck. *G. molesta* was accidentally introduced into South Africa

in about 1987, probably on peach budwood illegally brought into the country. It was first detected in 1990, and spread rapidly throughout all stonefruit growing areas. The warmer peach-growing areas support six generations of *G. molesta* per year, with four per year occurring in the cooler areas.

Initial control programmes comprised up to 13 applications of organophosphate insecticides. This heavy pesticide programme was expensive and disruptive to beneficial orchard fauna, and necessitated the application of at least two sprays against mites. In one particular area, the Tulbagh valley, shoot damage by *G. molesta* to canning peaches at the end of the 1990/91 season averaged 49%, ranging from 30% to 80% (Table 2). Although fruit damage in the valley was sporadic, other nearby areas had crop losses to *G. molesta* of up to 60%, despite rigorous spray programmes (W. Boonzaaier *unpubl. report*).

As a result of the inability at that stage to control *G. molesta* chemically, some growers had started to remove productive peach orchards in favour of fruit kinds not susceptible to *G. molesta*. In the light of these factors the future of canning fruit industry was considered to be in jeopardy. As a consequence, the South African Preserving Company (SAPCO, a canning company in Tulbagh), and the Canning Fruit Board approached the authors for assistance, particularly with respect to the situation in the Tulbagh valley. We suggested the possibility of mating disruption using Iso-mate-M, the only *G. molesta* mating disruption product on the market at that time. Out of this was born the Tulbagh valley mating disruption project, which we undertook to plan and coordinate.

The South African pome fruit industry comprises approximately 20 000 ha of apples and 12 000 ha of pears. The key pest on pome fruit is codling moth, *Cydia pomonella* L. In South Africa's warm climate it has a high reproductive potential, and two and a half to three generations per year. Azinphos-methyl has been used almost exclusively and very effectively for its control for about 35 years. However, laboratory bioassays have shown that codling moth has recently developed a high degree of resistance to azinphos-methyl and synthetic pyrethroids in some orchards (M. Addison *pers. comm.*). Up to 12 sprays per season are common, and despite this some orchards suffer 30% or more codling moth damage. Resistance to these insecticides, the possibility of cross-resistance to others, and a limited number of products registered against codling moth was placing a severe strain on the pome fruit industry. A project was therefore initiated to evaluate mating disruption as a means of controlling codling moth with reduced insecticide usage, which could thereby contribute to slowing the development of resistance.

In this article we describe the two projects, and discuss factors influencing the use of mating disruption in South Africa.

## Materials and methods

### Oriental fruit moth

The area-wide *G. molesta* mating disruption project was initiated in the Tulbagh/Wolseley valley in August 1991. The valley comprised 2 800 ha of mixed fruit; 1 200 ha of peaches (mainly canning cultivars) and nectarines, and 1 600 ha of plums, prunes, apricots, almonds, pears and grapes. It is a discrete fruit production area and was therefore very suitable for an undertaking of this nature. The valley is about 30 km long and 10 to 15 km wide, mostly surrounded by mountains, and comprises two distinct fruit-growing areas some 10 km apart. Sixty growers were involved.

Isomate-M (Shin-Etsu Chemical Co., Tokyo), containing 93% (Z)-8-dodecen-1-yl acetate (Z8-12Ac), 6% (E)-8-dodecen-1-yl acetate (E8-12Ac), and 1% (Z)-8-dodecen-1-ol (Z8-12OH), at 75 mg/dispenser was applied to all 1 200 ha of peach and nectarine orchards in the valley. It was also applied as five-row borders to all plum, prune, apricot, almond and pear orchards. All dispensers were applied by 15 August, at the recommended density of 1 000 dispensers/ha, and in the top third of the tree canopy. The second batch of dispensers was applied 90 days after the first application. SAPCO appointed two of its staff full-time to the project, who ensured that the application of dispensers was carried out strictly according to recommendations, and took responsibility for monitoring actions.

One supplementary spray of azinphos-methyl against the first moth flight had been planned for all peach and nectarine orchards. However, this was not carried out.

The status of *G. molesta* populations in the treated area was monitored with Pherocon 1C sex pheromone wing traps baited with Trécé 1-mg lures, and with terpinyl acetate bait traps. Both were deployed at one trap/10 ha throughout the treated area, *i.e.* 120 traps of each. They were installed over a period of a week starting on 15 August. All traps were monitored every 4 to 8 days; the pheromone traps until March 1992, and the bait traps for a shorter period as little success was obtained with them. Three pheromone traps were also placed in the canning factory bulk bin stack in mid-September. All traps were serviced and maintained according to standard recommendations. Due to the size of the project and limited human resources, no attempt was made to determine the mating status of females caught in the bait traps.

Routine visual inspections were made of shoot tips and fruit. Due to the size of the area and the limited number of monitors, inspections were not intensive, but were made approximately every month until harvest, covering all areas under Isomate-M. Fruit was inspected while still on the tree. All consignments of fruit sent to the canning factory at harvest were subjected to normal quality control during which fruit damaged by insects was set aside and inspected to determine the cause.

Another peach production area some 15 km away, the Breeriver valley, was used as a comparison. All orchards in this area were conventionally treated with insecticides, starting on 15 August 1991. Typically, two applications of acephate 750 g/kg

SP (1 kg/ha) were followed by five to 10 applications of azinphos-methyl 350 g/kg WP (1.75 kg/ha), depending on the harvest date of the cultivar. All sprays were applied 14 days apart and at high volume (8X). Nine Pherocon 1C 1-mg pheromone traps were deployed in different orchards in the valley on 20 August, and were accurately monitored every 4 to 8 days until early November. Accurate trap counts ceased on this date due to excessive *G. molesta* catches.

All statistics given for *G. molesta* were taken from an unpublished, informal report by W. Boonzaaier (SAPCO).

### Codling moth

Mating disruption of codling moth was started in 1993 on the farm Oak Valley Estates in the Elgin area.

*First season (1993/94)*. Three adjoining orchards forming a block of 11.7 ha, and another nearby isolated orchard of 3 ha, were selected. The cultivars comprised Granny Smith, Golden Delicious and Starking. They were mature trees of 3 to 5 m in height. The mating disruption product used was Isomate-C (Shin-Etsu Chemical Co., Tokyo), containing 51.8% (*E,E*)-8,10-dodecadien-1-ol (*E8,E10*-12OH); 29.1% dodecanol (12OH); 6.0% tetradecanol (14OH) at 165 mg/dispenser. The dispensers were applied according to recommendations at 1 000 dispensers/ha, the first batch before the emergence of the first spring moths and the second batch after 85 days. Pherocon 1C wing traps baited with Trécé 10-mg lures (10 mg *E8,E10*-12OH on rubber septum) were deployed in the top metre of the trees at a density of one trap/ha. They were inspected weekly and the septa changed monthly.

The full compliment of 4 sprays of azinphos-methyl 350 g/kg WP (1.25 kg/ha) was applied against the first generation of codling moth. Thereafter, azinphos-methyl was only applied to those orchards where trap catches exceeded 1 moth/trap/week for more than three consecutive weeks. All sprays were applied at low volume (4X).

At the end of the first two generations, and again a week before harvest (early March), fruit were visually inspected for codling moth damage on 100 trees per each of five 3- to 5-ha sample blocks, 64 trees in the border area and 36 trees in the inner area. Twenty fruit were randomly inspected from the top and 20 from the bottom of each tree without removing them from the tree, giving 4 000 fruit/sample block.

*Second season (1994/95)*. The same four orchards used during the previous season were used again. However, 10.5 ha was added to the 11.7 ha orchard, making a total of 25.2 ha, of which 22.2 ha were contiguous. The mating disruption product used was Isomate-C Plus (52.9% *E8,E10*-12OH, 29.7% 12OH, 6.0% 14OH). Dispensers were applied as described for the first season, and a second batch was applied after 121 days. Monitoring traps were deployed and inspected as in the first year, and the septa replaced fortnightly instead of monthly. Insecticide application and the method of fruit sampling was as during the first season, except that seven 3- to 5-ha sample blocks were used.

*Third season (1995/96).* The orchards used were the same as those in the second season, with the exception that the 3-ha orchard was excluded from the trial. Isomate-C Plus was again used, the second batch being applied after 100 days. Monitoring was carried out as during the second season. Sprays were applied only to those orchards where traps recorded more than 2 moths/trap/week, or 1 or more moths/trap/week for two consecutive weeks, or where fruit damage remained above 0.1% at the end of any generation. Flufenoxuron 100 g/L DC (0.75 L/ha) was applied against the first generation, chlorpyrifos 250 g/kg WP (3.75 kg/ha) and fenoxycarb 250 g/kg WP (1.4 kg/ha) against the second generation and azinphos-methyl (as in the first season) against the third generation. Fifty trees/block in eight 3- to 5-ha blocks were sampled, 32 trees from the border area and 16 from the inner area, inspecting at random 20 fruit from the top and 20 from the bottom of each tree (2 000 fruit/sample block). The sampling times were as during the first and second seasons.

In all three seasons, data from a conventionally treated orchard in the vicinity, was used for comparison. This orchard was sprayed only with azinphos-methyl during the first and second seasons, and with the four insecticides mentioned above during the third season. Monitoring was with Pherocon 1C wing traps baited with Trécé 1-mg lures (1 mg *E8,E10-12OH*) which were replaced every 4 to 6 weeks.

## Results

### Oriental fruit moth

*Pheromone traps.* Pheromone trap catches in the Isomate-M treated area and the conventionally treated Breeriver valley are given in Table 1. Between 23 August and 30 October 1991, 49 *G. molesta* adults were trapped in the 120 traps covering the 1 200 ha of orchards treated with Isomate-M. After this date, no further moths were trapped anywhere in the treated area for the rest of the season. The three traps in the bulk bin stack caught 20 *G. molesta* adults up to 7 November 1991, and no further moths were trapped after that date.

In the Breeriver valley, where insecticide sprays were applied every 14 days until harvest, very high catches of *G. molesta* were recorded; *e.g.* 952 were recorded from nine traps on 7 November at which point accurate records ceased.

*Bait traps.* By 30 October, only five *G. molesta* adults had been recorded from the 120 bait traps in the treated area. The use of these traps was therefore stopped.

*Shoot damage.* The percentage shoot damage at the end of the season preceding Isomate-M treatment (March 1991), and at the end of the Isomate-M season (March 1992), is shown in Table 2. In March 1991 shoot damage ranged from 30% to 80%. At the end of the 1992 season, shoot damage was virtually non-existent. In one orchard where Isomate-M was applied late, 6% shoot damage was recorded. The only other reported shoot damage was three shoots from a 4-ha orchard of Neethling peaches and "a few shoots" (W. Boonzaaier *unpubl. report*) from an orchard of Black

peaches. In contrast, between 15% and 40% shoot damage was reported from the Breeriver valley at the end of the 1991/92 season.

**Table 1** Number of *G. molesta* males trapped with 1-mg pheromone lures in the pheromone-treated area (Tulbagh valley) and insecticide-treated area (Breeriver valley), during the 1991/92 season

Date	Number of <i>G. molesta</i> males trapped	
	Tulbagh valley <sup>a</sup>	Breeriver valley <sup>b</sup>
23/08	0.04	1
27/08	0.24	22
04/09	0.08	91
11/09	0	39
19/09	0	90
25/09	0.01	99
02/10	0	59
10/10	0	2
17/10	0.02	3
24/10	0.01	4
30/10	0.02	31
07/11	0	106
14/11	0 <sup>c</sup>	- <sup>d</sup>

<sup>a</sup> Average from 120 traps; <sup>b</sup> average from nine traps; <sup>c</sup> no further moths trapped during the season; <sup>d</sup> no further records kept

**Table 2** Percentage shoot and fruit damage to nectarines and canning peaches by *G. molesta* in the Tulbagh and Breeriver valleys at the end of the 1990/91 and 1991/92 seasons (Tulbagh valley: mating disruption treatment in 1991/92; no additional insecticide sprays applied)

Cultivar	% shoot damage		Breeriver 1991/92	% fruit damage	
	Tulbagh 1990/91	1991/92		Tulbagh <sup>a</sup> 1991/92	Breeriver 1991/92
Nectarines	40	6 <sup>b</sup>	- <sup>c</sup>	0	-
Kakamas	50	0	40	0	12
Woltemade	30	0	-	0	-
Oom Sarel	30	0	-	0	-
Oom Sarel	50	0	-	0	-
Neethling	60	<0.001	-	0	-
Black	80	<0.001	-	0	-
Malherbe	-	-	>20	-	15

<sup>a</sup> Orchard and canning factory inspections; <sup>b</sup> Isomate-M applied later than recommended date; <sup>c</sup> not recorded

*Fruit damage* at the end of the Isomate-M season (March 1992) is shown in Table 2. Not a single fruit infested by *G. molesta* was recorded from the treated area, either during in-season orchard inspections or during canning factory inspections of each load of fruit as it entered the factory. This is especially significant considering that no supplementary insecticide sprays were applied.

In the Breeriver valley, fruit damage of between 12% and 15% was recorded at the end of the 1991/92 season, despite rigorous spray programmes.

### Codling moth

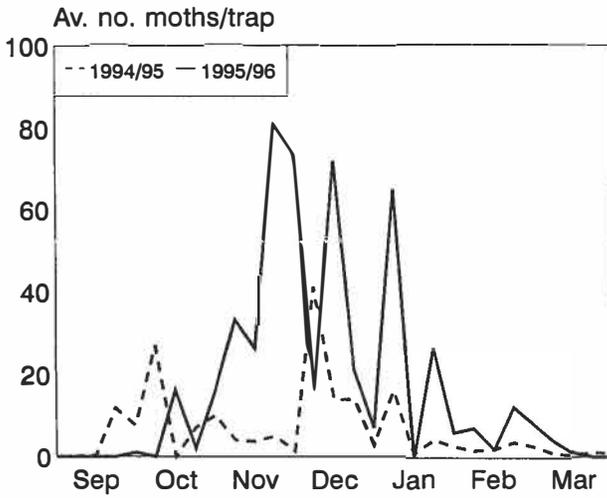
Pheromone trap catches from traps in the conventionally treated orchard and in the mating disruption block during the second (1994/95) and third (1995/96) seasons are given in Figures 1 and 2. Details of the extent of codling moth damage and the number of sprays in the mating disruption and conventionally treated blocks, are given in Table 3.

*Pheromone traps.* Trap catches in the conventional orchard indicated high codling moth populations outside the mating disruption block (Figure 1). The 10-mg traps inside the mating disruption block showed a pattern typical in these circumstances: a short period of low-level attraction immediately after lure replacement, followed by a sharp drop in catches as the pheromone concentration of the lure dropped below the high background concentration of mating disruption pheromone (Figure 2).

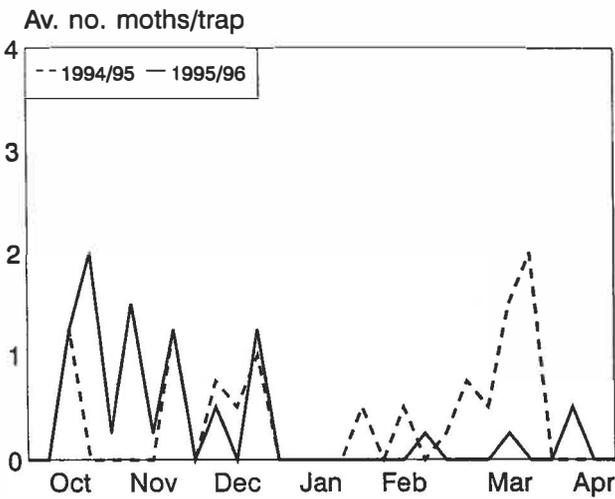
During the second season, trap catches indicated a build-up in codling moth populations in the mating disruption block towards the end of the season, suggesting that chemical intervention may have been inadequate. In contrast, at the end of the third season trap catches suggested that populations were substantially lower, perhaps pointing to better overall control.

**Table 3** Average percentage codling moth damage and number of insecticide sprays at the end of three successive seasons of mating disruption in apples.

Treatment	% damage (range)	Number of sprays (range)
	First season (1993/94)	
Conventional	2.7	11
Mating disruption	1.1 (0.2 - 3.6)	7.8 (6 - 11)
Second season (1994/95)		
Conventional	34.0	11
Mating disruption	0.3 (0.05 - 0.5)	7.8 (4 - 11)
Third season (1995/96)		
Conventional	6.3	11
Mating disruption	0.3 (0 - 1.5)	7.5 (6 - 11)



**Figure 1** Number of codling moth males caught over two seasons in traps baited with 1 mg *E8,E10-12OH* in an insecticide treated apple orchard close to the mating disruption block.



**Figure 2** Number of codling moth caught in traps baited with 10 mg *E8,E10-12OH* during the second and third season of mating disruption in apple orchards.

*Fruit damage.* The percentage codling moth damage in the conventionally treated orchard varied from 2.7% to 34% over the three seasons (Table 3). In the mating disruption block codling moth damage decreased over the three seasons from an average of 1.1% in the first season to 0.3% at the end of the third season. The range of damage in different mating disruption orchards also showed a downward trend in successive seasons, to the point where no codling moth damage was recorded from some orchards at the end of the third season.

*Number of sprays.* In all three seasons, 11 sprays were applied against codling moth in the conventionally treated orchard (Table 3). The average number of codling moth sprays applied in the mating disruption block remained relatively constant during the three seasons, between 7.5 and 7.8. This represents an average reduction of up to 32% in the number of sprays. The range in number of sprays hardly varied between seasons, with a lower limit of four (second season) and an upper limit of 11 (all three seasons).

## Discussion

### Oriental fruit moth

That very large populations of *G. molesta* existed in the Tulbagh valley at the end of the season preceding the application of mating disruption, cannot be doubted. Despite this, the area-wide use of Isomate-M to control *G. molesta* in the Tulbagh valley was a great success, as testified by the total absence of *G. molesta* fruit damage from 1 200 ha of fruit. As a result of this, and the extremely low incidence of shoot damage at the end of the 1991/92 season, it was suggested that the use of Isomate-M dispensers should be reduced to only one application at the beginning of the following season (P. Kirsch *pers. comm.*).

Unfortunately, mainly due to cost considerations, few growers took advantage of the huge benefit afforded by the mating disruption project and most of them returned to chemical control. Nevertheless, four years after the Tulbagh valley project, *G. molesta* is better under control than it was immediately before the project. About 30% of growers in the valley still use mating disruption, although compliance with recommendations is poor (F. Theron *pers. comm.*). This is largely due to very poor technical support on mating disruption from the stonefruit industry and from distributors of mating disruption products.

We consider the following factors to have been crucial to the success of the Tulbagh valley project:

*Fruit industry involvement.* Four organizations worked closely together before and during the project. SAPCO and the Canning Fruit Board among them organized grower meetings in Tulbagh at which the area-wide mating disruption project was proposed and planned; financed a fact-finding mission to California by their representatives and an entomologist to gather information on *G. molesta* and mating disruption.

tion; identified key growers who would support and promote the project amongst some reluctant growers; provided bridging finance to Tulbagh valley growers to assist them in purchasing mating disruption dispensers; and supervised the application of dispensers and subsequent monitoring actions.

*Research and extension backup.* The role played by the Stellenbosch Institute for Fruit Technology, in the persons of the two authors, was in basic planning and coordination of the project throughout its duration; providing information at grower meetings on the pros and cons of mating disruption and on application recommendations; promoting the project in the media; acting in an advisory capacity; and conducting separate 10-ha trials with Isomate-M dispensers to register the product for commercial use against *G. molesta*.

*Support by product distributor.* Pacific Biocontrol, in the person of Philipp Kirsch, made a number of visits to the area to provide information at grower meetings on mating disruption, Isomate-M dispensers and their application, and to refine aspects of the project methodology. He also provided real-time back-up on *G. molesta* behaviour and the use of Isomate-M during the course of the project.

*Participation by all growers.* It was considered imperative to avoid pockets of stonefruit orchards within the Tulbagh valley that were not treated with pheromone, as the large populations emanating from these orchards would have placed pressure on the pheromone-treated area. The eventual participation of every one of the 60 growers was cardinal to the success of the project.

*In situ supervision by SAPCO.* The vested interest by SAPCO in the success of the project guaranteed their commitment. They ensured strict compliance with application recommendations, and facilitated regular monitoring over the whole area.

### Codling moth

The results of the codling moth mating disruption project should be seen in the context of the high reproductive potential of codling moth in South Africa, and its resistance to insecticides. Furthermore, spray management on the farm on which the trial took place was sub-optimal. Trees on this farm were generally large and dense, and the farm had too few spray machines for the area under fruit. The result was inadequate spray coverage.

The level of codling moth damage in the mating disruption block compared very favourably with the conventionally treated orchard, especially during the second and third seasons when poor control was achieved with a straight insecticide programme.

The average percentage codling moth damage in the mating disruption block remained at 0.3% for both the 1994/95 and 1995/96 seasons. Nevertheless, the reduced second and third generation trap catches during 1995/96 (Figure 2) suggests that populations were much lower during this third season. The total absence of codling moth damage in some orchards at the end of the third season, and the fact that it was possible in some of the orchards to omit sprays against the second and third genera-

tions, supports this contention.

The high codling moth populations in the conventional orchards surrounding the mating disruption block placed pressure on the mating disruption treatment, which partly accounted for the fact that the average number of sprays in the mating disruption orchards could not be further reduced during 1995/96. Although there were some orchards in which only four sprays were needed during the season, some orchards required the full compliment of eleven sprays. This is ascribed partly to the poor spray management on the farm — large, dense trees and too few spray machines — and partly to the high codling moth populations outside the mating disruption block.

Despite this, the project is considered a success by virtue of the progressive improvement in codling moth control by mating disruption in successive seasons, under a reduced insecticide programme. Greater success can be expected in orchards with less dense trees and which are under better spray management. The project has now entered its fourth successive season, in which it is hoped to reduce the number of sprays even further.

#### Factors limiting the increased use of mating disruption

In our opinion the use of mating disruption in South African stone and pome fruit orchards is limited by the following factors:

*Biotic factors.* A hot climate resulting in more-than-usual Oriental fruit moth and codling moth generations per year, and in accelerated dispenser depletion; high codling moth reproductive potential; many orchards with large, dense trees; the probable necessity for simultaneous control of secondary pests.

*Management factors.* Large farms (on the average 50 ha); clashes in other orchard activities when mating disruption needs attention; increased levels of monitoring and sampling; the necessity for post-harvest control of Oriental fruit moth and codling moth (sprays for Oriental fruit moth, orchard sanitation for codling moth).

*Human factors.* Inadequate knowledge of mating disruption by growers and technical representatives; inadequate technical support by distributors of mating disruption products; perceived unrealistic profit-taking by distributors; lack of sophistication of some growers; resistance by growers to change and increased effort.

*Cost factors.* This is probably the greatest limitation in mating disruption usage. Mating disruption of codling moth currently costs between 590 and 780 US\$/ha/season, depending on the extent of chemical intervention. Mating disruption of Oriental fruit moth costs slightly less. These high costs are due to all mating disruption products having to be imported into the country. By contrast, a full spray programme costs between 190 and 275 US\$/ha/season, depending on the insecticides used. However, the latter costs do not take into account the cost of the development of resistance and associated crop losses.

Many of the management and human factors can and must be addressed and improvements made where possible. However, most of the biotic factors cannot be

changed. It is unlikely that the cost of mating disruption will become more affordable in South Africa, unless the products are manufactured in the country. The likelihood of this is uncertain.

### **Acknowledgements**

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## **Confusion amongst codling moth fellows continues: a commercial perspective on the implementation of codling moth mating disruption in North America**

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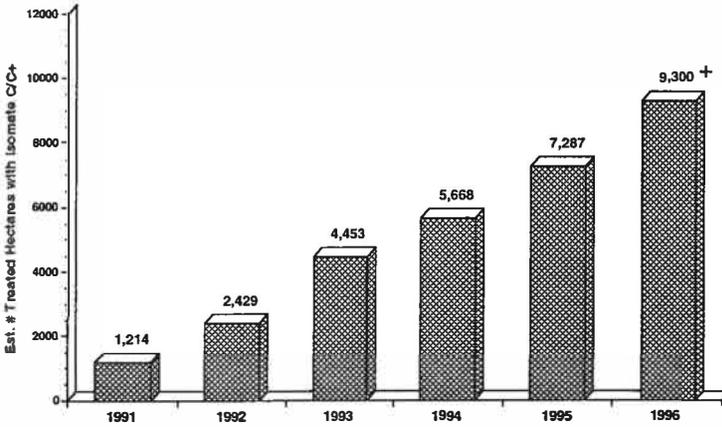
**Abstract** - Mating disruption technology is increasingly being used for the control of codling moth, *Cydia pomonella*, in pome fruit production areas around the world. Some of the countries where codling moth mating disruption is used commercially include Argentina, Australia, Canada, Italy, South Africa and the United States. The successful commercialization of mating disruption technology will depend in large part on the development and implementation of a pheromone-based integrated pest management (IPM) systems approach. In a pheromone-based IPM system, mating disruption is the major tactic used to control the key pest. The subsequent reduction or elimination of insecticides for control of the key pest will result in orchard environments that are able to sustain higher populations of natural enemies and thus enhance the biological control of both key and secondary pests. The major impediments to the implementation of a pheromone-based IPM systems approach include grower concerns over costs, efficacy and outbreaks of secondary pests.

**Key words** - sex pheromone, mating disruption, integrated pest management, implementation, *Cydia pomonella*, Tortricidae, Lepidoptera

### **Introduction**

There is general agreement within and among government agencies, research institutions, industry and grower organizations that there is a need to incorporate effective and more ecologically and environmentally acceptable technologies and strategies into agricultural pest management systems and thereby reduce or eliminate the input of broad spectrum highly toxic insecticides. Pheromone-mediated mating disruption is a viable technology that is central to the development of pest management strategies that meet these criteria for several important agricultural insect pests including Oriental fruit moth, *Grapholitha molesta* (Rice & Kirsch 1990), pink bollworm, *Pectino-*

*phora gossypiella* (Carde & Minks 1995) and codling moth, *Cydia pomonella* (Judd & Gardiner 1991; Gut & Brunner 1994; Knight 1995). In 1991, Isomate C (Pacific Bio-control, Vancouver, WA, USA.) was the first commercial formulation of codling moth pheromone to be registered in the United States. Total pome fruit acreage in the United States treated with Isomate C/C<sup>+</sup> dispensers has increased from approximately 1200 ha in 1991 to over 9 000 ha in 1996 (Figure).



**Figure** Pome fruit acreage treated with Isomate dispensers for mating disruption of codling moth, *C. pomonella*, in the USA from 1991 to 1996

In 1996, Washington State with plantings of 80 000 ha of pome fruit, had approx. 5 500 ha of pome fruit treated with Isomate C<sup>+</sup> (108 mg of *E*8,*E*10-12:OH, 60 mg of 12:OH, 12.3 mg of 14:OH) for the control of codling moth. Codling moth is the key pest of pome fruits and is widely distributed throughout Washington State. There are two complete and sometimes a partial third generation per season. Growers apply on average more than three applications of insecticide per season to control codling moth (Brunner 1989). The success of codling moth mating disruption applied to thousands of ha of pome fruit has helped create orchard environments that are able to sustain higher populations of natural enemies.

Improved biological control in codling moth mating disruption treated orchards often but not always correspond to a reduction in the number of insecticide applications needed for the control of secondary insect pests such as aphids (*Aphis pomi*, *Dysaphis plantaginea*) and leafminers (*Phyllonorycter elmaella*) (Gut & Brunner 1994). However, there is a strong potential for populations of other secondary pests such as pandemis leafroller, *Pandemis pyrusana*, and obliquebanded leafroller, *Choristoneura*

*rosaceana*, to increase to economically damaging levels in the absence of codling moth cover sprays (Judd & Gardiner 1991; Gut & Brunner 1994; Knight 1995).

The ongoing biological success of codling moth mating disruption will depend in large part on the continued development of monitoring and sampling techniques in conjunction with economic thresholds to accurately assess the biological relationships between key and secondary pests and their natural enemies. The information derived will help determine the need for supplementary controls to ensure the effective management of coding moth and the various secondary pests. The approach of using mating disruption for control of codling moth in conjunction with the enhanced biological control of secondary pests is referred to as a pheromone-based IPM systems approach.

### **Impediments to the adoption of pheromone-based IPM**

The most important impediments to the commercial acceptance of pheromone-based IPM include the cost of the technology and its implementation, concerns over efficacy and outbreaks of secondary pests such as leafrollers.

Thirty nine people employed in the discipline of insect pest management in Washington and California were surveyed for their opinions on the cost of codling moth mating disruption technology relative to the cost of conventional insecticides (Weddle, Hansen & Associates 1993). Fifty one per cent stated that they found the cost of mating disruption technology to be high compared to conventional insecticides; 31% and 8% indicated that they found the cost of codling moth mating disruption to be very high and extremely high, respectively. Only 8% of the respondents indicated that the cost of codling moth mating disruption was reasonable.

In small plot trials conducted in pear orchards over a six-year period in California, the average cost of a pheromone-based IPM program was 40% higher than a standard insecticide program (Weddle, Hansen & Associates 1994). Two applications of mating disruption dispensers are required to control codling moth in California, thereby substantially increasing costs. A study conducted in Washington State found that the cost of a pheromone-based IPM program when adjusted for material, labour and machinery costs was \$133/ha higher than a conventional insecticide program (Williamson *et al.* 1996). The disparity in costs provides a strong disincentive to adopt a pheromone-based IPM system approach.

Although mating disruption has been used successfully to control codling moth in thousands of ha of pome fruits, there are still concerns about efficacy. Mating disruption will not provide commercially acceptable levels of control in orchards with high resident populations of codling moth without the application of supplemental insecticides. Other factors known to limit the efficacy of mating disruption for control of codling moth include the size of the treated area, the degree of slope, the size and unevenness of the canopy and the isolation of the orchard from external sources of codling moth. Given the above, the selection of suitable orchards is difficult and in-

variably every year efficacy problems occur in some ill-suited orchards. The problems are compounded by the lack of reliable monitoring techniques to monitor the level of codling moth control within season. Therefore it is essential that candidate orchards are selected carefully.

The successful deployment of codling moth mating disruption and the subsequent reduction or elimination of insecticides to control codling moth often creates orchard environments where leafroller populations can rapidly increase to economically damaging levels. In 1993, a 485-ha apple orchard in central Washington State was successfully treated in its entirety with Isomate C dispensers. Supplemental insecticides were only applied to border areas. Forty eight sex pheromone traps captured 6 748 obliquebanded leafroller males. In 1994, the orchard was again treated in its entirety with Isomate C<sup>+</sup> and again supplemental insecticides were only applied to local border areas. Trap captures of obliquebanded leafroller increased four fold to 24 124. Increased applications of insecticides were required to prevent serious economic injury to the fruit. This example clearly illustrates how the successful use of codling moth mating disruption can increase the control problems of certain secondary pests and thus provide a disincentive to the adoption of the technology. The costs associated with the increased use of insecticides for leafroller control or the economic losses due to fruit injury can outweigh the benefits and savings derived from the biological control of other secondary pests.

The above mentioned impediments to the adoption of a pheromone-based IPM systems approach present serious obstacles to the commercial success of this technology. Yet as illustrated in Figure, codling moth mating disruption is increasingly being adopted by apple and pear growers in the western United States. There are many reasons why this is occurring but they cannot all be discussed in this paper. However, the following case study documents how one grower was able to successfully implement a pheromone-based IPM systems approach over a three-year period in a way that not only dealt with his concerns about efficacy but ultimately met his economic concerns.

### **Case study: economics and efficacy - the implementation of pheromone-based IPM**

In 1994, 60 ha of a 240-ha apple orchard in central Washington State were treated for the first time with Isomate C<sup>+</sup>. The table provides an analysis of some of the costs associated with the implementation of a pheromone-based IPM systems approach over a three-year period. The strategy employed by the grower was to minimize the risk from codling moth during the transition by integrating pheromones and insecticides in the most cost effective manner. The total costs per hectare were based on the number of applications times the approximate cost of materials and their application. A local distributor of agricultural chemicals and the grower provided the information on material and application costs.

**Table** Material and application costs associated with the implementation of a pheromone-based IPM systems approach in a 240-ha apple orchard in Washington State over three years

	1994			1995			1996		
	25 ha <sup>a</sup>	35 ha	180 ha	25 ha	115 ha	100 ha	25 ha	140 ha <sup>b</sup>	75 ha <sup>c</sup>
<i>Number of Applications</i>									
Isomate C <sup>+</sup> (1 000/ha)	1	1	-	2	1	-	1.5	0.5	1
Guthion (2.2 kg/ha)	-	1	3	-	1	3	-	1	1
Ryania (16.8 kg/ha)	8	-	-	2	-	-	-	-	-
Orchex Oil (1%)	-	-	-	6	-	-	6	-	-
Cost (\$/ha)	1 197	373	192	1 136	373	192	760	218	373
Fruit attack (%)	20	0	0	0.5	0	0	0.25	0	0

<sup>a</sup> Organic block; <sup>b</sup> Central area of conventional orchard; <sup>c</sup> Border area of conventional orchard;

In 1994, a 25-ha section (under organic production for three years) of the conventional orchard was treated with 1 000 dispensers/ha of Isomate C<sup>+</sup> prior to the first flight of codling moth (biofix) and then supplemented with multiple applications of the organic insecticide, ryania. The other 35-ha section was treated with 1 000 dispensers/ha prior to biofix and then supplemented with one application of the organophosphate insecticide azinphos-methyl (Guthion®) at first cover timing. The remaining 180 ha were treated with three applications of azinphos-methyl. The control of codling moth in the azinphos-methyl treated blocks and the areas treated with the mating disruption/azinphos-methyl program was excellent. The grower was willing to spend the extra money on Isomate C<sup>+</sup> to treat the 35-ha area because continued codling moth control problems in this area (three applications of azinphos-methyl and 3 to 5% damage in 1993) would have prevented shipment of the 1994 harvested crop to Japan where strict quarantine regulations were in place. In the organic block, codling moth fruit injury ranged from 15 to 25%. The grower estimated that approximately \$300 000 in harvestable fruit was lost due to codling moth injury. The results clearly showed the limitation of a mating disruption/ryania program to control high initial populations of codling moth.

In 1995, the grower expanded the mating disruption/azinphos-methyl program from 35 to 115 ha. The organic block was treated with two applications of Isomate C<sup>+</sup> at 1 000 dispensers/ha and supplemented with 2 and 6 applications of ryania and Orchex® horticultural oil, respectively. The remaining area was treated with a standard convention program. At harvest, the level of codling moth injury was non-detectable in the azinphos-methyl and mating disruption/azinphos-methyl treated blocks, and below 0.5% in the organic block. As a result the overwintering populations of codling moth in the orchard were substantially reduced.

In 1996, almost the entire orchard was treated with Isomate C<sup>+</sup>. However, as codling moth populations had been substantially reduced, Isomate C<sup>+</sup> was applied to

the middle 140 ha at 500 dispensers/ha and supplemented with one application of azinphos-methyl. The border areas (75 ha) were treated with one application of Isomate C<sup>+</sup> at 1 000 dispensers/ha and supplemented with one application of azinphos-methyl. The organic block was treated with 1 applications of Isomate C<sup>+</sup> at 1 000 dispensers/ha prior to biofix and a half rate of Isomate C<sup>+</sup> prior to the start of the second flight and supplemented with six applications of horticultural oil. At harvest, codling moth damage was almost non-detectable in all areas.

This case study illustrates how a grower implemented a pheromone-based IPM program over the course of a three-year period. By the third year, the growers was able to substantially reduce costs by reducing the application rate of Isomate C<sup>+</sup>. The supplemental application of one azinphos-methyl spray at first cover timing ensured a high level of codling moth control and additionally helped to keep leafroller populations under control. The overall reduction in the input of azinphos-methyl over the three years helped to enhance biological control of secondary pests although no attempt to quantify the cost savings is presented in this analysis. In addition, by adopting pheromone-based IPM other benefits often difficult to quantify were derived such as decrease potential for insecticide resistance, less expense associated with the implementation and administration of worker protection regulations and reduced costs associated with the management of the daily activities of orchard employees. At the end of the third year the grower felt that the cost and performance of a pheromone-based IPM systems approach had considerable advantages over the standard program. In 1997, the grower intends to further reduce rates of Isomate C<sup>+</sup> in the middle of the orchard and to eliminate the supplemental use of azinphos-methyl wherever possible so as to enhance biological control of secondary pests while further reducing costs.

## Conclusion

The adoption by growers of a pheromone-based IPM systems approach in pome fruit will depend upon how well the system can meet grower concerns about efficacy and cost. More research is needed into the mechanisms of mating disruption to make codling moth control more robust and reliable. The development of monitoring and sampling techniques in conjunction with economic thresholds are essential in order to accurately assess the biological relationships between key and secondary insects and their natural enemies and to apply supplementary controls if required. Pheromone-based IPM should be presented to growers as a long-term approach and commitment to pest management. Growers should be encouraged to define yearly objectives and then identify the strategies and tactics needed to achieve those objectives with a focus on optimizing efficacy and keeping costs reasonable.

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## **Ecopom dispensers for mating disruption in apple orchards**

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**Abstract** - Mating disruption has become a valuable alternative to insecticide treatments, and growers and agricultural organisations increasingly demand reliable products in order to implement the method. Industries were hence prompted to supply enough material both to investigate the agronomic aspects of mating disruption and to enable large-scale applications. The work done in the past years made it possible to identify the limiting factors of the mating disruption technique, mainly with respect to orchard organisation and the size of insect populations. Since 1987 trials have been carried out to evaluate mating disruption as a control method against codling moth, *Cydia pomonella* L., and leafroller species in apple orchards, using Isagro's Ecopom and Ecopom Combi dispensers. On the basis of these trials we were able to modify and optimise several factors, such as the amount of active ingredient/ha, the number of dispensers/ha, the amount of active ingredient/dispenser and the number of applications per season. The persistence of pheromone release in the field and the reproducibility of the manufacturing process of the dispensers became the most important elements to be defined. I here report the results of Isagro's efforts towards the development of an effective dispenser for mating disruption in apple orchards.

**Key words** - sex pheromone, mating disruption, dispensers, release rates, *Cydia pomonella*, leafrollers, Tortricidae

### **Introduction**

Our research aims at the development of mating disruption methods for the control of insect pests. Today, mating disruption is used, in combination with or instead of chemical treatments, to control insecticide-resistant populations of codling moth, *Cydia pomonella*, and to obtain fruit with no detectable pesticide residues at harvest, which will be processed into baby food. The objective of our studies is to provide a reliable mating disruption dispenser which can be utilised directly by the growers under various agronomic conditions.

## Dispenser design

We conceived a simple dispenser that can contain large amounts of active ingredient and is especially suitable for the release of codling moth sex pheromone (Rama *et al.* 1990; Arsurra *et al.* 1992; Charmillot *et al.* 1993). The dispensers are made of biodegradable, resin-treated filter paper and their shape makes it possible to easily hang them to the branches, thus avoiding the use of plastic or metal wires. Dispensers distributed by Isagro under the trade name Ecopom®, for the mating disruption against *C. pomonella*, contain codlemone, (*E,E*)-8,10-dodecadien-1-ol (*E8,E10*-12OH). Ecopom® Combi dispensers contain in addition (*Z*)-11-tetradecenyl acetate (*Z11*-14Ac), a pheromone component of a number of leafroller moths.

**Table 1** Mating disruption of codling moth and leafrollers with different dispenser formulations and application rates (Trentino, Italy)

Year	Dispenser	Active ingredient			Dispenser/ ha	Half-life (d)	Damage (%)
		Application (g/ha)		mg/ dispenser			
		1st	2nd				
Codling moth							
1987	Ecopom <sup>a</sup>	21	26	175	120 + 150	50	2.1
1988	Ecopom	50	30	250	200 + 120	39	1.5
1989	Ecopom	80	80	400	200 + 200	56	0.8
1990	Ecopom Combi <sup>b</sup>	90	94	300	300 + 300	34	0.4
1991	Ecopom Combi	97	97	350	280 + 280	81 <sup>c</sup>	0.1
1992	Ecopom	88	-	320	275	34	1.1
1993	Ecopom	100	100	320	312 + 312	56	0.7
1994	Ecopom	75	75	250	300 + 300	20	0.4
1995	Ecopom	75	-	250	300	52	0.2
1995	Ecopom Combi	75	-	250	300	46	0.2
Leafrollers							
1990	Ecopom Combi	116	-	300	400	-	3.1
1991	Ecopom Combi	83	83	300	275 + 275	-	1.4
1994	Ecopom Combi	75	-	250	300	110	1.0
1995	Ecopom Combi	60	-	200	300	72	0.9

<sup>a</sup> Codlemone; <sup>b</sup> Codlemone + Z11-14Ac; <sup>c</sup> Applied April 10, for control of *Pammene rhediella*

Trials have been carried out since 1987 (Rama *et al.* 1996) to optimise several factors, such as the quantity of attractant/ha, the number of dispensers/ha, the amount of active ingredient/dispenser, and the number of applications per season (Table 1).

The persistence of dispensers in the field determines not only the success of the method, but also the cost of control by mating disruption. The half-life of the dispenser is affected by several variables such as size, nature of the support, pheromone load, inert adjuvants and, of course, by the agroclimatic conditions. In order to minimise these last effects, most studies have been carried out also in greenhouses at more or less constant temperature and wind speed.

During the experiments in 1994, a rapid emission of codlemone made it necessary to increase the life of the Ecopom dispenser (Table 1). In 1995, the formulation was modified by addition of a new release modulator which brought about a persistence of about 90 days (Figure), compared to only 30-40 days in 1994.

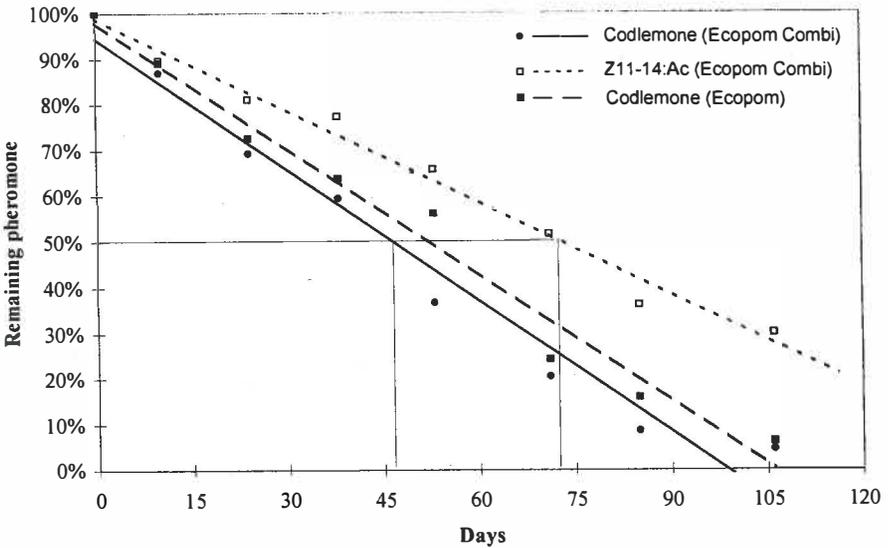


Figure Release rates from Ecopom and Ecopom Combi dispensers at Trentino 1995

Other important parameters are the linearity of pheromone release and the amount released per ha. Some authors claim a threshold of 10 mg/ha/hour as the lower limit to achieve good disruption against codling moth. Both Ecopom and Ecopom Combi dispensers show a rather constant release of 0.07 to 0.1 mg codlemone/hr during ca. 100 days, which would result in 20 to 30 mg codlemone/ha/hr at the recommended dispenser density of 300/ha. The average release rate for Z11-14Ac is slightly lower at 0.05 to 0.07 mg/hr.

Each dispenser was loaded with 250 mg of codlemone and, in the case of the

Combi version, with 200 mg of Z11-14Ac; the dispensers contained also an antioxidant, a UV-screener and a release modulator.

In order to ensure a constant quality of the dispenser material, it was important to improve the reproducibility of the manufacturing process. The variability in pheromone charge among the different batches of dispensers was greatly improved by replacing a manual impregnation procedure ( $261 \pm 34$  mg codlemone/dispenser) with a mechanical semi-automatic procedure ( $253 \pm 18$  mg). In addition, scaling up the volume of production resulted in a better standardisation of the single dispensers.

### Field trials

During the last years, experimental field trials on mating disruption against *Cydia pomonella* and apple leafrollers were carried out directly by extension organisations and farmers' associations, in order to test the potential and flexibility of the method. In 1995, more than 600 ha throughout Europe were protected by Ecopom or Ecopom Combi mating disruption dispensers.

The dispensers were applied twice in most orchards, in April/May and in June/July, at an average density of 300 dispensers/ha, external protection borders not included. The results of the more important applications are discussed below.

#### Trentino (Italy)

About 200 ha of apple orchards, over ten different sites, were treated with a single application of Isagro's dispensers Ecopom Combi. The protection against codling moth was almost complete, with damages below the economic threshold (Table 2).

**Table 2** Mating disruption of codling moth and leafrollers with with Ecopom Combi dispensers (Trentino, Italy, 1995)

Site	Area (ha)	Damage (%)		Insecticide treatments
		Codling moth	Leafrollers	
Aldeno	67	0.03	0.02	2 (2 ha)
Mattarello	47	0.22	0.33	2 ( <i>G. molesta</i> )
Pietramurata	8	0.20	0.12	1 (0.4 ha; border)
Fasse di Bono	4	0.32	0.26	1 (0.1 ha)
Toss	13	0.00	0.10	-
Segno	15	0.00	0.10	-
Livo	13	0.20	0.46	-
Cembra	6	0.97	0.35	1 ( <i>C. pomonella</i> )
Caldonazzo	6	0.53	2.20	-
Mezzocorona	8	0.00	5.00	1 (leafrollers)

The application of mating disruption reduced the number of insecticide treatments, from an average of three in 1994, to occasional curative treatments in 1995. The control by mating disruption was comparable with that obtained by insecticide sprays in the control plots.

Even the attack of apple leafrollers *Adoxophyes orana* and *Pandemis heparana* was satisfactorily contained below 1%; only at two sites it was necessary to spray insecticides due to a high pest population: at Caldonazzo and Mezzocorona, 7% and 15% of shoots infested after blooming led to a damage at harvest of about 2.2% and 5%, respectively.

In Alto Adige, the area treated with a single distribution of Ecopom Combi was limited to 11 ha, divided into 9 farms. No chemical were sprayed either before the application of mating disruption or during the season. The results indicate an excellent protection against codling moth, with damage levels below 0.3%.

## France

The growing resistance of codling moth in Southern France to the most commonly used insecticides has been of great concern over the last few years. Our trials started in 1993 in a few selected areas, where the population of codling moth was known to be substantial and required frequent insecticide sprays to be controlled.

After the positive results obtained in the first year, the treated area increased in 1994 and 1995, expanding also to regions where the pressure of codling moth was not so critical. The results obtained from trials done in cooperation with G.R.C.E.T.A. (Basse Durance) in the last three years are shown in Table 3 (Navarro *et al.* 1995). It is noteworthy that satisfactory control of codling moth was achieved even in spite of the drastic reduction of insecticide sprays.

In the same region, further trials were carried out in collaboration with the Cooperative Sicoly (Table 3). Two applications of 300 dispensers/ha were done each year, using Ecopom Combi in 1993, and Ecopom in 1994 and 1995.

The damage recorded at Les Horizons in 1994 was mainly due to the short persistence of dispensers of about 30-40 days. With the new formulation in 1995 the attack was reduced below the economic threshold, without any chemical treatment while the control plot received twelve insecticide sprays (Table 3). Interesting results were obtained also at two new sites in 1995, where mating disruption was applied for the first time: at Laroche in the previous year the damage had been held below the threshold by five chemical treatments, while at Chaurchaire the history of the orchard showed a high pressure by codling moth which required seven chemical sprays with a damage at harvest of about 25%. The application of mating disruption decreased the damage to 3.7% without any preliminary chemical treatment, despite the average of 2.17 larvae per cardboard traps caught in the autumn. Three sprays of phosalone were applied in August due to the early exhaustion of dispensers which showed, according to GC analysis, a lower pheromone content than expected.

**Table 3** Mating disruption of codling moth and leafrollers with Ecopom dispensers in apple orchards of the G.R.C.E.T.A. and the Sicoly cooperatives (France) during three consecutive years, 1993-1995

Site	Area (ha)			Dispenser applications			Damage (%)			Insecticide treatments (in control plots)		
	'93	'94	'95	'93	'94	'95	'93	'94	'95	1993	1994	1995
Grand Vignes	1.5	4.0	3.9	3	3	2	1.5	0	1.4	2 (5)	1 (10)	3 (14)
Barjac	0.9	9.0	2.4	3	3	2	0.5	0	0.7	3 (10)	4 (14)	1 (6)
Chaîne		3.1			2			0			3 (15)	
Grand Mas		6.6			2			0.6			9 (20)	
La Glacière		3.5			2			0			2 (14)	
La Courtoise		5.3			1			0.5			7 (11)	
M. Blanc		3.3			3			0			1 (15)	
Mas de Roy		4.6			2			0			3 (17)	
Mas de Nages		2.7			2			1.3			4 (14)	
Les Clapiers		4.6			2			0.3			6 (15)	
Les Horizons	4.8	4.8	3.8				0.1	4.3	0			0 (12)
Milliat		2.0						0.6				
Chavas		1.3						1.5				
Laroche			0.3					0.5	0.4		(5)	3 (8)
Chaurchaire			0.4				5	25	3.7		(7)	3 (10)

In 1995, further trials were coordinated by the Mallemort Experimental Station P.A.C.A., the results are shown in Table 4 (Deschanel & Florac 1996). The highest damage was recorded in a pear orchard at La Barthelasse, where the pressure of codling moth was very strong: in the control plot, pheromone traps caught a total of 533 moths requiring 13 chemical treatments for a 2% damage at harvest. At Ventron-Cavaillon, 219 codling moths were trapped in the control plot, the damage at harvest could have been due to an insufficient pheromone release during the last part of the season.

At all sites, the mating disruption treatment led to a highly significant reduction of insecticide sprays, while a high degree of protection was maintained.

#### The Netherlands

Experiments were also done in The Netherlands for two years in cooperation with ProAgro and IPO-DLO (van Deventer *et al.* 1996). Ecopom Combi dispensers were used at 300 dispenser/ha and two applications in 1994; and at 400 dispenser/ha with a single application in 1995. The results are shown in Table 5. Protection against codling moth was comparable with that obtained with the traditional pest management program, consisting of three insecticide sprays.

**Table 4** Mating disruption (MD) of codling moth with Ecopom dispensers in apple orchards of the P.A.C.A. cooperative (France, 1995)

Site	Area (ha)	Damage (%)		Insecticide treatments	
		MD	Control	MD	Control
Verdier-Cavaillon	4.0	0.1	1.0	4	15
Brunel-St. Remy	2.4	0.75	0.1	1	6
Cestier-Eyragues	3.8	0.2	0.1	2	12
Giacqui-St. Gilles	3.9	1.4	0.1	3	14
Gautier-Velorgues	2.9	1.45	-	6	12
Ventron-Cavaillon	2.4	6.1	-	7	14
Manguin-La Barthelasse	2.8	16.3	2.0	5	13

The attack by apple leafrollers, in 1994, was slightly above the economic threshold of 1% and thus not completely satisfactory, even if part of this damage could have been due to *Spilonota ocellana*. This species uses Z8-14Ac as main pheromone component (Arn *et al.* 1992, 1996), which is not included in Ecopom Combi dispensers.

**Table 5** Mating disruption (MD) of codling moth and leafrollers with Ecopom Combi dispensers in apple orchards (The Netherlands, 1994-1995)

Site	Area (ha)	Technique	Damage (%)		Sprays
			Codling moth	Leafrollers	
Alphen a/d Maas - South (1994)	1.4	MD	0.2	0.7	-
Alphen a/d Maas - North (1994)	3.0	IPM-treated	0.15	0.5	3
Deil - East (1994)	2.0	MD	0.15	1.3	-
Deil - West (1994)	4.0	IPM-treated	0.05	0.75	2
Deil - East (1995)	2.0	MD	0.4	0.3	-
Deil - West (1995)	4.0	IPM-treated	0.3	0.7	3
Geldermalsen - South (1995)	2.0	MD	4.6	0.6	-
Geldermalsen - North (1995)	5.0	IPM-treated	14.3	0.2	4

In 1995, the number of dispensers was raised to 400 per ha and this, in conjunction with the second, consecutive use of mating disruption, improved the results at Deil keeping the attack by leafrollers below 1%. A second orchard was treated with pheromones in 1995: at Geldermalsen the population of codling moth was larger, but no initial chemical treatment was used. At mid-July, no fruit penetration was recorded in both plots, while in August the attack ranged from 0 to 1.2% in the mating disruption and from 2 to 3.4% in the control plot. The damage then increased and at

harvest it ranged between 3 and 6.6% in the mating disruption plot, while the control plot showed an attack between 5.2 and 19.2%.

## Conclusions

The practical trials carried out in three European countries clearly demonstrate that mating disruption using Isagro's Ecopom dispensers kept fruit damage by codling moth below the economic threshold in most cases. The current formulation is designed to be applied twice per season, but in some cases, even a single application could be sufficient. Further studies to extend dispenser life are going on.

It should be reminded that mating disruption is not an appropriate method under all circumstances; its correct application requires a high degree of preparation of both the advisory staff and the growers, in order to evaluate the biological, ecological, topographical, and technical limitations. Mating disruption is not a general method of protection from insect pests, and should be used only if the characteristics of the orchard fulfil well-defined requirements and if all necessary precautions are adopted.

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## **Successful employment of pheromones in apple: exemplary results from Europe**

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**Abstract** - The paramount importance of population density for the mating disruption technique is discussed and demonstrated by results obtained with summerfruit tortrix, *Adoxophyes orana*, where population density is often neglected.

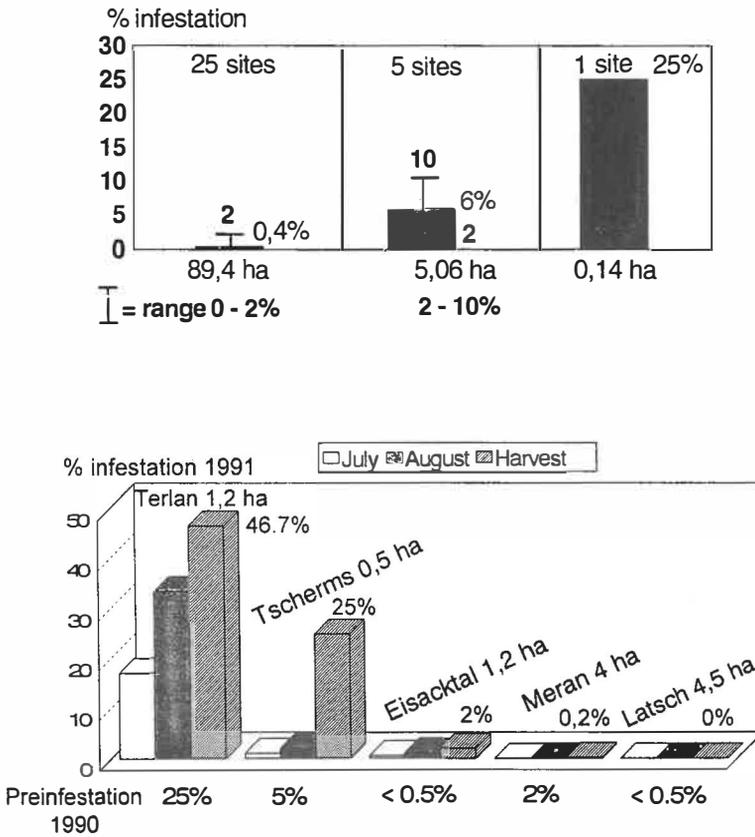
**Key words** - sex pheromone, mating disruption, controlled release dispensers, technology transfer, apple, pome fruit orchard, *Cydia pomonella*, *Adoxophyes orana*, Tortricidae, Lepidoptera

### **Introduction**

Meanwhile it has been recognised, that mating disruption by pheromones – besides for pests of grapes and peaches – can also be used successfully to control the major pests of apple. The number of communications on this subject presented at the Montpellier meeting is proof enough. Using exemplary results, I am particularly going to discuss the complex and difficult-to-define factor "population density", which is most important for successful applications of the mating disruption technique.

### **Codling moth (South Tyrol)**

First of all I would like to mention the work carried out by the South Tyrolean Advisory Service for Fruit- and Winegrowing (W. Waldner) with BASF dispensers (RAK 3+4) in 1991. The RAK 3+4 dispenser contains 420 mg *E8,E10-12OH* and 420 mg *Z11-14Ac*/dispenser and is being applied at 500 dispenser/ha. It was on the basis of the results obtained with codling moth, *Cydia pomonella* (Figure 1; Neumann *et al.* 1992), that the Advisory Service recommended the mating disruption technique for commercial use.

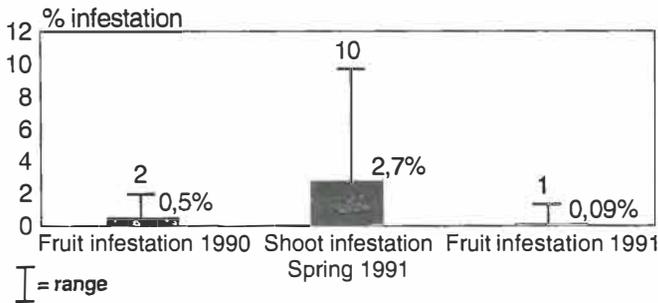


**Figure 1** Control of codling moth, *C. pomonella*, by mating disruption in South Tyrol. Top: Average infestation of apples on a total area of 94.6 ha (1991). Bottom: Fruit attack in relation to infestation levels during the previous season (1990/91)

Quite obviously, the success of the mating disruption technique depends to a large extent on the population density. A good indication for the population density of adult moths is the degree of larval attack during the previous generation. It is therefore not surprising that mating disruption was not successful in an unsprayed plot with a previous attack of 25% (Figure 1). Even with a previous attack rate of 5% the results were poor, despite low levels of infestation according to first larval counts. As a general rule, the threshold infestation level for successful control of codling moth by mating disruption is 1%. The reason for this could be a continuous emergence of moths over a longer period of time, which is often observed with codling moth, and which would lead to a critical level of the population density.

### Summerfruit tortrix (South Tyrol)

Results from South Tyrol on mating disruption of the summerfruit tortrix, *Adoxophyes orana*, with RAK 3+4 may be of general interest, since there is some doubt whether Z11-14Ac is efficacious for control of summerfruit tortrix (Waldner *unpubl. res.*; Figure 2). The shoot infestation in 1991 reaches 10% in some cases, indicating a rather high population density. Threshold levels for shoot infestation should probably not exceed 1%, according to our experience with Oriental fruit moth, *Grapholita molesta*, where only one larva/100 shoots is allowed (Audemard *et al.* 1989). Nevertheless, in none of the plots the fruit infestation exceeded 1%.



**Figure 2** Control of summerfruit tortrix, *A. orana*, by mating disruption (South Tyrol, 1991)

The trial in South Tyrol demonstrates that a level of fruit attack of 2% or less (1990; Figure 2) leads to the erroneous impression of an extremely low population density.

### Summerfruit tortrix (Germany)

Results from a trial in Jork, Germany, show a similar picture (Figure 3). A shoot infestation of up to 10% resulted in a fruit infestation below the economic damage threshold – but probably at the same time in an overall population density which would have been too high to be controlled by mating disruption in the following season. This means that mating disruption would have no longer decreased shoot infestation to a level of below 10%, resulting subsequently in insufficient protection against fruit infestation – especially if at the same time there is a regional increase in population density, as this was in my opinion the case in the South Tyrol.

This year the mating disruption technique has been employed for the first time on large areas in Germany. Before starting, the farmers were asked about the level of attack in 1995. No one was able to answer this question! Naturally none of them knew about the levels of shoot tip infestation or leaf attack either.

Pheromone traps in the mating disruption plots registered several times high captures of codling moth, but generally a corresponding degree of damage was not observed. It seems reasonable to assume that there had been a pronounced migration of male moths.

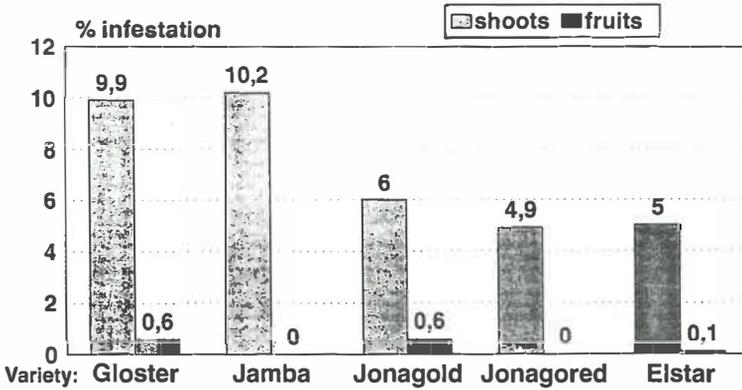


Figure 3 Control of summerfruit tortrix, *A. orana*, by mating disruption (Jork, Germany, 1990)

Another example of mating disruption against summerfruit tortrix is shown in Figure 4. An initially good control of codling moth and summerfruit tortrix turned into a rapid increase in infestation in September, once the experimental formulation (120 g/ha E8,E10-12OH, 130 g/ha Z11-14Ac) with too high release rates was exhausted. Fruit infestation increased to 2.9% in the case of summerfruit tortrix; the corresponding leaf infestation was probably much higher.

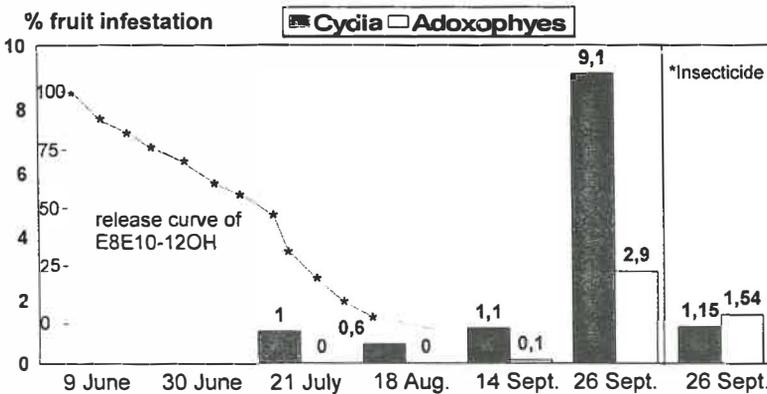


Figure 4 Control of codling moth, *C. pomonella*, and summerfruit tortrix, *A. orana*, by mating disruption. Fruit infestation (%; bars); amount of codlemone remaining in dispenser (%; line)

Figure 5 shows a typical example. With summerfruit tortrix, a better result was obtained with pheromone than with insecticides, although the differences are not very important. The threshold of damage was exceeded in all cases. The damage to the apples in the mating disruption plots would certainly still be acceptable for the fruit-grower, but with respect to successful mating disruption employment in the following season the prognosis is negative, since it must be assumed that there was an high degree of leaf infestation. If the mating disruption trials were to be continued in the corresponding plots, additional measures would have to be taken to lower the population density.

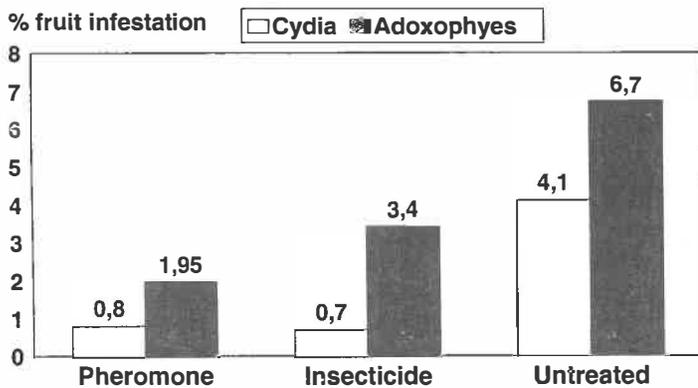


Figure 5 Control of codling moth, *C. pomonella*, and summerfruit tortrix, *A. orana*, by mating disruption (Kriftel, 1988)

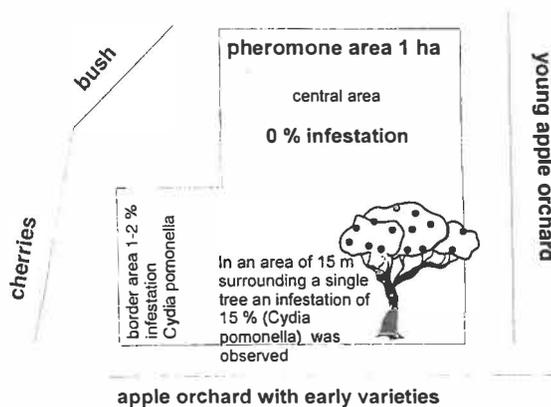


Figure 6 The influence of high trees on mating disruption of codling moth, *C. pomonella* (Öhningen, 1989)

Last not least, the presence of large apple trees in the neighbourhood of pheromone-treated orchards should be taken into account. Codling moths tend to fly out of such orchards towards tall trees (Zelger, *pers. comm.*); the results shown in Figure 6 suggest that even dispersal of mated females from these trees into the orchard can take place.

## Conclusion

I hope that the points I have made will help to shed some light on the problems which have been observed, particularly in the case of summerfruit tortrix. In my opinion it is, also in the case of summerfruit tortrix, the population density, as evidenced by infestation levels of both fruits *and* foliage, which remains the key factor, but is often underestimated.

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## **Mating disruption of codling moth, *Cydia pomonella*: experiences from the U.N.C.A.A. Arbotech Pool**

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**Abstract** - Confronted with the increased destructiveness of the codling moth, *Cydia pomonella* L., in French orchards, the Arbotech Pool cooperatives have integrated the search for novel control strategies into their experimental program. The assessment of control by mating disruption was carried out in parallel with the development of conventional insecticide strategies. This paper presents experiments carried out by three Arbotech Pool cooperatives: C.A.P.L., Alpesud and Union Set in 1995, using Ecopom pheromone dispensers. The results demonstrate that the mating disruption method, when properly used, has considerable potential for the control of codling moth, although it is still in the experimental stage. Mating disruption is also more of a technique than a product, and training of the distributors and users is therefore required to avoid failures. In addition, far from being a universal cure-all, it should be integrated into a global pest control strategy in orchards.

**Key words** - sex pheromone, mating disruption, technology transfer, integrated pest management, apple, pome fruit, codling moth, *Cydia pomonella*

### **Introduction**

Despite a growing use of insecticides, the damage caused by codling moth in Provençal apple orchards has steadily increased from 1990 to 1993. Since then, difficulties to control this pest have been encountered in all other regions. Crop damage has now exceeded the 2%-threshold in, for example, more than 20% of the orchards in the Garonne valley and in 15% of the orchards in the Loire valley.

Laboratory studies have confirmed the development of resistance and cross-resistance of codling moth to all insecticides used for conventional control. Fortunately, resistant strains are yet not widely spread in the field. Besides, there may be important differences in the sensitivity of codling moth to the various active ingredients, even

within each chemical family. However, some insecticide failures have been all too hastily attributed to assumed resistances, while the real causes are quite often related to non-compliance to prophylactic measures, "holes" in the treatment calendar, lack of protection against the first generation or poorly regulated spraying.

Confronted with the growing importance of codling moth, fruit growing is placed in a fragile state of equilibrium. Control programs must be understood as a function of orchard characteristics and history, and they should involve strategies for the prevention of resistance by maintaining the use of a range of chemicals. The Arbotech Pool cooperatives have therefore directed their efforts not only to the research and development of insecticides, but also to a prospective assessment of the mating disruption technique. At present, mating disruption products for codling moth are not registered in France.

### Materials and methods

Four mating disruption trials were carried out by three cooperatives within the U.N.C.A.A. Arbotech Pool: by C.A.P.L. at Vignères, by Alpesud at Laragne, and by Union Set at Saint Aubin and at La Bourdaisière Chenu.

At least two orchards of 2 to 2.5 ha were treated at each site; one with insecticides and one with Ecopom dispensers (Isagro, Novara, Italy). The Ecopom dispenser is a resin-treated cellulose pad, loaded with  $250 \pm 25$  mg (*E,E*)-8,10-dodecadien-1-ol (*E8,E10-12OH*, codlemone) (Rama 1997).

The dispensers were applied twice per season, at 300 dispensers/ha inside the orchard, and at 400 dispensers/ha along the border. During the trial at La Bourdaisière Chenu, an insecticide treatment against the first generation was combined with a single dispenser application against the second generation.

The trials were monitored by codlemone-baited traps; dispenser life and release rates were estimated according to degree-days. In each orchard, 1 000 apples were checked for visible damage. The controls were done on a daily basis, extending from the first occurrence of second-instar larvae until harvest.

### Results

The trials carried out at Vignères (Vaucluse) and Saint Aubin/La Bourdaisière Chenu (Loire valley), under completely different climatic conditions, clearly demonstrate the efficacy of the mating disruption technique.

At Vignères, two pheromone-treated orchards showed a very low rate of fruit attack (0.5 and 0.3%, respectively), which was equivalent to the insecticide-treated control (0.4%; Table). It should be noted that the amount of insecticide applied in the control orchard was quite typical for the Provence region: 17 specific anti-codling moth sprays, including 9 with organophosphates.

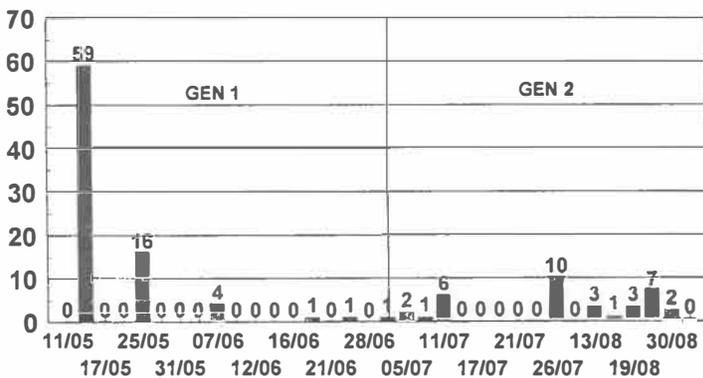
**Table** Mating disruption (MD) of codling moth, *C. pomonella*, in four different locations in France (1995)

	C.A.P.L. <sup>a</sup>		Alpesud <sup>b</sup>		Union Set <sup>c</sup>		Union Set <sup>d</sup>	
	Control	MD	Control	MD	Control	MD	Control	MD <sup>e</sup>
Dispenser application	-	May 5 July 7	-	May 15 July 21	-	May 3 July 7	-	- Aug. 1
No. insecticide treatments	17	0	3	0	5	0	7	5
Fruit attack (%)								
1 <sup>st</sup> generation	0	0	0	0.7	0	0	0.5	0.5
2 <sup>nd</sup> generation	0.4	0.2	5	7.1	0	0	-	-
At harvest	0.4	0.4	16.3	13.4	4.6	0.6	5.1	0.7

<sup>a</sup> Vignères, Provence; <sup>b</sup> Laragne, Hautes Alpes; <sup>c</sup> Saint Aubin, Loire valley; <sup>d</sup> La Bourdaisière Chenu, Loire valley; <sup>e</sup> Insecticide treatment against first generation

In the Loire Valley, the Union Set trial (Table) showed a 0.6% fruit damage in the mating disruption orchard, while insecticide control in the reference orchard was not satisfactory. This orchard was sprayed only 5 times, resulting in an insufficient control at high population levels.

The trial carried out at Laragne by Alpesud (Table) has two points of interest. First of all, it illustrates that mating disruption is not reliable at high population densities. Monitoring traps showed unusually high catches at the onset of the first flight (Figure), probably due to immigration from a nearby abandoned pear orchard.



**Figure** Attraction of codling moths, *C. pomonella*, to monitoring traps at Laragne (1995)

It also shows that even insecticide treatments fail, if not applied properly. The farmer obviously underestimated the codling moth risk and sprayed only three times. In addition, one of the products was erroneously underdosed at one-tenth. It should be added that visual controls were not carried out often enough to detect the high level of infestation.

This test demonstrates that both insecticide treatments and mating disruption are not infallible techniques. Critical conditions, such as high population densities or immigration of moths, have to be taken into account and methods must be made available to predict these.

The efficacy of mating disruption was found to be excellent in the Union Set trials carried out at Saint Aubin and La Bourdaisière Chenu, where the first generation was treated with insecticides and the second by the application of dispensers (Table). The combined use of conventional methods and mating disruption may prove to be a future strategy to ensure better control of the codling moth.

## Conclusions

Mating disruption is, when properly used, a promising method for the management of codling moth populations. The Arbotech Pool is continuing experimental work in 1996 in order to specify the dispenser life as a function of temperature and to integrate mating disruption into the framework of an overall pest control strategy.

However, it is necessary to emphasize that mating disruption against codling moth is still in the experimental stage. This technique may in no case be thought of as an ordinary plant protection product; training is required to ensure its appropriate use.

And, the mating disruption technique is not a universal cure-all. It will hopefully soon be made available to the farmer, but it will have to be integrated within an overall treatment strategy. This obviously complies with the concept pioneered by the fruit farmers themselves: *la lutte intégrée*.

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*Mating disruption in vineyards*

## Adoption of mating disruption for controlling the grape berry moth, *Endopiza viteana* (Clemens) (Lepidoptera, Tortricidae) in Ontario, Canada

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**Abstract** - The grape berry moth, *Endopiza viteana* (Clemens), is the most important insect pest of grapes in eastern North America. Sex pheromone-mediated mating disruption has proven highly effective for controlling this pest and a pheromone dispenser for this purpose has been registered in Canada since 1992. However, mating disruption has not been used for grape berry moth control on more than 4% of the 5 400 ha of commercial vineyard in Ontario. Possible reasons for the low rate of adoption of mating disruption and recommendations for increasing its use are discussed.

**Key words** - Sex pheromone, mating disruption, controlled release dispenser, viticulture, *Endopiza viteana*, Tortricidae, Lepidoptera

### Introduction

The grape berry moth, *Endopiza viteana* (Clemens), is the most important insect pest of grapes in eastern North America (Taschenberg *et al.* 1974) and has been recognized as a pest in the Niagara peninsula of Ontario since 1917 (Roberts & Simpson 1982a). In the Niagara region, *E. viteana* has three generations of actively feeding larvae per year. The first, or spring generation, attacks flowers or newly set clusters of berries; the next two generations feed on developing fruit (Roberts & Simpson 1982a). There is considerable variation in the size of *E. viteana* populations within the Niagara peninsula and consequently from two to six applications of organophosphorous insecticides have traditionally been used to control this pest (Roberts & Simpson 1982b).

### Development and evaluation of mating disruption

The sex pheromone of the grape berry moth was identified as (*Z*)-9-dodecenyl acetate (*Z*9-12Ac) (Roelofs *et al.* 1971) and the first studies on the feasibility of controlling *E. viteana* by sex pheromone-mediated mating disruption were conducted from 1972 to 1987 in New York State vineyards (Dennehy *et al.* 1990).

In Canada, the efficacy of mating disruption for controlling *E. viteana* was evaluated from 1987 to 1993 in the Niagara peninsula of Ontario. In the initial experiments, 1987 to 1989, the technique was tested in 0.7 to 1.4-ha plots in commercial vineyards with high-, moderate-, and low-density grape berry moth populations using several versions of a polyethylene, capillary-tube pheromone dispenser (Pacific Biocontrol Ltd., Vancouver, Oregon) (Trimble *et al.* 1991). In 1987 and 1988, each dispenser was loaded with 365 mg of *Z*9-12Ac, 40 mg of (*Z*)-11-tetradecenyl acetate (*Z*11-14Ac) and 45 mg of stabilizer. The dispensers had a field life of 4-5 months and were deployed at the recommended density of 250/ha. In 1989, each dispenser was loaded with 57 mg of *Z*9-12Ac, 6 mg of *Z*11-14Ac and 7 mg of stabilizer. The field life of these dispensers was also 4 to 5 months and they were deployed at the recommended rate of 1 000/ha.

In a subsequent experiment, the technique was tested in ca. 1.5-ha plots of vines at two Niagara farms during five consecutive growing seasons, 1989 to 1993, using Isomate® GBM pheromone dispensers (Pacific Biocontrol Ltd., Vancouver, Oregon) (Trimble 1993, Trimble *unpubl.*). In 1989, each dispenser was loaded with 135 mg of *Z*9-12Ac, 15 mg of *Z*11-14Ac and 20 mg of stabilizer. The field life of these dispensers was 4 to 5 months and they were deployed at the recommended rate of 1 000/ha. During 1990 to 1993, each dispenser was loaded with 57 mg of *Z*9-12Ac, 6 mg of *Z*11-14Ac and 7 mg of stabilizer. The field life of these dispensers was 2 months and they were deployed at the recommended rate of 1 000/ha twice each growing season.

The efficacy of mating disruption was evaluated by comparing captures of male moths in pheromone-baited traps and berry moth damage to grape clusters in plots treated with pheromone and in control plots treated with insecticide.

In the first experiments, average pheromone dispenser release rates ranged from 20 to 32 mg/ha/hr and pheromone-baited trap catches were reduced by 92% or more in plots treated with pheromone. Treatment with pheromone significantly reduced damage (*i.e.* percentage infested clusters) compared with an untreated control in each of two tests, and provided control as good as or better than an insecticide control programme in two of four tests. In the five-year experiment, the average rate of release of pheromone ranged from 29 to 37 mg/ha/hr and pheromone-baited trap catches were reduced by more than 99% in plots treated with pheromone. At one farm (Farm 1), insecticides provided better control than pheromone during 1989, but during the next four years, 1990 to 1993, only small differences were observed between methods (Table 1). At the other farm (Farm 2), mating disruption provided control as good as or better than insecticides during the 5-year-study (Table 1).

The evaluation of another pheromone dispenser, Decoy® GBM (Agrisense, Fresno, California), for use in controlling *E. viteana* by mating disruption was completed in Niagara peninsula vineyards by Coopermill Ltd. of Madoc, Ontario, from 1987 to 1991 (JB Hastings *pers. comm.*). However, the results of this evaluation have not been published in the scientific literature.

**Table 1** Percentage grape clusters infested with *E. viteana* at harvest in pheromone- and insecticide-treated plots at two Niagara peninsula farms, 1989 to 1993

Year	Farm 1		Farm 2	
	Pheromone	Insecticide	Pheromone	Insecticide
1989	45.8	20.6	4.8	16.9
1990	3.0	1.7	1.5	3.8
1991	4.5	3.3	8.4	19.1
1992	1.1	0.0	0.7	4.5
1993	0.4	0.2	7.8	11.9

### Registration and adoption of mating disruption

Decoy® GBM, a polymer-matrix-gel pheromone dispenser (Agrisense, Fresno, California) was registered in Canada in 1992 for controlling *E. viteana* by mating disruption. During the four-year period 1992 to 1995, 23 to 26 growers used this product. The total area treated with Decoy GBM during this period did not exceed 200 ha, or 4% of the ca. 5 400 ha of commercial vineyard in the Niagara peninsula. During the 1996 growing season, 18 growers treated a total of ca. 140 ha of vineyard (JB Hastings *pers. comm.*).

The most probable reasons for the low rate of adoption of mating disruption for controlling *E. viteana* are the relative costs of insecticide and Decoy GBM, the method of applying the pheromone and the method that has been used to promote the use of mating disruption. The cost of the insecticide required for a three spray control programme on one ha of vineyard using one of the six currently recommended insecticides (Anonymous 1996) ranged from \$ Can 45 to 210 during the 1995 growing season (Table 2.).

Niagara peninsula grape growers are discouraged from using the pyrethroid insecticides permethrin or cypermethrin, and the carbamate insecticide carbaryl, because they are toxic to predaceous mites, and their repeated use can result in outbreaks of the European red mite, *Panonychus ulmi* (Koch). Therefore, azinphosmethyl, phosmet and diazinon are the most commonly used insecticides for controlling *E. viteana*. The cost of using Decoy GBM on one ha of vineyard during the 1995 growing season was from \$ Can 196 to 249, depending on whether the grower purchased

the pheromone dispensers with or without application and monitoring services (JB Hastings *pers. comm.*) (Table 2).

**Table 2** Cost of *E. viteana* control during the 1995 growing season by a three-spray insecticide programme and by mating disruption, using Decoy® GBM

Insecticide	Cost (\$ Can/ha)	Mating disruption	Cost (\$ Can/ha)
aziphosmethyl	77 - 81	Pheromone dispensers	196
phosmet	106	Application (by supplier)	25
diazinon	71	Monitoring (by supplier)	28
carbaryl	210	<i>Total</i>	249
permethrin	89		
cypermethrin	45		

Therefore, during the 1995 growing season, the cost of the pheromone dispensers required for use in mating disruption was 2.4 to 3.5 fold greater than the cost of the insecticide for a three-spray grape berry moth control programme. Decoy GBM must be applied by hand at a rate of 1000 dispensers/ha and the cost of performing this task, whether by the supplier or the grower, is viewed as an additional cost compared to using insecticide. This is because insecticides for *E. viteana* control are generally applied as a sprayer tank mix with fungicides for disease control. The use of sex pheromone-baited traps as an indirect measure of the effectiveness of mating disruption, *i.e.* monitoring (Table 2), is an important component of the grape berry moth mating disruption programme (JB Hastings *pers. comm.*). However, this component of the programme is also regarded as an additional cost compared to using insecticides. Although sex pheromone-baited traps are used to acquire information that is used to time the application of insecticide sprays, this service has been provided free of charge by the Ontario Ministry of Agriculture, Food and Rural Affairs.

Another reason that mating disruption has not been widely adopted is that it has been promoted as an environmentally friendly alternative to the use of insecticides for controlling *E. viteana* and grape growers, in general, have not regarded this as a benefit to their grape growing business.

### Recommendations for increasing the adoption of mating disruption

The use of mating disruption for controlling the grape berry moth in Niagara peninsula vineyards would likely be more widely adopted if the technique was cost-competitive with using insecticides and if it was promoted as an insecticide resistance-management tool for use in a sustainable, cost-effective pest management programme. The cost of using mating disruption would be reduced if it could be applied using

conventional pesticide application technology. Microencapsulated formulations of pheromone can be applied with many types ground spraying equipment (Hall & Marrs 1989). The availability of a sprayable grape berry moth pheromone would enable growers to apply pheromone as a tank mix with the fungicides that are applied at approximately fortnightly intervals under Niagara peninsula growing conditions (Anonymous 1996).

Organophosphorous insecticides like azinphosmethyl, phosmet and diazinon have been used almost exclusively to control *E. viteana* in Niagara vineyards for the last 30 years. Although there are no published reports of insecticide resistance in this pest, organophosphorous insecticide-resistance has been reported in other tortricid fruit pests such as the codling moth, *Cydia pomonella* (L.) in Australia (Thwaite *et al.* 1993) and the western U.S.A. (Varela *et al.* 1993; Knight *et al.* 1994), and the Oriental fruit moth, *Grapholita molesta* (Busck) in South Africa (Barnes & Blomefield 1997). The fungus *Botrytis cinerea* Pers. has developed resistance to fungicides in Niagara peninsula vineyards (Northover & Matteoni 1986; Northover 1988) and therefore Ontario grape growers are aware of the potential for economic loss often associated with pesticide resistance. For this reason, mating disruption would be more readily accepted as a control strategy for grape berry moth control if it was promoted as part of a proactive, insecticide resistance-management programme instead of as a replacement for insecticides.

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## Effets de la confusion sexuelle contre l'Eudémis *Lobesia botrana* sur les populations d'autres ravageurs et d'auxiliaires dans le vignoble Bordelais

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**Abstract** - *The effect of mating disruption against the grape moth, Lobesia botrana, on populations of other pests and beneficials in Bordeaux vineyards.* Experiments to control the grape moth by the mating disruption technique were carried out in the Sauternes region. A 15-ha vineyard has been treated with BASF dispensers since 1989. Progressive decrease of insecticide and acaricide use made it necessary to carefully monitor populations of the green leafhopper, *Empoasca vitis*, the red mite, *Panonychus ulmi*, and its principal predator, *Typhlodromus pyri*. After seven consecutive years of mating disruption, the red mite and the green leafhopper are no longer serious pests in this vineyard. There are four possible explanations for the control of red mite by *T. pyri*: (1) the existence of native populations since 1990; (2) a favorable environment (woods, hedges, flowering plants); (3) pest management programs including a progressive reduction of insecticides and acaricides, and higher economic thresholds; (4) the use of fungicides lowly toxic to *T. pyri*. The reduction of green leafhopper damage can be explained by increased populations of beneficials such as *Chrysopa carnea* and *Anagrus atomus*, due to the reduction of insecticide use. This study clearly demonstrates that mating disruption of grape moth has a favourable effect on the control of secondary pests by their natural enemies.

**Key words** - sex pheromone, mating disruption, phytophagous mites, *Panonychus ulmi*, green leafhopper, *Empoasca vitis*, *Typhlodromus pyri*, *Chrysopa carnea*, population dynamics, damage threshold

### Introduction

Les expérimentations de lutte par confusion sexuelle contre l'Eudémis effectuées depuis 1989 à l'aide de diffuseurs BASF dans un vignoble du Sauternais ont largement démontré l'efficacité de cette méthode (Tableau; Stockel *et al.* 1994). Elles ont conduit à l'homologation du procédé en France dès le printemps 1995 (Lecharpentier *et al.* 1995). L'arrêt progressif des interventions insecticides et acaricides à partir de 1990 a

entraîné une surveillance attentive des populations de la cicadelle verte, *Empoasca vitis*, de l'acarien rouge, *Panonychus ulmi* et de son principal prédateur *Typhlodromus pyri*.

**Tableau** Résultats annuels de l'emploi de la méthode de confusion contre *Lobesia botrana*

Année	Niveaux de populations (dégâts ou chenilles/100 grappes)								
	Confusion sexuelle			Référence non traitée			Référence traitée <sup>a</sup>		
	G1 <sup>b</sup>	G2 <sup>b</sup>	G3 <sup>b</sup>	G1	G2	G3	G1	G2	G3
1989	- <sup>c</sup>	4	5	13	39	100	- <sup>d</sup>	20	100
1990	10.5 <sup>e</sup>	0.8	14.3	36.7	26	255	-	21	38.1
1991	15.4 <sup>e</sup>	9.8	12	26.6	40	300	23.5	22.5	14
1992	2.5	0.5	3.3	7	6	12	N <sup>f</sup>	N	8
1993	2.9	0	0	11.3	0	1.3	N	N	N
1994	0.7	1.3	7.3	3	5	25.3	N	N	N
1995	5.2	2.5	9.5	11.1	19.3	76	5.5	9	N
1996	3.7	1.3	8.4	30	7.5	51	N	N	N

<sup>a</sup> Référence traitée avec insecticides; <sup>b</sup> 1ère, 2ème et 3ème génération; <sup>c</sup> traitement curatif avec insecticides; <sup>d</sup> non évalué; <sup>e</sup> traitement curatif avec insecticides sur la moitié de la surface; <sup>f</sup> non traitée

## Matériels et méthodes

### Vignoble expérimental

Les essais avaient lieu à Preignac, dans l'Appellation Sauternes (AOC), au château d'Armajan des Ormes. Dès 1989, 8 ha furent équipés de diffuseurs BASF. L'année suivante, 4 ha supplémentaires étaient également placés sous confusion sexuelle et une zone voisine de 2 ha fut utilisée comme référence traitée et non traitée, pour un suivi phytosanitaire classique des populations de ravageurs.

### Techniques d'échantillonnage

A chaque espèce de ravageurs et d'auxiliaires correspond une technique adaptée :

*Acariens*. Les oeufs sont dénombrés sur 2 bourgeons de 50 sarments par parcelle à l'aide d'un stéréomicroscope (Gx25). On détermine ensuite le pourcentage de bourgeons occupés par 1 oeuf au moins, puis par plus de 5 oeufs. Ces résultats sont reportés sur un abaque de décision établi par le Service de la Protection des Végétaux en vue d'estimer le risque printanier.

Les formes libres en saison sont dénombrées sur des échantillons de 25 à 50 feuilles prélevées au hasard (une feuille par cep), sur 3 rangs au minimum, dans chaque parcelle de 0,75 à 1 ha. On procède de deux manières différentes: (1) observa-

tion directe sur les feuilles à la loupe binoculaire frontale (Gx2) (méthode de terrain); (2) observation indirecte au stéréomicroscope (Gx25), après extraction au laboratoire par brossage, technique de Berlèse ou trempage-rinçage-filtration (méthode utilisée depuis 1992).

*Cicadelle verte*. L'échantillonnage consiste à observer in situ, (loupe binoculaire frontale), les formes larvaires sur la face inférieure de 100 feuilles prises au hasard dans les mêmes parcelles.

*Auxiliaires*. Les parasites d'Eudémis étaient dénombrés à partir des prélèvements individualisés de chenilles effectués dans les différentes zones au cours des 1ère et 3ème générations. Les prédateurs du genre *Chrysopa* étaient récoltés sur le feuillage à l'aide de l'aspirateur "D-VAC" puis identifiés au laboratoire.

### Seuils de nuisibilité retenus

*Acariens*. Seuils préconisés par le Service de la Protection des Végétaux: 70% de feuilles occupées au printemps et 30% en été. La formule établie par Piganeau (1979) permet d'obtenir la correspondance entre ces pourcentages de feuilles occupées et le nombre de formes mobiles (f.m.) par feuille: 6.1 f.m. au printemps, 0.6 en été.

*Cicadelle verte*. Seuils définis par l'ACTA et l'ITV (Anonyme 1980): 100 larves/100 feuilles en 1ère génération; 50 larves/100 feuilles en 2ème génération.

Pour chacun des trois ravageurs étudiés, Eudémis, Acariens et Cicadelle verte, en cas de dépassement des seuils, des interventions insecticides étaient prévues à l'aide respectivement de téflubenzuron, de benzoximate et de vamidothion.

### Resultats

*Acariens*. Dans les parcelles en confusion depuis 1989, leurs populations n'ont jamais atteint les seuils de risque. En revanche, dans certaines de celles qui ont reçu des diffuseurs de phéromone depuis 1990, les dépassements de seuils observés cette même année ont nécessité une intervention spécifique (Figure 1). La zone de référence (Figure 2) ne mérite cette dénomination que jusqu'en 1992. C'est jusque là en effet qu'elle a reçu quelques traitements acaricides et insecticides dont les effets secondaires n'ont pourtant pas empêché le développement des typhlodromes.

*Cicadelles*. En 1989 et 1990, les populations étaient maîtrisées par les applications insecticides destinées à permettre l'installation de la confusion. En 1992, l'absence de traitements spécifiques dans les zones sous confusion n'a pas entraîné de grillures préjudiciables bien que les seuils de risques aient été encore dépassés dans certaines parcelles (Figure 3). Parallèlement, on observe dès 1991 la présence de Névroptères appartenant à l'espèce *Chrysopa carnea* Stephens (Delbac *et al.* 1996b).

*Parasitoïdes d'Eudémis*. On observe chaque année en première génération que plus de 50% des chenilles récoltées dans les zones sous confusion sont parasitées par des Ichneumonidae.

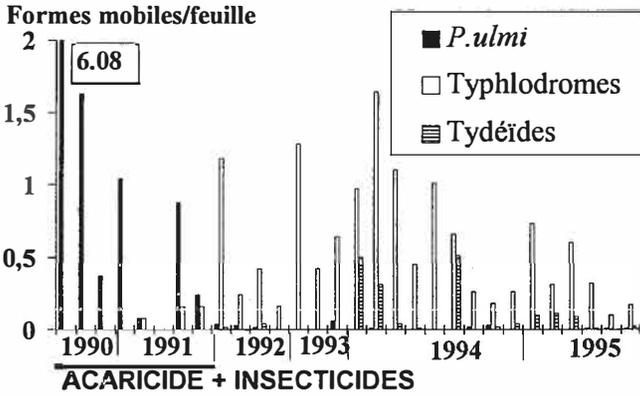


Figure 1 Evolution des populations d'acariens dans les parcelles sous confusion depuis 1990

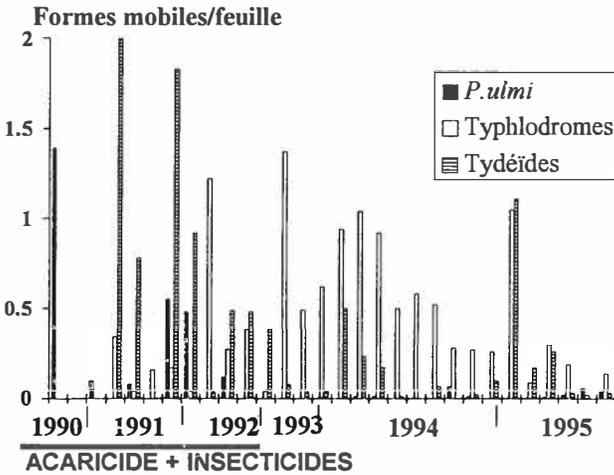
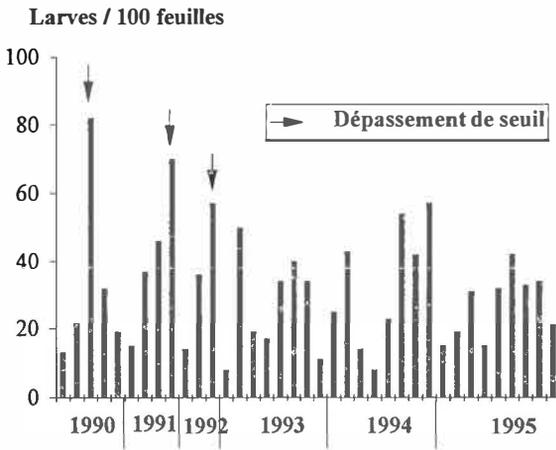


Figure 2 Evolution des populations d'acariens dans la zone de référence traitée avec insecticides depuis 1990

**Discussion et conclusions**

Après sept années consécutives d'emploi de la confusion contre l'Eudémis sur un site où la présence de l'acarien rouge et de la cicadelle verte étaient jusque là redoutées, il s'avère que les niveaux de population de ces trois ravageurs n'entraînent plus de dégâts préjudiciables à la qualité de la vendange.



**Figure 3** Evolution des populations d'*E. vitis* en zones sous confusion depuis 1990

La régulation des populations d'acarien rouge serait essentiellement due, dès la 3ème année d'expérimentation, au développement de populations de *Typhlodromus pyri* (Delbac *et al.* 1996a) sous l'action combinée de 4 facteurs: (1) l'existence de populations indigènes; (2) la présence d'un environnement (bois, haies, essences ornementales) favorable à leur hébergement; (3) l'aménagement du programme phytosanitaire par l'abandon progressif des insecticides et acaricides; (4) l'emploi de matières actives fongicides peu toxiques vis-à-vis de *T. pyri* (Groupe d'étude sur les auxiliaires en viticulture 1995).

L'absence de grillures dues à la cicadelle verte semblerait liée à l'effet de populations d'autres auxiliaires telles que *Chrysopa carnea* (Vidano *et al.* 1985), *Anagrus atomus* (Vidano *et al.* 1988) et aux seuils de nuisibilité du ravageur à redéfinir d'autre part.

Les effets bénéfiques à moyen terme de la confusion sexuelle sur la maîtrise des populations d'Eudémis, d'acariens phytophages et de la cicadelle des grillures attestent l'intérêt d'intégrer cette nouvelle méthode de lutte dans les programmes phytosanitaires en viticulture.

### Remerciements

Les auteurs tiennent à remercier la société BASF pour la fourniture des diffuseurs de phéromone et M. Perromat pour son accueil au Château D'Armajan Des Ormes et sa collaboration aux expérimentations décrites.

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## Mating disruption in vineyards: determination of population densities and effects on beneficials

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**Abstract** - Population densities, migration and long-term effects of mating disruption of *Lobesia botrana* were investigated in German vineyards during several years. Furthermore, the effect of beneficial organisms on pest control was studied.

**Key words** - sex pheromone, mating disruption, viticulture, population density, *Lobesia botrana*, *Eupoecilia ambiguella*, *Sparganothis pilleriana*, Tortricidae, Lepidoptera

### Introduction

In German wine-growing areas, the grape moth *Lobesia botrana* Schiff. and the grape berry moth *Eupoecilia ambiguella* Hbn. are the most widespread pests. During the last ten years there has been a distinct shift in the abundance of these two species: the population density of *L. botrana* has increased considerably in many regions, while the density of *E. ambiguella* has decreased concurrently.

In 1986, the pheromone dispenser RAK 1 Plus, containing (Z)-9-dodecen-1-yl acetate as active ingredient, was officially registered for mating disruption of *E. ambiguella* in Germany. In 1994, the RAK 1+2 dispenser, with (E,Z)-7,9-dodecadien-1-yl acetate and (Z)-9-dodecen-1-yl acetate as active ingredients, was registered for mating disruption of both species. In 1996, 17% of all German vineyards were treated with pheromone for control of *E. ambiguella* and *L. botrana* by mating disruption. During several years, various aspects of *L. botrana* population dynamics and the effect of beneficial organisms on control by mating disruption have been investigated.

### Population densities of *L. botrana*

By conventional trapping methods, for example pheromone traps, it is impossible to determine absolute moth densities. A sampling system based on a non-luring principle was developed which enabled us to determine the real density of male and female moths. For this purpose, a 2 x 5 m gauze tent was used, which was easily set up anywhere within a vineyard. After the moths had been disturbed by shaking the foliage within the tent, they gathered mostly at the tent-roof from where they could be sampled by a vacuum cleaner (Feldhege *et al.* 1994). Based on measurements on 10 m<sup>2</sup>, we estimated the number of moths/ha. In addition to the described sampling method, we also used a suction-trap, light traps and liquid bait traps for specific investigations.

The tent-system proved to be very suitable for recording the number of moths during the second generation. At the onset of the flight period of the second generation, the number of males was superior to the number of females (1:0.36), whereas at the end of the flight period females dominated (1:3.84). In total, the sex ratio was roughly balanced.

During the second generation, the densities of moths in the border zones of the vineyard differed widely compared to those found in the centre of the same vineyard. For example in 1994, the estimated density of *L. botrana* in the border zone was 210 000 moths/ha; compared to 98 000 moths/ha in the centre. In 1995, sampling with a suction-trap revealed a density of about 260 000 moths/ha in the border zone and only 10 000 moths/ha in the centre. In general, the moth density correlated significantly to the density of eggs and larvae counted in the respective vineyard zones. Therefore, monitoring of oviposition and first appearance of larvae in border zones of vineyards should have first priority.

During the first generation, the density of moths in vineyards which were not treated with pheromones, was rather low with a maximum of 3 500 moths/ha. It seems that the low density of moths during the first generation in the vines was due to the sparse vine foliage at this early stage - high numbers of female *L. botrana* moths were detected within greencover plants on the vineyard ground and in the adjoining hedges. This may also explain increased *L. botrana*-attacks in border zones of vineyards adjacent to gardens.

### Dispersal

In 1993, dispersal of *L. botrana* was investigated in a 50-ha vineyard. The vines had been removed during winter and along with them all *L. botrana* pupae. Using bait and pheromone traps, it was shown that mainly male moths flew up to 450 m into the centre of this area, during the first year of recultivation. Already in the second year, the density of female moths in the center of the recultivated vineyard was the same as in the surrounding vineyards. A surprising result was obtained from release-recapture experiments. Two female moths covered a distance of about 2 600 m within one day.

With modified light traps we investigated the migration of *L. botrana* moths between pheromone-treated and untreated areas. The light traps were equipped with four funnels pointing in four different directions. The trapping results showed that a significant number of male moths was attracted to the pheromone atmosphere, whereas no significant migration of female moths was detected. Higher densities of male *L. botrana* moths in the border zones and consequently higher rates of mating could explain the increased infestation levels observed in the border zone of pheromone-treated vineyards. Under German cultivation conditions, it seems to be absolutely necessary to apply pheromone dispensers to a border zone of 30 metres within adjacent, untreated vineyards in order to avoid higher infestation rates.

### Critical population density

The tent system was also used to obtain an estimation of the "critical density of moths" in pheromone-treated vineyards, above which mating disruption is no longer effective. In 1994, nearly no matings or oviposition occurred at densities of up to 4 000 males/ha and 4 000 females/ha in the experimental area. When the number of moths exceeded the critical threshold of 8 000 moths/ha, nearly all female moths were mated and there was also a significant increase in the number of eggs on the grapes (Feldhege *et al.* 1995).

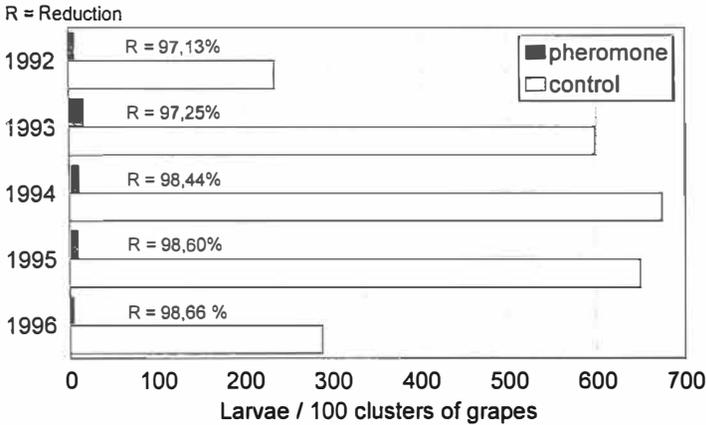
### Long-term effects of pheromone application

The results obtained between 1992 and 1996 on an area covering 60 ha revealed very clearly long-term effects of the mating disruption treatment. RAK 1+2, for mating disruption of *L. botrana* and *E. ambiguella*, was applied at 500 dispensers/ha. Further vineyard plots which were not sprayed with insecticides were set in distances of 150 m up to 500 m to the pheromone-treated area. In some years the density of larvae grew considerably in these control plots. In the second generation of 1994, for instance, there was an average of 6.7 larvae per cluster of grapes (Figure). In the pheromone-treated area only *L. botrana* appeared. However, in the pheromone treated area the density of moths decreased significantly and we gained stable degrees of effectiveness up to 98.7%. This leads to the conclusion that pheromone treatment applied over many years obviously reduces the population density of *L. botrana*.

### Beneficial organisms

Another long-term project focussed on mating disruption and its side effects on beneficial organisms in viticulture. In a three-year-study it was proven that, in pheromone-treated plots, the European red spider mite, *Panonychus ulmi* Koch, did not occur anymore due to high densities of the predator *Typhlodromus pyri* Scheuten. Plots which

were treated with the insecticide Deltamethrin showed high densities of *P. ulmi*, but only low numbers of *T. pyri*. Nowadays, treatments with acaricides is the exception in Germany, due to the long-term use of pheromones or due to the application of pesticides without negative side-effects on beneficials as *T. pyri*.



**Figure** Control of the grape moth *L. botrana* by mating disruption with pheromone in Neustadt/W.-Haardt. Infestation rates during the second generation in pheromone-treated and control plots

From 1987 to 1990, further experiments focussed on arthropods in insecticide- and pheromone-treated plots. In total, more than 34 000 individuals were recorded and investigated. Representative for all other studies one result will be presented here.

The experiments were conducted in four replicates. Compared to the insecticide treated plots, the pheromone-treated sites showed more than twice the number of individuals at the vine trunks. Most of the species belonged to the order Araneida. In addition to a larger number of individuals, the diversity of species was much higher in the pheromone-treated plots (Schirra *et al.* 1991).

From 1991 to 1994, another project was carried out to study the effects of beneficial organisms in pheromone-treated areas on the pest populations. It was not definitely proven that predators have a regulating influence on pests because the density of *E. ambiguella* and *L. botrana* was too low due to the high efficiency of the pheromone treatment. At the vine trunks, the araneid species *Salticus scenicus* and *Marpissa muscosa* were predominant. A regulation of the immigration of larvae of pests in the vine-trunk section by predators seems conceivable.

In contrast to the predators, there was a significant effect of parasitoids on grape moths. Up to 43% of the overwintering grape moth pupae were infested with parasitoids. The ichneumonid species *Itoplectis alternans* was predominant among the para-

sitoids attacking the grape moths; Braconidae and Calcidoidae were found to a lesser extent.

Remarkable densities of another grape moth, *Sparganothis pilleriana* Schiff. were recorded in the investigated areas. However, the density of *S. pilleriana* in vineyards treated with pheromone against *L. botrana* and *E. ambiguella* was not significantly higher than in insecticide-treated plots. The rate of pupae of *S. pilleriana* infested by parasitoids was 28% (1 917 pupae examined) in the pheromone-treated sites. In addition, up to 25% of *S. pilleriana* pupae had been prey of predators. In laboratory assays it was established that *Forficula auricularia*, a most important predator, was responsible for the specific damage of the *S. pilleriana* pupae (Schirra & Louis 1995).

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*Other control applications of pheromones*

## Confusion sexuelle des males de *Zeuzera pyrina* (Lepidoptera, Cossidae) en vergers de pommiers

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**Abstract** - Mating disruption of *Zeuzera pyrina* (Lepidoptera, Cossidae) in apple orchards. The development of biological control of the codling moth in apple and pear orchards will soon be imposed by the resistance of this insect to various pesticides. Several secondary pests, which were previously controlled by the chemicals applied against this key pest, will need specific control, too. Mating disruption trials against the leopard moth, *Zeuzera pyrina* L., were therefore conducted in three orchards of the Rhône Valley near Avignon. The dispensers (RAK 8, BASF) were loaded with the main pheromone component, (*E,Z*)-2,13-octadecadien-1-yl acetate and placed at different densities in the orchards at the end of May. The pheromone release ranged from 53 to 131 mg/ha during the 18-week test period, and was closely correlated with temperatures. The effectiveness of mating disruption was evaluated by recording the primary larval infestation of shoots in summer and the secondary infestation of trees in autumn. Tree infestation in two orchards decreased from 100% to 17.8%, and from 28% to 6.7% after the first season, at a dispenser release of 131 g/ha. The population was maintained at the same level during the following season with 80 and 53 g/ha pheromone released in these two orchards. A single trial in another orchard allowed the control of a higher infestation than in the previous orchards at a pheromone release of 106 g/ha. It appears that moderate populations of this species can be controlled by a release of 15 mg/ha/hr. These first trials allowed the definition of experimental conditions and sampling methods to evaluate the mating disruption method against *Z. pyrina*.

**Key words** - sex pheromone, (*E,Z*)-2,13-octadecadien-1-yl acetate, dispenser release rate, mating disruption, sampling, pome fruit orchard, *Zeuzera pyrina*

### Introduction

La lutte contre les lépidoptères des vergers par des méthodes spécifiques peut favoriser le développement de ravageurs secondaires. C'est le cas notamment aux USA et en Italie pour les tordeuses de la pelure et les mineuses des feuilles lorsque les vergers

de pommiers sont protégés contre le carpocapse *Cydia pomonella* L. par confusion sexuelle. Il en est de même dans les vergers français, où s'ajoutent aux deux groupes de ravageurs précités des dégâts réguliers de *Zeuzera pyrina* L. Des diffuseurs chargés du composé principal de la phéromone de cette espèce, le (*E,Z*)-2,13-octadécadien-1-yl acetate (*E2,Z13-18Ac*), ont été évalués comme moyen de lutte par confusion pendant deux années consécutives dans deux vergers des environs d'Avignon.

## Matériel et méthodes

### Vergers expérimentaux

Les essais sont implantés sur deux sites des environs d'Avignon (Tableau 1). (1) A Avignon, sur le domaine St Paul (INRA). La parcelle, de 0.5 ha, est plantée d'une variété résistante à la tavelure. Elle est bordée au nord et au sud de haies composites et jouxte un verger de poiriers de 0.5 ha. (2) A Les Vignères chez un arboriculteur (R. Mestre). L'implantation est réalisée sur deux vergers contigus (M12, variétés Golden Smoothee et Grany Smith; M13, variété Golden Delicious). Les parcelles sont séparées par des haies monospécifiques de cyprès et de peupliers, et entourées d'autres vergers de pommiers sur la même exploitation.

Les vergers des deux sites sont protégés par confusion sexuelle en 1991 et 1992 contre la sésie du pommier, *Synanthedon myopaeformis* Borkh, ainsi que contre *C. pomonella* et la tordeuse de la pelure *Pandemis heparana* D.&S. en 1991. La lutte contre *C. pomonella* en 1992 est réalisée avec une préparation de virus de la granulose.

### Diffuseurs

La phéromone de synthèse est diffusée par des ampoules RAK 8 (BASF, Ludwigshafen) de couleur brun rouge, chargées en 1991 à 234 mg et en 1992 à 160 mg, à 85% de pureté du composant majoritaire de la phéromone sexuelle de *Z. pyrina*, le *E2,Z13-18Ac* (Frérot *et al.* 1986).

Les diffuseurs sont installés en une seule pose: en 1991, le 29 mai, avant le début du vol de *Z. pyrina* (234 g de phéromone/ha); en 1992, les 22 et 25 mai (première capture au piège lumineux le 1er juin) à raison de 80 à 160 g/ha selon la parcelle (Tableau 1). Le dispositif est établi de manière à garantir une diffusion au moins égale à 15 mg/ha/h.

Sur les deux sites, des diffuseurs sont placés en protection sur les haies et sur les vergers contigus, à la même densité que sur les essais: sur la totalité du verger de poiriers sur le site INRA, sur une zone tampon de 40 m à Les Vignères.

### Evolution de la diffusion

Elle est mesurée par pesée hebdomadaire des 20 mêmes ampoules pendant une période de 18 semaines en 1991 (du 4 juin au 8 octobre) et de 19 semaines (du 26 mai au

19 octobre) en 1992. En fin de campagne (8 octobre et 6 octobre en 1991 et 1992), le contenu des diffuseurs utilisés pour la pesée ainsi que cinq autres diffuseurs prélevés au hasard dans le verger sont analysés par chromatographie en phase gazeuse (A. Buschmann, BASF).

**Tableau 1** Caractéristiques des vergers d'essai et quantité de phéromone installée

Parcelle	Superficie plantée (ha)	Arbres /ha	1991		1992	
			Diffuseurs /ha	E2,Z13-18Ac (g/ha)	Diffuseurs /ha	E2,Z13-18Ac (g/ha)
Avignon INRA	0,44	625	1000	234	750	120
Vignères M12	0,71	1665	1000	234	500	80
Vignères M13	1	830	1000	234	1000	160

#### Evolution des populations de *Z. pyrina*

Les populations initiales sont évaluées en 1991 par comptage des dépouilles nymphales en cours de vol et en 1992 par dénombrement des larves actives (rejet de sciures au printemps). L'évolution des populations est suivie par contrôle visuel des attaques primaires sur pousses en été, puis des attaques secondaires sur troncs et charpentières à l'automne.

L'examen porte sur tous les arbres de la parcelle INRA, au nombre de 252, et sur un échantillon de 540 arbres des parcelles Les Vignères (totalité des arbres des deux rangées centrales et de deux rangées de chacune des bordures nord et sud, soit 273 arbres en M12 et 227 arbres en M13).

En 1991, les contrôles visuels estivaux sont destructifs, les pousses infestées étant sectionnées. En 1992, le contrôle est non destructif. Chaque arbre porteur d'une pousse infestée au moins est marqué. Il est considéré comme atteint et n'est pas examiné lors du contrôle suivant. Les coordonnées de chaque arbre attaqué sont relevées afin de cartographier l'évolution de l'infestation.

## Résultats

### Diffusion de la phéromone

En 1991, la perte de poids hebdomadaire des diffuseurs chargés à 234 mg varie entre 0.8% (18ème et dernière semaine) et 5.4% (9ème semaine) de leur charge initiale. Si l'on excepte la dernière semaine correspondant à des basses températures, après la fin

du vol de *Z. pyrina*, le taux minimal de diffusion est de 1.2% de la charge, soit 16.7 mg/ha/h, en 3ème semaine. La quantité de phéromone diffusée est fortement corrélée avec la température. D'après cette méthode gravimétrique, 44,4% de la charge totale resterait dans les ampoules à l'issue des 18 semaines de diffusion, correspondant à 2809 degrés-jours. L'analyse chimique du contenu des diffuseurs révèle qu'en fin d'expérimentation le taux de *E2,Z13-18Ac* est en fait de 18% de la charge initiale. La méthode gravimétrique conduit donc à une sous estimation sensible de la diffusion.

En 1992, la quantité de phéromone restant dans les diffuseurs (modèle différent du modèle 1991) à l'issue des 19 semaines d'expérimentation est évalué à 33.5% de la charge initiale d'après la méthode gravimétrique. Le taux de diffusion hebdomadaire varie de 1.6% (1ère semaine) à 6% (11ème semaine) de la charge initiale. Compte tenu du nombre de diffuseurs à l'hectare, la quantité de phéromone émise varie donc de 7.6 à 28.6 mg/ha/h dans la parcelle M12, 11.4 à 42.9 mg/ha/h dans la parcelle INRA, et 15.2 à 57.1 mg/ha/h dans la parcelle M13. Elle est inférieure au seuil de 15 mg/ha/h dans la parcelle M12 pendant les trois premières semaines ainsi qu'en 12ème et 18ème semaine, sans doute également pendant plusieurs jours des cinq semaines ou le seuil est juste atteint. Dans les deux autres parcelles la diffusion reste supérieure au seuil, pendant la période de vol de *Z. pyrina*.

#### Evolution des populations de *Z. pyrina*

*Année 1991.* Les estimations des populations initiales de *Z. pyrina* par le comptage des attaques secondaires à l'automne 1990 et des dépouilles nymphales pendant la campagne 1991 sont convergentes (Tableau 2), avec une infestation plus forte à l'INRA que sur la parcelle Les Vignères.

**Tableau 2** Evolution des populations de *Zeuzera pyrina* en vergers protégés par confusion sexuelle

Parcelle	1990	1991		1992			
	Infestation secondaire (%)	Dépouilles nymphales /ha <sup>a</sup>	Infestation primaire (%)	Infestation secondaire (%)	Population initiale <sup>b</sup>	Infestation primaire (%)	Infestation secondaire (%)
Avignon INRA	100	11,4	52,1	17,8	6,8	47,4	20,7
Vignères M12	28	6	15,8	6,7	5,9	16,1	2,6 <sup>c</sup>
Vignères M13					35	29,1	8,8

<sup>a</sup> Comptage le 25/07/96; <sup>b</sup> Larves actives par hectare au printemps; <sup>c</sup> Opération de taille des arbres avant l'observation

Les attaques primaires sur rameaux en 1991 sont en conséquence plus nombreuses sur la parcelle INRA, avec un dépassement du seuil d'intervention fixé à 10% de rameaux infestés dès la mi-juillet. Le taux cumulé d'infestation primaires atteint 52% à l'issue de la saison. L'infestation reste inférieure à 10% des rameaux sur la parcelle M12, où le taux cumulé d'arbres subissant une attaque primaire est de 15.8% sur l'ensemble de la saison. Sur les deux sites, l'infestation secondaire à l'automne 1991 est en régression sensible par rapport à celle de 1990.

*Année 1992.* Comparés aux infestations secondaires de l'automne 1991 les comptages effectués au printemps 1992 attestent d'une forte mortalité hivernale sur les deux sites, confirmant les résultats d'études antérieures dans la région (Audemard 1967). La parcelle M13, non protégée par confusion en 1993, présente la plus forte infestation initiale en 1992 justifiant l'implantation d'une plus forte densité de diffuseurs. Les populations initiales des parcelles INRA et M12 sont assez proches l'une de l'autre, mais correspondent à un taux d'arbres infestés très inférieur en M12 compte tenu de la densité de plantation.

L'infestation primaire sur la parcelle INRA dépasse les 10% dès le début août pour atteindre un taux cumulé de 47.4% à l'issue de la saison. De même l'infestation secondaire à l'automne présente une valeur proche de celle de 1991 (20.7%). Au regard de l'infestation primaire, la population apparaît également stabilisée par rapport à l'année précédente en M12. La faible valeur de l'attaque secondaire en novembre est en partie imputable à une taille précoce de la parcelle. Dans la parcelle M13, les infestations primaires et secondaires sont inférieures à celle de la parcelle INRA, malgré une population larvaire initiale supérieure.

La cartographie de l'infestation effectuée tout au long de la saison ne révèle d'effet de bordure sur aucun des deux sites, aussi bien au début des attaques primaires qu'après migrations automnales des larves.

## Discussion

Les résultats enregistrés pendant ces deux années d'essai confirment le potentiel des ampoules testées pour la diffusion du *E2,Z13-18Ac*. La quantité de phéromone libérée dépasse en effet le seuil de 15 mg/ha/h sur toute la période de vol de lorsque la dose installée est au moins égale à 120 g/ha.

Les résultats enregistrés pour la maîtrise des populations de *Z. pyrina* sont à moduler du fait de la lutte hivernale contre les larves âgées (pâte insecticide à base de phosalone appliqué à l'entrée des galeries), pratiquée dans la parcelle INRA pour réduire l'inoculum. La comparaison des résultats de 1991 et 1992 doit par ailleurs tenir compte du fait que les comptages destructifs de 1991 ont conduit à réduire l'infestation secondaire constatée à l'automne 1991. Compte tenu de ces réserves, la confusion sexuelle a néanmoins permis de réduire l'infestation en 1991, puis de la stabiliser en 1992 malgré une réduction de la dose de phéromone appliquée à l'hectare.

Dans la perspectives d'études ultérieures, cette première expérimentation a également permis de mieux définir la méthodologie d'évaluation de la lutte par confusion sexuelle contre la zeuzère: (1) l'échec relatif enregistré à l'INRA en 1992 (pas de réduction de la population malgré une quantité de phéromone émise jugée suffisante) peut être lié à une configuration défavorable de la parcelle (taille réduite et isolement déjà cités comme impropres à l'utilisation de la confusion sexuelle contre les Lépidoptères); (2) la méthode d'échantillonnage utilisée en 1992 est bien appropriée à ce type d'étude. Le dénombrement printanier des larves actives permet d'établir l'inoculum de départ des parcelles expérimentales, et peut être complété par un comptage des dépouilles nymphales qui rend compte plus fidèlement du vol que le piégeage sexuel (qui bien qu'aujourd'hui opérationnel pour cette espèce sera probablement inopérant dans les parcelles en confusion). Le cumul des relevés d'infestations primaires constitue l'estimation la plus précise de l'attaque de la parcelle. L'observation régulière de ces pénétrations permet par ailleurs de situer dans le temps les périodes où la confusion sexuelle a été mise en défaut. En 1992, la progression de l'infestation a paru régulière entre la fin du mois de juin et début septembre, ce qui peut être mis en relation avec la régularité de la diffusion de phéromone.

L'ensemble de ces observations reflète dans une large mesure l'impact sur la population de la méthode de lutte utilisée. Bien qu'il soit très difficile de disposer de parcelles de référence en tous points comparables aux parcelles expérimentales, l'observation de parcelles voisines apporterait des compléments d'information sur l'évolution des populations en l'absence de lutte spécifique contre la zeuzère. La lutte par confusion serait à évaluer comparativement, sur un même site, à la lutte par piégeage sexuel massif des mâles (Pasqualini *et al.* 1993) dont les premiers résultats dans le sud de la France paraissent encourageants.

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## Use of pheromones in biological control against *Zeuzera pyrina* L. on hazelnuts in Spain: mass trapping efficiency for different pheromone dispensers

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**Abstract** - *Zeuzera pyrina* L. (Lepidoptera, Cossidae) is a pest of hazelnuts (*Corylus avellana* L.) and apple (*Pyrus malus* L.) in Spain. A study of the methods of control against this pest was conducted in Tarragona (province of Spain) on hazelnuts since 1994. The results of mass trapping during 1995-1996 are given in relation to different pheromone dispensers. Success in this method of control is studied. Mass trapping *Z. pyrina* with pheromones on hazelnuts, improving dispensers, is a good method to control the pest.

**Key words** - sex pheromone, mass trapping, controlled release dispensers, *Zeuzera pyrina* L., hazelnuts, Catalonia

### Introduction

Since 1985, we are working about an integrated control of pests on apple orchards (*Malus sylvestris* Mill.) in Catalonia, NE Spain (Isart *et al.* 1988). In this country, the Lepidoptera *Synanthedon myopaeformis* (Borkhausen), *Zeuzera pyrina* (L.), *Cydia pomonella* (L.), the Aphids *Aphis pomi* (De Geer), *Eriosoma lanigerum* (Hausmann), and the Acari *Panonychus ulmi* (Koch) are important pests on apple orchards. *Z. pyrina* is also the most important pest on hazel nuts orchards (*Corylus avellana* L.) (Torrell & Barrios 1983). Now, we are developing a Project of the EU (AIR3-CT94-1607) which include the study of different pheromone dispensers to catch *Z. pyrina*, in order to use them after for mating disruption. First, during 1995-1996, we have tried the pheromone dispensers for mass trapping, in order to know which is the best one. Different authors (Arias & Nieto 1973, 1983; Fernandez *et al.* 1973; Villaronga 1986, and ourselves) have made preliminary studies about the behaviour of *Z. pyrina*.

## Materials and methods

"Netherlands Organisation for Applied Scientific Research TNO" provides the dispensers with *Z. pyrina* pheromone furnished by "DENKA International B.V.", from Holland. Special "Funnel Traps" for *Z. pyrina* furnished by "SERVIOS, s.r.l.", from Italy, were used.

Four hazel nut orchards were chosen in Catalonia, Tarragona province, NE Spain. Two of them (about 2 ha each) for mass trapping, and the others in order to control the population level and farm infestation.

The distribution of the population of *Z. pyrina* on hazel nuts is alike on apples (Pasqualini *et al.* 1994), but the sample size to pointed out for apple trees is not appropriate for hazel nuts shrubs. The population level before and after the season was made in all orchards, counting the number of active larvae in every infested shrub, and counting the number of infested shrubs.

In 1995, three different dispensers are tried, contrasted with a commercialized one (IPO in Table 1). In 1996, two different dispensers are tried, contrasted also with a commercialized one (ZP03606 in Table 1). In order to know the trap's role itself, in 1996, also a trap without pheromone dispenser was put on the field (none in Table 1). About twenty traps were put on the field in accordance with the orchard size (10 traps/ha), and making a rotation every week.

**Table 1** *Z. pyrina* catches in different orchards with the tested dispensers

	Dispensers	Hortes de Pedrera	Les Sorts
1995	1804-1	12	36
	1804-2	102	181
	1804-3	10	84
	IPO	9	27
1996	Zp-270310	10	137
	Zp-270311	10	63
	ZP03606	107	227
	None	0	0

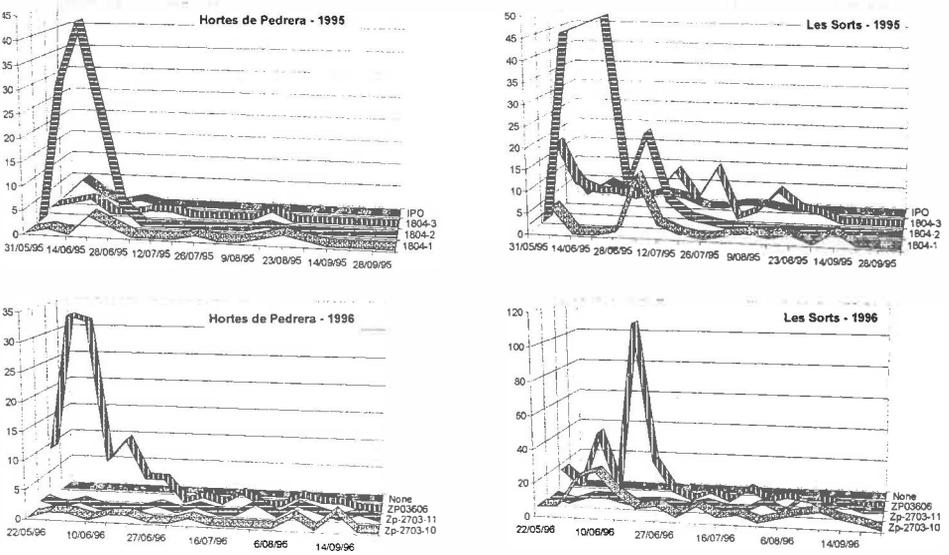
## Results and discussion

Table 1 and Figure show the catches of *Z. pyrina* along the season in different orchards during 1995 and 1996, with every tested pheromone dispenser. In 1995, the best dispenser was 1804-2. In 1996 it was for the Zp03606, but this dispenser was the commercialized one.

The most important in this case is the total catches per farm. All catches in the traps were males. Only two females were caught on the traps in 1996, but only in one farm (Orchard "Les Sorts"). The total catches in every farm was very good, in relation with other times. The great number of catches in "Les Sorts" (1996), must be because the big catches on 15.06.96 (125 males in one week). Probably the success for our method is the location of the traps (Isart *et al.* 1996). We can conclude that mass trapping *Z. pyrina* with pheromones is a successful method of control for this pest on hazel nuts in NE Spain (Table 2).

**Table 2** Number of hazel nut shrubs infested

Farm	Total shrubs in the farm	Observed	Infestation (%)		
			April '96	October '96	New infested shrubs
"Magriña" (Control)	283	203	59.6	56.6	1.5
"Les Sorts B" (Control)	311	311	21.2	23.8	12.9
Hortes de Pedrera	612	393	32.8	16.3	3.8



**Figure** *Z. pyrina* catches with different pheromone dispensers along the season, for 1995 and 1996

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## The use of sex pheromones against *Zeuzera pyrina* L. and *Cossus cossus* L. (Lepidoptera, Cossidae)

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**Abstract** - *Zeuzera pyrina* and *Cossus cossus* are damaging pest for many plants included fruit trees (apple, pear, peach). The chemical and biological control have always shown rare efficiency for both species. The use of synthetic sex pheromones applied according the mass trapping method has been experimented. A comparison between different dispenser types have been carried out. The pheromone blend used has been Z3-10Ac and Z5-12Ac in a 4:1 ratio for *C. cossus* and E2, Z13-18Ac and E3, Z13-18Ac in a 95:5 ratio for *Z. pyrina*. The trap type used has been the pyramidal funnel one without wings under the roof. Also the mating disruption method for *C. cossus* control has been evaluated for the first time. The concept of mating disruption is to permeate the air with a large amount of synthetic sex pheromone. In this condition the males are not able to locate the original source released by natural females. The biotechnological way with use of synthetic pheromones seems to be a real possibility of control even if their application have to be improved particularly for *Z. pyrina*.

**Key words** - sex pheromone, mass trapping, mating disruption, *Zeuzera pyrina*, *Cossus cossus*

### Introduction

*Zeuzera pyrina* is a palearctic pest insect mainly distributed in the Southern Europe with temperate climate, Northern Africa and Middle East. It is a damaging pest for many forest plant and various fruit orchards, among which apple, pear, peach, plum, olive trees and so on. The larvae, which make an axial centripetal gallery, take one or two years to mature. The damages consist of the dry up of the shoots and other vegetative parts, buds, or the death of entire branches. In nurseries or young implantation, the damages could be particularly massive (Liotta & Giuffrida 1965; Castellari 1986; Tremblay 1986).

*Cossus cossus* is a palearctic pest insect with a large euroasiatic and northern-african distribution. It attacks a great number of plants included fruit trees (apple,

pear, cherry, plum, quince, apricot, peach, olive, chestnut and vineyards too). The larva take two or three years to mature. They penetrate into the low parts of the plant, interesting both branches and trunks causing its death.

The chemical and biological control has always been problematic and often unresolved for both species. Therefore the use of synthetic pheromones applied according to the mass-trapping or mating disruption methods, seems to be a real possibility of control (Pasqualini *et al.* 1993, 1995, 1996).

In this report, the results of some experimentation carried out to test the efficacy of dispensers used for mass-trapping in both pests are reported, and the first results regarding the mating disruption method in *C. cossus* too.

## Materials and methods

Serbios funnel trap were used: with wings the ones for mass-trapping in *C. cossus*, without wings for *Z. pyrina*. The pheromone blend was Z3-10Ac and Z5-12Ac in a 4:1 ratio for *C. cossus*, E2,Z13-18Ac and E3,Z13-18Ac in a 95:5 ratio for *Z. pyrina*. We used to put the traps at 1.7 m with respect to the plant and 1 m over the plant for *C. cossus* and *Z. pyrina* respectively.

### Comparison between different dispensers for *Z. pyrina*

Six different dispensers were tested during 1995 (A-1804-1a, B-1804-2a and C-1804-3a from TNO; D from IPO; E from Russell '95 and F from ISAGRO), while four in 1996 (A-2703-10, B-2703-11 from TNO; C from Russell '96, D from Novapher and E from Russell '93). The pattern used was with randomised blocks.

### Comparison between different dispensers for *C. cossus*

Five different dispensers were tested in 1995 (A-1804-4-a, B-1805-4-a and C-1806-4-a from TNO; D from IPO and E from ISAGRO), while three in 1996 (A-2703-238, B-2703-239 from TNO and C from Novapher). The pattern used was with randomised blocks.

Every two weeks each type of dispensers from TNO in comparison was sent to the TNO Research Centre. In that Centre the release of the pheromone blend was detected through the gaschromatographic analysis. The sampling to the traps were effectuated week by week.

### Comparison between different number of traps per hectare for *C. cossus* and *Z. pyrina*

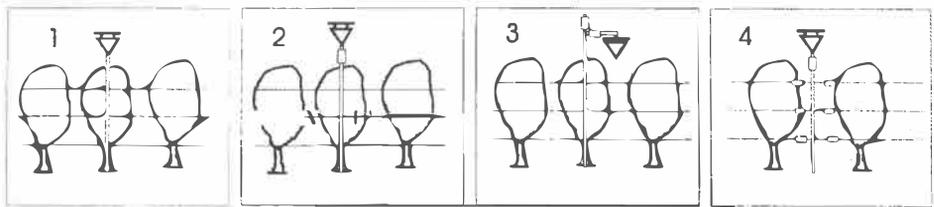
The comparison was effectuated between 10 and 5 traps per hectare in the same field trials. For *C. cossus* two repetitions has been effectuated; for *Z. pyrina*, three. The pattern was a double comparison.

### Mating disruption in *C. cossus* (1996)

The method consist of the application of a high number of dispensers to the orchard that doesn't make the males mate the females. About 500 dispensers per hectare, very easy to put on, have been collocated to the higher branches in reason of 2/3 on the border and the remaining 1/3 on the middle side of the field. The pheromone component was: Z5-dodecenyl acetate (200 mg/dispenser). The experimentation was carried out in two different field trials. Check traps, baited with Novapher dispenser, were collocated inside and outside the field trial at different high respect to the vegetation. The sampling to the traps were effectuated week by week. The samplings to the trunks were planned two times per season.

### Investigations about *Z. pyrina* adults trap approach

In previous and recent experiences some females were caught into the traps. This experience we thought to take information about pest approach to the trap, in particularly regarding females. For this reason some modified trap-systems was made: (1) funnel trap and pole (standard system); (2) funnel trap and pole with a sticky surface (about 10 cm in length just below the trap) around the pole; (3) funnel trap on a lateral support and a sticky surface (about 10 cm in length) both on proximal insertion of this one and on a prolongation of pole toward the high; (4) funnel trap and pole collocated between two plants with sticky surface on iron wires. All the traps used were Serbios model baited by a Russell 1996 dispensers.



**Figure 4.** *Z. pyrina*: experimental conditions

## Results

### Comparison between different dispensers for *Z. pyrina* and *C. cossus* (1995/96)

The data regarding the number of catches obtained in different field trials are reported (Tables 1, 2). They are expressed as average per week and elaborated through the variance analysis (ANOVA), and LSD test ( $p < 0.05$ ). The data have been transformed in

$\log(x+1)$  before. The results show that the better dispenser in 1995 was from Isagro, while in 1996 was the dispenser 2703-239 from TNO.

**Table 1** *Z. pyrina* dispensers comparison: catches obtained in 1995 and 1996

Thesis (1995)	Farm Conselice	Cavicchioli	Govoni	Total	p < 0.05
1804-1A	6	2	2	10	n.s.
1804-2A	9	2	3	14	n.s.
1804-3A	7	2	2	11	n.s.
IPO	7	2	0	9	n.s.
RUSSELL	8	2	1	11	n.s.
ISAGRO	2	0	0	2	n.s.
Thesis (1996)	Farm Conselice	Bornazzini	Fiumazzo	Total	p < 0.05
2703-10	2	0	3	5	a
2703-11	4	1	3	8	a
Russell 1996	17	5	4	26	b
Novapher	11	24	1	36	b
Russell 1993	4	43	10	57	(1 trap)

**Table 2** *C. cossus* dispensers comparison: catches obtained in 1995 and 1996

Thesis (1995)	Farm Govoni	Gavicchioli	Carlo	Total	p < 0.05
IPO	1	0	3	4	a
1806-4-a	2	2	2	6	ab
1804-4-a	3	9	4	16	ab
1805-4-a	4	11	4	19	ab
Isagro	9	9	17	35	b
Thesis (1996)	Farm Vignoli	Ballardini	Folli/Marani	Total	p < 0.05
2703-238	11	12	51	74	a
2703-239	26	30	116	172	b
Novapher	11	15	44	70	a

Comparison between different number of traps per hectare for *C. cossus* and *Z. pyrina*

The results of comparisons, in different field trials between 5 and 10 traps per hectare, are reported (Table 3). The data regard the number of catches obtained, are referred to different years, and are expressed as total and average per trap/week and elaborated through the variance analysis (ANOVA), and LSD test ( $p < 0.05$ ). The data have been transformed in  $\log(x+1)$  before.

**Table 3** *Z. pyrina* and *C. cossus*: catches obtained by different number of trap per hectare, 1994 to 1996

Thesis	1994		1995		1996	
	Total	Average/trap	Total	Average/trap	Total	Average/trap
<i>Z. pyrina</i>						
5 traps	-	-	40	1.14 <sup>a</sup>	40	1.14 <sup>a</sup>
10 traps	-	-	50	0.71 <sup>a</sup>	28	0.28 <sup>a</sup>
<i>C. cossus</i>						
5 traps	15	0.33 <sup>a</sup>	15	0.5 <sup>a</sup>	17	0.34 <sup>a</sup>
10 traps	25	0.27 <sup>a</sup>	33	0.55 <sup>a</sup>	10	0.1 <sup>a</sup>

<sup>a</sup> Differences not significant at  $p < 0.05$

The data regarding both *C. cossus* and *Z. pyrina* are in contrast probably because of the small number of catches, nevertheless there aren't statistical differences.

#### Mating disruption for *C. cossus*

Only one adult was caught in two field trials, while 6 have been caught in the external fields. For a first evaluation we have to wait the results from second sampling on trunks (the next winter).

#### Investigations about *Z. pyrina* adults trap approach

The catches obtained shown by the different experimental configurations shown in Figure were, from left to right: 42 males (d); 22 (ab); 14 (a); 32 (bc). Numbers followed by different letters are significantly different at  $p < 0.05$ .

Unfortunately any female was caught both into the traps and sticky parts on poles. Any males was entrapped on sticky surface confirming that they enter into the traps by flying. Between catches from different experimental configuration the lower ones were obtained by configuration 2, perhaps because of the mobility of the traps.

#### Discussion

The results obtained in the EU Project are substantially positive and encouraging, particularly concerning *C. cossus*. For that species a dispenser supplied by TNO has resulted more attractive than the best one available up to now, while the number of traps to set on each hectare could be reduced from 10 to 5. The results given during the first year about the mating disruption are encouraging. In fact in only one of the two experimental fields one adult has been caught even though the catches on the checked fields haven't been a lot. Because of the biological cycle of this species the first definitive

results could be available only in the year 1998.

The results of many other researches about *Z. pyrina* aren't unfortunately so much satisfactory because of the poor catches obtained with all compared dispensers. This is probably due to the pheromone blend or its release by the dispenser. Although the data obtained are insufficient to get any definite conclusion, it has been possible to observe that the catches are not different between 10 and 5 traps per hectare.

Regarding the fly approach trial, only males have been caught. Not one of them has been found on the sticky parts, therefore it seems the adults get into the trap by flying.

## Conclusion

In 1996 the EU Project AIR-3-CT94-1607 has come to the development of half prearranged program. At the moment, the information which come from the data processing, seem to show two parallel scenarios. The results are substantially positive and encouraging, concerning *C. cossus*. More experimentation has to be done with the mating disruption method, carried out for the first time in 1996. Waiting for new comforting data, two factors appear to condition the choice for one of the two methods: the specific environmental condition and the economic balance regarding the farm.

On the other hand, the results regarding *Z. pyrina* are not so exciting. The trap-dispenser systems tested till now, didn't work yet at the best. If it is possible to suggest the best trap type (Serbios without wings) and its collocation with respect to the plant (1 m over the vegetation), the comparison between different dispenser type hasn't shown good results yet. Probably more data regarding the dispenser, such as the time of release and the doses of pheromone blend, have to be acquired. Moreover it isn't well known the behaviour of this pest, and a specific investigation on the specific ethology should be done. In any case it appears opportune to continue with new investigations, because dispensers more effective could make the mass trapping method applicable with our farm condition (2-3 hectares dimensioned). New investigations should begun regarding the mating disruption method, never tested before in our areas.

## Acknowledgements

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## Mating disruption of corn stalk borer, *Sesamia nonagrioides* Lef. (Lep., Noctuidae)

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**Abstract** - New technologies for formulating sprayable pheromones allow the use of mating disruption techniques to industrial scale crops as cereals. Mating disruption should be considered as an alternative method for monitoring populations of a target insect. On maize crops *Sesamia nonagrioides* (Lef.) is a major pest in southern France and insecticide treatments have low impact on the reduction of population levels. Thus, development of such a method is of interest and can be developed due to apparition of new technologies for formulating sprayable pheromones. Field trials were conducted with a solid sprayable pheromone and with a liquid pheromone, both applied by helicopter over an area of more than 25 ha. The release rate and the quality of the released blend were studied by trapping effluvia, a technique based on dynamic head space. Results showed that the blend released was very different from the initial blend. Release of chemicals depended both on the molecular weight and on the polarity. Both sprayable formulations of pheromone presented lower release rates than pure pheromone, but in field conditions did not last as long as in laboratory conditions. During field tests male trapping showed that the sprayable solid formulation catches were inhibited for about one week. A correlation between efficiency and release rate was established, and it appeared that the dispensers released very high level of pheromone during the first week, whereas this level remained at a level too low to allow mating disruption. The liquid formulation applied twice completely inhibited male catches. Controls of damage showed an efficiency of 48% for the solid formulation compared with an insecticide treated plot and an efficiency of 86% for the liquid formulation. Such results demonstrated that mating disruption could be a good method to monitor populations of *Sesamia nonagrioides* but special attention must be paid to the release rate and to the quality of the blend released.

**Key words** - sex pheromone, mating disruption, head space, release rate, *Sesamia nonagrioides*, Noctuidae, Lepidoptera

## Introduction

The technique of reducing populations by disrupting pheromonal communication with synthetic sex pheromones offers the potential of controlling insect pests without incurring the problems associated with insecticide use. Moreover the use of mating disruption techniques provides an alternative means of reducing insect populations poorly controlled by insecticides. The corn stalk borer *Sesamia nonagrioides* (Lef.) which is a major pest on maize crops in the South of France and all around the Mediterranean area is insufficiently controlled by insecticides due to the fact that larvae develop as stalk borers and are therefore inaccessible to insecticide sprays. The difficulties to control populations by usual insecticide sprays make this insect a good candidate to develop population control by mating disruption in industrial crops.

In Europe mating disruption concerns mainly orchard and vineyard pests (Minks & Cardé 1995). The reason why no trials have been undertaken against industrial crop pests could be the lack of reliable sprayable dispensers which allow conventional spray equipment or helicopter to be used (Frérot 1990). Moreover in European farming methods, especially on large scale, manual application dispensers might be considered as unthinkable. On the other hand, dispenser longevity appears to have also inhibited the use of sprayable microencapsulated formulations (Charmillot & Vickers 1991). New technologies for formulating sprayable pheromones have renewed interest for the use of mating disruption techniques for industrial scale crops as cereals. Sprayable formulations of synthetic pheromone seemed more compatible with large scale agriculture. The method will create a multitude of artificial point sources of pheromones. Recently, Suckling & Angerilli (1996) demonstrated that mating disruption is achieved better if it is caused by an uniform cloud of sex pheromone, though Sanders (1982) showed in wind tunnel conditions that a few strong sources disrupted males more effectively than many diffuse sources. However, this has not been proved in field experiments and the fact remains that early experiments with microencapsulated sex pheromones were efficient (Hall & Marrs 1989). Sprayable dispensers or liquid pheromone formulations will renew interest for diffuse sources.

Successful use of this method requires the release of large amounts of pheromone over the target area during all the flight period of adult insects. Although the mechanism of disruption is still the subject of conjecture and might be multi mechanism (Cardé & Minks 1995), the quality of the released blend is also essential to success. Little information (Knight 1995; Svensson *et al.* 1995; Bradley *et al.* 1995; Witzgall *et al.* 1996) was available on the release rate of multicomponent pheromones released during mating disruption trials. Recent advances in field electroantennogram technique (Karg *et al.* 1990) allow the estimation of air permeation by synthetic pheromone, but it remains difficult to conclude about ambient pheromone concentration and moreover the blend released cannot be evaluated by this tech-

nique (Karg *et al.* 1994). Information about the quality and the concentration of the blend released during the trial is needed to understand success or failure.

Thus, our study was undertaken to determine the relative efficacy of potential communication disruptants of *Sesamia nonagrioides*. The control of release characteristics of both liquid and solid sprayable formulations, *i.e.*: quantity and quality of the blend released in the field during trials, was set up. Comparison of efficiency of the two sprayable formulations and first results on mating disruption against *S. nonagrioides* are presented in this paper.

## Materials and methods

### Field test lay out

The experimental area was situated in main region of maize production, Tarn (France). The field tests, located near La Gardiolle consisted of four blocks of maize field sharing the same characteristics of cultures: *i.e.* Sowing late April-begining of May, cv Cecilia, density: 49 500 to 76 400 plants/ha. The field did not receive insecticide treatment during the first generation of *S. nonagrioides*.

Disruption trials were conducted only on the second generation, on two plots immediatly adjacent - Plot I (14.5 ha); Plot II (12 ha). Plot I received a sprayable solid formulation of synthetic pheromone whereas Plot II was spread with liquid formulation of synthtetic pheromone. Treatments, whatever the formulation, were applied by helicopter at the same dose of pheromone:100 g/ha. A second spray was made at the dose of 50 g/ha for Plot II, 15 days after the first spray.

Two untreated control plots (UCP) were for the first (9 ha) located closed to experimental plots and for the second (2.5 ha) located 3 km away. Insecticide control plot (ICP - 25 ha) located 5 km away from the experimental plots received a single application by helicopter of Lambdacyhalothrin at 15 g active ingredient/ha (0.3 l/ha of Karate).

According to Mazomenos' sex pheromone identification of *S. nonagrioides* (1989) the following blend was used for the trials Z11-16Ac 69%, Z11-16OH 8%, Z11-16A1 8%, 12-Ac 15%. For plot I, the sex pheromone was first dissolved in a specific extender (Elf-Atochem) and then included in technical polymer microgranules (Elf-Atochem) of 80 µm diam. Miscellaneous adjuvants and inert clay were mixed and pulverisation was applied at the dose of 25 kg/ha for 100 g of pheromone.

Concerning plot II, synthetic sex pheromone was dissolved in the specific extender (QSP 1L) and was applied by helicopter at low volume (40 l/ha).

Traps, built specifically for sexual trapping of *S. nonagrioides* by Plant Protection Institute were used. They were baited either with virgin females or with synthetic lures. Three virgin females kept in wire mesh containers and renewed twice a week were placed in traps for Plot II, UCP and ICP. Each experimental plot and control plot contained a trap baited with a cap filled with 1000 µg of synthetic phero-

none. Pheromone caps were renewed each fortnight and traps were checked three times per week.

### Control of infestation

In the first generation, before mating disruption experiments, 1500 stalks (300 stalks in 4 areas chosen following a diagonal plus 300 stalks selected at random) per experimental plot were controlled and specific damage was noted on the plants.

In second generation, 500 plants per experimental and control plots were dissected in order to assess species of insect and larvae instars. The 500 stems were chosen in four spots following a diagonal plus 100 stems at random in each plot.

### Control of release rate

Evolution of quality and quantity of pheromone which was released during field trials was followed up during eight weeks. Sachets containing 5 g of the microgranule formulation used for treatment on plot I were hung in the field and sampled each week for analyses. Two replications per treatment per week were made. Each sachet represented 1/50 ha, *i.e.*: 200 m<sup>2</sup>.

Analyses were made by head space collection of air borne volatils on resin (Frérot *et al.*, 1992). Samples were placed in a cylindrical glass container (5 cm x 2.5 d) connected on one side with a trap filled with 5 g of Supelpack<sup>TM</sup> 2 (16/50 mesh ; purified résin Amberlite XAD 2, SUPELCO) and on the other side, downwind with an air purifier filled with 40 g of Amberlite XAD 16 (SUPELCO). The air flow (100 ml/min.) was generated for 24 h by a vacuum pump and flown through the device from the air purifier cartridge to the effluvia trap cartridge. Desorption of the resin contained in the trap cartridge was achieved by washing the resin in a minimal volume of CH<sub>2</sub>CL<sub>2</sub> (0.5 ml). After a few minutes of maceration and shaking, the solution was filtered over glass wool and then stored at -27°C before analyses. Effluvia sampling was obtained under the following conditions: 25 ± 2°C and 80 ± 5% R.H. and quantities of each sample introduced were equivalent to 92 mg of pure pheromone.

Quantity of the remaining pheromone was evaluated by complete extraction of solid samples in ethanol. The complete sachet was placed in 80 ml of ethanol (organic solvents are not compatible with the technical polymer). The resulting solution was shaken using a vortex for 10 min, then filtered over glass wool and analysed using the following GC conditions.

### Analytical procedure

Gas chromatographic analyses (GC) were carried out with a 5890 Hewlett Packard gas chromatograph fitted with a split-splitless injector (240°C) and a flame-ionization detector (260°C). The fused silica capillary column used was a CPSil8 CB (25 m x

0.32 ID, Chrompack) with temperature programming from 90°C to 140°C at 20°C/mn and from 140°C to 260°C at 5°C/mn. Helium was used as the carrier gas at 10 psi pressure. Two  $\mu$ l were injected.

Gas chromatographic-mass spectrometric (GC-MS) analyses were used to confirm identities of compounds. GCMS was carried out with a Nermag R10-10C quadrupole mass spectrometer coupled to a Girdel 32 gas chromatograph equipped with a split-splitless injector and a CPSi18 CB fused silica capillary column (25 m x 0.32 ID, Chrompack) with temperature programming from 90°C (2 min) to 240°C at 8°C/mn. Electron impact (EI) mass spectra were obtained at 70 eV, the instrument scanning from 40 to 350 amu. Calibration curves were obtained by GC for 12Ac and for Z11-16Ac and following equations were obtained for 12Ac Surf=(8.13732 x Quantity)-21.15515. The same equation was applied to Z11-16Al, Z11-16OH and Z11-16Ac Surf=(8.66254 x Quantity)-3.85434.

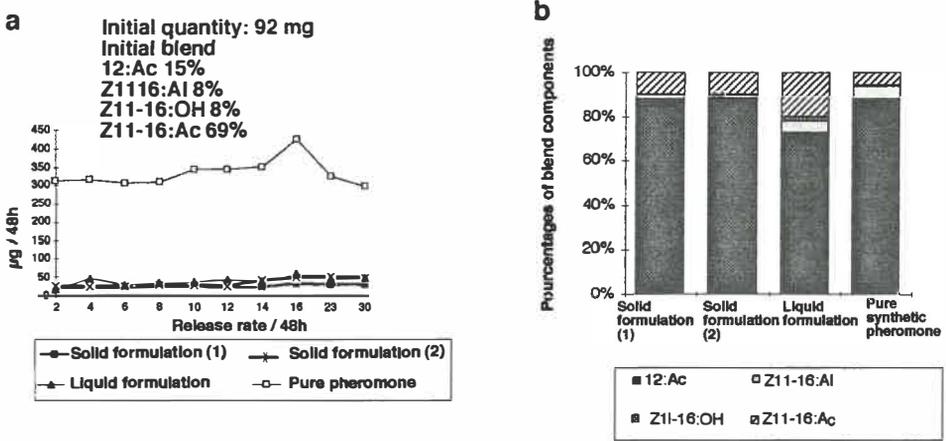
## Results

### Release rate of pheromone

Figure 1a shows results obtained in laboratory conditions by collecting compounds released by liquid and solid sprayable formulations compared with pure pheromone. It appeared that both solid and liquid formulations behaved similarly. The release rate of the pure pheromone was higher than that of the sprayable pheromone indicating that the specific extender and the technical polymer slowed down the release rate of the pheromone. However, Figure 1b shows that the blend released was different from the blend introduced. Release rate of pheromone components depended mainly on the molecular weight and the polarity of the molecule. 12Ac was the main compound released even if present only at the ratio of 15%. The main component of the pheromone blend Z11-16Ac was released at the same rate as Z11-16Al (2% to 5%) and the most polar compound the Z11-16OH represented only 0.1% to 0.5% of the released blend.

Laboratory results showed a constant release rate which lasted 30 days, but solid formulation kept in natural conditions and analysed after 30 days showed no detectable compounds, demonstrating that there was a disparity between natural and laboratory conditions. Thus, analyses of pheromone quantities remaining in sachet were made for the solid formulation and it was decided to apply a second spray.

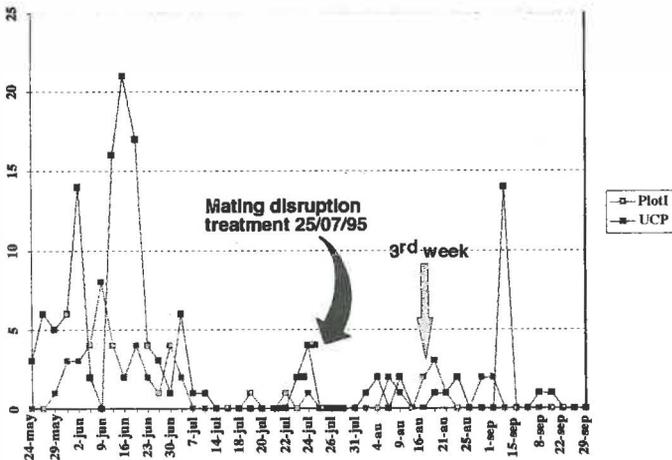
Results of release rate from the solid formulation showed that the release rate during the first week in the field was very high, *i.e.*: 309 mg/ha/hr. After one week 50% of the load had been released but in the following weeks the release rate decreased considerably and reached 17.5 mg/ha/hr the third week, a level under that which the mating disruption is known to be achieved. Regular emission was not obtained under natural conditions and was probably dependant on the ratio between synthetic pheromone, extender and technical polymer.



**Figure 1** Results of effluvia collections: a) release rate of solid and liquid sprayable formulations; b) quality of the blend released

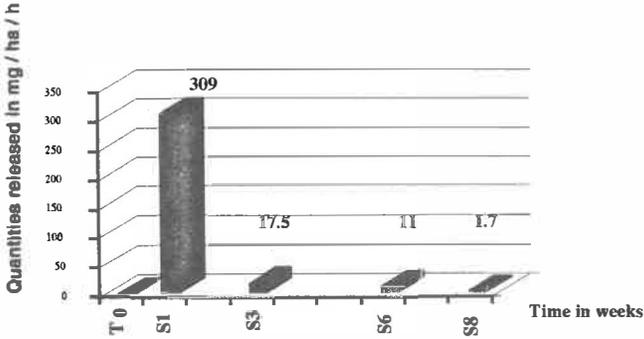
Field trapping and damage level before and after mating disruption experiment

Fourteen days before mating disruption treatment, damage rates per experimental plots Plot I and II, were assessed at 2.6 %, whereas untreated plots UCP were respectively 1.3 % and 4.1 % and insecticide treated field was 6.3 %. Field trapping with either virgin females or synthetic lures before mating disruption experiment evidenced

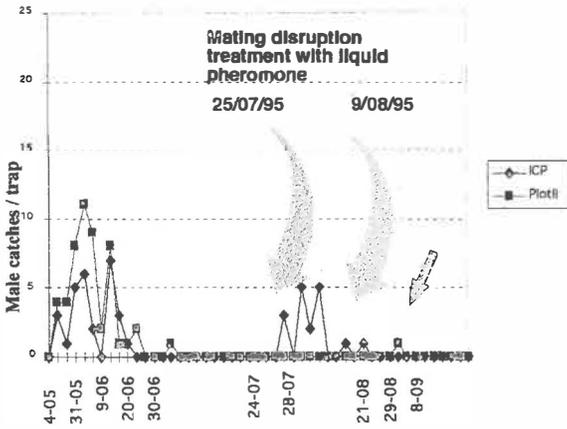


**Figure 2** Results of male trapping in plot I, treated with solid sprayable formulation and in the untreated control plot (UCP)

a high level of males caught in UCP (116 per trap) compared with ICP (27), Plot I (47) and Plot II (46), both almost equivalent (Figures 2 to 4). After mating disruption treatments, traps continued to catch males in both control plots (UCP, ICP). On plot I a reduction of catches was achieved during the first 12 days, then catches resumed showing that mating disruption was not achieved. These results corroborated the re-release rate results, demonstrating that after three weeks the amount of pheromone was too low to allow mating disruption. On plot II reduction of catches was achieved throughout the experiment. Comparison with control plots confirmed that the second half dose spray was necessary.



**Figure 3** Quantity of pheromone released in the field from solid sprayable formulation of pheromone. Results were obtained by complete extraction. During 8 weeks 64.1 g/ha were released, 35.9 g/ha remained in solid formulation



**Figure 4** Results of male trapping in mating disruption plot treated with liquid formulation (Plot 2) and in control plot (ICP)

Last control of damage (25/09) showed (Table 1) that Plot II was significantly different from other plots with the lowest level of damage attesting that pheromonal communication was disrupted.

**Table 1** Percentages of damage obtained after dissection of 500 plants on each plot. Different letters mean a significant difference ( $p \leq 0.05$ )

	% of damage on stalk	% of damage on corn cob
Plot I. solid sprayable formulation	6.8 a	0.4 a
Plot II. liquid sprayable formulation	1.8 b	0 b
ICP. insecticide control plot	13.2 a	0.6 a
UCP. untreated control plot 1	9.4 a	1.4 a
UCP. untreated control plot 2	31.5 c	2.5 c

Observations of instar larvae (Table 2) showed that in all the cases there were no young larvae L1 and L2. Plot II was the only one to have no L3 and no L5 evidencing that matings had not occurred late and early during the adult flight period. In Plot II the number of L4 was the lowest, even if not significant. The most abundant stages in all plots considered were L3 followed by L4. Considering that 30 to 35 days are necessary at an average temperature of about 20°C, which were the average field conditions, for eggs to reach the third and fourth instar larvae (Hilal 1978), mating had occurred around August 27<sup>th</sup> for L3 and around August 22<sup>nd</sup> for L4. This information added to results of field trapping allowed us to conclude that Plot II was fairly well protected until August 22<sup>nd</sup>. To reach fifth instar about 40 days were required under the current climatic conditions. Consequently L5 would have been oviposited around August 15<sup>th</sup>, period during which mating disruption was not achieved in plot I.

**Table 2** Mean numbers of each instar larvae per dissected plant

	L1	L2	L3	L4	L5	L6
Plot I. solid sprayable formulation	0	0	0.05 ab	0.03 a	0.01 ab	0 a
Plot II. liquid sprayable formulation	0	0	0 a	0.01 a	0 ab	0 a
ICP. insecticide control plot	0	0	0.14 b	0.06 a	0.1 ab	0 a
UCP. untreated control plot 1	0	0	0.09 ab	0.04 a	0.03 ab	0 a
UCP. untreated control plot 2	0	0	0.28 c	0.16 b	0.06 b	0.2 b

## Conclusions

Disrupting pheromonal communication with synthetic sex pheromone, formulated for application by helicopter was obtained against *S. nonagrioides* leading us to consider the method as promising against cereal pests (Table 3). The final assessment of damage showed a significant reduction of larvae population in plot II whereas plot I treated with solid formulation was not different from control plots. This negative result in plot II was directly correlated with the insufficient quantity of pheromone released after three weeks. These controls of the infestation appeared to be very important in order to reach a conclusion on the efficacy of the trials and validity of field trapping. Although field trappings gave information about insects flying in the field, they were poorly correlated with the level of damage.

**Table 3** Final results from field assessment data. Percentage of efficacy were calculated as follows:  $(\% \text{ of damage in the control plot} - \% \text{ of damage in the considered plot}) * 100 / \% \text{ of damage in the control plot}$ . \* mean difference significant  $p \leq 0.5$

	UCP I	UCP II	ICP
Plot I	27.6%	79%	48.5%
Plot II	80.9%*	94.4%*	86.4%*

The method used to control the release rate in terms of quantity and quality requires a minimum of specific equipment, but is necessary for the understanding of mechanisms and limitation of the mating disruption. Results obtained on the quality of the blend released indicate that special attention must be paid when the target insect produces a multicomponent sex pheromone. Release rate of compounds is directly related to molecular weight and polarity more than to quantity introduced. These physical properties could limit the development of such a method to monitor pest populations. In the case of *S. nonagrioides*, reinvestigation of the pheromone produced by the females (Frérot *et al.* 1996) did not evidence 12Ac and Z11-16Al either on the gland surface or in gland extracts. Further investigation will be undertaken to verify the effect of each component on chemical communication between males and females. In addition, the solid formulation will be modified and improved in order to calibrate and keep the release rate more regular.

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## Mating disruption field trials to control the olive moth, *Prays oleae* Bern: a four-year study

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**Abstract** - Mating disruption to control the olive moth, *Prays oleae* Bern (Lepidoptera, Yponomeutiidae) was applied for four consecutive years in an olive grove in Greece. The major sex pheromone component (Z)-7-tetradecenal was formulated in  $\beta$ -cyclodextrin and polyvinyl chloride polymers. Pheromone trap catches were reduced to 96 to 100% in the treated plots and fruit infestation remained at commercially acceptable levels. During the first year of the mating disruption programme, a treatment with *Bacillus thuringiensis* (var. *kurstaki*) was applied to reduce the first generation larvae. The effectiveness of the method depends on the availability of oviposition sites.

**Key words** - sex pheromone, mating disruption, (Z)-7-tetradecenal, olive moth, *Prays oleae*, Yponomeutiidae, Lepidoptera

### Introduction

The olive moth *Prays oleae* is one of the most serious pests of olives in the Mediterranean basin. This moth has three generations per year. The first generation appears in April and the females lay their eggs on the blossoms, the hatching larvae live and feed within the blossom and on the flower at the later stage of their development. The second generation emerging early June is the most economic damaging. The females lay the eggs on the small fruits close to the stem, that holds the fruit, the larvae bores within the stone of the olive fruit and when they complete their development in September, coming out from the fruits are causing spectacular fruit drop with major crop losses. The third generation attacks the leaves the emerging larvae mine the olive leaves in autumn hibernate as larvae and complete their development early next spring. Two to three sprays by chemical pesticides are required annually to prevent premature olive fruit drop.

The major sex pheromone (Z)-7-tetradecenal (Z7-14Ald) produced by the female olive moth was identified by Campion *et al.* (1979). Pheromone traps have been used to monitor the moth population and have been found to be very effective in trapping males (Ramos *et al.* 1988). An integrated pest management system that uses phero-

mone baited traps to monitor the olive moth population and to time the application of control measures has been reported (Ramos *et al.* 1989).

Pheromones are used in mating disruption trials to control several lepidopterous species (Cardé & Minks 1995). Here we present the results obtained when the mating disruption method was evaluated, for four consecutive years. The effectiveness of the mating disruption was assessed by male captures in pheromone traps and measuring fruit infestation levels in mating disruption plots compared with those treated with *B. thurigiensis*, or left untreated.

## Materials and methods

*Plot selection and description.* The area where the method was evaluated is located in the province of Attikis. The grove approx. 8 ha, is the main grove in this region surrounded by vineyards, pistatio and pasture fields. The grove was divided into 2 plots, a third olive field approx. 2 ha and 300 m apart was used as control this field was left untreated during this study.

*Pheromone formulations.* The major sex pheromone component Z7-14Ald was formulated in  $\beta$ -cyclodextrin ( $\beta$ -CD) (Mazomenos & Moustakali-Mavridis 1993), or black polyvinyl chloride (PVC) strings (AgriSence BCS Ltd., Pontypridd, UK).

*Mating disruption.* The pheromone dispensers were applied prior to the emergence of the moths that give rise to the second generation. The pheromone formulated in ( $\beta$ -CD) was dispensed from plastic bags. The release rate of the pheromone was measured by capillary GC and was ranged from 43.2 mg/h/ha initially to 12.5 mg/h/ha, 15 weeks later. The pheromone release rate was not measured for (PVC)-Z7-14Ald formulation, according to the producer's this formulation release sufficient amounts of pheromone for mating disruption for 45-50 days. Three bags or (PVC) strings were hanged at head height outside the canopy of each tree the pheromone concentration per hectare was 40 g.

## Results and discussion

### Trap catch

The total number of males captured in pheromone traps for each generation every year in pheromone treated, or untreated plots are presented in Table. During the first year of the experiment in 1992 the grove was treated with *B. thurigiensis*, when 10% of the flowers were open. In order to suppress the larvae population of the flower generation and minimise the potential failure of the mating disruption treatment.

The number of males caught in pheromone baited traps in the plot 2 that received only a *B. thurigiensis* treatment was slightly reduced compared to the males caught in the untreated plot. The  $\beta$ -CD pheromone dispensers installed in plot 1, prior to the emergence of the second generation inhibited male captures in the pheromone

traps by 99.2%. The number of males caught in the pheromone traps during the third generation September and October was also low. This indicated that the pheromone concentration within the treated plot remain at relatively high levels resulting in a high proportion of male disorientation. In 1993 the moth population was low in the pheromone treated plot during the first generation. All the plots were left untreated in this generation. At the second generation where plot 1 and 2 were treated with  $\beta$ -CD and PVC dispensers respectively, 98% reductions in trap catches were achieved. Reduction in trap catches was also observed during the third generation. In 1994 and 1995 the moth population in plots 1 and 2 was low in the first generation. The  $\beta$ -CD pheromone dispensers applied prior to the second generation completely inhibited pheromone traps catches. The level of male disorientation to pheromone traps was also high for the third generation and reached 99.4%.

**Table** Pheromone traps catches of *Prays oleae* males in plots treated with pheromone dispensers (mating disruption), *Bacillus thuringiensis*, and untreated.

Generation Plot	1 <sup>st</sup>			2 <sup>nd</sup>			3 <sup>rd</sup>		
	1	2	3	1	2	3	1	2	3
1992 <sup>a</sup>	794	862	873	3	290	553	17	168	283
1993 <sup>b</sup>	180	348	399	2	1	228	11	7	254
1994 <sup>c</sup>	133	123	370	0	0	425	2	3	249
1995 <sup>c</sup>	235	245	835	0	0	1284	3	2	545

<sup>a</sup> Plots 1, 2 were treated with *B. thuringiensis* during the 1<sup>st</sup> generation, plot 1 was treated with  $\beta$ -CD-Z7-14Ald dispensers during the 2<sup>nd</sup> generation; <sup>b</sup> plot 1 was treated with  $\beta$ -CD Z7-14Ald dispensers, plot 2 with PVC-Z7-14Ald dispensers during the 2<sup>nd</sup> generation; <sup>c</sup> plots 1, 2 were treated with  $\beta$ -CD-Z7-14Ald dispensers during the second generation.

### Fruit infestation

In 1992 the level of fruit infestation in plot 1, remained low 3.4% of the olives were infested while in *B. thuringiensis* and the untreated plots 33% and 26% of the fruit were infested. In 1993 although the male catches to the pheromone traps were reduced by 98%, the fruit infestation was 32% and 30% for plots 1 and 2 respectively compared to 38% found in the untreated plot. The high level of fruit infestation during this year is the result of limited oviposition substrate available to the females because of the year's very low olive fruits production. 1994 was a year of high fruit production and the level of fruit infestation remained below the 2% in the treated plot compared with the 28% observed in the untreated plot. In 1995 the fruit infestation in mating disruption plots remained at low level, only 3.7% of the fruits were infested compared to 27% found in the untreated plot, despite the fact that 1995 was a low fruiting year.

## Conclusion

The results presented here suggest that the mating disruption can be used successfully to control the olive moth. Both pheromone formulations used, proved to be very effective controlled release devices for the pheromone in the field and both provide adequate protection of the aldehyde molecule from rapid degradation. The pheromone release rate seems to be higher in the (PVC) dispensers and they need to be replaced at least once to provide satisfactory male disorientation for the three adult flight periods. The ( $\beta$ -CD)-pheromone complex maintained high pheromone release rate for longer period. It was also observed that the effectiveness of the mating disruption depends on the availability of ovipositional sites. In a low fruiting year, fruit damage is higher compared to that of a high fruiting year. Continuous application of these methods in the same olive groves progressively reduced pest population from one year to the next.

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## **Control of Douglas-fir tussock moth, *Orgyia pseudotsugata*, using a pheromone and virus treatment**

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**Abstract** - Virtually complete mating disruption of Douglas-fir tussock moth, *Orgyia pseudotsugata*, has been demonstrated by applying 72 g/ha of synthetic sex pheromone (*Z*)-6-heneicosen-11-one in polyvinyl chloride beads. This work marks the first successful demonstration of mating disruption of a forest pest in Canada. A similar result was obtained when the dosage was reduced below 20 g/ha. Application of a nuclear polyhedrosis virus is also known to be highly effective. Circumstances occur where a combined application of pheromone and virus may be more effective than either treatment alone. Examples are given based on practical considerations of the spray application, and on the possible biological properties of the applied spray developed from modeling.

**Key words** - sex pheromone, mating disruption, virus treatment, *Orgyia pseudotsugata*, Lymantriidae, Lepidoptera

### **Pest bionomics**

In British Columbia the Douglas-fir tussock moth, *Orgyia pseudotsugata* McDunnough, typically damages trees in the same area about every ten years (Brooks *et al.* 1978). Defoliation of its main host, Douglas-fir, *Pseudotsuga menzeisii* Franco var. *glauca* Beissn. is sudden, and severe damage occurs in the first year of a pest outbreak. At this time defoliated trees are in distinct groups, but these groups usually merge in following years. Some branches, especially near the tree top are killed at first, but during the course of a normal three-year outbreak many trees are totally defoliated and die. The pest outbreak ends as abruptly as it started, and coincides with an extensive fatal viral infection of the larvae.

Tree damage results from larval feeding in May, June and July, when fresh needles and then older needles are consumed. Most spread of the pest occurs at this stage as small larvae disperse widely on silken threads blown by the wind, and larger

larvae crawl to other trees. Following pupation in late July, adults emerge from their cocoons in early August. The female cannot fly, and emits a sex pheromone to attract the winged male to mate with her on her cocoon. She subsequently lays all of her fertilized eggs in groups on her cocoon. The eggs remain dormant throughout the winter, and hatch the following spring shortly after buds on the Douglas-fir trees have begun to burst.

### Two methods of pest control demonstrated in British Columbia

Apart from using chemical insecticides, two successful control treatments have been demonstrated in British Columbia, one based on mating disruption by applying synthetic sex pheromone, and the other based on a virus application.

#### Application of synthetic sex pheromone

Virtually total disruption of mating of *O. pseudotsugata* was demonstrated recently, by applying a synthetic sex pheromone (Hulme & Gray 1994). It is the first successful demonstration of mating disruption of a Canadian forest pest.

The main component of the sex pheromone of *O. pseudotsugata*, (*Z*)-6-heneicosen-11-one, was synthesized, impregnated into polyvinyl chloride beads 250-400  $\mu\text{m}$  in diameter, and applied using standard spray equipment. Application of 72 g/ha of the pheromone either from the ground or from the air completely prevented mating, measured by the number of male moths caught in sticky traps baited with feral females, or by the number of fertile eggs masses produced by wild females on trees in the sprayed areas.

When the pheromone was applied from the ground, substantial amounts of pheromone continued to be emitted one year after application (Gray & Hulme 1995). The ground application was conducted by simply applying the pheromone spray about 10 m up the trunk of each tree. These pheromone-impregnated beads were thus less exposed to weathering than aerially-applied beads which would accumulate on the outer foliage of the trees. One year after spraying, sufficient pheromone was still being emitted from the ground-sprayed beads to reduce the number of male moths caught in sticky traps baited with feral females by 80% compared with numbers caught in traps in unsprayed areas. In contrast, little of the aerially-applied pheromone continued to be emitted one year after application, since no significant reduction in numbers was found between trap catches in areas that had been treated aerially and those in unsprayed areas.

More recent work has shown that the dosage of applied pheromone could be reduced below 20 g/ha without significantly altering the biological effects observed applying 72 g/ha of pheromone (Hulme & Gray 1996). Furthermore, an elusive minor component of the sex pheromone of *O. pseudotsugata* has just been identified as (*Z,E*)-6,8-heneicosadien-11-one (Gries *et al.* 1996). Trap catch remained the same

when this newly identified dienic ketone was mixed with the monoenic ketone in the ratio 1:10 and the amount of pheromone in the lures was reduced 50-fold from that used with the monoene alone (Gries *et al.* 1996). Since excellent mating disruption has already been obtained with less than 20 g/ha of the monoene (Hulme & Gray 1996), prospects appear good for successfully disrupting mating using considerably less than 20 g/ha of the pheromone blend.

#### Application of virus

A second pest control method demonstrated in British Columbia is based on application of the nuclear polyhedrosis virus that is widely found naturally in larval populations near the end of a pest epidemic (Shepherd *et al.* 1984). This virus has been propagated in white-marked tussock moth, *Orgyia leucostigma* Smith, and an aqueous suspension of milled insect parts comprising about 5% polyhedral inclusion bodies (PIB) is prepared from lyophilized virus-infected larvae. A viral epizootic is initiated by spraying this suspension from an aircraft or from the ground. When 22 billion PIB/ha were applied from aircraft or 2 billion PIB per tree from a ground sprayer, pest control was so successful that no fertile eggs could be found (Shepherd *et al.* 1994).

The virus should be applied soon after eggs hatch to allow the virus time to replicate and spread through the larval population prior to pupation. Foliage is not protected in the year of application because the larvae still destroy foliage before they sicken and die from the viral infection. Early treatment is essential both to increase the presence of the epizootic and to reduce foliage loss. During the year of application, control using the virus is about 60%. The infected larvae assist in spreading the virus throughout the treated area to infect the next generation of larvae.

The virus is now applied operationally in British Columbia following routine monitoring of male moth populations using sticky traps baited with (*Z*)-6-heneicosen-11-one (Shepherd *et al.* 1985). This sensitive monitoring using synthetic sex pheromone is crucial to prepare for application of the virus in the first year of a pest outbreak, because populations of *O. pseudotsugata* erupt quickly.

#### Potential benefits of combining a pheromone and virus treatment

There are both logistical and biological reasons why a combined treatment may be more successful than either treatment alone. One example from each category is presented for illustration. In effect, one treatment obviates potential deficiencies of the other, resulting in synergy between the two treatments.

Logistics are a major concern of the viral treatment. Precise timing of the spraying is crucial, because the application must immediately follow egg hatch for maximum larvicidal effect. Weather can interfere in two main ways. The spraying may be conducted in ideal weather conditions but at the wrong time, because the development rate of the insect is unexpectedly altered by unpredicted weather changes after the spraying date has been set. Alternatively, spraying may be conducted at the correct

time but in the wrong weather conditions because persistent wet weather removes spray deposits from the foliage. Unfortunately, wet weather is more common at the time eggs of *O. pseudotsugata* hatch than when the larvae are fully grown. The end result in both cases is a lowering of the larvicidal effectiveness of the viral treatment. However, if pheromone is included with virus in the spray then unwanted adult moths produced from uninfected larvae would now be subjected to mating disruption. Synergy between the two treatments can obviate the logistical problems of using the virus alone.

Biological constraints may be a major concern for mating disruption. Depending on the mechanism of mating disruption, the method may not be effective when pest numbers are above a threshold value. Several disruption mechanisms have been proposed (Cardé & Minks 1995), and a recent theoretical study indicates that for some mechanisms the effectiveness of mating disruption should depend on the density of the pest population being treated (Barclay & Judd 1995). For these mechanisms, this largely heuristic study shows that effective disruption is not possible above a threshold value of pest numbers, regardless of the amount of pheromone applied. This lack of effectiveness may be particularly marked where pest outbreaks begin from epicenters or where the pest aggregates. Both these behaviors are shown by *O. pseudotsugata*. If one of these density-dependent mechanisms were found to apply to the mating disruption of *O. pseudotsugata*, then high pest numbers may need lowering in some circumstances before applied pheromone could be effective. A viral application could accomplish this decrease in pest numbers. The synergistic effect of both treatments would thus overcome the biological restrictions that may result from applying the pheromone alone.

## Conclusions

Highly effective methods of controlling populations of *O. pseudotsugata* have been demonstrated in British Columbia based on the application of a viral insecticide to destroy the larvae, or the application of synthetic sex pheromone to disrupt adult mating. While either method can be effective alone, there are circumstances where a combination of the two treatments should be more effective.

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## **Control of codling moth, *Cydia pomonella* L., by an attract and kill formulation**

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**Abstract** - Field trials show that the attract and kill technique has a different mode of action from mating disruption and that it is effective in controlling codling moth.

**Key words** - sex pheromone, attract and kill, *Cydia pomonella*, Tortricidae, Lepidoptera

### **Introduction**

The attract and kill technique developed by Ciba (Sirene® CM) consists of a viscous formulation containing 0.16% codlemone, (*E,E*)-8,10-dodecadien-1-ol, to attract the males and 6.0% permethrine to kill them. Males contacting a drop die within hours. Thus, reproduction is reduced. The formulation is applied twice per season, by hand with a specially developed application system (Hofer & Brassel 1992).

### **Materials and methods**

Two trials were carried out in 1995 in isolated orchards to control codling moth by applying 1200 and 2700 drops/ha. An average drop was 0.1 ml in size and contained 0.16 mg of codlemone and 6 mg of permethrine.

Another trial was carried out in 1995 to study the effect of 100 versus 5000 drops/ha and the addition or respectively lack of an insecticide in the formulation. The trial was evaluated based on male trap catches and copulation of tethered females.

Seven trials were carried out in 1996 in isolated orchards to control codling moth by applying 1600 and 5000 drops/ha. An average drop was 0.05 ml in size and contained 0.08 mg of codlemone and 3 mg of permethrine.

## Results

In the two trials to control codling moth in 1995, the larval attack of codling moth on fruit was below a threshold of 1% during all the season and also at harvest. The hibernating population stayed at a low level.

In the other trial using drops without insecticide, trap catches were reduced by 50% compared to the untreated check, independent whether 100 or 5000 drops/ha were applied. At both drop densities the copulation of tethered females was practically not reduced. Attract and kill formulations with insecticide reduced trap catches by 55% and 94% and copulation of tethered females by 54% and 84% at dose rates of 100 and 5000 drops/ha, respectively. Detailed results have been published (Charmillot *et al.* 1996).

In two orchards where attract and kill technique was applied for the second year, the larval attack of codling moth on fruit and the hibernating population was at a very low level. Among the five orchards where attract and kill was applied for the first time, a single plot with much too high an initial population (more than 20% fruit damage at 1995' harvest) had to be sprayed curatively. In the other four trials, fruit damage at harvest was 0.24%, 0.32%, 1.1% and 1.14%, respectively, and the density of hibernating larvae, as determined by corrugated bands, was lower than 1 larva/tree.

## Conclusion

These trials show that Sirene® CM in fact attracts and kills the males and therefore has a different mode of action from the disruption technique and is an effective agent in controlling codling moth.

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*Pheromone release and dispersal*

## **A novel controlled-release device for disrupting sex pheromone communication in moths**

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**Abstract** - The results of experiments using a novel controlled release system, called the "Metered Semiochemical Timed Release System", or MSTRS™, for disrupting mating or pheromone source location by males of three lepidopterous pests, are described. In this system, pheromone is emitted at rates ca. 20 times higher than existing dispensers. Fewer dispensers are therefore needed for effective disruption. Unlike existing systems, MSTRS allows the user not only to choose how frequently pheromone is discharged but also to regulate the diel periodicity of this emission to correspond to the time of activity of the adults of the targeted pest insect. In addition, the pheromone is protected from oxidation and UV degradation since it is housed in pressurized canisters.

**Key words** - sex pheromone, mating disruption, controlled release dispensers, aerosol, *Ostrinia nubilalis*, *Cadra cautella*, *Rhopobota naevana*, Lepidoptera

### **Introduction**

There has been much progress over the past decade in improving the release-rate characteristics of some of the commercially most successful pheromone mating disruption formulations. However, none of the existing controlled-release technologies allow the user to actively alter the release rate. The existing systems are all passive systems that emit pheromone continuously according to ambient wind and temperature conditions.

We recently described a new system, called "Metered Semiochemical Timed Release Systems", or MSTRS™ (Mafra-Neto & Baker 1996a), in which an aerosol canister containing pheromone is placed in a machine and an aerosol spray-burst is

emitted onto a large pad on a timed basis (*e.g.*, every 15 minutes). Pheromone is then emitted from the pad at extremely high rates, ca. 20 times higher than most existing dispensers. Fewer dispensers are therefore needed for effective disruption. A similar system was reported by Shorey *et al.* (1996).

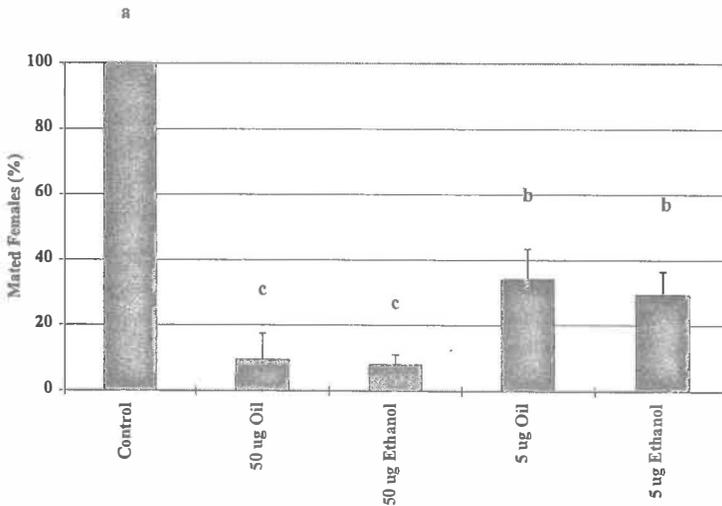
Unlike existing systems, ours allows the user not only to choose how frequently pheromone is discharged but also to regulate the diel periodicity of this emission to correspond to the time of activity of the adults of the targeted pest insect. Pheromone is not wasted by being passively emitted from the reservoir during periods of the day when the insects are inactive. In addition, the pheromone is protected from oxidation and UV degradation since it is housed in pressurized canisters. Although new to moth pheromone mating disruption research, aerosol dispensation of pheromone was examined in the early 1970s by bark beetle pheromone researchers for directing mass attacks of the southern pine beetle, *Dendroctonus frontalis*, onto targeted trees (Thomas *et al.* 1975). For unknown reasons, this work on bark beetles went completely unnoticed and the concept of using metered aerosols for moth mating disruption only emerged recently (Mafra-Neto & Baker 1996a; Shorey *et al.* 1996).

Here we summarize some of our results thus far for using MSTRS technology against the almond moth, *Cadra cautella*, which is a stored products pest, the European corn borer, *Ostrinia nubilalis*, a pest of corn, and the blackheaded fireworm, *Rhopobota naevana*, a serious pest of cranberries in North America.

### Almond moth, *Cadra cautella*

Laboratory-reared virgin males and females were released into 3 x 3 x 2.2 m rooms lined with white paper to make recovery of the moths easier. Two densities were tried, one low (15 females and 30 males) and the other one high (50 females and 150 males), the latter density for this sized room being above that for which successful disruption was achieved in previous studies (Sower & Whitmer 1977; Hodges *et al.* 1984; Hagstrum & Davies 1982). Depending on the experiment, either one or two MSTRS devices were placed in the corner(s) of the rooms, and the moths were allowed to fly freely. The machine-portion of the devices were purchased from Waterbury Companies, Inc. (Waterbury, Connecticut USA) and fitted with a spray-capturing pad 15 cm in diameter made of 1-cm-thick acrylic fiber. The machine sprayed pheromone from a pressurized canister containing either 0.4 g or 0.04 g of *C. cautella* pheromone, a blend of (*Z,E*)-9,12-tetradecadienyl acetate and (*Z*)-9-tetradecenyl acetate (Bedoukian Research Inc., Danbury, Connecticut) in a ratio of 10:0.9, onto the pad positioned 15 cm in front of the canister nozzle every 15 min, 24 hr a day. Two potential diluants were used in the cans, either ethanol or petroleum distillate. Treatments were replicated three times, with disruption being assessed by examination of females recovered from the rooms for the presence of spermatophores.

Significant disruption of mating, up to 100% for both the low-density and high-density populations of free-flying almond moths, was achieved over a 24-hr period with two MSTRS devices placed in opposite corners of the room at 1.3 m height, and emitting either 50  $\mu\text{g}$  or 5  $\mu\text{g}$  per spray-burst (Mafra-Neto & Baker 1996a). Disruption was greater than 90%, even at the high moth density over a 72-hr period (Figure 1). The emission rate from the pads from the 50  $\mu\text{g}$ -per-spray treatment with ethanol as the diluant exceeded 0.6  $\mu\text{g}/\text{min}$  after receiving 22 sprays. We found that just one device per room emitting 50  $\mu\text{g}$  of pheromone per spray resulted in greater than 90% disruption of mating over 24 hr at the high moth density (Mafra-Neto & Baker 1996a).



**Figure 1** Mean percentage of 50 free-flying female *C. cautella* that had mated after 72 hr in a 3 x 3 x 2.2 m room with 100 males ( $N = 3$ ). Rooms contained two MSTRS™ devices, one each in opposite corners, that every 15 min discharged either 50 or 5  $\mu\text{g}$  of pheromone, in solution with either ethanol or paraffin oil, onto an acrylic pad

Our observations gave some insight into the mechanisms of disruption under these conditions. First, in the pheromone-treated rooms, males were completely inactive after 24 hr, and over 90% of the females were observed to be sitting on the walls, calling. In the control rooms, males were observed flying around, and many mating pairs were seen sitting on the walls. In the pheromone-treated rooms, interestingly, when the MSTRS device discharged, males were seen to begin wing-fanning and take flight, with many of the males orienting toward the pad, and some even touching the pad. After a few minutes, this activity would subside. These observations implied that

the habituation that was occurring was not absolute, and that the higher emission rate temporarily produced by a fresh spray on the pads could overcome that level of habituation.

These suppositions were confirmed in another study, in which males were pre-exposed to various levels of pheromone sprayed into a jar in which they were housed. The males pre-exposed to pheromone in this fashion were then flown in a wind tunnel to various dosages of pheromone from a point-source plume in clean air (Mafra-Neto & Baker 1996b). Males pre-exposed to higher amounts of pheromone exhibited elevated thresholds of response to subsequent exposure to pheromone plumes in the wind tunnel. The results showed that the optimum dosage for eliciting upwind flight and source location shifted to significantly higher levels for males pre-exposed to higher levels of pheromone, and that dosages that would otherwise be unacceptably high for unexposed males now could become the optimal dosages for males pre-exposed to high amounts of pheromone (Mafra-Neto & Baker 1996b). Thus habituation of *C. cautella* males during disruption is not absolute, and significant levels of misdirected flight (false trail-following) to the disruptant dispensers seemed to contribute directly to the disruption and perhaps to the males' elevated levels of habituation.

#### European corn borer, *Ostrinia nubilalis*

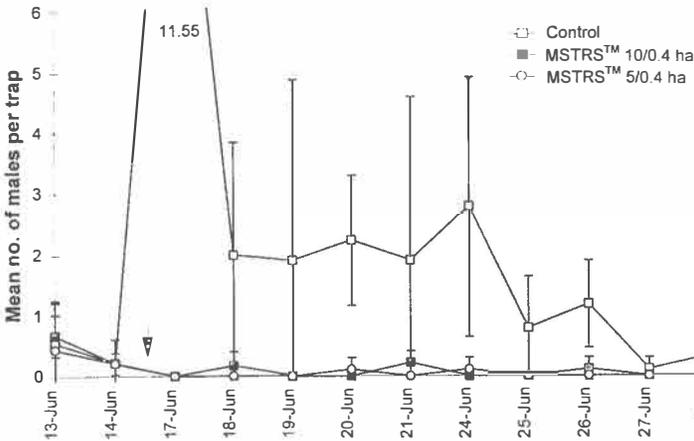
In a series of studies, Showers and colleagues (Showers & Reed 1976; DeRozari *et al.* 1977; Showers *et al.* 1980; Sappington & Showers 1983) reported that in Iowa, mating by *O. nubilalis* occurs mainly in grassy areas adjacent to corn fields, and not in the corn itself. They were able to demonstrate that treating the grass, either by mowing or with insecticide, resulted in significantly reduced damage to corn (Showers *et al.* 1980). Interestingly, there are no published reports of mating disruption for this species, and our objective was first of all to see whether we could disrupt mate location by males in small grassy plots. If this were to be successful, then in the ensuing years we would try to see whether we could prevent mating of feral females as well as reduce larval populations and damage to corn in neighboring fields.

We used MSTRS devices at two deployment densities in 0.4-ha grassy areas near Ames, Iowa, USA. Treatments were replicated three times, as were the 0.4-ha areas treated with twist-tie dispensers, also called "ropes" (Shin-Etsu Chemical Co. Ltd., Tokyo, Japan). The devices were identical to the ones used indoors for *C. cautella* (except for the pheromone blend employed) and were placed on stakes at a height of 1 m. The canisters contained either 4 or 8 g of the pheromone of the Iowa strain of this species (Klun *et al.* 1973), a blend of (*Z*)-11-tetradecenyl acetate (Z11-14Ac) and (*E*)-11-tetradecenyl acetate (E11-14Ac) (Bedoukian Research, Inc., Danbury, Connecticut) in a ratio of 97:3 *Z/E*. Devices containing the 4-g cans were placed at a density of 10/0.4 ha in 1-acre square-shaped grassy plots comprised of various

grasses including brome grass and foxtail grass. Machines containing the 8-g cans were deployed at a density of 5/0.4 ha in 3-acre plots of similar grass composition in a rectangular geometry. Machines discharged pheromone every 15 min, 24 hr/day onto the pad.

The Shin-Etsu ropes containing the Hamaki-con formulation (95:5 Z/E-ratio) were deployed in 0.4-ha, long, narrow plots, usually 5 to 10 m wide, situated in amongst the corn such as to form irregularly shaped islets. The grass was grabbed by hand to form a bunch, and a rope was twisted around the top of the bunch, with the researcher walking a prescribed number of meters between bunches to create a rope density of either 130, 400, or 1600 ropes/acre, making sure that the grassy strips received ropes along their edges.

Disruption was assessed by two wing-style sticky traps, (IPM Technologies Inc., Oregon) containing a 100-mg septum of the 97:3 blend used in the canisters, as well as by two wing traps containing five virgin females in a small cage plus a sucrose water source. The traps were placed at least 30 m from the nearest MSTRS device, and without regard to the location of the ropes. Release rates of Z11-14Ac from the pads of the 8-g machines after 14 days of emission was  $5.85 \pm 2.20 \mu\text{g}/\text{min}$  ( $N = 3$ ). Release rates from the pads from the 4-g machines after 14 days in the field were  $3.45 \pm 0.94 \mu\text{g}/\text{min}$ . ( $N = 3$ ). The Shin-Etsu ropes emitted pheromone at a rate of  $0.29 \mu\text{g}/\text{min}$ . after 6 days in the field, and  $0.30 \mu\text{g}/\text{min}$  after 30 days in the field ( $N = 3$ ).



**Figure 2** Mean capture of male *O. nubilalis* in wing traps containing 5 virgin females in grassy fields in which either 5 or 10 MSTRS™ devices/0.4 ha were deployed, each containing canisters loaded with either 8 or 4 g of pheromone, respectively. After two days of pre-treatment monitoring of all fields, the MSTRS devices were activated on June 15th (arrow). Bars indicate standard deviation ( $N = 3$ )

Disruption of males' ability to locate females or the synthetic pheromone dispensers averaged 98% for the 10/0.4 ha MSTRS treatment and 99.2% for the 5/0.4 ha MSTRS during the entire first flight (Figure 2). The capture rates of males as the flight began for the two days before the disruptant dispensers were deployed were equivalent in all plots. The capture levels and the levels of disruption caused by either the MSTRS or the Shin-Etsu ropes deployed at either 130 ropes/0.4 ha (94.9% disruption), 400 ropes/0.4 ha (95.5% disruption) or 1200 ropes/0.4 ha (98.8% disruption) were not significantly different ( $p > 0.05$ ; ANOVA,  $N = 3$ ).

The data show not only the potential for disrupting mating of this serious pest of corn using MSTRS technology, but also using Shin-Etsu twist-on dispensers. The geometry of the grassy areas varies considerably, and long narrow strips are not optimal for coverage by plumes from widely spaced, high-emission rate dispensers such as the MSTRS devices. Nevertheless, our data show that in large grassy plots having favorable geometry, a few high-emission-rate sources per ha can disrupt mate location by *O. nubilalis* males as effectively as hundreds of lower-emission-rate sources per ha having the same amount of pheromone on a per-ha basis.

### **Blackheaded fireworm, *Rhopobota naevana***

Significant work on disrupting mating of this serious pest of cranberries has been undertaken by Fitzpatrick *et al.* (1995), and has shown much promise for this technique, using Shin-Etsu ropes or Ecogen Spirals (Scentry/Ecogen, Billings, Montana) with a total application rate of ca. 70 g pheromone/acre. One problem with these dispensers, however, is that they must be retrieved at the end of the season due to the potential for the buildup of environmentally unacceptable levels of plastic in the cranberry marshes. The placement and retrieval of a high number of point sources on the cranberry beds would also result in unacceptably high foot traffic, which would damage the delicate, slow-growing plants. The use of MSTRS devices would be advantageous because only a few dispensers would be necessary per acre, mostly deployed around the perimeter of the beds where they could be fairly easily retrieved without incurring crop damage. Furthermore, the MSTRS can be stored for re-use in subsequent years.

We used MSTRS devices identical to those described above for *O. nubilalis*, except for the pheromone blend in the canisters, and affixed them to wooden stakes at a height of 20 cm above the cranberry plant canopy. The canisters contained either 8 or 20 g of *R. naevana* pheromone, which is a blend of Z11-14Ac, (Z)-11-tetradecenyl alcohol (Z11-14OH), and (Z)-9-dodecenyl acetate (Z9-12Ac) in a ratio of 9:3:1 (McDonough *et al.* 1987; Slessor *et al.* 1987). These components were purchased from Bedoukian Research Inc. Devices containing the 8-g cans were deployed at a density of 5/0.4 ha along and within cranberry beds or series of beds that averaged ca. 1.2 ha in total area. Two configurations were used for this density of

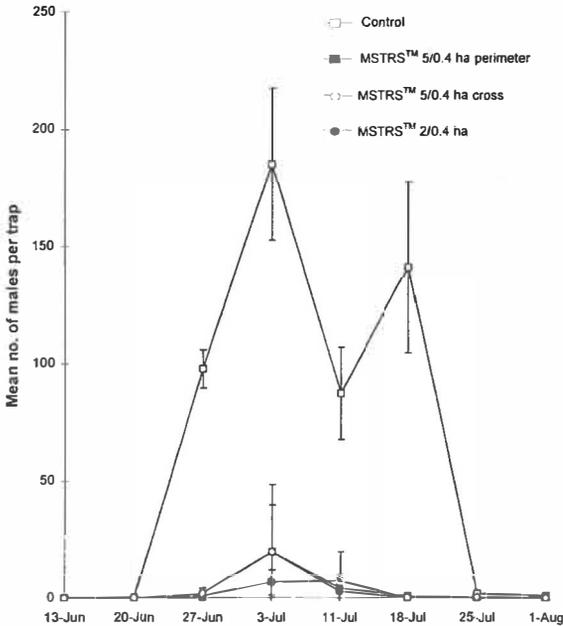
devices, one being a perimeter-only treatment with MSTRS spaced ca. every 30 m at the edges of the beds. The second consisted of the same density of devices and amount of pheromone per ha overall, but three devices were removed from the perimeter (remaining devices being more widely, but evenly spaced) and instead were deployed across the centers of the beds, bisecting them longitudinally. The 20-g cans were deployed at a density of 2/0.4 ha along the same sized beds, such that there were nine machines only around the perimeter of the 1.2-ha beds. Treatments as well as 1.2-ha control plots several hundred m from the treated beds were replicated three times in different locations within ca. 30 miles of each other in the cranberry growing region near Babcock, Wisconsin. Machines were programmed to discharge every 15 min, 24 hr/day.

Disruption was assessed by counting the number of males captured in wing traps baited with 10 µg of the above pheromone blend on a rubber septum, a lure that has been shown to be comparable in attractancy to females (Fitzpatrick *et al.* 1995). The wing traps were placed, three per 1.2-ha plot, at locations in the interior of the marsh, and not closer than 30 m from the nearest machine. The number of males captured was assessed weekly, the males removed, and trap bottoms replaced as needed.

Disruption averaged 99% in the first location, regardless of the MSTRS deployment pattern, and 95% in the second location throughout the first flight (detailed record for this location is shown in Figure 3), but averaged only 82%, 80%, and 57% for the 5/0.4 ha cross pattern, the 2/0.4 ha perimeter, and the 5/0.4 ha perimeter treatments, respectively, in the third location, which had a history of very high populations of fireworm and low yields compared to the industry average in the region. Captures in the control plots in the three locations averaged 52.3, 73.4, and 63.3 males/trap/week over the six-week flight period. Unlike the treated beds in the other two locations, the 1.2 ha comprising the treated areas for each of the three MSTRS deployment arrays in the poor disruption location were comprised of six, 0.2-ha beds separated by 1.5 m-high grassy banks functioning as dikes for water control. Thus, it is possible that the aerial transport of pheromone plumes from the MSTRS over the disruption areas could have been disturbed in these plots, resulting in lower efficacy of disruption. In all three locations, the MSTRS devices were deployed at the same time as a sprayable formulation of pheromone (Scentry/Ecogen) was applied directly to the cranberry beds; the MSTRS were as effective in disrupting pheromone source location as the sprayable formulation in all plots (Sheila Fitzpatrick, pers. comm.).

Our results show that a relatively few MSTRS per ha can effectively disrupt pheromone source location by *R. naevana* at levels of 98% disruption for an entire flight period on 1.2-ha cranberry beds. Again, as in the *O. nubilalis* studies, the geometry of deployment was important, and it must be considered that on smaller plots, there is a greater edge area to protect relative to the interior area of crop that is to be protected. In principle, the MSTRS technology should work better over a very large,

regularly shaped area where there will be fewer pheromone-plume-free holes along the edges.



**Figure 3** Mean capture of male *R. naevana* in wing traps containing 10  $\mu\text{g}$  of synthetic pheromone in 1.3-ha cranberry marshes at one of the three locations in Wisconsin in which either 2 or 5 MSTRS™ devices per 0.4 ha were deployed. The devices were activated before the first flight began and continued to release pheromone throughout the flight from either 20-g cans (2/0.4 ha) or 8-g cans (5/0.4 ha). Bars indicate standard deviation ( $N = 3$ )

Finally, it must be considered that the efficacy of widely-spaced dispensers such as these, whose plumes need to sweep for tens, and perhaps hundreds of meters horizontally over the crop canopy to both attract and habituate males sufficiently that they are prevented from mating, will likely be more dependent upon ambient meteorological conditions than will be numerous lower-emission-rate point sources spaced only meters apart throughout the crop. This vulnerability may be accentuated for species that mate during the daytime, when adiabatic lapse rates are highest, and unstable, rising air can carry plumes from disruptant dispensers up and away from the canopy.

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## Efficacy of mating disruption pheromones in paraffin emulsion dispensers

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**Abstract** - Pheromones of Oriental fruit moth (OFM), *Grapholita molesta*, peach twig borer (PTB), *Anarsia lineatella*, and San Jose scale (SJS), *Quadraspidiotus perniciosus*, were mixed into sprayable emulsions of water and paraffin and applied to stone fruit trees for mating disruption of these pests. Pheromone/emulsion blends were applied twice for OFM and PTB during the 1995 season, using 49.4 to 76.6 g active ingredient/ha/application; SJS pheromone was applied only once at 74 g active ingredient/ha. Efficacy of mating disruption was measured by pheromone trap (OFM, PTB) or sticky tape (SJS) collections and infested fruit. Results of these field trials showed that treatments using OFM pheromone were comparable to commercial mating disruption dispensers, with monitoring trap shutdown for 8 to 12 weeks, and reduction of fruit infestation of 80 to 90 percent. The PTB pheromone treatments were less effective, with increased collections of PTB moths in monitoring traps and no differences in infested fruit compared to untreated checks. Mating disruption of SJS using pheromone in paraffin emulsions showed some reduction in crawler populations for two generations after treatment, but the cost of pheromone is prohibitive for commercial use.

**Key words** - sex pheromone, mating disruption, sprayable dispenser, *Grapholita molesta*, *Anarsia lineatella*, *Quadraspidiotus perniciosus*

### Introduction

Mating disruption as a control strategy for insect pests has been the subject of considerable research and commercial interest in recent time (Ridgway *et al.* 1990). In these efforts, control of the Oriental fruit moth (OFM), *Grapholita molesta* Busck, in peaches and nectarines has been one of the more successful applications of this tech-

nique (Rice & Kirsch 1990; Vickers 1990; Pree *et al.* 1994). More recently, mating disruption of the peach twig borer (PTB), *Anarsia lineatella* Zeller, has been demonstrated in California (Rice & Millar 1992).

Although successful, these and other mating disruption programs have primarily used hand-applied plastic or polymer pheromone dispensers of various types and designs. Over time, it has become apparent that development of sprayable or other mechanical application system for disruption pheromones could lead to decreased application costs, improved placement of pheromones in tree crops, and possibly less environmental contamination from depleted plastic dispensers. In this report, we describe the development and application of a prototype sprayable, mechanically applied pheromone dispensing system for mating disruption of Oriental fruit moth and peach twig borer in orchard crops that is easy and potentially less costly to apply, and is biodegradable over a period of months.

## Materials and methods

### Oriental fruit moth and peach twig borer emulsions

Synthetic Oriental fruit moth pheromone (Biwer *et al.* 1979; Cardé *et al.* 1980) (93% (Z)-8-dodecenyl acetate, 6% (E)-8-dodecenyl acetate, 1% (Z)-8-dodecenol), and peach twig borer pheromone (Roelofs *et al.* 1975) (75% (E)-5-decenyl acetate, 18.7% (E)-5-decen-1-ol, 6.3% inerts) were obtained from Bedoukian Research Inc. (Danbury, CT, USA). The pheromones were mixed in water emulsions of paraffin and two different emulsifiers. The two emulsifiers used in the respective pheromone formulations were triethylamine (TEA) stearate or Span 60® (sorbitan monostearate), a commercial emulsifier used in cosmetic products. Paraffin (ParoWax®) blocks were melted on a laboratory heater and emulsifier was added. OFM or PTB pheromone was then added to the paraffin/emulsifier blend along with small amounts of soy oil and vitamin E. The liquid paraffin-pheromone blend was mixed during this process with a handheld kitchen blender. After mixing the pheromone and other materials into the paraffin, the emulsion was formed by adding water in a 60% water - 40% paraffin/emulsifier/pheromone ratio.

The finished paraffin emulsions were applied to peach, nectarine, plum, and almond trees using an Idico® stainless steel one-quart tree-marking paint gun (Forestry Suppliers; Jackson, MS, USA). It was calculated that each squirt from the paint gun would deliver approximately 1.26 ml of emulsion. In field applications, a portion of this amount was lost due to splatter from the target site on the tree (large scaffold limbs) or dripping from the nozzle of the gun. This loss was estimated at *ca.* 0.26 ml per shot, thus leaving approximately 1 ml of emulsion per shot on the tree, or 62.5 mg of pheromone per shot. Four squirts of pheromone emulsion would deliver 250 mg pheromone to each target tree. Emulsions formulated with TEA stearate were less viscous than emulsions using Span 60, which were creamier, more viscous, and formed a

more compact deposit when applied to scaffold limb surfaces. In addition, different batches of emulsion blends tended to have somewhat variable viscosities, making it difficult to control precisely the actual amount of pheromone applied.

The target rate of pheromone application for OFM disruption was set at 75 g pheromone/ha. Using this as a base application rate, the actual number of squirts and amount of pheromone needed per tree was then calculated based on the planting density and number of trees/ha. The target rate of PTB pheromone was targeted at 50 to 63 g/ha, comparable to the rate registered for commercial use.

After first emergence of Oriental fruit moth at the Kearney Agricultural Center in 1995, OFM pheromone in paraffin emulsion was applied to the south (downwind) 0.8 ha of a 1.6-ha plum orchard. OFM pheromone-paraffin emulsions were also applied to a 0.8-ha block of Fantasia nectarines and a 0.58-ha block of mixed stone fruits at Kearney. The plums received emulsion mixed with Span 60 emulsifier. The nectarines and mixed stone fruit orchards were initially treated with emulsions using the TEA stearate emulsifier; second applications used Span 60 emulsifier.

On March 6, 1995, OFM pheromone emulsion using Span 60 emulsifier was applied to a 0.8-ha portion of a large almond orchard. Pheromone monitoring traps were placed in the treated area, and in the same orchard 0.7 km from the treated area. Following applications of the pheromones to these four test plots, the emulsion deposits were subjected to unseasonably heavy amounts of rain and wind over the following three weeks, but these conditions appeared to have little or no effect on the emulsion deposits. OFM pheromone-emulsion blends were reapplied to the plums on June 7; to the nectarines on May 17, and to the mixed stone fruit block on May 16, 1995. A second application was applied in the almond orchard on June 3.

Peach twig borer pheromone in the paraffin emulsion blends was applied at *ca.* 25 g active ingredient/ha to the nectarines at Kearney on April 3, with a second application on May 10. The mixed stone fruit planting was treated with PTB pheromone emulsions on March 30 and May 10, and the almonds were treated for PTB mating disruption with pheromone emulsions on April 4 and May 10.

In all orchards, second applications of OFM and PTB pheromone-emulsion blends were mixed using Span 60 emulsifier.

### San Jose scale pheromone emulsions

Synthetic San Jose scale pheromone (Gieselmann *et al.* 1979) was obtained from PheroTech Inc. (Delta, B. C., Canada), in a blend of 22.8% (*Z*)-3,7-dimethyl-2,7-octadien-1-yl propanoate, 73.9% 3-methylene-7-methyl-7-octen-1-yl propanoate, and 3.3% impurities including 0.6% neryl propanoate. This pheromone blend was mixed in paraffin emulsion using Span 60 emulsifier with 312.5 mg pheromone/g emulsion. Delivered application rate of the SJS pheromone was targeted to be 75 g pheromone/ha as with the OFM pheromones. However, the delivered volume of pheromone emulsion was eight shots per tree (to deliver 0.25 g pheromone/tree) rather than the four

shots per tree with the OFM and PTB pheromones. The reason for the additional amount of emulsion per tree was to attempt greater dispersal of the SJS pheromone over a larger portion of the scaffold limbs because of the thought that the pheromone must be more widely distributed throughout the tree to disrupt orientation of walking males on tree bark in addition to males flying within the tree canopy.

On March 6, 1995, San Jose scale pheromone paraffin emulsion was applied to twelve contiguous Fantasia nectarine trees in a three-row by four-tree block. A similar block of untreated Fantasia check trees was located approximately 90 m crosswind from the twelve pheromone-treated trees. Within each treatment (pheromone and untreated check), five trees were randomly selected for monitoring with a SJS pheromone trap (Trécé Inc., Salinas, CA, USA) and two sticky tapes for collection of flying and walking male scale, and crawlers resulting from mating of virgin females.

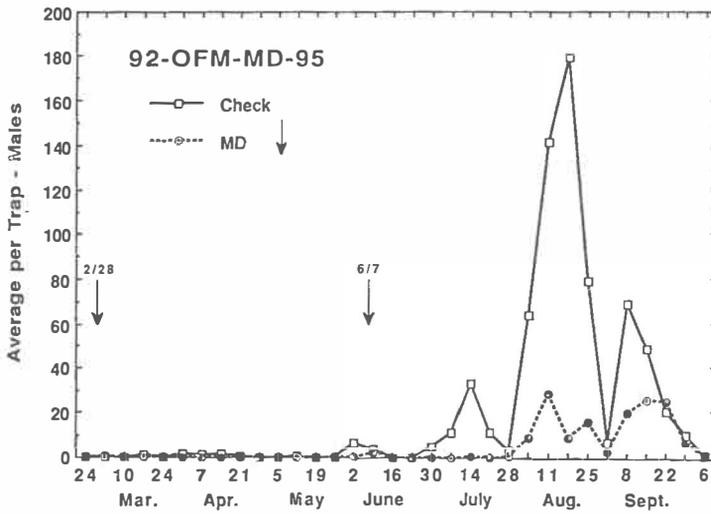
The first detected SJS male emergence occurred on March 7, 1995. This male biofix was established by collection of males on the sticky tapes rather than flying males collected on the pheromone traps. Thus it appears that the SJS pheromone emulsion was applied at the optimum time for disruption of male scale emergence and mating. The first male scale was collected on a pheromone trap in the untreated check trees on March 10. As in the OFM applications, the Span 60 deposits on nectarines showed no apparent affect from over 7.6 cm of rain on March 9 and 10, *e.g.* there was no loss of emulsion deposit from the tree limbs.

Following application, emulsion deposits on the trees would usually dry within one to four hours. Pheromone emulsion using the TEA stearate emulsifier produced a thinner deposit on the tree bark and also tended to discolor after application to the bark, taking on an oily, light brown appearance. Emulsions using the Span 60 emulsifier dried somewhat slower, but produced a more uniform deposit that tended to clump rather than run and spread on the bark. The Span 60 emulsifier blend held its form in a "toothpaste" blob, whereas the stearate emulsifier mix was too thin to form a thick paste and would run and drip readily if applied in only one small spot. The characteristics of the Span 60 emulsifier may be advantageous by slowing release of the pheromone from a more compact deposit rather than a thin, dispersed deposit as produced by the stearate emulsifier.

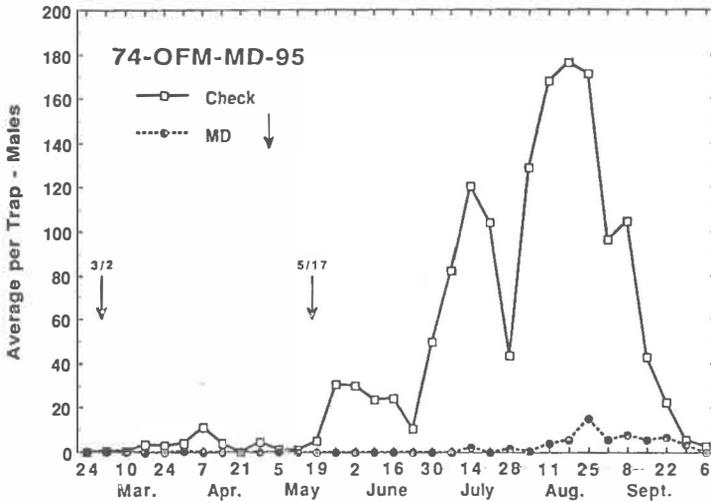
## Results and discussion

### Oriental fruit moth mating disruption

The effects of two applications of Oriental fruit moth pheromone mixed in the paraffin-emulsion blends applied in the 0.8-ha plum block are shown in Figure 1. Although only twelve OFM male moths were captured in the two pheromone monitoring traps in the untreated (north) portion of this orchard during the first flight in mid-March through late April, no moths were captured in the treated 0.8 ha of this block. During the second flight, from late May through late June, 21 moths were captured in the un-



**Figure 1** Pheromone trap collections of Oriental fruit moth in a 0.8-ha plum orchard treated with OFM pheromone/paraffin emulsion. Span 60 emulsifier used in both applications; 76.0 g and 76.3 g OFM pheromone/ha in first and second MD applications, respectively (Kearney Agricultural Center, Parlier, CA, 1995)



**Figure 2** Pheromone trap collections of Oriental fruit moth in a 0.8-ha nectarine orchard treated with OFM pheromone/paraffin emulsion TEA stearate emulsion with 50.0 g pheromone/ha used in first application; Span 60 emulsion with 75.3 g pheromone/ha in second application (Kearney Agricultural Center, Parlier, CA, 1995)

treated check area and five moths were caught in the treatment on June 2 and 6. This triggered the second application of pheromone on June 7. As a result, only one moth was captured in the treatment on July 14 during the third flight, while significant increase was seen in the untreated check. This trend with higher populations in the check was also seen in the fourth and fifth flights in August and September, while the populations in the paraffin-emulsion treatments remained relatively low in the treated area. Evaluations for OFM larvae in fruit at harvest were not taken in this orchard due to the extremely low or nonexistent incidence of OFM larvae in plum fruit.

The results of mating disruption with OFM pheromones in paraffin emulsions in the 0.8-ha block of nectarines are shown in Figure 2. Applications of the disruption pheromones on March 2 at the beginning of the OFM flight and again on May 17 at the beginning of the second flight resulted in complete trap shutdown in this orchard until July 14 at the peak of the third OFM flight. Oriental fruit moth population pressure in this orchard was considered relatively high for a commercial orchard with populations peaking at over 30 in the second flight, over 120 moths/trap/week in the third flight, and approaching 180 moths/trap/week in the fourth flight. Even under these conditions moth populations in the treated block remained acceptably low through the end of the fifth flight in late September and early October.

OFM infestation levels in mature nectarines in this orchard were determined at harvest dates of July 14 and July 21 and were compared to fruit taken from an untreated Fantasia block approximately 0.8 km upwind. One thousand fruit were taken from each orchard and examined for both OFM and PTB damage. Fruit in the mating disruption block showed 0.4% OFM infestation while fruit from the untreated check orchard showed 2.4% OFM infestation (Table).

**Table** Efficacy of mating disruption (MD) with pheromones applied in paraffin emulsion carriers

Location	Crop	Treatment <sup>a</sup>	% Infested Fruit	
			OFM	PTB
KAC 36	Peach	Check	3.5	3.1
		MD	0.3	3.5
	Almond	Check	-	2.7
		MD	-	3.8
KAC 74	Nectarine	Check	2.4	19.8
		MD	0.4	15.0
			SJS	
KAC 32	Nectarine	Check		19.8
		MD		26.1

<sup>a</sup> Two applications per season for Oriental fruit moth (OFM) and peach twig borer (PTB) (50.0 to 77.5 g a.i./ha/application). One application for San Jose scale (SJS) (75.0 g a.i./ha; March 6, 1995)

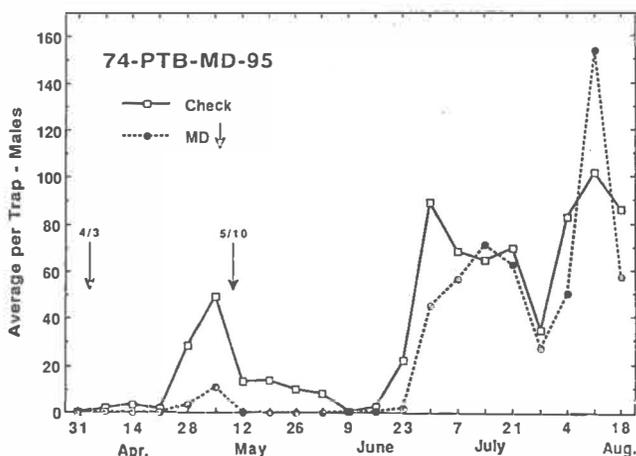
Pheromone monitoring traps in the mixed stone fruit orchard also showed extremely good monitoring trap shutdown in the mating disruption treatments following applications of pheromone on March 2 and May 16. No OFM moths were caught in either of the first two flights in this orchard and only a small number were collected in the disrupted orchard during the third flight in comparison to the upwind untreated check. Male moth collections in the fourth and fifth flights increased to much higher numbers in relation to the untreated checks but this is thought to be due to the small size of the pheromone-treated block and possible inflights of male moths from adjacent untreated almonds. One thousand Flamecrest peaches were harvested in this block on June 26 for evaluation of OFM and PTB damage and compared to 1 000 fruit taken from the nearby untreated check orchard. The OFM infestation level in the mating disruption block was 0.3% compared to 3.5% in the untreated check (Table).

The two applications of OFM mating disruption pheromones in paraffin emulsions applied to almonds also showed excellent trap shutdown through the first three flights. OFM is not usually considered a pest in almonds but populations can often be high enough to allow evaluation of various types of OFM treatments and controls. Because trap shutdown in the almonds following the first application of pheromone on March 6 was so good, it was determined not to apply the second application until the first moth was captured in monitoring traps in the pheromone-treated 0.8-ha block of this orchard. A single male OFM was captured in the pheromone treatment on June 2, resulting in the second application the following day. After this application, the monitoring traps in the pheromone treatment were again completely shut down through the third OFM flight and no moths were captured in the treatment until mid-August, during the major portion of the fourth OFM flight. Monitoring for OFM in almonds was terminated in early September due to the drying-down of the orchard and preparations for harvest. However, it was apparent that the pheromone treatments in this orchard had a significant effect on the ability of male OFM moths to orient to monitoring traps, compared to the untreated portion of this 50-ha orchard.

The efficacy of the pheromone-paraffin emulsion blends for mating disruption of Oriental fruit moth in these several orchards in 1995 indicate that the pheromone treatments were capable of shutting down the monitoring traps for at least the entire first OFM flight and perhaps a significant portion of the second flight as well. This period of trap shutdown of approximately 60 to 75 days is comparable to that observed with the plastic hand-applied mating disruption dispensers currently available. OFM trap shutdown for approximately 7 to 10 weeks following the second application of disruption pheromone in mid-May to early June was also quite encouraging, given the absence of prior history and experience with this pheromone-dispensing system. In addition, the reduction in fruit damage from OFM in the nectarines and peaches was also encouraging for the first evaluation of control of OFM with this pheromone system. These data show results comparable to what would be expected from the commercial mating disruption pheromone products presently available to growers.

## Peach twig borer mating disruption

The first applications of PTB pheromones in paraffin emulsions were made at the Kearney Agricultural Center during the first week of April 1995. In nectarines the emulsions were applied on April 3 but within three weeks moths were being trapped in the pheromone treatment as well as in the untreated nearby check orchard (Figure 3). The second application of PTB pheromone was applied in this orchard on May 10, resulting in PTB collections in monitoring traps being brought down to zero levels during the latter portion of the first PTB flight. With the onset of the second flight in early June, however, monitoring traps in the pheromone-treated orchard again showed increasing collections comparable to those in the untreated check orchard. These collections indicated that the field residual and longevity of the PTB pheromone in either the TEA stearate or Span 60 paraffin-emulsion formulations was relatively short compared to OFM pheromones in the same formulations. PTB infestations in fruit harvested on July 14 and 21 showed an infestation level of 15% in the mating disruption pheromone treatment, which compared to a 19.8% infestation level in the untreated check orchard (Table).



**Figure 3** Pheromone trap collections of peach twig borer in a 0.8-ha nectarine orchard treated with PTB pheromone in paraffin emulsion. Span 60 emulsifier used in both applications; 59.8 g and 62.0 g pheromone/ha in first and second applications, respectively (Kearney Agricultural Center, Parlier, CA, 1995)

Pheromone trap data from the mixed stone fruit block again showed an unacceptably short residual of PTB pheromone in the paraffin emulsions resulting in breakdown of the disruption treatment within three to four weeks after pheromone application. Male

PTB moth collections in this orchard during the second and third flights in July through August showed monitoring trap collections higher in the pheromone treatment than in the untreated check orchard traps. Fruit harvested on June 26 showed a PTB infestation level of 3.5% compared to 3.1% in fruit harvested from the untreated check orchard on the same date (Table).

Data from pheromone monitoring traps in the almond orchard also showed rapid breakthrough of male PTB moths to monitoring traps in the disruption treatment following the first application on April 4. This occurred only three weeks following the first application. Following a second application of pheromone on May 10, PTB moth collections were reduced in the pheromone treatment for a period of approximately five weeks compared to the untreated check portion of the orchard. However, with the onset of the second flight in late June, moth collections in the pheromone treatment were similar to the untreated check and continued at high levels in both treatments through the remainder of the season.

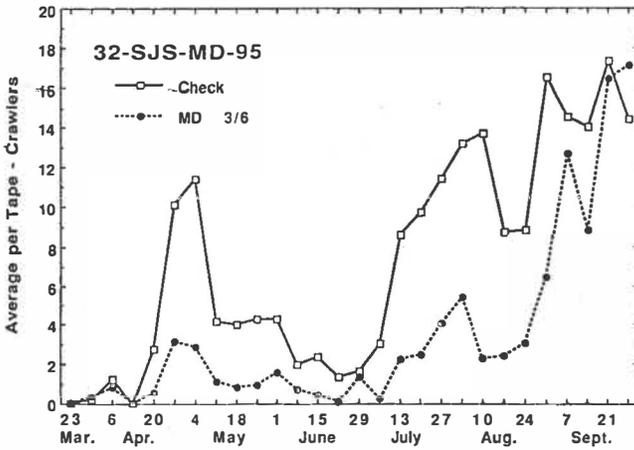
Peach twig borer infestation levels in the almonds were evaluated by harvesting 1 000 nuts at random from within both the 0.8-ha pheromone-treated block and the area of the untreated orchard containing monitoring traps. These nuts were collected immediately following commercial shaking of the nuts to the ground in the respective treatment areas. Because these almonds were a hard-shell cultivar, PTB presence was evaluated only on the basis of hull infestation and showed a 3.8% infestation level in the mating disruption treatment compared to a 2.7% infestation in the untreated check portion of the orchard (Table). PTB pheromone was not applied to the plum orchard at Kearney because this orchard had been treated prior to bloom with dormant sprays for PTB and SJS control. Consequently it was felt that pheromone application and monitoring for PTB in this block would not yield useful data for PTB mating disruption.

The results of the treatments with PTB pheromone in paraffin emulsion indicate that acceptable mating disruption was not achieved with the paraffin-emulsion formulations used in field trials in 1995. Both the pheromone trap data and fruit infestation data indicate relatively low or no mating reduction in these several treatments. This is in contrast, however, to the results observed with the OFM mating disruption formulations used in the same orchards in 1995.

#### San Jose scale mating disruption

Pheromone treatments in paraffin emulsions for SJS in Fantasia nectarines were evaluated by counting crawlers trapped on sticky tapes on treated trees versus untreated trees and by evaluation of scale infesting mature fruit. Following the single application of pheromone to these trees on March 6 (beginning of male scale emergence and potential disruption) crawler collections on sticky tapes during the first generation of crawler emergence showed a considerable reduction in populations on treated trees versus untreated trees (Figure 4). The small initial peak of crawler collections on April 6, showing no difference between the check and mating disruption treatment, is

believed due to crawler emergence from mated, overwintered females that were not affected by the pheromone treatment.



**Figure 4** Sticky tape trap collections of San Jose scale crawlers in untreated check nectarine trees compared to trees treated with 75.0 g SJS pheromone/ha in paraffin emulsion (Span 60 emulsifier) (Kearney Agricultural Center, Parlier, CA, 1995)

The lower collections of crawlers from April 13 through June 22 are thought to be a result of reduced male orientation to calling virgin female scale and less mating during the first male flight. This initial reduction of scale crawlers seemed to carry over into the second adult generation in June and early July, again resulting in a lower population of crawlers in the mating disruption treatment during the second generation in July and August. However, this difference in populations disappeared during the third crawler generation in late August and early September.

Fruit harvested in mid-July from both the pheromone treated trees and the untreated check trees showed no significant difference in infestation levels on fruit, with the mating disruption treatment having 26.1% infested fruit and the check trees showing 19.8% infested fruit. The fruit infestation data is contradictory to the data collected on the sticky tapes for total crawler populations. The fruit data, however, may be somewhat misleading as no attempt was made during the harvest evaluation to distinguish between only a single crawler per fruit versus several or more crawlers per fruit.

The results of the single application of SJS pheromone to nectarines suggest that there may be some potential for mating disruption and control of SJS using pheromones in paraffin-emulsion formulations. Based on the single trial in 1995, however, it would be premature to either reject or accept the potential for mating disruption of

scale using synthetic pheromones on tree fruit. The crawler collection data on sticky tapes suggest that further trials for mating disruption of SJS are warranted, but this will depend upon both availability and cost of the synthetic SJS pheromones.

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## **Codling moth mating disruption field trials with TNO dispensers**

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**Abstract** - In five consecutive years (1991 to 1995) TNO has tested the method of mating disruption for codling moth with a new type of dispenser in apple orchards in Spain, The Netherlands, Switzerland, France, Italy, Hungary, California, Washington, Israel and South Africa. Codling moth dispensers in the form of flat square wafers releasing 70 g codlemone/ha/season were applied at a density of 500 dispensers/ha. Release profiles of field exposed dispensers have shown a very gradual release over a period of four to five months and excellent protection of codlemone. In almost all trials, the mating disruption application resulted in season-long satisfactory control. In cases where high (initial) populations of codling moth were present, supplementary insecticide treatments were necessary occasionally.

**Key words** - sex pheromone, mating disruption, controlled release dispensers, (*E,E*)-8,10-dodecadien-1-ol, pome fruit orchards, *Cydia pomonella*, Tortricidae, Lepidoptera

### **Introduction**

Practical use of insect pheromones in pest control is primarily determined by the availability of controlled release devices. Requirements for suitable pheromone dispensers are high: protection of the (expensive) active ingredient(s) from preliminary environmental degradation and delivery of effective concentrations of pheromone for prolonged periods of time are both highly desirable. For monitoring dispensers release rate of pheromone in its natural composition should neither be too low nor too high. For mating disruption dispensers, the effective release period preferably should be long enough to cover the flight season of the target insect.

Our laboratory has developed a novel controlled release technology that can be used for a large variety of volatile substances (Derks 1991; Smit *et al.* 1991). During the last five years prototype dispensers for a variety of insect pests have been prepared and tested in the field for their release profile and biological efficacy (Klijnstra & de

Vlieger 1994). Major effort has been put on the development of a pheromone dispenser that can be effectively used for mating disruption of the codling moth, one of the most devastating orchard pests occurring worldwide. Preliminary field tests with the first prototypes were carried out in 1990 to 1992 in Spain in cooperation with Servei Proteccion dels Vegetals in Barcelona and in The Netherlands in cooperation with IPO-DLO, Wageningen and Denka International, Barneveld. The results of these trials were reported at the IOBC meeting in Chatham, UK (de Vlieger & Klijnstra 1993).

From 1993 onwards, TNO-technology based mating disruption dispensers for control of codling moth have been tested on more than 120 ha at various locations all over the world. Partners involved with these field tests were: L. Anshelevich, Volcani Center, Bet Dagan, Israel; A. K. Minks, IPO-DLO Wageningen, The Netherlands; P.W. Weddle, Hansen & Associates Consultants, California, USA; A. Knight, Yakima Agricultural Research Laboratory (USDA-ARS) and J. F. Howell, Superior Ag Products, Inc., USA, Washington; E. Navarro, GRCETA, France; C. Ioriati, Instituto Agrario, Trento and A. Pacini, Sipcarn S.P.A., Italy; P. J. Charmillot, Federal Agricultural Research Station, Changins, Switzerland; Babolna Bioenvironmental Center, Budapest, Hungary; Sandoz Agro, South Africa. Here, a summary of the results of these field tests with regard to dispenser performance is reported.

## Materials and methods

The novel technology is based on crosslinked polymeric matrices prepared by radiation curing (UV or  $\gamma$ -radiation) of acrylated prepolymers. The pheromone components and, if necessary, protective agents like UV-absorbers and anti-oxidants, are added to the pre-polymer (mixture) prior to polymerization. After curing a solid, so-called monolithic matrix is obtained. Variation in matrix composition, *i.e.* the crosslink density of the matrix is the leading principle in designing appropriate dispensers for specific pheromones or purposes. In addition, the release rate can be adjusted by variation in pheromone loading and surface/volume ratio of the dispenser. Dispensers can be loaded with pheromones up to 60% w/w and can be produced in various configurations.

Codling moth dispensers were prepared in flat sheets of 0.3 cm which, after curing with  $\gamma$ -radiation, were cut in small square wafers measuring 2.5 x 2.5 cm. The wafers were stitched to plastic clips which could be hung on branches of the trees. Dispenser density in each plot was approx. 500 dispensers/ha. Pre-test chemical analyses showed that each dispenser contained 200 to 230 mg of codlemone (> 97 % (*E,E*)-8,10-dodecadien-1-ol). In all experimental plots, field exposed dispensers were recollected at a regular scheme for determination of residual pheromone content by means of Soxhlet extraction and GC analysis.

## Results and discussion

In 1993, field collected dispensers from four different locations (Washington, Wageningen, Israel and Switzerland) showed consistent, almost linear release profiles over a periods of more then 140 days. A season long release was thus obtained. Biological performance of the dispensers varied with the location, good control was achieved in Wageningen and Israel whereas in Washington and Switzerland control was not optimal due to various reasons.

In Israel, comparative trials were carried out in 1993 in apple and pear orchards. Table 1 gives the results of these tests. In pheromone treated plots, trap catch reduction was clearly observed and both pheromone systems tested gave good control without any chemical treatments during the season.

**Table 1** Results of codling moth mating disruption trials in Israel (plots with TNO dispensers measured 10 ha)

Location Orchard	Plot	Dispenser	Chemical treatment	Catch/trap		% infested
				before	after	
Galed, 1993	Pheromone	TNO	2 x before	80	0	0.1
Pears	Pheromone	Shin-Etsu long life	0	26	0	0.2
Zuma, 1993	Pheromone	TNO	2 x before	90	12	0.1
Apples	Pheromone	Shin-Etsu usual	0	0	5	0.5
	Control	-	6 x total	72	61	0.1

In 1994, TNO codling moth dispensers were tested in field trials on 52 ha in total. The results are given in Table 2. In almost all plots monitoring traps caught significant numbers of moths. In California during the previous season (1993) codling moth damage approximated 2.5% in the 1994 mating disruption plot. Therefore, a combination program of azinphosmethyl and mating disruption was carried out to reduce this population. Reduced trap counts in later generations indicated that this was indeed accomplished. Fruit damage at harvest was less than 1% in both types of plots. TNO dispensers showed an average release of approximately 1 mg/dispenser/day over 176 days.

In Hungary, trials were started approx. five weeks after start of moth flight. Also here, during the season an additional treatment with azinphosmethyl was necessary. At harvest, the combined pheromone and insecticide treatment showed substantially reduced damage when compared with insecticide alone. An average release of 0.8 mg/dispenser/day was found.

From the Italian trials in 1994 no trap catch data were received. Damage inside the plot was very low, but was very high at some particular spots along the border.

In France, mating disruption trials were started one month after beginning of moth flight. One insecticide treatment was applied prior to the start of the trial, later on three partial treatments were necessary. Adjacent control blocks received multiple chemical treatments during the season. Trap shut down was complete in the pheromone block, damage level at harvest was very low. Release data from the first 45 days indicate an average release of 2.2 mg/dispenser/day.

**Table 2** Results of field trials in 1994 (control plots were chemically treated)

Plot	Treatment	Trap catch (moths/trap)	Damage (%)
California, 6 ha	Pheromone	27	0.7
	Control	39	0
Hungary, 4 ha	Pheromone	2	0.4
	Control	32	2.7
France, 2.5 ha	Pheromone	0	0.5
	Control	90	0
Italy, 2.5 ha	Pheromone		0.1
South Africa, 3 x 9 ha	Pheromone	27	0.8
	Control	77	0.8

In South Africa trials in three comparable plots were initiated just prior to beginning of moth flight. Two of the three sites contained high moth levels and received additional insecticide treatments. The third block received pheromone application only. In this last block, trap shut down was complete. Because of the long flight season in South Africa and very high day temperatures anticipated, a second set of dispensers was applied after approximately 4 months. Average release appeared to be 0.8 mg/dispenser/day. Fruit damage was found to be minor (in the first two plots) to zero (in the final plot).

In 1995 mating disruption trials were carried out at a total acreage of 45 ha. The results are shown in Table 3.

In California trials were conducted in the same blocks as in 1994. No trap shut down was observed with 10-mg lures. Numbers of moths caught were quite high. Nevertheless, in the pheromone block only one chemical treatment was required, instead of four in 1994, to give adequate control. The control block received two sprays. Overall harvest damage was less than 1%, similar to 1994. Average release of dispensers was 0.9 mg/dispenser/day.

In Hungary, the infestation level in experimental plots was again quite high as can be deduced from trap catches in Table 3. No trap shut down was observed in traps containing 10-mg lures. Mating disruption combined with IGR treatment showed substantially reduced damage when compared to conventional treatment alone. Damage

levels in 1995 were higher than those in 1994. Dispenser release on average was found to be 0.8 mg/dispenser/day.

**Table 3** Results of field trials in 1995 (1x and 10x indicate 1-mg and 10-mg lures)

Plot	Treatment	Trap catch (moths/trap)	Damage (%)
California, 8 ha	Pheromone	176 (10x) 9 (1x)	1.0
	Control	215	0
Hungary, 4 ha	Pheromone	88 (10x)	2
	Control	92 (10x)	7.4
France, 4 x 2.5 ha	Pheromone	13 (10x) 2 (1x)	>2
Italy, 5 ha	Pheromone	1	0
	Untreated	1	0
	Untreated	18 (10x) 41 (1x)	1.2
Switzerland, 4 ha	Pheromone	3	0.7

Also in France, trap catches in the pheromone plots were found to be higher than those of 1994. Organophosphate treatment was applied at mid-season. Damage level also appeared to be higher than in 1994, reaching 5.5% at some particular spots. Average release of dispensers amounted to approximately 1 mg/dispenser/day.

In Italy trap catch data in an untreated plot suggest that 1-mg lures are more attractive than 10-mg lures. Data were based on one trap of each type only, therefore a factor like trap placement may have had a significant influence as well. Damage figures in this trial relate to mid-season damage which appeared to be low in all three plots. Even in the untreated plot, with significant trap catch, damage was not higher than 1.2%. Average release of dispensers approximated 1.1 mg/dispenser/day.

In Switzerland initial populations were low, no preventive insecticide treatments were necessary. Trap catch as well as fruit damage appeared to be low: 0.7% is well below economic threshold. Average release rate of dispensers was found to be 1.3 mg/dispenser/day.

Release profiles of field exposed dispensers have shown a very gradual release over a period of 4 to 5 months which, for most countries in the world, covers the whole flight season of codling moth. During this period excellent protection of codlemone was found. The results of all trials generally indicate the proper performance of TNO dispensers for sustained release of codlemone and sufficient biological efficacy. Also at higher initial pest populations good control can be obtained when application of mating disruption is combined with supplementary insecticide treatment prior to or

during the season. From technical point of view the TNO system is ready for commercialisation and market introduction. The system is very versatile and is suitable for preparing dispensers with a variety of other pheromones and volatile compounds (Klijnstra & de Vlieger 1994; de Vlieger & Klijnstra 1994). A production scenario of dispensers is currently under development and the technology is available for licensing.

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## **The role of foliage in mating disruption in apple orchards**

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**Abstract** - Apple foliage affects atmospheric concentrations of pheromone by absorption and release, according to electroantennogram (EAG) measurements, behavioural studies with *Epiphyas postvittana* (Lepidoptera: Tortricidae), and chemical analysis. This paper reviews the behavioural evidence for the impact of apple leaves on mating disruption. Release of marked naive male moths into blocks following the removal of polyethylene dispensers resulted in significantly lower trap catch, compared to untreated blocks. Catch of wild male moths was similarly reduced in the blocks which had previously been treated with dispensers. The level of disruption was the same for naive and wild male moths (63% disruption). A similar experiment using synthetic pheromone lures (tenfold the standard lure) produced a similar level of disruption in the treated area (72% disruption) following the removal of dispensers. These results indicate that apple leaves can absorb and release sufficient pheromone to enhance mating disruption

**Key words** - sex pheromone, mating disruption, release rates, foliage absorption, apple orchard, *Epiphyas postvittana*, Tortricidae, Lepidoptera

### **Introduction**

Mating disruption relies on maintaining a sufficiently high pheromone concentration over the treated area, to disrupt intraspecific communication. Plant foliage has been recognized for some time to be capable of pheromone uptake and release (Wall *et al.* 1981). However, the potential importance of absorption and release of pheromone by plants on the temporal and spatial distribution of pheromone in the context of mating disruption has not been widely investigated.

Karg *et al.* (1990) and Karg & Sauer (1992) showed the importance of grape vine foliage to the mean pheromone concentration in a pheromone-treated vineyard. Hrdy *et al.* (1990) recovered pheromone from stonefruit foliage. Karg *et al.* (1994) demonstrated foliage uptake and release of pheromone by apple leaves. These results

led us to hypothesise that leaves could affect the atmospheric pheromone concentration and its temporal distribution, and thereby enhance the success of mating disruption in apple orchards. Here we summarise two behavioural experiments on the influence of foliage on disruption, following the removal of polyethylene dispensers.

### Materials and methods

Dispensers contained 54.9 mg (*E*)-11-tetradecen-1-yl acetate, 2.5 mg (*E,E*)-9,11-tetradecadien-1-yl acetate, 19.7 mg (*Z*)-11-tetradecen-1-yl acetate, as well as 16.8 mg of other substances such as stabilisers (Shin-Etsu Chemical Co., Tokyo). These dispensers have been used in large scale trials of disruption (Suckling & Shaw 1995). Delta traps were baited either with rubber septa containing 1 000 µg of 100/5 *E*11-14Ac/*E*9,*E*11-14Ac (Bellas *et al.* 1983), or virgin female moths in gauze cages. Traps were checked daily. Disruption was calculated from catch in the treatment/(treatment plus control).

In experiment 1, the first behavioural experiment of the effect of foliage on disruption (Suckling *et al.* 1996), we released 52 to 125 naive laboratory-reared moths ca. 1 hr after the removal of dispensers (700/ha), which had been in a 0.05 ha apple block for 48 hr. Feral moths, which had been present in the area during the treatment, were also trapped. The experiment was repeated on four nights, each time in a different apple block. Nine delta traps baited with virgin females were used in each block, which had similar numbers of moths released into a similar untreated control block, about four hours before moth flight at dusk. Traps were hung after the dispensers were removed.

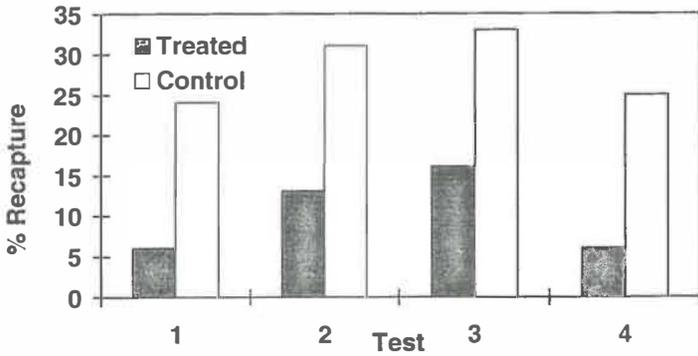
In experiment 2, the treated area was similar to the above, but dispensers were placed into the four replicated blocks for two days, and removed, as above. Feral moths, which had been present in the area during the treatment were trapped in nine delta traps baited with 1 000 µg were used in each block. This lure strength (equivalent to tenfold the standard monitoring lure) was used to ensure high moth catches, after previous trapping had shown increased catch in treated and untreated apple blocks with high-dose lures (Suckling & Karg 1996). Control blocks were operated as above.

### Results and discussion

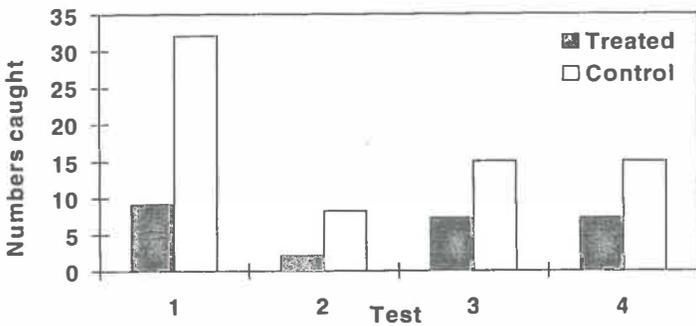
The recapture of naive male moths in traps baited with virgin females was lower in the pre-treated blocks than in the equivalent control blocks (Figure 1), and was equivalent to 65% disruption. These results were obtained when dispensers were no longer present, and when the sole source of pheromone for disruption was from the apple trees. Since it has been demonstrated elsewhere that apple foliage can absorb and release pheromone (Karg *et al.* 1994, Suckling *et al.* 1996), it can be argued that the

most probable source of pheromone in this experiment was the apple foliage, which is covered by leaf waxes (Baker 1982). The use of naive moths prevents the possibility of pre-exposure.

The same level of disruptive effect was observed for feral moths (Figure 2), which had been present in the blocks before dispensers were removed.



**Figure 1** Rate of recapture of unexposed male *E. postvittana* in four paired apple orchard blocks, with moth release after removal of disruptant dispensers (after Suckling *et al.* 1996)



**Figure 2** Catch of wild male *E. postvittana* in four paired apple orchard blocks, after removal of disruptant dispensers (after Suckling *et al.* 1996)

The proportion of moths caught in the previously-treated blocks was the same (Table), and again significantly lower ( $P < 0.05$ ) than the expected proportion of the total catch (0.5). This finding suggests that the potential pre-exposure of the feral moths was not sufficiently lasting to cause greater disruption than occurred for the naive moths.

The next experiment, which used synthetic lures (at 10 fold the standard lure strength) produced a similar result, with a proportion of 0.22 of all moths caught in the blocks which had been previously treated (Table). This result indicates that the magnitude of the estimated effect of disruption from foliage sources was independent of lure. A similar effect of disruption for one night, despite removal of dispensers, was reported using synthetic lures at the standard rate (Suckling *et al.* 1990).

**Table** Effect of pheromone from foliage on trap catch of *E. postvittana* in 0.05-ha plots on the night after removal of polyethylene disruption dispensers (after Suckling *et al.* 1996 and unpublished data)

	Naive	Wild	Wild
Catch in treatment/total <sup>a</sup>	0.26	0.26	0.22
SEM	0.06	0.04	0.10
Disruption	65%	65%	78%
Males caught	156	95	106
Lure	virgin female	virgin female	1000 µg

<sup>a</sup> Treatment/(treatment + control)

Apple foliage is capable of absorbing and releasing pheromone in biologically important amounts, and is therefore likely to play a role in aiding disruption. While the level of disruption caused by foliage following the removal of dispensers is less than would be desirable from the perspective of insect control (ca. 60-70% disruption), dispensers are normally left in place. The buffering effect we have demonstrated is likely to supplement pheromone from dispensers, by raising the background concentration in the air. Pheromone trapped on foliage in this way would remain in the block, to be released later, rather than being immediately lost from the system. Pheromone concentrations in foliage are a function of atmospheric concentration, and decline logarithmically with distance from dispensers (Suckling *et al.* 1996). The buffering effect would therefore be expected to be most important in the vicinity of dispensers. It would be least effective in orchards with missing foliage, or during times of limited leaf area density, such as in the spring.

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## **Pheromone release by codling moth females and mating disruption dispensers**

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**Abstract** - Codling moth females (*Cydia pomonella* L.) released codlemone, (E,E)-8,10-dodecadien-1-ol, at an average rate of  $6.1 \pm 1.3$  ng/hr during the first two hr of calling. A ten-day-old red rubber septum, loaded with 100 µg of codlemone, released ten times more than a calling female ( $60.5 \pm 18.0$  ng/hr). Codlemone release from Ecopom and Isomate C+ mating disruption dispensers, containing 250 mg and 90 mg codlemone, decreased from  $>20$  µg/hr on the first day to  $5.7 \pm 0.1$  µg/hr and  $2.9 \pm 0.2$  µg/hr after 64 days, respectively. The proportion of the codlemone isomers (E,Z-, Z,E-, and Z,Z) released from these dispensers ranged from 6.0 to 9.1%. In an apple orchard treated with Ecopom dispensers, releasing approximately 12 mg/hr/ha, the aerial concentration of codlemone was estimated to be  $1.1$  ng/m<sup>3</sup> by air sampling.

**Key words** - sex pheromone, mating disruption, female effluvia, dispenser, release rate, air sampling, *Cydia pomonella*, Tortricidae, Lepidoptera

### **Introduction**

This study attempted a comparative analysis of the release rates of codling moth sex pheromone, (E,E)-8,10-dodecadien-1-ol (E8,E10-12OH; codlemone) (Roelofs *et al.* 1971), from calling females, trap lures, and mating disruption dispensers. The aerial concentration of codlemone in a mating disruption orchard was determined by air sampling, concurrently with field-EAG measurements (Koch *et al.* 1997).

Such data is needed for the further development of the mating disruption technique, including optimization of pheromone dosage and dispenser densities under different climatic and topographic conditions, and the interpretation of the induced behavioural modifications.

## Materials and methods

Effluvia from ten individual female codling moths were collected in an air-flow apparatus (Witzgall & Frérot 1989). The glands of these females were extracted after two hr of calling. Female glands were also extracted at various times before and after the onset of calling ( $N = 10$ ). The release from ten-day-old red rubber septa (Thomas Scientific, Illinois), loaded with 100  $\mu\text{g}$  of codlemone, was measured using the same apparatus as for the calling females ( $N = 5$ ; Bäckman *et al.* 1997).

The release of codlemone was measured from field-aged Ecopom (Isagro Ricerca, Novara, Italy) and Isomate C+ (Shin-Etsu Chemical Co., Tokyo) mating disruption dispensers, containing initially 250 mg (Rama 1997) and 90 mg codlemone, respectively. The same dispensers ( $N = 3$ ) were suspended in stoppered glass flasks for 0.5 to 2 hr, at 0, 8, 32 and 64 d after field exposure, and in the case of Isomate C+ also after 1 yr. The flasks were washed with solvent, which was analysed by gas chromatography (GC) (see Bengtsson *et al.* 1994). For measurements of isomeric composition, the extracts were concentrated until the *E,Z*; *Z,E*; and *Z,Z* isomers of codlemone were detectable.

The concentration of codlemone in orchard air (1000 Ecopom dispensers/ha) was determined by air sampling on Tenax filters (Bäckman *et al. in prep.*). The filters were carefully rinsed and sealed before use. Two weeks after dispenser application, 2.5 to 3.5  $\text{m}^3$  of orchard air was drawn through a filter during 4 to 5 hr. Hexane filter extracts were evaporated under a stream of nitrogen and each extract was injected on two different GC columns. Tridecanol was used as internal standard.

## Results and discussion

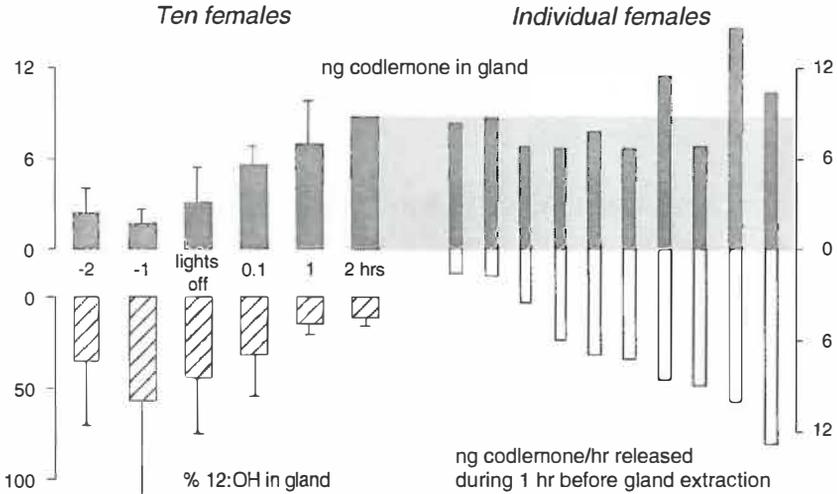
### Female gland extracts

The amount of codlemone in female glands increased after lights off, and peaked at  $8.8 \pm 2.7$  ng after 2 hr of calling. This increase was accompanied by a decrease in gland proportion of dodecan-1-ol (12OH), from  $56.0 \pm 52.6$  %, 1 hr before light off, to  $10.7 \pm 5.0$  % after 2 hr of calling (Figure 1; Bäckman *et al.* 1997).

Dodecanol and tetradecanol (14OH) have been identified as minor pheromone components (Arn *et al.* 1985; Einhorn *et al.* 1986; Bartell *et al.* 1988; Causse *et al.* 1988). Isomate C+ dispensers contain these two compounds together with codlemone. The behavioural role of the saturated alcohols is still controversial (McDonough *et al.* 1993, 1994, 1995). The proportional decrease in female glands would argue against a behavioural role of 12OH, since behaviourally active minor components are expected to be produced in rather constant ratios. According to biosynthetic studies, 12OH stems from reduced codlemone precursors (Löfstedt & Bengtsson 1988), and the high proportion of 12OH before calling was probably due to an accumulation in the gland during an early phase of pheromone production.

Female effluvia

The female release of codlemone was on average 6.7 ng/hr between 1 and 2 hr of calling. The individual variation was quite large (Figure 1), although all females were calling continuously for 2 hr without moving, with their glands close to the orifice of the glass capillary tube used for pheromone collection. The comparison of codlemone gland titer and release suggests a continuous production during calling, at a turnover rate of approximately 1 hr (Figure 1).



**Figure 1** Average codlemone and 12OH gland titer (left side), at 2, 1 and 0 hr before onset of the scotophase, and at 0.1, 1, and 2 hr after onset of female calling ( $N = 10$ ). Codlemone gland titer after 2 hr of calling, split into individual females (shaded, narrower bars; right side), and codlemone release by these females during 1 hr before gland extraction (empty bars) (data from Bäckman *et al.* 1997)

Release from rubber septa

Red rubber septa, loaded with 100  $\mu\text{g}$  of codlemone and aged in the field for 10 days, released  $60.6 \pm 15.7$  ng/hr ( $N = 5$ ). Such septa, used for monitoring of codling moth males, released almost ten times more codlemone than a calling female.

Dispenser release

The release rates of codlemone from fresh Ecopom and Isomate C+ dispensers were the same: this could be an artefact, due to rapid saturation of the glass walls with

pheromone. The release rates then gradually decreased over 64 days; the decrease was slower in Ecopom than in Isomate C+ dispensers (Table). Ecopom dispensers are loaded with 250 mg of codlemone, the recommended density for codling moth control is 300 to 400 dispensers/ha; Isomate C+ dispensers are loaded with 90 mg/dispenser, the recommended density is 1000 dispensers/ha. All release rates were measured in static atmosphere, and are expected to be higher under air flow.

Compared to a calling female, fresh Ecopom mating disruption dispensers released approx. 3000 times, and a 64-day-old 600 times more codlemone. In one-year-old Isomate C+ dispensers, the release was still above 1 µg/hr (Table). It is important to note that dispensers left in orchards release enough codlemone to affect male behaviours during the following season, even if the orchard is treated with fresh dispensers.

The proportion of codlemone isomers (*EZ*, *ZE*, *ZZ*) was quite stable over 64 days; 1-yr-old Isomate C+ released  $24.1 \pm 2.8$  % of the other isomers (Table). Both fresh and aged dispensers, including 1-yr-old Isomate C+, were attractive to codling moth males in the field (Bäckman *et al. in prep.*). The equilibrium blend of 61 % codlemone and 39 % of its geometric isomers has been reported to inhibit male attraction in the wind tunnel (McDonough *et al.* 1993).

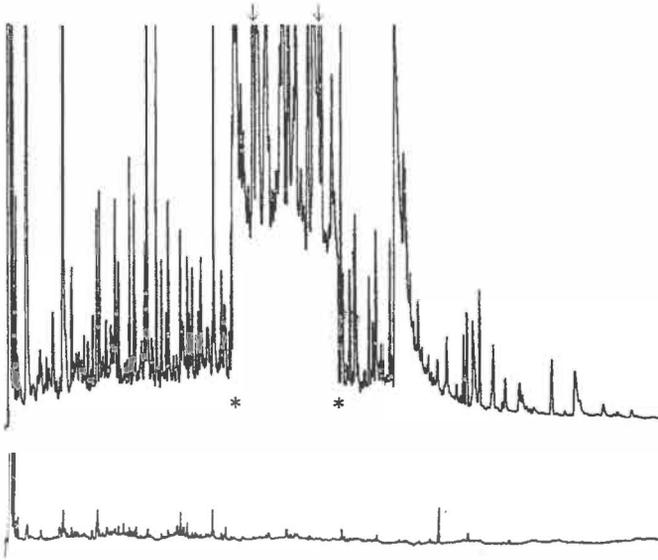
**Table** Release of codlemone and its geometric isomers (*EZ*, *ZE*, *ZZ*) from Ecopom and Isomate C+ mating disruption dispensers ( $N = 3$ )

Dispenser age	0	8	32	64 days	1 year
<i>Ecopom</i>					
codlemone (µg/hr)	$21.9 \pm 1.0$	$15.8 \pm 0.6$	$11.0 \pm 0.2$	$5.7 \pm 0.1$	-
other isomers (%)	$6.4 \pm 0.3$	$6.4 \pm 0.2$	$6.9 \pm 0.4$	$9.1 \pm 0.2$	-
<i>Isomate C+</i>					
codlemone (µg/hr)	$21.8 \pm 1.6$	$7.9 \pm 0.9$	$3.6 \pm 0.1$	$2.9 \pm 0.2$	$1.4 \pm 0.1$
other isomers (%)	$7.6 \pm 0.3$	$6.0 \pm 1.1$	$6.4 \pm 0.2$	$7.2 \pm 0.1$	$24.1 \pm 2.8$

#### Pheromone concentration in field air

Orchard air contains a large number of volatile compounds, at high amounts (Figure 2). This made GC-analysis of codlemone collected from field air difficult, since some of these compounds elute close to codlemone. According to the successful measurements (codlemone peak visible on both polar and nonpolar capillary column in each sample), the aerial concentration of codlemone in an apple orchard with 1 000 Ecopom dispensers/ha, releasing ca. 12 mg/hr/ha (14 days old), was estimated to be in the order of 1 ng/m<sup>3</sup> ( $1.1 \pm 0.2$ ;  $N=3$ ). The air-sampling was done simultaneously with field-EAG measurements (Koch *et al.* 1997). The dispenser density at the site used

for air sampling was 2.5 times higher than the recommended density for control of codling moth (Rama 1997).



**Figure 2** Gas chromatogram of 2.5 m<sup>3</sup> orchard air sampled during 2.5 hr on a Tenax filter. First arrow: internal standard (10 ng 13:OH); second arrow: codlemone. Asterisks indicate a change in signal amplification (attenuation)

Figure 2 and Figure 4 in Koch *et al.* (1997) illustrate the selective perception of minute amounts of codlemone by the male antenna in orchard air, containing numerous other volatile compounds at much higher amounts. Nanogram concentrations of pheromone in this "odour environment" induce behavioural modifications leading to disruption of mating.

The aim of parallel air sampling and field-EAG measurements was to calibrate the antennal response measured in mV (Koch *et al.* 1997) with aerial pheromone concentrations. The field-EAG apparatus allows on-line measurements at a high temporal resolution - air sampling on filters over several hours produces only average measurements. It should be desirable to include a range of concentrations in the calibration measurements, but the field-EAG technique is not applicable at higher concentrations — and air sampling for longer time intervals at lower pheromone concentrations is not possible either, due to the accumulation of plant volatiles on the filter. Simultaneous calibration measurements will therefore be pursued in clean air.

## Acknowledgements

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## **Pheromone measurements by field EAG in apple orchards**

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**Abstract** - Pheromone measurements using a field EAG system were made in apple orchards treated for mating disruption of codling moth, *Cydia pomonella*. We present the first example of simultaneous pheromone concentration measurements, using a field EAG system and gas chromatographic analysis of air filters. This allows an absolute calibration of the antennal response to pheromone dilutions in oil. Original recordings are shown illustrating the interaction of pheromone and non-pheromone stimuli in the EAG signal, and the instantaneous reaction to the transition from pheromone-free to a pheromone-loaded air current.

**Key words** - sex pheromone, mating disruption, field EAG, aerial pheromone concentrations, pome fruit orchard, *Cydia pomonella*, Tortricidae, Lepidoptera

### **Introduction**

Measurements of aerial pheromone concentrations can be an important contribution to the further development of the mating disruption technique. Investigations of wind effects, plant-surface interactions and concentration profiles in vertical and horizontal dimensions all require the acquisition of many measurements in time intervals which can be considered short in comparison to the time course of changes in temperature or wind situation.

Methods using air sampling and subsequent analysis by gas chromatography (*e.g.*, Flint *et al.* 1993) offer absolute average concentration values, but they are not fast enough to yield a sufficient number of measurements in the required time. Field electroantennogram (EAG) measurements of pheromone concentrations can offer reproducible concentration values on a relative scale. The field EAG measurement system developed in Kaiserslautern has been used to measure pheromone concentrations in vineyards (Milli 1990; Sauer 1991; Färbert 1992, 1995; Karg 1992; Koch *et al.*

1992; Termer 1992; Karg & Sauer 1995), in cotton fields (Cardé *et al.* 1993; Färbert & Koch 1993; Färbert 1995; Färbert *et al.* 1996), in pea fields (Bengtsson *et al.* 1994) and in apple orchards (Milli 1993; Karg *et al.* 1994; Suckling *et al.* 1994; Milli *et al.* 1996). The newer versions of our system make use of a signal superposition technique to suppress the influence of plant odors and other non-pheromone airborne stimuli on the pheromone concentration measurements.

Up to now, field EAG measurement results had only been given in relative units, defined by the properties of the calibration sources in our system. In collaboration with the Chemical Ecology Group at Alnarp (*see* Bäckman 1997) we have simultaneously measured pheromone concentration with the field EAG and the classical filter adsorption technique. This permits to transfer the relative concentration units to absolute concentration values. In addition, we present measurement examples with highly dynamic changes in airborne pheromone concentration values demonstrating the linear superposition of pheromone- and non-pheromone-generated EAG signals.

## Materials and methods

### Field EAG System

The field EAG system used in these experiments has been described in detail (Färbert *et al.* 1996a,b). It consists of an antenna holder, which is attached to the bottom of a vertical tube in which a steady current of air (14 ml/s) is maintained using a suction pump. A charcoal filter placed at the tube upper entrance removes all stimulating odor components from the incoming air. Three calibration sources, consisting of glass syringes, containing a vial with a pheromone/oil mixture (Sauer 1989), are connected to the tube in such a way that activation of the syringe piston generates an air puff (0.25 ml, 0.6 s duration) with defined pheromone content which is injected into the main airstream. An excised antenna of codling moth, *Cydia pomonella*, placed into the antennal holder, was used for measurements of airborne codlemone, (*E,E*)-8,10-dodecadien-1-ol.

The antennal responses to puffs from the calibration syringes with pheromone concentrations in three decadic steps are used to construct a dose response curve characterizing the properties of the antenna. When the charcoal filter is removed from the tube, outside air reaches the antenna and produces a rise in the EAG signal similar to a step function. The height of this step is caused by background odors as well as pheromones, and cannot be used as a measure for pheromone concentration. While the filter remains open, additional calibration pulses are released. The additional response of the EAG signal to the superimposed calibration puffs is used to calculate the airborne pheromone concentration: a small or disappearing additional response indicates high pheromone concentration, whereas an EAG response to the superimposed stimulus which is almost as strong as in the calibration mode indicates a very low level of ambient pheromone concentration, close to the reaction threshold of the antenna.

The relative pheromone concentration units used up to now in all our experiments are defined as follows: a concentration of  $10^{-6}$  relative units is the concentration present in the headspace of a calibration syringe containing a vial with  $10^6$  parts of paraffin oil (Merck No.7161) and one part of pheromone.

The EAG measurement system including pumps, calibration syringes and associated step motor drives is mounted on a compact probe which is fully remote controlled and can be operated on a pole up to 5.5 m high. Wind velocity and direction are recorded in 40-ms intervals by two sensitive vector anemometers, one mounted on the EAG probe, the other at 5.7 m height.

#### Parallel air adsorption measurements

These experiments were done in an experimental apple orchard at Alnarp (Sweden). The tree height was 2 m, tree spacing was 2.5 m, and the rows were 2 m apart. A surface of 0.5 ha had been treated with dispensers (Isagro, Novara) loaded with 250 mg of codlemone and 250 mg of codlemone acetate, (*E,E*)-8,10-dodecadien-1-yl acetate, placed at a density of 1000 dispenser/ha.

The first air sampling experiment was made on July 8, 1996 (Bäckman 1997). The air filter was positioned in the center of the orchard, at equal distance from the closest dispensers, at 2.0 m height. The field EAG probe was positioned at the same height. The EAG probe and the air filter were positioned on a line perpendicular to the wind direction, 0.5 m apart. While the orchard air was drawn through the filter, measurements were done continuously with the field EAG. The individual readings of the EAG system were corrected for temperature dependence of the pheromone vapor pressure in the calibration syringes and then averaged to yield a representative overall pheromone concentration reading representing the time span during which the air sampling had taken place.

#### Interaction of pheromone and background

These recordings were made in an orchard at Solnäs, 10 km north of Lund (Sweden). Trees (2.20 m high) were spaced 1.6 m apart, at a row distance of 3.3 m. A total of approximately 14 ha had been treated with Ecopom dispensers (Isagro; 250 mg codlemone/dispenser) on May 27 at a density of 400 dispensers/ha. The field EAG recordings were taken on June 6, 21 hrs, on a transect parallel to the rows leading to the southern edge of the orchard.

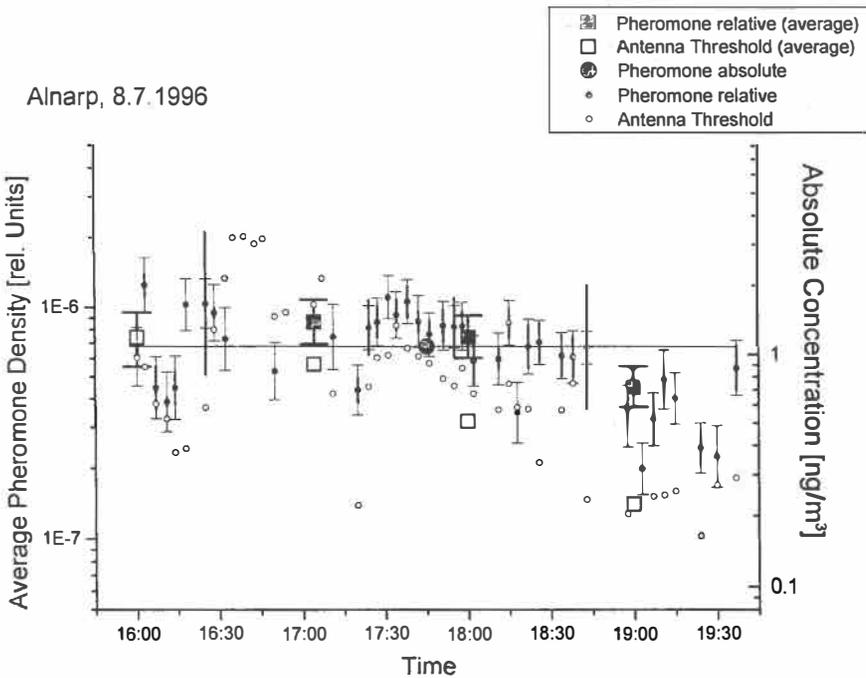
## Results

#### Simultaneous EAG and absorption measurements

Figure 1 shows the results of the field EAG measurements made on July 8, concurrently with a filter adsorption measurement. The pheromone concentration readings

were averaged in four sections. During each section the temperature was sufficiently constant. The four averages were then individually corrected for the temperature dependence of the pheromone vapor pressure in the calibration syringes. Finally, the four averages were again averaged to yield one overall average concentration:  $6.8 \pm 0.5 \cdot 10^{-7}$  relative units. The result of the adsorption measurement was  $1.1 \text{ ng/m}^3$  (Bäckman 1997);  $1.0 \cdot 10^{-6}$  relative units of codlemone are accordingly equivalent to  $1.6 \pm 0.3 \text{ ng/m}^3$ .

The repetition of this measurement (12:30 to 17:30 hrs) took place on July 12 at the same location (Figure 2). Our overall averaged relative concentration reading was

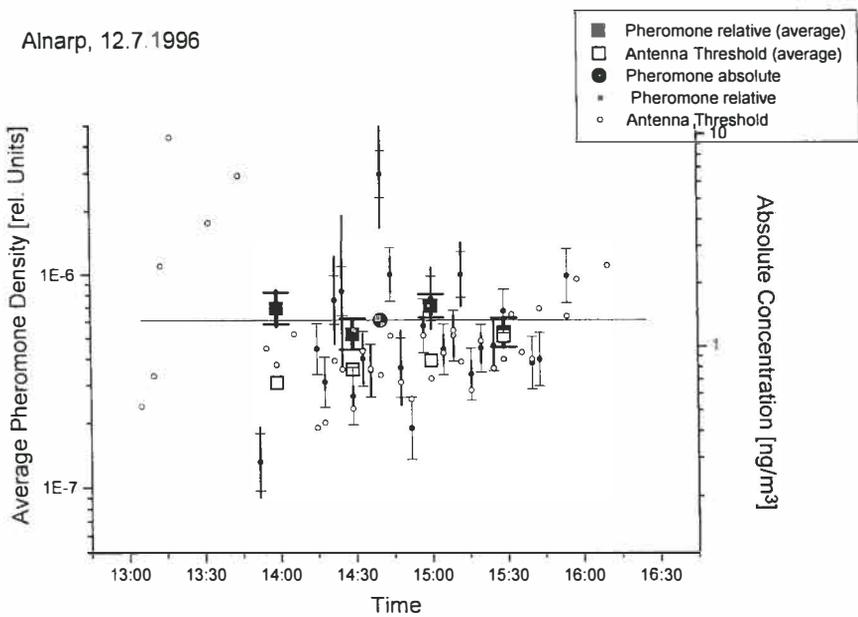


**Figure 1** Pheromone concentration measured in relative units (for definition see text) plotted versus time. Between 16:10 and 19:57, the orchard air was pumped through a pheromone-adsorbing filter (Bäckman 1997). Thin line error bars indicate the confidence interval of an individual concentration reading. Thick line error bars (without delineator) indicate standard error generated by averaging individual pheromone concentration readings. Open circles indicate the sensitivity threshold of the antenna in use. Large filled squares are averages taken over four time sections during which temperature was sufficiently constant, with temperature correction applied. The large filled circle indicates overall average of pheromone concentration in relative units. The right vertical axis was adjusted in such a way that the absolute concentration measured by adsorption technique reaches the same position as the overall average of the field EAG

$6.1 \pm 0.5 * 10^{-6}$  relative units. The result of the adsorption measurement was  $1.3 \text{ ng/m}^3$  (Bäckman 1997):  $1.0 * 10^{-6}$  relative units of codlemone are accordingly equivalent to  $2.1 \pm 0.4 \text{ ng/m}^3$ . The measurements of July 8 and 12 are in agreement within the range of the errors.

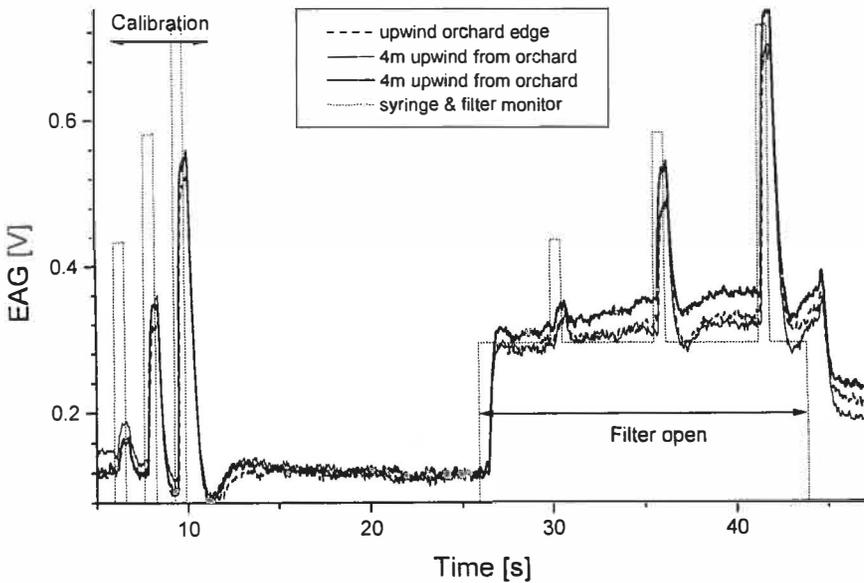
Pheromone-background interaction

During most of these measurements, the wind came from southern directions, thus the southern edge of the orchard received pheromone-free air coming from the neighbouring sugar beet field. Figure 3 shows three superimposed EAG traces recorded 4 m upwind from the orchard and at the orchard edge. As there is no pheromone in the air, the plateau response in Figure 3 is caused only by background odors. Note that although the position was not changed, the plateau height changed significantly. This must have been caused at least in part by a change of background odor concentration. In contrast, the additional response to the superimposed stimulus remains the same, indicating the unchanged pheromone concentration near the threshold of the system.



**Figure 2** Repetition of the measurement of Figure 1 at the same location, 4 days later. The equivalence between relative and absolute concentration units found in Figure 1 is confirmed within the confidence intervals of both experiments

Figure 4 shows three superimposed EAG traces taken at different positions along the transect, oriented in north-south direction. At 4 m upwind from the orchard edge, the calibration pulses superimposed on the plateau (generated by opening the charcoal filter) were almost the same height as during the calibration. At the edge and inside the orchard 30 m downwind from the edge, we observed strong fluctuations of the EAG signal. Whenever the superimposed pulses occurred during a strong upward excursion of the EAG signal, the net response to the pheromone stimulus was strongly reduced - but the absolute height of the peak of the response to the superimposed stimuli remained the same (see arrows in Figure 4). This antennal behavior is consistent with the concept of linear superposition of the EAG voltages generated by background stimuli and pheromone stimuli.



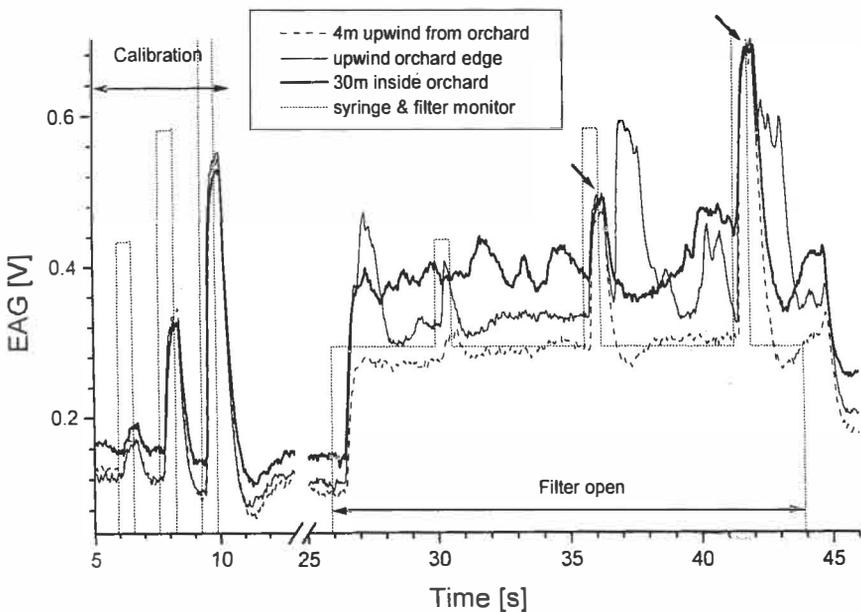
**Figure 3** EAG signals recorded at the upwind edge of an apple orchard treated for mating disruption. Solid and dashed traces: EAG voltage; dotted trace: activation of calibration syringes (columns of different height indicate activation of syringe with different pheromone content and opening of charcoal filter; plateau signal). The left part of the figure shows the calibration phase, in which the syringes are activated while the charcoal filter is in place. The right part shows the reactions to ambient air and superimposed calibration stimuli. Since the air in this measurement situation is pheromone-free, the plateau appearing on the opening of the charcoal filter is generated by background odors. Note that although the plateau height increases for the thick solid trace, the net reaction to the syringe stimulus stays constant

Another measurement is shown in Figure 5. Here, the probe was positioned 6 m upwind from the orchard edge. Initially, only background signals were recorded, until the wind direction shifted by about  $120^\circ$  (as shown by the traces of the vector anemometer). After the wind was coming from the treated orchard, the air was loaded with pheromone, leading to an EAG trace with a very high plateau, in which both low dose syringe pulses disappeared completely. The evaluation of the pheromone concentration readings yields  $5.5 \cdot 10^{-8}$  or  $0.10 \text{ ng/m}^3$  and  $1.8 \cdot 10^{-6}$  or  $3.3 \text{ ng/m}^3$  for the two traces in Figure 5. This is a 33-fold increase in concentration without a change of the position of the probe, at a time lag of only 50 s between the two measurements.

## Discussion

### Simultaneous measurements

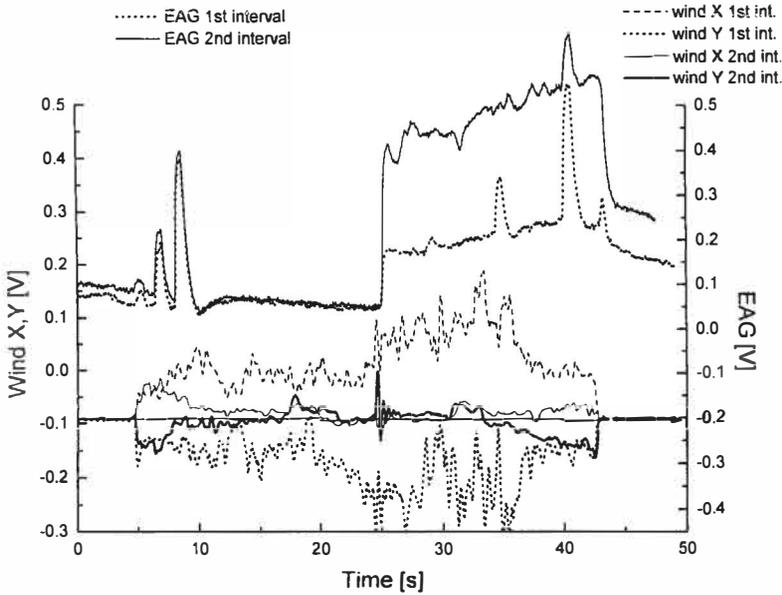
The calibration of the field EAG system *via* sampling of airborne pheromone on filters represents a substantial improvement. Up to now, attempts were only made to determine the absolute pheromone concentration in the headspace of our calibration syringes (Sauer 1991; de Kramer *pers. comm.*; Koch & Cardé *in prep.*). Even if a precise knowledge of the syringe headspace concentration was available, a calculation of



**Figure 4** EAG signals recorded at different positions in the orchard. The response peaks to the calibration stimuli remain at the same absolute height; but the traces between these stimuli show strong variations, resulting from pheromone-loaded air packets arriving at the probe

the overall sensitivity of our system would require data on the mixing ratio between the syringe puffs and the ambient air entering the measurement system.

The values determined here represent the performance of the field EAG system as a whole, and therefore offer a much higher reliability. The feasibility of aerial pheromone measurements by filter adsorption offers the possibility to experimentally test the sensitivity of the field EAG to different types and levels of background signals.



**Figure 5** Comparison of responses to pheromone-free (dotted line) and pheromone-loaded (solid line) air occurring within 50 s due to a sudden shift in wind direction. Top traces: EAG voltage; bottom traces: vector anemometer x and y components. The y-component (thick line) of the anemometer output changes sign in the second interval, indicating a shift in wind direction by approximately  $120^\circ$ . This causes air from the treated orchard instead of clean air to reach the probe. Note that due to the high pheromone concentration, the responses to the low and medium dose syringes disappear in the pheromone plateau

#### Pheromone-background interactions

The signal superposition technique used in our field EAG measurements rests on the assumption that the EAG signal voltages generated by pheromone add linearly to the EAG signal voltages generated by background odors. Recently, Rumbo *et al.* (1995) presented recordings in which the validity of this assumption was questioned. The cir-

cumstances of the recordings of Rumbo *et al.* were not documented to full extent; it is possible that the level of pheromone stimulation was so high, that saturation of the EAG response occurred. In addition, the air speed was extremely high, and they did not use an isolated antenna, but a whole-animal preparation. Furthermore, we have also found antennae which had a moderate pheromone response but an extremely strong response to background stimuli. In these cases, a linear superposition cannot be achieved, these antennae were discarded. The small amount of interference documented in Figures 3 and 4 results in a slightly higher pheromone concentration reading. But since the readings resulting from records as in Figure 3 are close to the threshold of the antenna, they do not enhance the uncertainty of measurements in this concentration range.

The results presented in Figure 5 clearly show examples of the highly dynamic effects occurring in the field EAG signal and underline the importance of concomitant recordings of wind speed and wind direction at a high time resolution.

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*Insect behaviour*

## Understanding mating disruption in the pink bollworm moth

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**Abstract** - Field wind tunnels set out over cotton were used to gauge the efficacy and mechanisms of mating disruption in the pink bollworm, *Pectinophora gossypiella*. Shin-Etsu rope formulation (PBW-Rope) containing synthetic pheromone was hand applied at the standard, commercial-use density of 1 000 dispensers/ha. Laboratory-reared males were marked and released at dusk at the downwind end of the wind tunnels. Efficacy of disruption was estimated from hourly catches throughout the night in both pheromone-baited traps in treatment and check (disruptant-free) tunnels. Exposure of males to pheromone prior to release allowed the contribution of sensory adaptation/habituation to disruption of attraction to be estimated. Male flight tracks as well as other behaviors near Shin-Etsu ropes and pheromone-baited rubber septa were videotaped. Analyses of data revealed that several mechanisms contributed to disruption of attraction: sensory adaptation/habituation, competition, camouflage, advancement in time of the males' window of pheromone response, and arrestment of upwind anemotaxis.

**Key words** - pheromone, mating disruption, habituation, sensory adaptation, orientation, pink bollworm, *Pectinophora gossypiella*

### Introduction

In most cotton-growing regions of the world the pink bollworm (*Pectinophora gossypiella*) is a key pest. Its larval habitus of internal feeding makes foliar sprays of insecticide generally ineffective and consequently in many management programs numerous aerial sprays of insecticide are directed against the adult. Such applications frequently occur as often as weekly, from the setting of the first cotton bolls until close to harvest. Not unexpectedly, these sprays often trigger outbreaks of secondary pests which otherwise would have been regulated by natural enemies. The availability since

1978 of synthetic pheromone formulated to disrupt mating of *P. gossypiella* has fostered alternative management strategies (Baker *et al.* 1990). These include the application at the beginning of flowering ("pin-square stage") of dispensers releasing pheromone over the remainder of the growing season. The Shin-Etsu plastic "rope" dispenser (PBW-Rope<sup>®</sup>), for example, is typically deployed at the pin-square stage at the base of the cotton plant at a rate of 1 000/ha with a total application of 75 g of pheromone per hectare. Alternatively, catch of males in sentinel, pheromone-baited traps can be used to decide whether a population is dense enough to warrant an application of pheromone. If so, formulations with a longevity of generally 7 to 10 days are applied aerially. The Scentry hollow fiber (NoMate<sup>®</sup>), is an example of a low rate, short-life formulation: aerial application of 10 000 fibers/ha deposits a total of 2.8 g of pheromone per hectare, mainly on the upper portion of the cotton canopy. As measured by an electroantennogram-based system (Färbert *et al.* 1997), these two formulations generate different airborne concentrations of pheromone: the Shin-Etsu ropes emit about 1000-fold more pheromone near and within the canopy than the Scentry fibers.

These disparate deployment strategies entail considerable differences in application costs: season-long dispensers have a high, one-time expenditure. Aerially applied formulations cost less per application than hand-applied, long-life dispensers, but the former approach requires season-long monitoring with traps, and, typically, multiple applications.

A number of mechanisms have been proposed as contributing to mating disruption (Bartell 1982; Cardé 1990; Minks & Cardé 1988; Cardé & Minks 1995). When the disruptant is a copy of the natural blend, three mechanisms are believed to predominate. (1) Either sensory adaptation of the peripheral receptors or habituation of response at the central nervous system level either eliminates pheromone response or impairs mate finding performance. (2) In competition males spend time flying upwind along plumes from point sources of synthetic pheromone. Therefore, males have less time available to locate females. The reduction achieved in the proportion of females mating depends on the ratio of artificial pheromone sources to calling females and the comparative attractiveness of synthetic sources and females. (3) Formulated pheromone can camouflage pheromone plumes from calling females; the greater distance a male is from the female the more likely it is that the female's plume will be rendered imperceptible amongst the background of synthetic pheromone. The omnipresence of pheromone also may blur the plume's fine-scale structure, thereby lowering a male's probability of locating the pheromone's source (Mafra-Neto & Cardé 1994; Vickers & Baker 1994).

Other mechanisms may contribute to disruption. The presence of formulated pheromone could advance the timing of a male's rhythm of response, so that he initiates upwind search before females call (Cardé *et al.* 1993). Males may become "arrested" in high concentrations of pheromone (*e.g.*, Baker & Cardé 1979), failing to proceed upwind.

The proposed mechanisms need not be mutually exclusive; rather, their effects may be additive or even synergistic. For example, if the formulation consists of point sources of pheromone attractive to males, competition should be a principal disruptive mechanism. Disruption levels could be enhanced, however, by a precocious response of males to the artificial sources of pheromone before the initiation of female calling. Males that have responded to these artificial plumes may be either less apt or able to navigate a female's plume later in the night when natural calling commences. This example also underscores that the type of formulation (its emission characteristics, distribution, and position in the crop) dictates the mechanisms of disruption, the interactions among mechanisms, and therefore the efficacy of mating disruption. A formulation's efficacy also can be contingent on a pest's population density, which of course varies between sites (Cardé & Minks 1995).

Laboratory bioassays with many moth species (*e.g.*, Traynier 1970; Bartell & Lawrence 1973; Sanders 1996) have provided indirect evidence that each of these mechanisms could contribute to mating disruption in the field, but the extent to which such laboratory observations equate to behavior of free-ranging males in the field remains inferential. To duplicate in bioassays the range of conditions that a native male would encounter in a crop treated with disruptant and to have a record of a male's prior exposure to pheromone is an intractable problem; indeed, we simply do not know enough about the behavior of males in the field to allow us to recreate these parameters in a bioassay. We have attempted to mimic field conditions by employing large, walk-in wind tunnels set out over cotton plants in cotton fields (Cardé *et al.* 1993). We observed the plume-following behavior of individual moths released either in tunnels set in cotton fields treated with disruptant or in check tunnels placed in fields free of disruptant. A second valuable approach used an electroantennogram-based system to measure the airborne concentrations of pheromone in treated cotton fields over fractions of a second (Färbert *et al.* 1997). Measurements determined the spatial pattern of airborne pheromone generated by a given formulation as well as a formulation's longevity of emission. A combination of these two approaches has shown promise in establishing how mating disruption works (Cardé *et al.* 1993).

## Materials and methods

Our field wind tunnels have a working section that is 6.2 m long, 2.5 m wide and 1.85 m high. Air is pulled through the tunnel at 0.7 to 0.8 m/s by a fan mounted at one end; the air drawn into the tunnel is laminarized by a sheet of Hexel<sup>®</sup> cells. The tunnel consists of a frame over which a sheet of nearly clear polyethylene is set out at dusk and removed at dawn. The ground serves as the tunnel's floor and the tunnel is positioned lengthwise along two rows of cotton. Tunnels can be moved readily to new sites.

Levels of attraction and its disruption are assessed in several ways. Laboratory-reared males are marked to indicate day of release and treatment with color-coded

fluorescent powders. Males are released at ground level at the tunnel's downwind end immediately after sunset. Levels of attraction are gauged by catches in two pheromone-baited sticky traps (Delta), each positioned at the upwind end of one of the two rows of cotton, just below the top of the canopy. Males flying along a plume to one of the two monitoring traps must navigate a 6 m course through and around cotton foliage. Before release males can be pre-exposed to pheromone. Trap catches are tallied hourly from sunset to dawn.

When males are released into a tunnel just after sunset, many are immediately attracted to the traps, well before their normal time of attraction in the field (*e.g.*, Beasley & Adams 1994; Schouest & Miller 1994). Such an alteration in the timing of attraction raises the possibility that the navigation behaviors of released and wild males are not comparable; this is an especial concern if a male's history of exposure prior to release is a dependent variable. Therefore, in one test we used an alternative release strategy designed to mimic a natural pattern of exposure to field conditions. Males are released at dusk into screen cages 2 x 0.75 x 0.5 m high set over two rows of cotton. The screen cage is positioned across the width of the tunnel's downwind end. On the following night at dusk the cage's upwind and downwind sides are removed, and the cage is hoisted on cables to the wind tunnel's ceiling. This procedure allows males during the day before assay to enter fissures in the soil (Flint *et al.* 1975), where they presumably encounter relatively little pheromone. Males thus are "exposed" for 24 hr in the field to disruptant formulations before bioassay. This method also eliminates handling of males immediately prior to release, a procedure which may alter the timing of response to pheromone.

In several tests we recorded on video the reactions of males to formulations placed in the cotton canopy. We obtained tracks of males flying upwind and records of male reactions near point sources of formulations.

The formulation used in the following tests was the PBW-Rope containing *ca.* 75 mg of *P. gossypiella* pheromone. Ropes were tied around the base of cotton plants, approximately 3 m apart in alternate rows of cotton; this pattern is the standard commercial application rate of 1 000 dispensers/ha.

## Measurement of disruption using field wind tunnels

### Camouflage of a plume

Our first use of field wind tunnels (Cardé *et al.* 1993) showed that rope and fiber formulations severely disrupted male *P. gossypiella* orientation to sticky traps baited with pheromone, compared to attraction to traps in a check tunnel. A rope formulation in a wind tunnel placed in an untreated cotton field only disrupted trap catch along one side of the tunnel, the row of cotton in which the three dispensers were placed. The other row was free of disruptant dispensers, and many males were caught in its monitoring trap. A wind tunnel with the same 3-rope configuration placed in a field

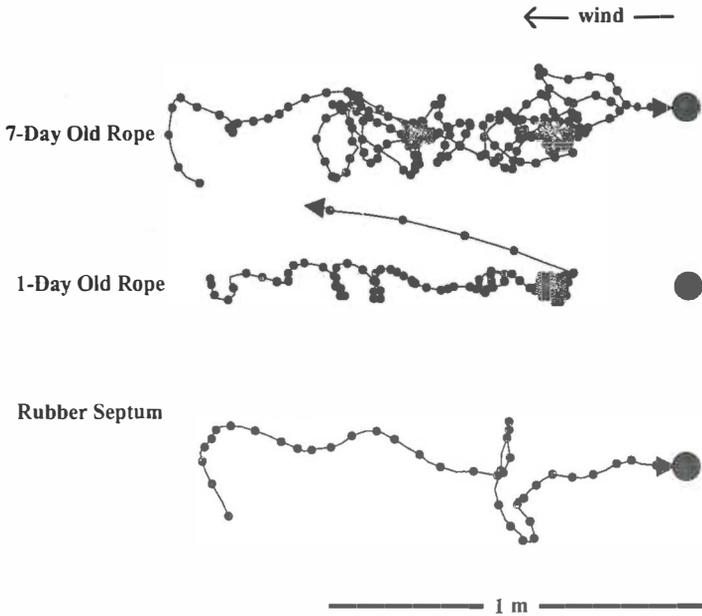
treated with rope, however, had low trap catches that were disrupted equally on both sides of the tunnel.

Measurements of airborne concentrations of pheromone by the electroantennogram system (Färbert *et al.* 1997) showed that in the 3-rope tunnel in an untreated cotton field, pheromone was present mainly on the treated side of the tunnel (some pheromone, of course, is emitted from the monitoring trap on both sides). Equal concentrations of pheromone, however, were measured along both rows of cotton in the tunnel within a rope-treated field (Cardé *et al.* 1993). The high level of disruption along the untreated cotton row in this tunnel was due, therefore, to pheromone-laden air from the treated field being drawn into the tunnel and along both rows of cotton. We interpret the high level of disruption documented on the untreated side of the tunnel as being due to a camouflage effect, although it is probable that the additional mechanisms described below augment this process.

#### Attraction to rope formulation

The PBW-Rope formulation emits pheromone at substantially higher rates than pink bollworm females. Flint *et al.* (1993) estimated that a rope exposed in the field for 30 to 60 days releases about 370 ng/min, whereas Haynes *et al.* (1987) measured mean emission from females at 0.16 ng/min. Given this wide disparity and the arrestment of upwind flight noted in other moth species at high pheromone emission rates (*e.g.*, Baker & Cardé 1979), it was not expected that *P. gossypiella* males would be routinely attracted to ropes. Males flying upwind along pheromone plumes generated by a rope dispenser which had been field aged for 7 days or from a rubber septum baited with 4 mg of pheromone produce generally comparable flight tracks (Figure 1), except that flight along the plumes from 7-day-old ropes is more torturous and slower than flight along plumes from septa. A rope aged in the field for only 24 hr also evokes upwind flight, but males generally do not approach, or land on, fresh rope formulation, and arrestment of upwind anemotaxis often is followed by flight downwind, and either another flight upwind or flight away from the plume.

Analyses of male activity within 0.5 m of a 7-day-old rope shows that a considerable proportion of released males arrive in its vicinity, wing fan for several min, eventually becoming quiescent. We also have found in late season observations in untreated cotton that an individual, week-old rope is quite attractive. Together these observations of flight tracks to ropes and behavior in their vicinity suggest that competition is a mechanism of disruption in rope-treated fields. The quiescence that ensues after attraction also verifies sensory adaptation/habituation as a factor. It will be of interest to learn if the threshold of response of such males has been raised (Cardé 1990; Mafra-Neto & Baker 1996).

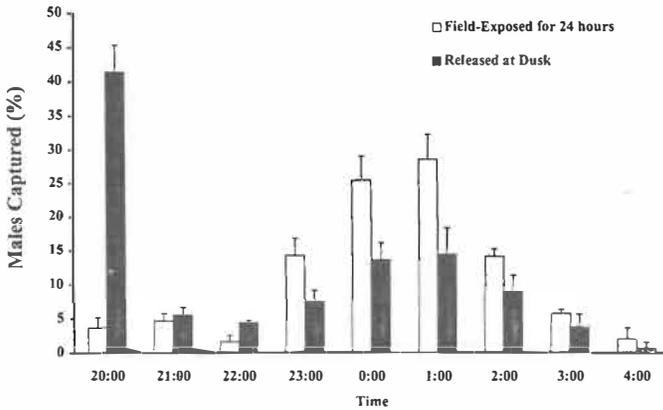


**Figure 1** Representative flight tracks of *P. gossypiella* males flying to three different pheromone sources in field wind tunnels. Wind flow was 0.7 to 0.8 m/s from the right to the left. Large dots represent the position of the pheromone sources and small dots represent the position of the moth every 0.07 s. Groups of 20 moths were released downwind, and flight within 2 m of the source was recorded from above using a Sony RSC 1050 rotary-shutter video camera. Ambient light was supplemented by a 25 w dark red tungsten lamp directed downward from the tunnel's ceiling. Moths flying upwind usually landed on either the 7-day-old rope or on the rubber septum; however, males exposed to a 1-day-old rope were arrested in flight and terminated the approach. In general, flight tracks were slower and more tortuous as the dose of pheromone in the dispenser was increased

#### Effects on the rhythm of response

When males are released from their holding cups at dusk, many of them initiate flight immediately and proceed upwind along plumes generated either from the rope formulation or the two monitoring traps. At the time of year and temperatures of our tests, male attraction would normally commence from about 24:00 to 02:00 (Beasley & Adams 1994; Schouest & Miller 1994). A precocious rhythm raises two questions. First, is the orientation behavior of males released from cups disrupted in the same way as that of males that have spent the previous 24 hr in the treated field where they potentially were exposed to disruptant? Second, can this wind tunnel method be used to evaluate the possibility of formulated pheromone altering the timing of males present in the field?

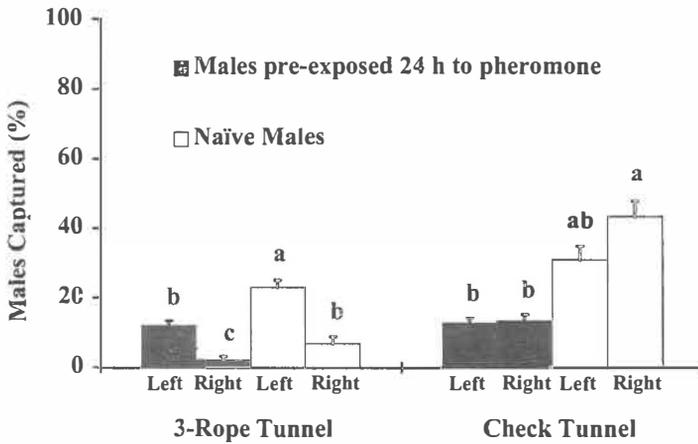
We compared the timing of attraction of males released at dusk with males of the same age held in large field cages for the previous 24 hr (Figure 2). No disruptant was used in this comparison. A pronounced flush of males in the traps shortly after their release from cups is evident. In contrast, males held in cages for 24 hr exhibit a rhythm of attraction similar to that described for native males (Beasley & Adams 1994; Schouest & Miller 1994), except that a small proportion is attracted between dusk and midnight (an interval when few native males would be attracted) and the peak of male attraction seems advanced to earlier in the night. We suggest that the small advance in time of attraction of some males held for 24 hr in the field is induced by the presence of the two pheromone-baited traps at the tunnel's upwind end. The overall levels of recapture averaged across the three nights of the test are equivalent ( $37.8\% \pm 4.1$  S.E. for males released at dusk on the night of capture versus  $34.5\% \pm 3.3$  S.E. for males caged in the field for 24 hr before release). Such comparability indicates that measuring the disruption of navigation to lures yields an equivalent outcome with either of these release protocols.



**Figure 2** Hourly percentages of *P. gossypiella* males that were either field-exposed for 24 hr or released at dusk. Males were captured in Delta traps baited with 4 mg of pheromone in a rubber septum. Fluorescent-powder-marked *P. gossypiella* males were held in groups of 50 in closed 1.8-dl containers. Field-exposed males were released at dusk on the night before the test into 1.8 x 0.75 x 0.5 m high screen cages placed across two rows of cotton. The following day the wind tunnel was positioned over the two rows of cotton such that the downwind end of the tunnel was over the screen cage. At dusk the upwind and downwind screen panels of the screen cage holding the 24-h-field-exposed males were removed and the cage was hoisted to the ceiling of the wind tunnel. Simultaneously, the cups with males that had just been brought to the field were introduced into the downwind end of the same wind tunnel and opened, thereby allowing both groups of males to move upwind. The only sources of pheromone in the tunnels were the two Delta traps in the upwind position. The experiment was replicated three times. Bars represent the mean and line bars represent the standard error of the mean ( $N = 420$  for each treatment)

## Effects of pre-exposure on subsequent response

Confinement of males in a holding cup with a 4-mg pheromone rubber septum for 24 hr before their release into tunnels showed that such prior exposure lowered subsequent attraction by about one-half compared to control males (Figure 3). The magnitude of reduction was similar both in the check tunnel with only the two monitoring traps and in the tunnel treated with three ropes. The lower levels of attraction in the three-rope tunnel reflect disruption of attraction on the treated right side, with some spillover of disruptive effect to the tunnel's untreated left half. When the covers of the cups containing the 4-mg lure were removed, many males took flight within 10 to 60 s, and in the check tunnel many of these males proceeded directly upwind to the traps.



**Figure 3** Percentages of pre-exposed and naïve *P. gossypiella* males captured in Delta traps baited with 4 mg of pheromone in a rubber septum. Fluorescent-powder-marked *P. gossypiella* males were held in groups of 50 for 24 hr prior to test in closed 1.8-dl cups. Cups for naïve males were pheromone-free, whereas the ones for the pre-exposed males contained a rubber septum loaded with 4 mg pheromone, attached with a pin to the internal surface of the container's lid. The two groups of males were held in separate environmental control rooms for 24 hr, and transported to the field in separate, temperature-regulated containers. Males were released in the wind tunnel at ground level at sunset (approx. 20:00). Lids and attached rubber septa were removed and capture of males was tallied hourly until sunrise (approximately 05:00 of the following day).

The check wind tunnel had no source of pheromone other than the two monitoring Delta traps in the upwind end of the tunnel. In the treatment tunnel 3 Shin-Etsu PBW-Ropes were attached 3 m apart to base of cotton plants on the right side of the tunnel. The percentage of naïve males responding in the check wind tunnel establishes the base levels of response against which disruption is measured. The experiment was replicated three times. Bars represent the mean and line bars represent the standard error of the mean ( $N = 450$  for each treatment). For each wind tunnel, bars having no letters in common are significantly different (ANOVA,  $p = 0.05$ , LSD comparisons)

This short interval indicates that some males have a very brief refractory period between prior exposure to high concentrations of pheromone and recovery of responsiveness.

## Conclusion

Behavioral experiments with the pink bollworm moths in field wind tunnels have verified that when PBW-Ropes are applied to a cotton field, diverse mechanisms contribute to mating disruption. Applied at the standard rate, this formulation releases sufficient pheromone to camouflage a pheromone plume within several meters of its point-source origin. This effect is reflected both in reduction in trap catch and in the airborne concentrations of the plume and ambient pheromone as measured by EAG (Cardé *et al.* 1993).

Direct behavioral observations in a field wind tunnel have established that some males fly upwind to these high-dose formulations, and many of these become arrested in flight. Other males may reach the rope dispenser, walk on it and wing fan nearby; within minutes, many of these males become quiescent within 50 cm of the ropes. Such reactions support both competition and sensory adaptation/habituation as contributing to disruption. Pre-exposure of males to high concentrations of pheromone before release in the tunnel results in a reduction in the proportion of males responding, again supporting a contribution of sensory adaptation/habituation to disruption. This pre-exposure test also documents that some males retain their ability to navigate a pheromone plume to its source. Males that presumably are exposed continually to pheromone in the field appear to advance their rhythm of pheromone response, which contributes to a competition effect and likely increases the probability of those moths subsequently becoming adapted/habituated.

Pink bollworm males in a cotton field treated with rope thus are subject to multiple, interactive mechanisms of disruption. A male's fate may be dependent on factors such as the level of his exposure to pheromone before his rhythm of attraction is expressed and his individual vulnerability to habituation. Our present understanding of the mechanisms of disruption remains fragmentary, in part because many other factors remain to be clarified. The effects of foliage, for example, on the fine-scale distribution of disruptant and on its adsorption and re-release (Färbert *et al.* 1997) may alter how disruption is achieved. If so, these effects will vary with the growth stage of cotton.

Mating disruption by broadcast application of pheromone clearly regulates pink bollworm populations and thereby the damage that this pest inflicts on cotton. Several commercial formulations achieve this end, but they do so with substantially different application rates and spatial distributions of emitted pheromone. We conclude that an examination of the behavior of males in cotton treated with different types of formulation will show substantial divergences in how disruption of mate finding is

achieved. The levels of contribution of the various mechanisms of disruption and their interactions will differ with the dispersion pattern of the formulation, and the spatial distribution of disruptant achieved within and near the canopy. Understanding these behavioral and dispersion processes will allow us to optimize deployment strategies and resultant efficacy.

### Acknowledgements

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## **Delay of mating of codling moth in pheromone disrupted orchards**

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**Abstract** - Studies were conducted to demonstrate the occurrence of delay of mating and its effect on the fecundity of codling moth in apple orchards under mating disruption. A delay in mating of two days was demonstrated with sterile moths released in pheromone-treated versus conventional orchards. In laboratory tests a two-day delay in mating resulted in a 40% reduction in fecundity.

**Key words** - sex pheromone, mating disruption, reproductive behaviour, pome fruit orchard, codling moth, *Cydia pomonella*

### **Introduction**

Adoption of mating disruption for management of codling moth, *Cydia pomonella* L., has proceeded at a rapid rate in several fruit growing regions of the United States following registration in 1991. Factors that affect the successful implementation of mating disruption (economics, moth population density, orchard topography) have been identified, and most successful uses of mating disruption have developed an intensive monitoring program and are integrating mating disruption with limited insecticide spray programs. Mating disruption for codling moth has been most successful in large contiguous orchards that have some degree of isolation.

The assumption of the various practioners has been that disruption of mating is the major mechanism affecting the success of their program. Since 1994 I have explored the potential of other factors associated with mating disruption-treated orchards that may contribute to grower's success in managing codling moth. For example, levels of egg predation were evaluated in eight orchards under mating disruption and conventional programs during 1995. These results found that egg mortality, primarily by earwigs, bugs, and spiders is ca. 20% higher in mating disruption orchards. Other

studies have examined the impact of mating disruption on moth behavior, and moth distribution and movement within and between treated and untreated orchards. Using a passive interception trap (Weissling & Knight 1994) I found that 45% of the female codling moths were mated during the first adult flight within replicated pheromone-treated orchards in 1994 (unpubl. data). Herein, I report new findings on the mating status of female codling moths in mating disruption orchards during the first generation, and from experiments to determine whether mating is delayed in disrupted orchards and what impact a delay in mating could have on the reproductive potential of codling moth.

### Materials and methods

Laboratory experiments were conducted to evaluate the effect of female age on fecundity and fertility. Moths from a laboratory colony were maintained at  $22 \pm 2$  °C, 50 to 65% relative humidity, and a 16:8 (L:D) photoperiod within 250-ml waxed paper cups. Adults were supplied with a cotton wick saturated with a 10% honey solution. Groups of 25 individual female moths <1 to 10 days old were placed in cups with two male moths 1 to 2 days old. Males were removed after one scotophase. Cups were checked daily for dead moths. Females were dissected to determine their mating status and the number of eggs; the number that hatched were counted two weeks after the female died.

Six solar-powered light traps and six groups of 18 interception traps were placed on April 27, 1995 within three orchards treated with Isomate-C+ dispensers (Shin-Etsu Chemical Co., Tokyo) at 1 000/ha on April 22 for codling moth. Light traps were run from 17:00 to 20:00. Traps were checked twice per week and interception traps were replaced weekly. All traps were removed on June 23.

Sterile codling moths were provided by Dr. Ken Bloem from the rearing facility in Osoyoos, British Columbia and transported chilled (0 to 2°C) to Yakima. Groups of 6 000 moths were released at ca. 15:00 on the first day of each experiment by sprinkling the chilled moths on the ground beneath trees in a 0.25-ha plot in the middle of four selected orchards. Two orchards were treated with Isomate-C+ (1 000 dispensers/ha applied in late April) and two were untreated but used a conventional spray program. Experiments were conducted from July 25 to August 28, 1996. Sixty interception traps (two per tree) were evenly spaced within the designated 0.25-ha plot. Traps were checked each day and panes were replaced every three days. Tests were run for six nights.

### Results

Under laboratory conditions female codling moth 1 to 2 days old laid an average of 135 eggs and 75% of these eggs hatched (Table 1). Both the number of eggs laid and

fertility declined for moths when they were mated at an age older than 2 days. These two factors combined for >40% drop in the number of viable offspring produced by females >2 days old.

**Table 1** Laboratory study to evaluate the number of offspring produced as a function of the age at which female codling moth were mated ( $N = 25$ )

Female age when mated (d)	Female longevity (d)	Females mated (%)	Eggs laid per female ( $N$ )	Eggs hatched (%)	Viable offspring per female ( $N$ )
1	12.5	72	132.2	73.6	97.4
2	12.4	68	138.7	76.6	106.2
3	10.9	80	80.5	48.6	39.1
4	11.7	72	115.7	59.8	69.2
5	11.5	68	56.9	30.3	17.2
6	11.8	52	58.5	22.0	12.9
7	14.5	52	78.8	29.5	23.2
8	11.5	60	52.8	35.6	18.8
9	16.6	36	83.2	30.7	25.6
10	15.2	44	57.1	24.4	13.9

Interception and light traps caught predominately male moths (4:1 male/female ratio) during the first moth flight in three mating disruption-treated orchards in 1995. No females were caught during the first week of moth flight. The proportion of mated females captured was fairly consistent over six weeks and averaged 55%.

**Table 2** Percentage of mated female codling moths recaptured on interception traps following release of 6 000 sterilized moths into an orchard treated with Isomate-C+ dispensers (MD) or an untreated check orchard (July 25 to August 28, 1996)

Treatment	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup> night
MD	0	21.1	19.0	40.0	41.2	37.5
Check	42.9	76.9	87.5	100.0	82.1	81.8

In 1996, the number of moths recaptured on interception traps was slightly higher for males (54:46) and substantially lower for females (39:61) in the mating disruption vs the check orchards. Only 2.6 and 0.6% of the estimated 36 000 male and female moths were recaptured in these six releases, respectively. Recapture of moths dropped 98% from the first to the sixth night. The male/female ratio of captured sterile moths was

3.7 and 5.2 in the mating disruption and check orchards, respectively. Over 40% of the females recaptured on passive traps in the untreated plot were mated by the first night (Table 2).

Level of mating in the untreated orchards peaked after two nights and ranged from 77 to 100%. All females recaptured on the first night in the mating disruption orchards were virgin. On the second and third night ca. 20% of the females captured were mated. From the fourth to sixth night the level of mating was consistent and averaged 40% (Table 2).

## Discussion

Laboratory and field studies have demonstrated that delay in mating can occur and could have a significant affect on the population build-up of codling moth in mating disruption orchards. Field studies that could quantify this relationship are needed for both the spring and summer moth flights. The effect of moth density on mating success and delay in mating also needs to be investigated. Next season, I plan to compare the temporal patterns of mated female codling moth egg load within mating disruption and untreated orchards with high population densities. Field studies investigating mating success with low population densities are problematic, and may be best addressed experimentally.

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## Mating disruption of pea moth, *Cydia nigricana*, and codling moth, *C. pomonella*, using blends of sex pheromone and attraction antagonists

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**Abstract** - The sex pheromone of pea moth, *Cydia nigricana*, is (*E,E*)-8,10-dodecadien-1-yl acetate (*E8,E10*-12Ac). Small amounts of the geometric isomers *E,Z*-, *Z,E*-, and *Z8,Z10*-12Ac strongly inhibit male attraction to *E8,E10*-12Ac. In pea fields treated for mating disruption, males are attracted to fresh dispensers releasing >98% pure *E8,E10*-12Ac. After formation of >6% of the antagonistic geometric isomers in the dispensers, males are no longer attracted, but fly out of the treated fields. The main pheromone compound of the codling moth, *C. pomonella*, is (*E,E*)-8,10-dodecadien-1-ol (*E8,E10*-12OH; codlemone). The nonpheromonal isomers do not prevent attraction to codlemone; males are attracted to mating disruption dispensers releasing an equilibrium isomer blend of 66% *E,E*, 17% *E,Z*-, 13% *Z,E*-, and 3% *Z8,Z10*-12OH. Codlemone acetate, *E8,E10*-12Ac, significantly inhibits male attraction to codlemone, but treatment with a 1:1-blend of codlemone and codlemone acetate does not have a repellent effect on males emerging within the orchard. However, males are not attracted from surrounding orchards - in contrast to mating disruption treatments with codlemone alone.

**Key words** - sex pheromone, attraction antagonist, mating disruption, *Cydia nigricana*, pea, *Cydia pomonella*, pome fruit orchard, Tortricidae, Lepidoptera

### Introduction

Public concern about the use of toxic chemicals in fruit production and increasing resistance against insecticides demand the development of new techniques for control of

codling moth, *Cydia pomonella* L. Mating disruption is the most promising method for environmentally safe control of this key pest of pome fruit, and is being applied on more than 20 000 ha worldwide. For widespread use, however, it needs to be made more reliable and cost-effective. At high population densities, additional insecticide sprays are still necessary and even multiple insecticide applications against resistant strains are usually cheaper than the pheromone treatment (Charmillot 1990; Vickers & Rothschild 1991; Barnes *et al.* 1992; Howell *et al.* 1992; Pfeiffer *et al.* 1993; Barnes & Blomefield 1997; Becid 1997; Rama 1997; Thomson 1997; Waldner 1997).

Orchard treatments with the main pheromone component (*E,E*)-8,10-dodecadien-1-ol (*E8,E10*-12OH; codlemone) alone or in combination with the saturated alcohols dodecanol (12OH) and tetradecanol (14OH) enhance search flights of male moths in tree crowns and even attract males from untreated orchards (Witzgall *et al.* 1996b; Bäckman *et al.* in prep.). This may lead to matings particularly along borders and in tree tops, where lower aerial concentrations and less homogenous dispersal of synthetic pheromone are to be expected.

In the pea moth, *C. nigricana* F., a blend of sex pheromone, (*E,E*)-8,10-dodecadien-1-yl acetate (*E8,E10*-12Ac) and the antagonistic isomers *E,Z*-, *Z,E*-, and *Z8,Z10*-12Ac repels male moths from pea fields and is thus efficient to prevent matings even at high population densities (Witzgall *et al.* 1993, 1996a; Bengtsson *et al.* 1994).

We have therefore investigated the effectiveness of pheromone/antagonist blends for disruption of mating in codling moth. Codlemone acetate, *E8,E10*-12Ac, is the most potent antagonist of male attraction to codlemone, *E8,E10*-12OH (Hathaway *et al.* 1974) and has been shown to be an effective disruptant on its own (Hathaway *et al.* 1979, 1985). The geometric isomers *E,Z*-, *Z,E*-, and *Z8,Z10*-12OH have a moderate inhibitory effect on male attraction (McDonough *et al.* 1993), blends with codlemone gave better communication disruption than codlemone alone (McDonough *et al.* 1994, 1996).

## Materials and methods

### Pea moth, *C. nigricana*

Pea moth pheromone, *E8,E10*-12Ac (>99.1% isomeric and 99.7% chemical purity, by gas chromatography), was formulated in polyethylene tubes (Shin-Etsu Chemical Co., Tokyo) and puzzlepiece trilaminar dispensers (Hercon Environmental Co., Emigsville, Pennsylvania) at 50 mg/dispenser. Dispensers were placed in two unsprayed pea fields (0.6 ha and 3 ha) at 600 dispensers/ha (30 g *E8,E10*-12Ac/ha) and 333 dispensers/ha (17 g *E8,E10*-12Ac/ha), respectively.

Dispenser release rates were measured by glass adsorption in a static atmosphere. Aerial pheromone concentrations were determined by field-EAG and air sampling. Communication disruption was monitored by field trapping with 100 µg *E8,E10*-12Ac on rubber septum or 3 mg "propheromone" (Streinz *et al.* 1993) on filter

paper; behavioural observations during pea moth diel flight in the late afternoon; and larval counts after the seasonal flight period (Bengtsson *et al.* 1994; Witzgall *et al.* 1996a).

#### Codling moth, *C. pomonella*

Resin-treated paper dispensers (Isagro Ricerca, Novara; Rama 1997) were placed at rates of 400/ha and 1000/ha in five unsprayed apple orchards (2 to 15 ha) in 1995 and 1996. These dispensers contained (1) 250 mg codlemone, *E8,E10-12OH* (99.3% isomeric and 99.7% chemical purity, by GC); (2) 250 mg of an equilibrium blend of codlemone and its geometric isomers (26% *E,Z*; 20% *Z,E*; 5% *Z,Z*, relative to *E8,E10-12OH*); (3) 250 mg codlemone plus 250 mg codlemone acetate, *E8,E10-12Ac* (96.4% isomeric and 97.9% chemical purity).

Polyethylene tubes (Shin-Etsu Chemical Co., Tokyo) containing ca. 100 mg *E8,E10-12OH* (unknown isomeric and chemical purity of formulated compound), 56% dodecanol (12OH) and 20% tetradecanol (14OH), were applied at 1000 dispensers/ha in two of these orchards (5 ha and 15 ha) in 1994.

Male flight behaviours were observed during the diel flight period between 20:00 and 23:00, over three seasons. Trap captures were recorded in treated and untreated orchards (tetra traps; 100 µg and 1 mg *E8,E10-12OH*/septum). The effect of the geometric isomers of codlemone on male attraction was determined by trap tests in untreated orchards (Bäckman *et al.* in prep).

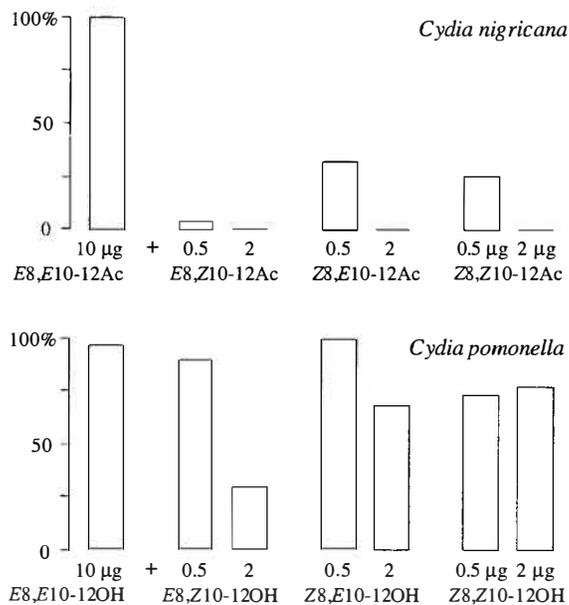
#### Pea moth, *C. nigricana*

In the pea moth, male attraction to sex pheromone, *E8,E10-12Ac*, is strongly inhibited by all three non-pheromonal geometric isomers. A 5%-addition reduces trap catch significantly (Figure 1; Witzgall *et al.* 1993), even captures in the control trap seemed to be affected by neighbouring traps with isomer blends. *C. succedana* D.&S. and *C. jungiella* Cl. have a similar diel flight period and they feed on Leguminosae; they are attracted to blends of *E,E/E,Z* and *E,Z/Z8,E10-12Ac*, respectively (Witzgall *et al.* 1996c).

Pea moth males were attracted to fresh rope and trilaminar dispensers, formulated with rather pure *E8,E10-12Ac*. Already after two days of field exposure, these dispensers released 6% of the other isomers: this blend of pheromone and attraction antagonists had a repellent effect on male moths, they were observed to fly out of the treated fields.

At a density of 330 and 600 dispensers/ha, three and no males at all, respectively, were attracted to monitoring traps throughout the season, compared to >200 males/trap in control fields. The absence of males in treated fields was corroborated by visual observations, including cages with calling females. Male pea moths respond to synthetic pheromone up to one hr before the onset of female calling. For mating

disruption, it may be effective to repel males by attractant/antagonist blends during this pre-mating flight period, rather than to attract them (Bengtsson *et al.* 1994; Witzgall *et al.* 1996a).



**Figure 1** Attraction of *C. nigricana* (above; data from Witzgall *et al.* 1993) and *C. pomonella* males (below) to traps baited with sex pheromone and 5 or 20% of the non-pheromonal geometric isomers *E,Z*; *Z,E*; and *Z,Z* ( $N = 10$ )

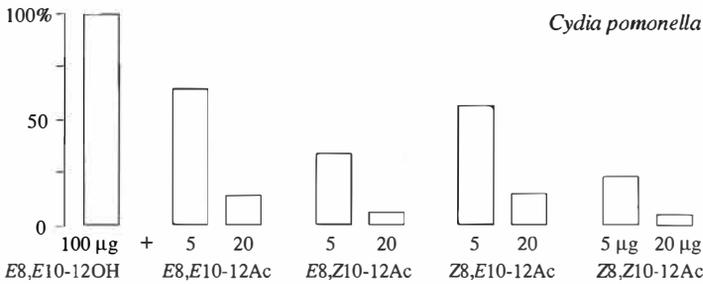
### Codling moth, *C. pomonella*

Of the geometric isomers of codlemone, only a 20%-addition of *E8,Z10-12OH* reduced trap catch with codlemone (Figure 1); mating disruption dispensers formulated with an isomer blend (100% *E,E*-, 26% *E,Z*-, 20% *Z,E*-, 5% *Z8,Z10-12OH*) were attractive to male codling moths (Bäckman *et al.* in prep.). None of the species closely related to codling moth is known to use the isomers of codlemone as sex pheromone (Arm *et al.* 1997b).

It is not possible to release isomerically pure codlemone from currently available dispenser material. Two commercial dispensers, formulated with rather pure codlemone, release several percent of the other isomers immediately after field application. However, even after field exposure of up to one year, the isomer blend proportion re-

mains well below the equilibrium blend (Brown *et al.* 1992; McDonough *et al.* 1994; Knight 1995; Knight *et al.* 1995; Bäckman 1997).

Codlemone acetate, *E8,E10-12Ac*, and its geometric isomers significantly diminished male attraction to codlemone, at a 20%-addition (Figure 2; Witzgall *et al.* 1996b), trap catch with a 1:1-blend was reduced to 3%, compared to codlemone (unpubl. res.). Codlemone acetate is the sex pheromone of pea moth (see above), and pear moth, *C. pyrivora* Danil., a sibling species of codling moth. Females of the beech tortrix, *C. fagiglandana* Z., produce a blend of codlemone and codlemone acetate (Witzgall *et al.* 1996c).



**Figure 2** Attraction of *C. pomonella* males to traps baited with codlemone *E8,E10-12OH* and 5 or 20% of codlemone acetate *E8,E10-12Ac* and its geometric isomers *EZ*, *ZE*, and *ZZ* ( $N = 10$ ; data from Witzgall *et al.* 1996b)

For mating disruption, three orchard treatments were compared: *E8,E10-12OH*; an isomer blend ( $\Delta 8,\Delta 10-12OH$ ); and a 1:1-blend of *E8,E10-12OH* and *E8,E10-12Ac* (Bäckman *et al.* in prep.).

Communication disruption by the isomer blend was not significantly different from the codlemone/codlemone acetate blend, according to trap catch and visual observations (Bäckman *et al.* in prep.). Further trials on larger surfaces will be needed to determine whether such blends could be better suited for codling moth mating disruption than codlemone alone (see also McDonough *et al.* 1994, 1996).

In orchards with high overwintering populations, none of the two pheromone/antagonist-treatments had a pronounced repellent effect. *E8,E10-12Ac* and *E8,Z10-12OH* are only moderate antagonists of codling moth attraction - the nonpheromonal acetates had a much stronger effect on pea moth (Figures 1, 2).

Male flight activity was much enhanced by all three treatments, compared to untreated plots. The males "searched" mainly the upper half of the trees by flying about branches and top shoots, where they frequently landed on leaves - and where matings occurred. Males terminated searching by flying off downwind or across the wind line,

usually well above the trees. It was impossible to determine how far these males travelled, but there was no noticeable decrease in the density of male moths in any of the treatments.

It is important to note that our observations did not allow to distinguish males emerging within the orchard from those arriving from outside. Field wind tunnels (Cardé *et al.* 1993, 1997) allow to investigate the behavioural effects of long-term exposure to different disruptant chemicals. In comparison, pea moths emerge outside the pheromone treatment, as peas are sown in different fields each year.

Codling moth males were attracted upwind to codlemone treatments from nearby untreated orchards, over at least 50 m, while long-range attraction to E8,E10-12OH/E8,E10-12Ac-treatments was not observed.

## Conclusions

The behavioural modifications leading to disruption of mating result from the chemicals used - permeating the atmosphere with pheromone blends, single components, incomplete or off-blends will induce different male behaviours (*e.g.*, Flint & Merkle 1983; Chisholm *et al.* 1984; Palaniswamy *et al.* 1984; Palaniswamy & Underhill 1988; Bengtsson *et al.* 1994; Suckling & Burnip 1996).

The chemicals used for mating disruption and their purity must therefore be known. This is the *sine qua non* for reproducible experiments, comparative analysis of applications, and for the interpretation of behavioural studies (see also Arn *et al.* 1997a; Millar *et al.* 1997). Tests with the pea moth underline that it is necessary to monitor the dispenser release by chemical analysis: isomerization of formulated pea moth pheromone reverses its behavioural effect within a few days.

Which chemicals should be used for disruption of mating? A complete pheromone blend should be best suited to preclude male perception of females (see Charlton & Cardé 1981; Sanders 1981; Minks & Cardé 1988; Cardé 1990; Suckling & Clearwater 1990; Cardé & Minks 1995; Felland *et al.* 1995). The behavioural activity of the main pheromone component is much enhanced by the addition of pheromone synergists: the same amount of active ingredient will produce a stronger behavioural effect (Linn *et al.* 1986) and this may even compensate for the cost of additional compounds.

It will rarely be possible to apply the complete pheromone blend at the female-released proportions. Incomplete blends, however, may still lead to attraction of males from surrounding, untreated crops. These males may be capable of distinguishing female signals from the synthetic background, as long as they haven't become adapted or habituated, and this may account for matings especially along treatment borders. Attraction of males can be overcome by area-wide mating disruption, or by pheromone antagonists, as in pea moth.

Male codling moths are attracted to codlemone-treated orchards, but not to treatments with blends of codlemone and codlemone acetate. It is therefore surprising that this pheromone/antagonist blend enhanced search flights within the orchard. A tentative explanation is selective sensory adaptation or central habituation to codlemone acetate, perhaps in combination with a synergistic interaction of codlemone and plant volatiles. The addition of the codlemone isomers or codlemone acetate to codlemone dispensers seems to have little effect on males emerging into the treated orchards.

### Acknowledgements

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## *Monitoring and detection*

## **The use of pheromone traps in spruce budworm integrated pest management**

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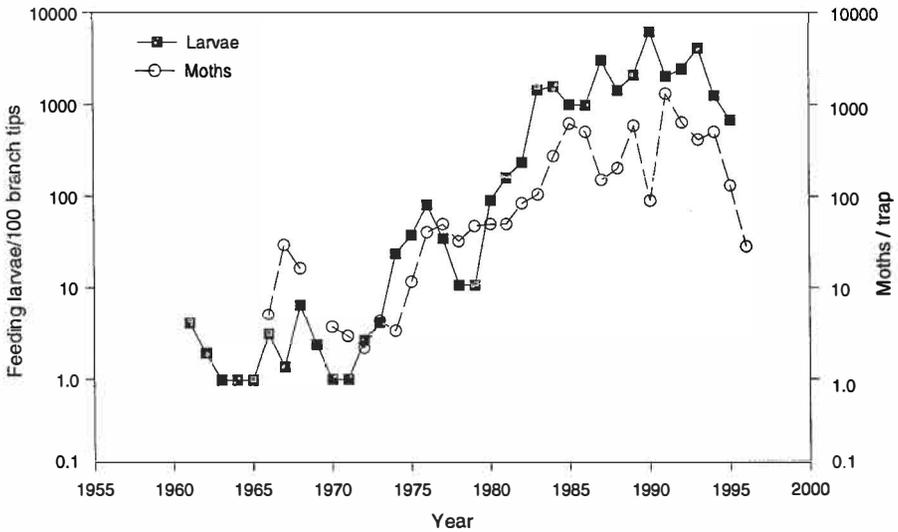
**Abstract** - Sex pheromone traps are extremely efficient and effective for monitoring low density populations of spruce budworm, *Choristoneura fumiferana* Clem., when more conventional sampling techniques are too time consuming and expensive. They have now been deployed at more than 700 sites throughout North America for ten years as a method of monitoring low density populations to determine when more intensive larval sampling is warranted. On a local scale (forest management units) catches of over 100 moths/season are used as a threshold to trigger more intensive larval sampling. On a provincial and national scale, trap catches are converted from point sample data to contour maps by a geostatistical process called 'kriging'. The resultant maps are being compared with those from previous years by use of geographic information systems (GIS) to locate areas where populations are showing significant changes in density.

**Key words** - sex pheromone traps, monitoring, integrated pest management, geographic information systems, spruce budworm, *Choristoneura fumiferana*, Tortricidae, Lepidoptera

### **Introduction**

In contrast to most agricultural pests which are chronic problems recurring each year, most forest pests are cyclical, erupting into outbreaks at intervals separated by several years of vanishingly low densities. Most fluctuate on cycles of approximately ten years, but the spruce budworm, *Choristoneura fumiferana* Clem., fluctuates on an unusually long cycle of 35 to 40 years (Royama 1984, also see Figure 1). Up until now, increasing population densities leading to outbreaks have gone undetected until trees become visibly defoliated, by which time the only management option is to spray insecticides to keep the trees alive along with accelerated harvesting to salvage the trees before they are unusable. Early prediction of when and where outbreaks will occur would provide management with lead time to relocate harvesting operations or, possi-

bly, to suppress the severity of an outbreak by slowing down population growth rates by the judicious use of insecticides.



**Figure 1** Relationship between spruce budworm, *C. fumiferana*, larval densities and subsequent moth catches in pheromone baited traps at Black Sturgeon Lake in northwestern Ontario, Canada

Between outbreaks spruce budworm populations sink to extremely low densities - less than 1 larva/100 branches. At these densities larval sampling is impractical because it is far too time consuming and expensive. However, sex pheromone traps have considerable potential for monitoring changes in insect numbers at low population densities. In one location in northwestern Ontario, pheromone baited traps (using virgin females before synthetic pheromone became available) have been deployed every year since 1966. The trap catches together with larval sampling (Figure 1) show graphically the potential of pheromone traps for tracking changes in population density, particularly at low densities.

### Materials and methods

The sex pheromone of the spruce budworm has been identified as a 95:5 blend of E- and Z-11-tetradecenal (Sanders & Weatherston 1976; Silk *et al.* 1980). After extensive testing, the following combination of trap and lure is now recommended for monitoring changes in spruce budworm population densities throughout its range across North America.

### Trap

The recommended trap is the Multi-pher I<sup>®</sup> (Sanders 1986, Jobin *et al.* 1993) manufactured by Le Groupe Biocontrôle (Ste-Foy, Québec), and available through a number of suppliers. The Unitrap<sup>®</sup> (International Pheromone Systems, Wirral, United Kingdom) performs equally well and is an acceptable substitute. Both types of trap have the capacity to hold several thousand moths without losing their effectiveness, thereby making them suitable for monitoring a wide range of population densities.

### Lure

The necessary criteria for the lure formulation are protection of the synthetic pheromone from chemical degradation (usually oxidation, which is hastened by exposure to UV radiation), and release of the synthetic pheromone at a predetermined and relatively constant rate that spans the flight period of the spruce budworm plus a few weeks to permit the lures to be deployed in advance of the flight period. The chosen release rate is 100 ng/hr, slightly higher than the natural release rate of a female moth (Silk *et al.* 1980, Morse *et al.* 1982). This is sufficient to catch some males at very low densities, and yet it causes no aberrant behavior in the males, which is a possibility at excessively high release rates. The duration of release is specified to be at least eight weeks. This permits traps to be deployed during larval sampling, which may occur three to four weeks before moth flight, and yet it still spans the moth flight period (about 3 weeks in any one location). The lures currently recommended are Biolures<sup>®</sup> (Consepm Membranes Inc., Bend, Oregon), with a loading of 2.8 mg of synthetic pheromone/lure.

Each new batch of lures should be calibrated against the previous batch to ensure that they are of equal potency and correction factors should be applied where necessary (Sanders 1996). In Quebec, the Ministère des Forêts has used a different approach. There, correction factors are applied using the relationship between trap catch and density of overwintering second instar larvae (Boulet 1992).

### Insecticide

In contrast to many of the traps used for the detection and timing of moth flight, the Uni-trap and Multi-pher traps contain no sticky surface, and if the moths are not immobilized in some way they will fly around inside the trap, damaging both themselves and other moths and making identification and counting difficult. The moths are therefore killed by an insecticide, for which registration is required. The insecticide selected for this purpose is the fumigant dichlorvos (DDVP). There are several products on the market with DDVP impregnated in plastic, but only one is registered for use in pheromone traps in Canada and the U.S.A.: namely Vaportape II<sup>®</sup> (Hercon Environmental Corp., Emigsville, Pennsylvania; Canadian Registration No. 21222; US Registration EPA No. 8730-32).

## Deployment

To ensure that trap catches are representative of budworm populations, certain protocols must be met. Traps are deployed in mature forest stands (a minimum of 10 ha in area) containing at least 50 percent white spruce, *Picea glauca* [Moench] Voss, and/or balsam fir, *Abies balsamea* [L.] Mill. Each trap is hung at eye level on a dead branch at least 50 cm from the stem of the tree, free from any obstruction that might prevent it from swinging freely in case the trap becomes snagged at an angle that allows moisture to enter and causes the moths to rot. As suggested by Jobin *et al.* (1993), hinged brackets can be fastened to trees in permanent sample plots. This ensures that the traps are hung in exactly the same position each year. Because trap catches are more variable at the edge of a stand, traps should be positioned at least 40 m from the edge.

One trap will provide almost as reliable an estimate as will a cluster of traps. However, three traps, arranged in an equilateral triangle with 40 m between traps is recommended. This allows for vandalism and damage to traps by bears.

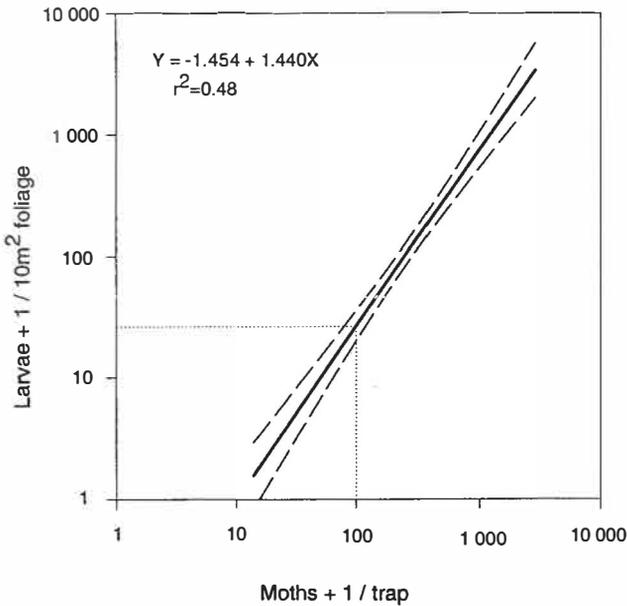
## Application in forest management

### Index of population density

The correlation between trap catch and larval density was determined between 1989 and 1993 from about 40 sites in northwestern Ontario that cover a wide range of budworm densities. Pheromone traps were deployed each year throughout the moth flight period and the following winter branch samples were taken to determine the densities of the overwintering second instar larvae. The regression of larval counts against moth catch (Figure 2) shows that a catch of 100 moths corresponds to a density of 25 L<sub>2</sub>-larvae/10 m<sup>2</sup> branch surface area, or about three larvae/branch, in the subsequent generation. This is approximately the threshold density at which sampling of the overwintering larvae becomes practical.

Therefore, in the boreal mixedwood stands of central Canada where these data were gathered, a trap catch of 100 can be used as a trigger to initiate a more intensive assessment of the situation. This can be done by a combination of more intensive pheromone trapping supplemented by larval sampling.

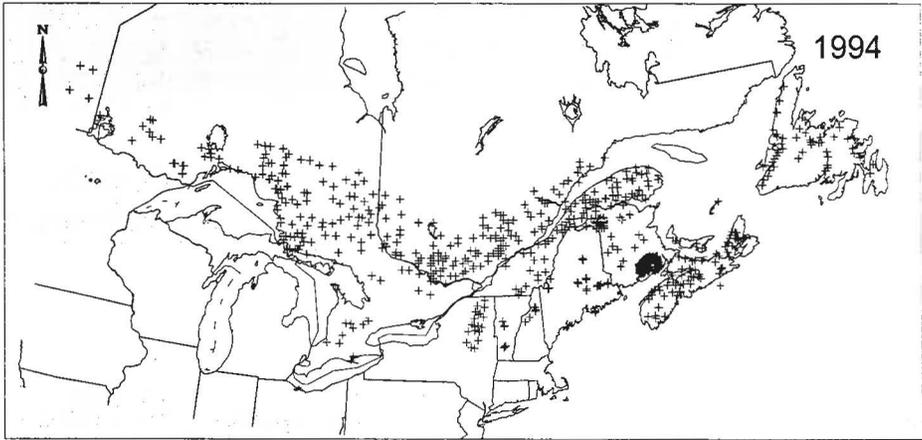
Trap catch is a reflection of the number of insects per unit area of forest. This of course varies with host tree density and size. In pure stands of mature host trees, such as white spruce stands in river bottoms in Alberta or balsam fir forests in Maine and the Maritime provinces of Canada, there will be far higher populations of spruce budworm per hectare than there will be in the mixedwood stands of Ontario that contain only 50 percent spruce and fir. Therefore, relationships between larval density and trap catch will have to be established for the major stand types involved in each region.



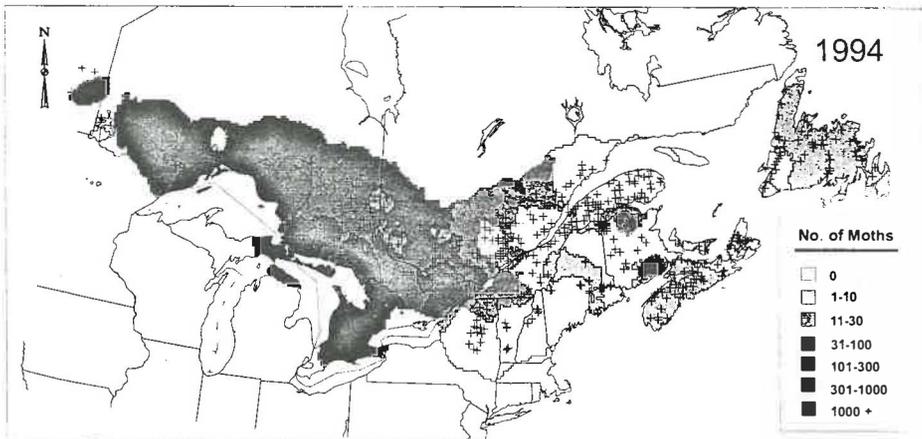
**Figure 2** Relationship between spruce budworm larval densities and catches of male moths in Multi-pher sex pheromone traps as determined from 40 locations in Ontario during the period 1989 to 1993. The dashed lines denote the 95% confidence intervals. The dotted line shows that a moth catch of 100 corresponds to a larval density of about 25 overwintering larvae ( $L_2$ )/10 m<sup>2</sup> of tree foliage, or about 3 larvae/branch

### Monitoring population changes

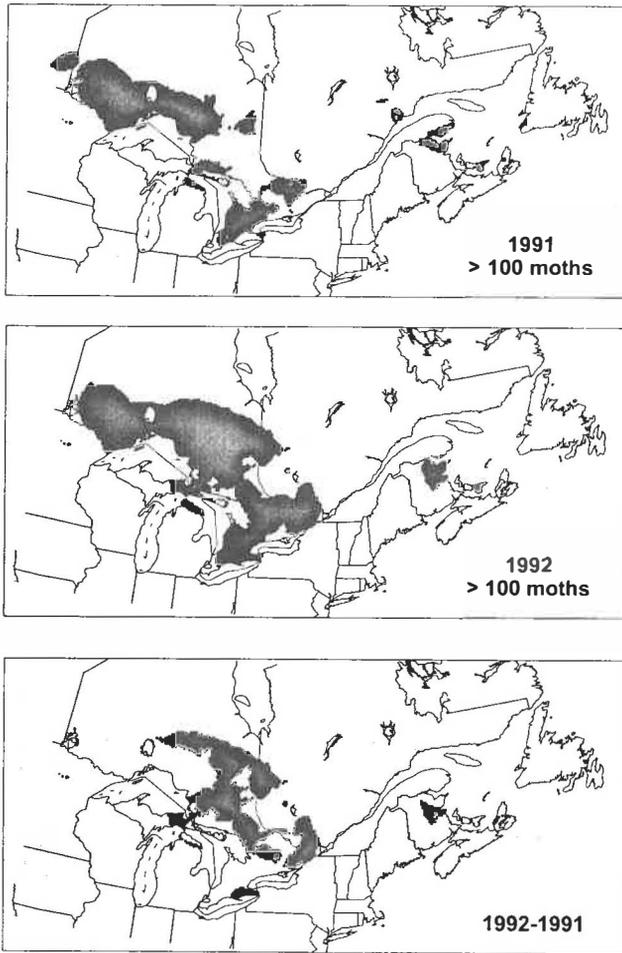
In order to monitor long-term trends in population fluctuations, including changes at low densities, traps are now deployed annually in approximately 700 sites throughout the range of the spruce budworm from Alberta to Newfoundland and across the six northern U.S. states (Figure 3). In order to analyze the data, however, it is first necessary to convert the point sample data provided by the traps into contour maps. A technique known as 'kriging' has been used for this. This technique, which was developed for mapping geological formations, has been applied to other point sample pest data (Liebhold *et al.* 1993). Briefly, kriging calculates a weighted average of trap catches for each pixel, based on the recorded catches from all locations falling within a defined radius. Details of the technique as it has been applied to the spruce budworm situation, together with details of the supporting software are described by Lyons *et al.* (1996). A sample map, spruce budworm moth density for 1994, is shown in Figure 4.



**Figure 3** Map of eastern North America showing locations of spruce budworm sex pheromone trapping locations. The two areas of dense locations in New Brunswick are where intensive trapping was carried out to evaluate optimum distances between trapping locations



**Figure 4** Map of spruce budworm moth catches for 1994 as determined from the trap catch data by the geostatistical technique of kriging



**Figure 5** Maps showing areas where catches of male spruce budworm moths exceeded 100 in a) 1991 and b) 1992, and by subtraction of a) from b), those areas c) where catches exceeded 100 in 1992 but not in 1991, which are areas of potentially new infestation.

These maps can then be used to determine changes in density from year to year, and can be superimposed on maps of cover type etc. to determine where the greatest risk of future damage is likely to occur. For instance, maps from successive years can be compared by geographic information systems (GIS) to show where trap catches have risen above the threshold level of 100 moths/trap during the past year as shown in Figure 5. More intensive sampling can then be carried out in these areas to delineate

the boundaries of a potential outbreak, which provides forest managers with several years of lead time before defoliation first becomes visible.

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## Use of sex pheromone traps for monitoring of European corn borer in Romania

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**Abstract** - The use of pheromone traps for monitoring the flight period of European corn borer, *Ostrinia nubilalis*, has been investigated. Trap captures with blends of (Z)- and (E)-11-tetradecen-1-yl acetate, at a ratio of 97:3 or 3:97, have shown that the Z-pherotype is predominant all over Romania. The traps attract also a number of other species, their specificity should be improved. Pheromone traps are a valuable tool to optimize the time of release of *Trichogramma* spp. or sterile males and will become an important component of integrated control programs.

**Key words** - European corn borer, *Ostrinia nubilalis* Hb., sex pheromone, monitoring, pest control, Romania

### Introduction

In Romania, the European corn borer, *Ostrinia nubilalis*, is considered to be the most important pest of maize after panicle apparition, being spread throughout the country. The damage caused by this pest on corn can reach up to 40% (Paulian *et al.* 1961). Multiannual data indicate averages of 44% plants attacked, resulting in a yield loss of 550 kg/ha or 7.5% (Paulian *et al.* 1976). *O. nubilalis* feeds also on hemp and sorghum crops, as well as other species of the wild flora.

The actual method to forecast European corn borer population levels consists of controlling, in at least three fields with different corn hybrids in each district, the number of attacked plants and the number of larvae per attacked plant. The warning system is also based on the registration of the flight period by light traps.

The sex pheromone of *O. nubilalis* consists of (*Z*)- and (*E*)-11-tetradecenyl acetate (*Z*11-14Ac, *E*11-14Ac) (Klun & Brindley 1970); the occurrence of pheromone strains, using predominantly either the *Z*- or *E*-isomer, has been studied in different parts of the world (Kochansky *et al.* 1975; McLeod *et al.* 1979; Stockel 1982; Roelofs *et al.* 1985).

A simple and efficient tool for monitoring of corn borer populations is a prerequisite for integrated pest management programs (Rosca *et al.* 1985, 1990, 1991), it has been investigated whether pheromone-baited traps could be used to this purpose.

### Materials and methods

From 1990 to 1995, the specificity and efficacy of synthetic sexual pheromone lures and traps produced by the Chemistry Institute (Cluj Napoca, Romania) has been studied. Pheromone traps, suspended 1 m above ground, separated by 50 m, were placed into corn fields. Lures were changed at two-week intervals, the sticky bottoms when it was necessary. The number of corn borers and of other lepidopteran species trapped was recorded weekly. Field tests were conducted from June to September.

With the development of an integrated management program in mind, corn borer dispersal and flight range was studied by release/recapture of marked males. Insects were marked by adding an emulsification mixture of Calco oil red N-1700 (150 mg/kg diet) and sunflower oil to the semisynthetic diet used for lab-rearing (Barbulescu & Rosca 1993). Lab-reared corn borer males were released in the field and recaptured with pheromone traps placed at various distances from the release sites, in the four cardinal directions. Traps were placed at 25, 100 and 200 m in 1993; at 100, 200 and 300 m in 1994; at 300, 600, 900, 1200 and 1500 m in 1995; and at 1000, 2000, 3000, 4000 and 5000 m in 1996.

### Results and discussion

The most efficient pheromone lures had been identified during preliminary studies from 1982 to 1987: The *Z*-lure contains a 97:3 blend of *Z*11-14Ac/*E*11-14Ac, the *E*-lure contains a 3:97 blend of these compounds and tetradecyl acetate (Rosca *et al.* 1991).

The results in Table 1 show the numbers of *O. nubilalis* males captured with the *Z*-lure throughout the trapping period. Trap catch varied between localities and years, most males were trapped at Turda, in 1992.

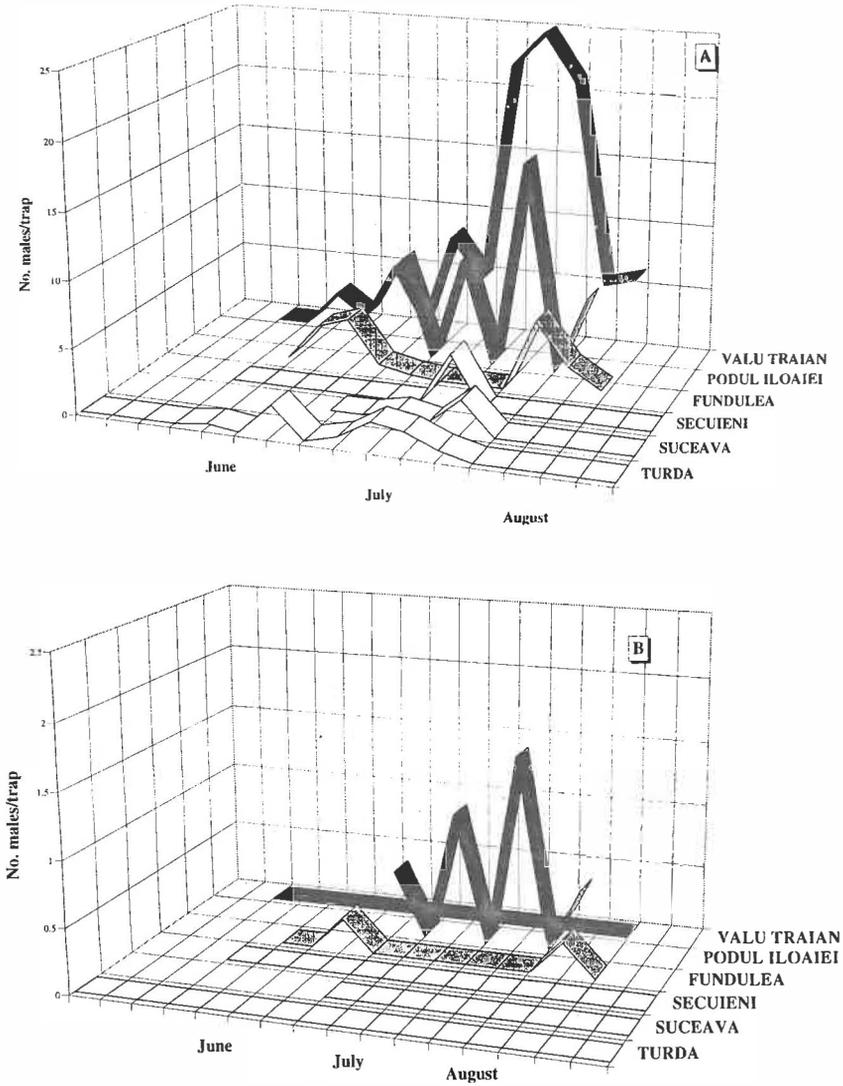


Figure Male flight of European corn borer populations in Romania, as monitored by Z-lures (a) and E-lures (b)

In addition, it has been shown for the first time that both the Z- and E-phenotypes of European corn borer occur all over Romania; before the E-type had not been recorded in the center and the north-east of the country (Rosca *et al.* 1991). *Ostrinia nubilalis* populations consist on the average of 92% Z-type and 8% E-type males, this ratio

varies between years and localities (Table 2, Figure), but up to 14% *E*-males occurred in some fields.

**Table 1** Number of European corn borer males captured/year/Z-trap

Locality	1990	1991	1992	1993	1994	1995
Suceava	1.75	1	-	19.5	45.5	5
Podul Iloaiei	- a	19	13.8	19	21.3	37.5
Scuieni	0.5	-	1.5	0.3	12	4
Valu Traian	-	-	13	17	32.3	104
Fundulea	8.3	14.8	23.3	29.5	35.3	17.2
Oradea	5	13.5	9.8	54.3	12.1	4
Turda	45	53	172	49.5	64.9	7.4

a not detected

**Table 2** European corn borer males captured with Z- and E-blend

Pherotype	1990	1991	1992	1993	1994	1995
<i>E</i>	6.3	9.5	34.7	11.2	19.1	7
<i>Z</i>	60.5	101.3	233.3	189.1	223.4	179.1

The specificity of pheromone formulations is still not optimal, since other Microlepidoptera such as *Tortrix viridana* L., *Etiella zinkenella* Tr. and *Emelia trabealis* Scop. are frequently trapped. However, these species are easily distinguished from the target species.

The flight period of *O. nubilalis*, as monitored by pheromone traps is shown in the Figure. Our results indicate that there is even a small second generation at the end of August or early September.

Release and recapture experiments showed that the males did not fly further than 300 m/day under the local climatic conditions. The recapture rates were 5.9% in 1993, 3.9% in 1994, 0.2% in 1995 and 0.3% in 1996. Tests in 1995 and 1996 showed that the males did not travel further than 3000 m from the point of release.

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## Sex pheromone traps as a tool for the study of population trends of the predator of a scale insect and for the identification of potential predators for biological control

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**Abstract** - The capture of adult *Elatophilus hebraicus* (Heteroptera, Anthocoridae) in traps baited with the sex pheromone of the Israeli pine bast scale, *Matsucoccus josephi* (Homoptera, Matsucoccidae), enabled the study of population trends of the predator as related to those of its prey. Traps were exposed at monthly intervals in stands of *Pinus halepensis* and *P. brutia* ssp. *brutia* for a period of 27 months. Fourier series were used to modulate population trends. The population density of *M. josephi* increased in March-April and in August and October. A steep increase in trap catch of *E. hebraicus* was noticeable during May and June. The rise of the predator population related positively, but only to a limited extent ( $r^2=0.40$ ) to the increase in prey density in the previous spring. On an annual base, an inverse relationship was found between the mean densities of *M. josephi* and *E. hebraicus*. Population trends of both prey and predator varied slightly between regions, but not between host plant species. We also used pheromone-baited traps in additional areas of the Palaearctic region where other *Matsucoccus* species occur. It was found that the range of *E. hebraicus* and *M. josephi* coincides with that of *P. brutia* ssp. *brutia* in the East Mediterranean. In *Pinus pinaster* stands in Portugal, two other predators, *Elatophilus crassicornis* and *Hemerobius stigma* (Neuroptera, Hemerobiidae) were caught in traps baited with the pheromone of *M. josephi*. These predators are now under appraisal to augment the natural enemy fauna of *M. josephi* in Israel.

**Key words** - *Elatophilus*, *Matsucoccus*, *Pinus*, sex pheromone, population monitoring, kairomone, biological control

### Introduction

The Israeli pine bast scale *Matsucoccus josephi* Bodenheimer and Harpaz is the major pest of pine in Israel (Mendel *et al.* 1994a). In the Eastern Mediterranean, the scale occurs in the natural stands of *P. brutia* ssp. *brutia* at moderate densities, and damage is practically nil. Conversely, high population densities have been observed in plantations and natural forests of *P. halepensis* in Israel, Jordan and Turkey (Mendel 1992; Mendel *et al.* 1994a) and in plantations of *P. brutia* ssp. *eldarica* in Israel (Golan *et al.* 1983; Mendel *et al.* 1994b) leading to severe injury in these forests.

The predator *Elatophilus hebraicus* Pericart (Heteroptera, Anthocoridae) is the most common natural enemy of *M. josephi* in Israel. Like other members of the genus, *E. hebraicus* feeds only on *Matsucoccus* and reproduces only on pine (Mendel *et al.* 1991).

Direct sampling of *Matsucoccus* on adult trees is tedious and impractical, especially in the case of a multivoltine species such as *M. josephi*. Because the occurrence of distinct symptoms of injury appear after the scale population has reached its peak, the level of injury does not provide a reliable measure for its population density. Sampling of *E. hebraicus* in adult pine forests is even more complicated. Hence, one of the major topics regarding the study of pine bast scales of economic importance concerns the development of simple and reliable techniques for monitoring populations trends. Pheromone-baited traps are considered as an efficient tool for monitoring and constitute an important component in any management program for the control of insect pests. In view of this, efforts have been made in the last decade to identify and make available the female sex pheromones of the three pernicious pine bast scales *M. josephi*, *M. feytaudi* and *M. matsumurae* (*M. resinosa*) (Lanier *et al.* 1989; Einhorn *et al.* 1990; Dunkelblum *et al.* 1995).

It is known that some natural enemies of insect pests respond to sex or aggregation pheromones of their prey. There are several examples of kairomonal response by egg parasitoids of lepidopteran pests and by parasitoids of armored scales to the sex pheromones of their hosts (e.g. McClain *et al.* 1990; Noldus *et al.* 1991). However, until now, there has been no evidence that insect predators use sex pheromones of their prey as kairomones. Recently, we have shown that males and females of *E. hebraicus* use the sex pheromone of *M. josephi* as a long-range kairomone (Mendel *et al.* 1995). Furthermore, we have found that *E. hebraicus* is also attracted to the sex pheromones of the allopatric *M. feytaudi* and *M. matsumurae* (Dunkelblum *et al.* 1996).

The availability of the sex pheromone of *M. josephi* has allowed us to use the pheromone trap as a tool for studying the population trends of *E. hebraicus* in pine forests and for defining its geographical distribution. Taking into account that *E. hebraicus* is attracted to the sex pheromones of other pine bast scales, these traps have been also used to identify potential predators of *M. josephi* occurring in the natural area of other *Matsucoccus* spp. The objective has been to select and subsequently to introduce into Israel those predators which display kairomonal response to the sex pheromones of *M. josephi*.

## Materials and methods

### Pheromone traps and insect capture

Traps baited with a 56% *E*:44% *Z* mixture of the racemic sex pheromone of *M. josephi*, (2*E*,6*E*,8*E*)-5,7-dimethyl-2,6,8-decatrien-4-one and (2*E*,6*Z*,8*E*)-5,7-dime-

thyl-2,6,8-decatrien -4-one) (Dunkelblum *et al.* 1996), were exposed at monthly intervals in stands of *P. halepensis* and *P. brutia* ssp. *brutia* during 27 months, in several regions in Israel. White plastic triangular traps with a replaceable sticky bottom were equipped with a rubber dispenser (Maavit Products, Tel Aviv, Israel) loaded with 500 µg of the racemic pheromone mixture. Four traps were attached to the north side of living trees for about 4 weeks in each plot. The trapped insects were examined under magnification at the Volcani Center. The number of *M. josephi* males/trap/day and of *E. hebraicus* individuals/trap/day was determined for each plot and sampling interval.

Considering that both insects are multivoltine, with several overlapping generations, Fourier series were applied to the time series data and used to modulate population trends. Fourier analysis is based on a theory proposed by J. B. Fourier that any periodic wave can be represented as a sum of harmonic terms with an angular frequency (Bigger 1973). Numbers of annual generations of prey and predator were determined by plotting the annual accumulated degree-days with those required to complete a single generation of *M. josephi* (Bodenheimer & Neumark 1955) and *E. hebraicus* (Carmi 1990). Generations of the scale were counted from the first population peak in April and generations of the predator from the first population peak in March.

#### Geographic distribution of *E. hebraicus* and attraction of other potential predators of *M. josephi*

Traps baited with the sex pheromones of *M. josephi* were tested in other areas of the Palaearctic region where other *Matsucoccus* spp. had been previously recorded. At least five traps were used on each site, mostly during spring or early summer. Traps were distributed in the following countries: Japan (1, *M. matsumurae*), China (2, *M. matsumurae*), Jordan (4, *M. josephi*), Lebanon (5, *M. josephi*), Cyprus (4, *M. josephi*), Turkey (3, *M. josephi*), Greece (mainland 1, *M. pini*; Crete, 2 *M. josephi*), Italy (2, *M. feytaudi*), France (2, *M. feytaudi*), Spain (3, *Pinus halepensis* stands with no record of *Matsucoccus*), Portugal (4, *M. feytaudi*) and England (1, *M. pini*). Numbers of study sites and *Matsucoccus* species occurring in each country are in brackets. The sticky bottoms with trapped insects were shipped to Israel and examined in our laboratory.

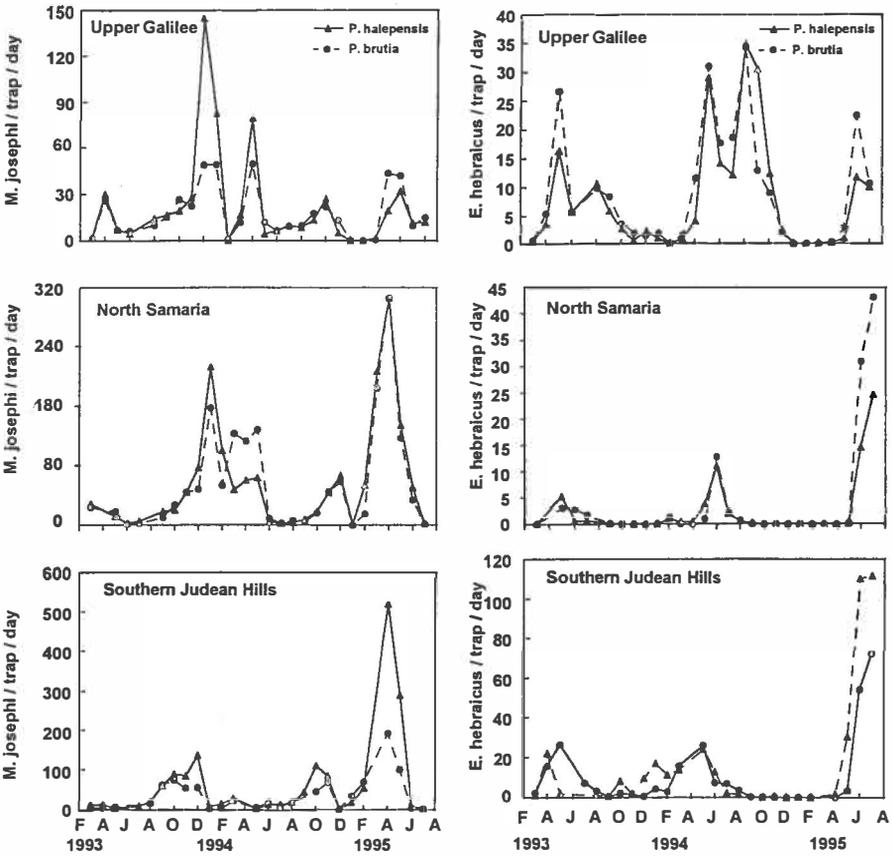
## Results and discussion

### Population trends of *M. josephi*

Seven overlapping generations for each year were calculated for *M. josephi*. Results showed that the population density increased in March/April, mid October and early December, and remained low during the rest of the year. This general pattern was consistent in the different regions and for stands of different pines (Figure 1). The average number of males varied between locations, although modulation of the scale population, according to Fourier series, shows that the seasonal fluctuations are ap-

proximately the same among pine species in the various regions of Israel (Figure 2).

Although *M. josephi* is a multivoltine species and is active throughout the year, our results indicate two main periods of high reproduction, as demonstrated by peaks of male catch. The two waves of male flight are probably related to the period of cambial activity of *P. halepensis* and *P. brutia* ssp. *brutia* in spring (March/June) and autumn (September/October) (Lipshitz *et al.* 1984). Growth ceases in mid-summer and mid-winter. (Oppenheimer 1945; Lipshitz & Lev-Yadun 1986). Width growth activity of both pine taxa in Israel is similar (Lipshitz & Mendel 1989).



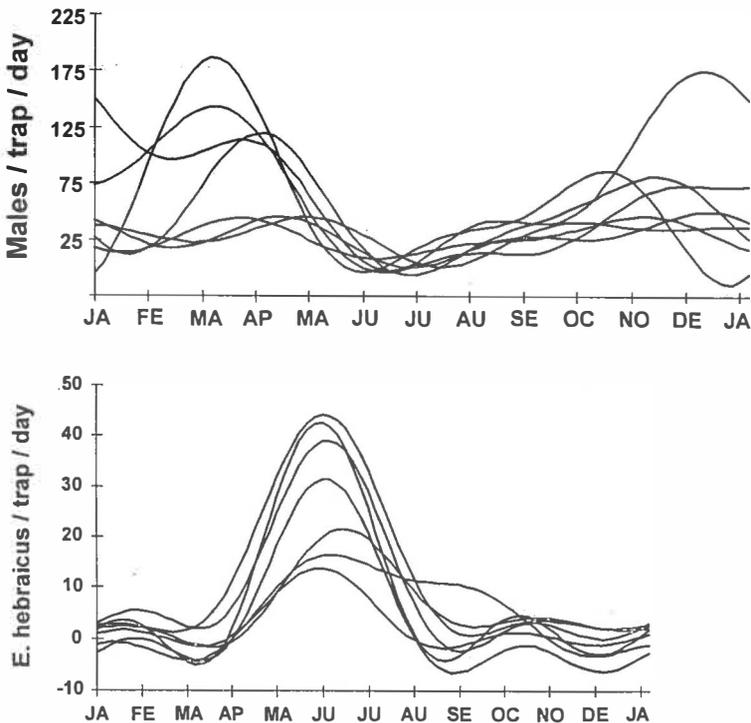
**Figure 1** (left) Population fluctuations of males of *M. josephi* as determined by pheromone traps in three regions in Israel during 1993 to 1995 in stands of *P. halepensis* (Δ) and *P. brutia* (○)

**Figure 3** (right) Population fluctuations of adults of *E. hebraicus* as determined by pheromone traps in three regions in Israel during 1993 to 1995 in stands of *P. halepensis* (Δ) and *P. brutia* (○)

Attraction of *E. hebraicus* to pheromone traps and population trends.

Adults alone were caught in traps baited with *M. josephi* sex pheromone traps. The behavior of the individual bugs approaching traps showed that most of the bugs aggregated on the stem, in the vicinity of them, and were caught on the sticky bottom after landing on the external surface of the trap. As a rule, bugs entered by walking or jumping, but not by flying directly into the trap. In all sampling plots and seasons, the sex ratio of the trapped individuals was markedly male-biased, with males making up of 74 to 90% of the catch. Despite the high numbers of larvae that could be easily located on stems and crowns, these were never observed among the aggregating bugs on the stem near the trap, nor were they caught in the trap.

Our findings suggest that despite the similarity of feeding behavior and habitat of larvae and adults of *E. hebraicus*, only adults respond to the sex pheromone of *M. josephi*. The male-biased sex ratio may be explained by the lower flight capability of the females. The female mates soon after the emergence from the fifth larval instar. The development of the ovaries, as indicated by the increased volume of the abdomen,



**Figure 2** Fourier analysis of population trends of *M. josephi* in seven regions in Israel (above)

**Figure 4** Fourier analysis of population trends of *E. hebraicus* in seven regions in Israel (below)

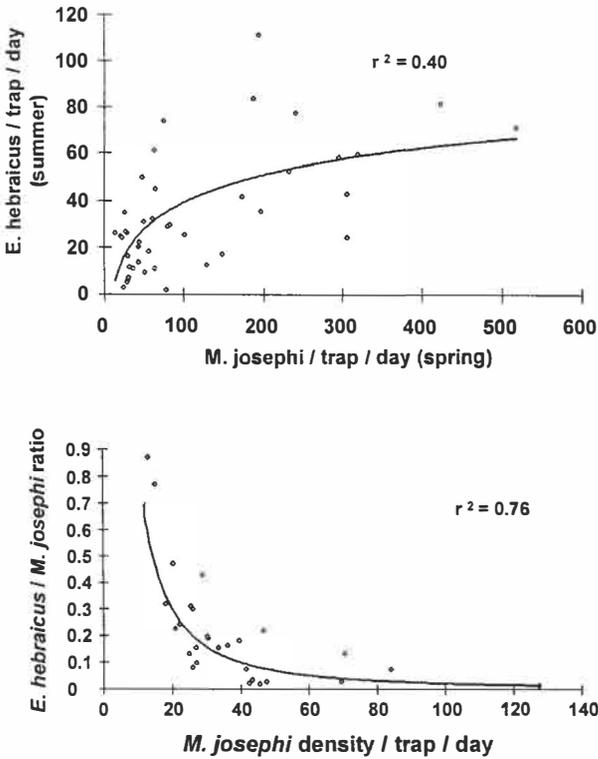
occurs within a few days, with an apparent increase in body weight. No changes in body dimensions were observed for males (Carmi 1989). The oviposition period may extend between 1 and 3 months and corresponds to almost the entire life span of the female. Under controlled conditions, the life span of males is similar to that of the females (Mendel *et al.* 1995).

#### Population trends of *E. hebraicus*

There are 8-9 annual generations of *E. hebraicus* at low altitudes and 6-7 generations at 700m or more above sea level in the Upper Galilee. Population trends of the predator slightly varied between regions but not between host plant species, despite the distinct differences in susceptibility to *M. josephi* of the examined pine species (see Figure 3). Modulation, according to Fourier series (Bigger 1973), showed that population density of *E. hebraicus* increased only in the first half of the summer (mid-May to mid-July) (Figure 4). Excluding the Upper Galilee, where the bug population remained relatively high in the second half of the summer, very few *E. hebraicus* were caught between August and March in all study areas.

The relationship between spring peak (March) of *M. josephi* (males/trap/day) and that of *E. hebraicus* (adults/trap/day) in early summer (May) seemed to be only partially related ( $r^2=0.40$ ) (Figure 5). However, based on the mean annual catch, the ratio *E. hebraicus*/*M. josephi* was inversely related ( $r^2=0.76$ ) to the density of *M. josephi* (males/trap/day) (Figure 6).

The above findings indicate that the pheromone trap of *M. josephi* is a useful device for studying the population trends of its main predator *E. hebraicus*. Furthermore, the pheromone traps enabled us for the first time to study the seasonal history of the predator population, and to estimate the effect of geographical factors and pine species on its populations. The *in situ* study of populations of predatory bugs in general, and species of *Elatophilus* in particular, is limited due to the lack of reliable sampling procedures. The trap has a special advantage for a multivoltinuous species such as *E. hebraicus*. Other congeneric species have a smaller number of annual generations: *Elatophilus nigricornis* Zetherstedt has three generations in Italy (Covassi *et al.* 1991) and *E. inimica* Drake and Harris has only two generations in the Southeastern USA (Lussier 1965). It seems that the increase of the population of *E. hebraicus* is related to the concomitant increase of the prey population in spring, whereas the predator population density remains low during the second half of the summer and autumn, not responding to the population buildup of *M. josephi*. We assume that the bug population is adversely affected by the high temperatures during the summer at low altitudes, whereas lower temperatures at higher elevations account for the relatively high population in Upper Galilee. Based on the density relationship between prey and predator, it appears that the response of the bug population to the change, *i.e.* increase, in the scale population is in fact limited. Therefore *E. hebraicus* is unable to prevent the heavy damage caused by *M. josephi*.



**Figure 5** Relationship between peak density of *M. josephi* males in March and peak density of *E. hebraicus* adults in May (above)

**Figure 6** Relationship between mean annual density of *M. josephi* males and *E. hebraicus*/*M. josephi* ratio (below)

#### Geographic distribution of *E. hebraicus*

The predator, *E. hebraicus*, was always caught together with its prey *M. josephi*. It was found in *P. brutia* ssp. *brutia* stands in western Turkey, Lebanon, and Cyprus and Crete. On *P. halepensis*, *E. hebraicus* was trapped only in Israel and Jordan, but not in mainland Greece or the West Mediterranean. Further, it was never caught outside the natural range of *P. brutia* and *P. halepensis*. The range of *E. hebraicus* most probably overlaps the natural distribution of *M. josephi* and its principal host *P. brutia* ssp. *brutia*. Therefore, it may be found also in pine forests on the offshore islands along the Anatolian coast, in Syria, northern Lebanon and in natural relicts of northern Iraq and eastern Turkey. Our findings suggest that *E. hebraicus* does not occur in southern Eu-

rope (mainland Greece, Spain, Southern France, Italy) or North Africa due to the absence of the scale. We assume that its occurrence in the *P. halepensis* forests in Israel and Jordan is the result of its introduction from Cyprus together with *M. josephi* (Mendel *et al.* 1994a) or of its natural spread from northern Lebanon.

#### Attraction of potential enemies of *M. josephi* to its sex pheromone

Pheromone traps exposed in the area of distribution of *M. josephi* did not attract other predatory species other than *E. hebraicus*. Traps baited with the sex pheromones of *M. josephi*, *M. feytaudi* or *M. matsumurae* (Dunkelblum *et al.* 1996), exposed in various countries, outside the range of *M. josephi* did not attract any known or potential predators of *Matsucoccus* with the exception of Portugal. Traps, baited with these three pheromones, exposed in stands of *P. pinaster* in Portugal attracted significant numbers of two predators: *Elatophilus crassicornis* Reuter and *Hemerobius stigma* Stephens (Neuroptera, Hemerobiidae). We have recently observed that adult *E. hebraicus* are also attracted to the sex pheromones of *M. feytaudi* and *M. matsumurae* despite the fact that *E. hebraicus* is associated only with *M. josephi* (Dunkelblum *et al.* 1966).

The association of *Hemerobius* spp. with *Matsucoccus* (Bean & Godwin 1955; Siewniak 1976; Ming *et al.* 1983; Covassi *et al.* 1991) and *Elatophilus* (Mendel *et al.* 1991; Lattin & Stanton 1993) is well documented. Here, we have shown for the first time that these predators respond to kairomones in order to find their prey. Furthermore, it seems that the mode of attraction of *E. hebraicus*, *E. crassicornis* and *H. stigma* is the same, and that all three predators are strongly attracted to the sex pheromones of the three studied *Matsucoccus* spp. The attraction of actual and potential prey species to the sex pheromones of different *Matsucoccus* spp. may be based on chemical similarity of the pheromones, or on the fact that the kairomonal response of the predators evolved during speciation of the genus *Matsucoccus* (Dunkelblum *et al.* 1995, 1996).

The predators *E. crassicornis* and *H. stigma* are now under appraisal as a means to augment the enemy fauna of *M. josephi* in Israel. Since *E. hebraicus* is also attracted by the sex pheromone of the allopatric scales, *M. feytaudi* and *M. matsumurae*, it may, therefore, be considered as a candidate to improve the biological control in the pine forests injured by these pests. Future biological control projects will benefit from the use of traps baited with the sex pheromones of *Matsucoccus* spp. in order to identify potential predators and introduce them into forests endangered by *Matsucoccus* pests.

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## *Outlook*

## **Pheromones of Lepidoptera - Research and business in the Dutch way: a retrospect**

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### **Introduction**

Pheromone research at Wageningen started at the then called Laboratory for Research on Insecticides (LIO) in 1967. Leafroller moths were chosen as subject for our studies, because they formed at that time a major problem in pome fruit orchards in the Netherlands. After we had shown that live female traps worked effectively, we set our first goal, namely the chemical identification of the sex pheromone of the summerfruit tortrix moth, *Adoxophyes orana*. The publication of the pheromone identification of the closely related red-banded leafroller, *Argyrotaenia velutinana*, by Roelofs & Arn in 1968 came right on time and helped us in convincing our research leaders to give green light to go ahead with our research. A fruitful collaboration between LIO and TNO at Delft (F.J. Ritter & C.J. Persoons) was initiated and continued until 1980. Pheromone identifications of several leafrollers and other moth species followed, starting with that of *A. orana* in 1971 (see further Table 1A).

In addition to the identification studies, we developed our own lines of research: a chemical (S. Voerman) and an entomological research line (A.K. Minks). Our major objective was to make pheromones usable in agricultural practice, according to the institutional policy, which we could endorse fully. We both preferred a "no-nonsense" approach in our research, probably due to our Friesian origin, and we have tried to keep things straightforward *e.g.* by taking our chemicals directly into the field for activity testing. During all these years we kept our believe in the great practical potential of pheromones. These substances are unique in their species specificity and extremely low toxicity, which are very favourable characteristics for pest control agents in integrated control systems. The great potential has been illustrated by the large number of pheromone traps used nowadays for monitoring. During more than 25 years tens of

thousands of traps per season have been used worldwide in many different cultures and this number is still growing.

Pheromone research at Wageningen has made a significant contribution to that development in chemical respect (through the systematic production of potential pheromone compounds of high quality), as well as in entomological respect (through making methods of indirect and direct control with pheromones applicable to agricultural practice). We are glad that we can add that a consistent research policy could be maintained, despite budget cuts and changes in the organisational framework: LIO was renamed IOB (Institute for Pesticide Research) in 1979. On its turn the activities of IOB were terminated in 1990 and the pheromone research group became part of IPO-DLO.

### **Chemical research and the IPO pheromone bank**

Chemical research eventually resulted in the development of the so-called pheromone bank, which became one of the best known and most valuable assets of our present institute IPO-DLO.

Soon after the first successful identifications of lepidopterous sex pheromones in the early 70's had been completed, it became apparent that only a rather limited number of compounds was involved. These pheromones comprised almost exclusively unsaturated alcohols, esters and aldehydes with 10 to 18 carbon atoms in the aliphatic chain. Mono-unsaturated acetates and alcohols played an important role. It became also clear that there were certain relationships between chemical structure of the pheromone and the taxonomic position of groups of moths.

This triggered the idea to make a collection of these compounds by synthesizing the complete series of mono-unsaturated acetates and alcohols, later followed by a selected group of poly-unsaturated compounds. It became known as the pheromone bank and was originally meant as a service to colleagues. Compounds were delivered to be used as reference compounds for identification problems in gaschromatography and electroantennography. A special argentation chromatography set-up was developed to obtain substantial quantities of end products with a high isomeric purity: an essential requirement in pheromone chemistry. The pheromone bank made it also possible to quickly find effective attractants via EAG- and field screening (see Table 1B).

In more recent years, when the financing of our institute became more and more problematic, the pheromone bank developed gradually into a commercial enterprise. We found ourselves back selling pheromone dispensers in increasing numbers, for an increasing number of moth species and for increasing prices. It was a niche in the market and we could serve a large group of clients in many countries. We still synthesize most of the compounds by ourselves, but, when it is cheaper, we also buy pheromones from the industry. Yet, this is not as simple as it looks at first sight, as there are only few reliable addresses available. A fortunate example concerns the

pheromone of the codling moth, *Cydia pomonella*, which is available for an acceptable price and at a good quality. Pheromone components that are not for sale must be synthesized by ourselves in our own laboratory, such as those of the potato moths, *Phthorimaea operculella* (*E4,Z7-13Ac* and *E4,Z7.Z10-13Ac*; identified 20 years ago) and the recently discovered mixtures (*E3-14Ac* and *E3,Z7-14Ac*) for *Symmetrischema tangolias* and (*E3,Z8-14Ac* and *E3,Z8,Z11-14Ac*) for *Scrobipalpuloides absoluta* (Table 1A). And there are more simple looking compounds for which only tedious and time consuming methods exist. They form a real challenge for the pheromone chemist and keep him alive.

**Table 1** List of Dutch identifications of sex pheromones and sex attractants of Lepidoptera

A. Identifications of sex pheromones by means of chemo-analytical research, confirmed by field screening

<i>Adoxophyes orana</i>	Meijer <i>et al.</i> 1972
<i>Clepsia spectrana</i>	Minks <i>et al.</i> 1973
<i>Archips podana</i>	Persoons <i>et al.</i> 1974
<i>Phthorimaea operculella</i>	Persoons <i>et al.</i> 1976
<i>Cryptophlebia leucotreta</i>	Persoons <i>et al.</i> 1977
<i>Spodoptera exigua</i>	Persoons <i>et al.</i> 1981
<i>Syndemis musculana</i>	Persoons <i>et al.</i> 1984
<i>Symmetrischema tangolias</i>	Griepink <i>et al.</i> 1995
<i>Scrobipalpuloides absoluta</i>	Griepink <i>et al.</i> 1996

B. Number of sex attractants, found via field screening of compounds from the IPO pheromone bank, arranged per lepidopteran family (until 1995)

Acrolepiidae (1)	Noctuidae (10)
Argyresthiidae (6)	Pyralidae (8)
Drepanidae (1)	Sesiidae (4)
Gelechiidae (2)	Tineidae (3)
Glyphipterigidae (1)	Tortricidae (49)
Gracillariidae (8)	Yponomeutidae (3)

The IPO pheromone bank has great significance for our clients, both the dispenser users for practical purposes and for the chemists, the reference compound users. We firmly believe that our institute should maintain and even extend the pherobank, since it certainly has a much higher potential to serve clients than is being used now. Collaboration with pheromone sellers in other markets should be sought and further developed.

## Entomological Research

Entomological research first concentrated on the estimation of the efficiency of sex pheromone traps and on the development of quantitative monitoring with sex pheromone traps through studies of the relationship between trap catches and moth numbers actually present in the field. In the 80's our attention shifted to mating disruption and we were mainly occupied in efficacy testing of various commercially available pheromone dispensers, first of all orchard pests. Recently, after excellent greenhouse facilities became available to our institute, we spent most of our time on pheromone control of noctuid pests in greenhouse cultures. The renewed collaboration with TNO, this time the TNO Plastics and Rubber Research Institute (J.J. de Vlieger & J. Klijnstra), should be mentioned here, since TNO polymer dispensers are being used in all our greenhouse tests. Highly fruitful and very exciting is also our cooperation with Syn-tech (J.v.d. Pers), where portable EAG and SSR equipment is being developed that enables direct measurement of pheromones in ambient air.

## The IOBC/WPRS Working Group on Semiochemicals

We always have felt a close relationship with this working group. We were both present at the meeting of the IOBC/WPRS Working Group "Integrated Pest Management in Fruit Orchards", held at Wädenswil in November 1973, where at least 30% of the programme was devoted to pheromones. From the pheromone colleagues assembled at the meeting the idea arose to start "their own" working group. The proposals were readily accepted by the IOBC/WPRS Council and in October 1975 the first meeting of the Working Group "The Use of Insect Pheromones in Integrated Control" took place at Wageningen. There were 32 participants from 8 countries. The major goal of this first meeting was to get an idea of what was done in Europe in the rapidly expanding field of pheromone research. The participants found the meeting very rewarding, as most of them realized that they were badly informed about each others work.

Table 2 shows that since then the Working Group has met at 12 other occasions, including the recent meeting at Montpellier. Its meetings developed into an important forum for pheromone workers in Europe in the first place, but we are happy to state that they were often attended by colleagues from countries outside the WPRS region, such as the USA, Australia, Canada and Japan. It looks a bit contradictory, but a major problem of the Working Group has always been the overwhelming interest. Most meetings were attended by more than one hundred participants and showed rather the character of a conference, which was not really in line with the meetings of most other IOBC/WPRS Working Groups, where joint projects were planned, executed and the results discussed at the next meeting. However, there is no doubt that the significance of the Semiochemical Working Group has been great and that numerous informal collaborative projects have been initiated and carried out in the past 20 years as a result of its activities.

**Table 2** Meetings of the IOBC/WPRS Working Group on Semiochemicals since 1975

Convenor: Albert K. Minks (1975-1985)			
Wageningen	NL	1975	Introductory meeting, various topics
Harpenden	UK	1977	Chemistry and biological activity
Wädenswil	CH	1979	Fundamental and applied aspects
Nyon	CH	1982	Mating disruption in fruit and grapes
Hamburg	D	1984	Pheromone and attractant chemistry
Balatonalmádi	H	1984	Joint meeting with EPRS Working Group
Convenor: Heinrich Arn (1985-1995)			
Neustadt/Weinstrasse *	D	1986	Mating disruption, behaviour
Avignon *	F	1988	Insect monitoring and attractants
Granada	E	1990	Use in Mediterranean pest management
San Michele all'Adige *	I	1992	Mating disruption
Chatham *	UK	1993	Basic and applied aspects
St. Peters Insel	CH	1994	Use of mating disruption in practice
Convenor: Peter Witzgall (since 1995)			
Montpellier *	F	1996	Technology transfer in mating disruption

\* The proceedings of these meetings have been published as IOBC/WPRS Bulletins.

When glancing through the reports of the early meetings, it is striking to see that some of the items are still present on the programme of today, which does not mean that no progress has been made in this field. On the contrary! It is also interesting to note that already in the meetings in 1977 and 1979 proposals for a pherobank and a pheromone list came forward. We described above that the pherobank idea became reality and developed to a highly successful operation. The same can be said about the pheromone list. After a rather amateurish attempt by the first author of this contribution, which appeared in 1984 as IOBC/WPRS Bulletin VII/1, Heinrich Arn published, together with Miklós Toth and the late Ernst Priesner, the "List of Sex Pheromones of Lepidoptera and Related Attractants", as an IOBC/WPRS Brochure in 1986. The book became a great success and serves now as the vademecum of every pheromone researcher. A 2nd edition appeared in 1992, which can still be obtained from the Federal Research Station at Wädenswil in Switzerland. Nowadays the regularly updated list can also be consulted on the Internet.

These examples show that the Working Group has played an important role in the coordination and stimulation of pheromone research in Europe. It is still much alive. We sincerely hope that it will continue its activities for many more years to come.

### Some concluding remarks

After almost 30 years in pheromone research it is time to say goodbye. We have really enjoyed working together with you in this exciting area of research. Many highlights have passed along in all those years and we consider ourselves as very lucky that we could play our part. One important recommendation emerges from our experience: close collaboration between chemists and entomologists, preferably in the same institute is essential for success in this research area!

So it is time to stop now: Simon will do this completely and Albert will hang around for another year or so by participating in some small projects. And he certainly will continue with his editorial work.

Finally, we are happy to announce that the management of our institute has recently decided to continue with pheromone research. Some months ago a successor for Simon Voerman has been appointed: Dr. Frans Griepink, a young and bright chemist, who has to meet great expectations. Although his position is secure for one year only, we are optimistic about further developments. It is further agreed that in the coming autumn Albert will be replaced by one of the IPO entomologists via an internal reshuffle.

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## **“Amazing daze: confusion brings change”**

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**Abstract** - In the commercialization of pheromones, we are striving to modify pest behaviour to our advantage through the release of specific chemicals at specific rates. For the purposes of this discussion, our pheromone is like the wine, and our controlled release device is the skin. In most established programs the wine is not changing, but we are forever testing new skins. Fundamental to commercialization is the balance that we strike between wine and skin, and progress is dependent on finding and proving new skins that preserve, protect and release the wine at rates optimum to our needs. For the past three decades we can see the tantalizing benefits of pheromone based pest management, but high levels of pest control are not consistently attainable. This paper will present a global summary of commercial semiochemical application and address issues in pheromone commercialization. The author concludes that significant progress has been made. Mating disruption is alive and well in 1996, and considerable promise exists for the commercial implementation of new formulations and use strategies.

**Key words** - sex pheromone, mating disruption, integrated pest management, commercialization

### **Issues in the development framework for semiochemical products**

Conceptually, the commercialization of semiochemicals takes place within several spheres of influence, and it is important to manage product development to overcome the impact of expectations and barriers that these spheres present. In a diagram, these spheres of influence can be presented like concentric rings, with society being the outside sphere and the controlled release formulation the inner core.

#### **Application and society**

Commercial pheromone-based products continue to offer considerable promise and solutions to modern issues in pest management strategies. Safety and specificity are the most obvious. Efficacy with very low rates of active ingredient is another. However, widespread implementation of insecticides has presented western society with worm-free apples for the past three decades, and it requires considerably more know-

ledge and careful management to attain toxicant based quality standards with a behaviour modifying chemical.

Semiochemicals are shadowed with the expectations asked of conventional pest control materials. These products need to compete with chemicals in registration requirements, efficacy standards, cost structure and ease of application.

*Registration requirements.* By definition, in most countries semiochemical products are classified as pesticides as soon as claims are made that they can mitigate a pest. Progress in implementation of North American pheromone products has resulted from the U.S. Environmental Protection Agency's (EPA) aggressive revision of semiochemical product registration data requirements, including the following: (1) Establishment of a toxicology based tier testing data requirement for biochemicals (longer term toxicology studies are only triggered by adverse results in acute testing); (2) establishment of a completely separate division, the Biopesticide and Pollution Prevention Division, to manage the registration of semiochemicals and other biologically based products; (3) exemption of arthropod pheromones as a class from the requirements of tolerance (companies are no longer required to obtain a food use clearance for each compound); (4) exemption of inert ingredients in removable formulations (*i.e.* hand applied, discrete point sources) from the requirements of tolerance (companies now have the flexibility to continuously improve and modify formulations without needing to seek new registrations for each modification); (5) exemption from the requirements of an Experimental Use Permit for arthropod pheromone testing on areas less than 250 acres (testing area has been increased from less than 10 acres, to less than 250 acres).

The biggest development in the recent time has been removal of the distinction between retrievable (*i.e.* mostly hand applied) and non-retrievable (*i.e.* mostly sprayable) formulations. EPA have accepted the argument and extended the exemptions to include all semiochemical formulations for the following reasons: there is no real difference in aerial exposure to pheromone concentrations from either retrievable or non-retrievable formulations, and likewise there is no difference in the crop residue (the inert ingredients were already exempted for food use when use in retrievables, so the exemption could be expanded to include all formulations).

Smashing the North American regulatory glass ceiling was only achieved through the concerted efforts of a partnership of pheromone scientists, crop protection entomologists, semiochemical companies and aggressive lobbying by farmers' organisations. It is now time to make a similar concerted effort seeking such regulatory relief on a global basis.

*Efficacy standards and economics.* Semiochemical products are often burdened with a premium price. It is difficult to get a commercially acceptable return on investment for products predisposed for low usage due to pest specificity and activity at molecular rates, while at the same time dependent on synthesis and production of exacting molecules. A product for codling moth will only be sold for apple and pear pest management. However, a premium price demands premium protection, especially

when in competition with conventional chemicals, and this leaves little room for wormy apples or compromise in product efficacy.

If we charge high prices for mating disruption, the products have to work, and we better understand the conditions that preclude acceptable efficacy. Further progress in commercial implementation will require ongoing advancement in our basic and applied knowledge of the behavioural mechanisms of semiochemical control. I would suggest that the models of mating disruption will need to be configured for each pest, each semiochemical blend and each formulation. Smashing this glass ceiling requires a harmonious marriage of chemistry and entomology.

*Ease of use.* Widespread implementation of semiochemical products will only be possible if application is cheap and easy. The semiochemical industry has developed a wide palette of formulations that can be sprayed, twisted, clipped, pumped and puffed. Innovative application technique will facilitate rapid acceptance and implementation of commercial products. A good example is the transfer of the plastic bag closure from consumer products into codling moth and other tree fruit mating disruption applications. Orchards can be treated in 75% less time using the closure and a pole applicator, when compared to the prior technique of climbing a ladder and twisting each dispenser individually around the branch.

#### Ecology of the crop and environs

Semiochemical product performance is considerably influenced by environmental factors. Behaviour of the molecules is influenced by interactions between the crop and the climate.

For example, elegant European research has carefully documented the differences in pheromone concentration and distribution in vineyards as canopies mature over the season. Similarly, Japanese colleagues were able to document different pheromone concentrations in tea canopies and relate this to differences in the levels of mating between field edges and the field center.

While it is difficult to change the environment, we can now select treatment sites that favor successful implementation of semiochemical products, and we can predict those locations where successful implementation will require the integration of other control strategies. An ideal orchard will have a uniform crop canopy, with no missing trees or rows, an even slope and trees of the same age and type. Missing trees, and even slope can modify the distribution of both pest and pheromone, and such orchards often require supplementary insecticide applications to field edges, or hot spots within the block.

#### Pest behaviour and ecology

In developing any pheromone product, it is critical to research and understand the behaviour and biology of the target insect as completely as possible. Important factors include the following: Time of day for mating; duration of female calling behaviour;

quantity of female pheromone release rate; location of female calling within crop; male searching behaviour.

**Table 1** Commercial application of semiochemicals by pest control sectors

Sector	Monitoring and detection <sup>a</sup>	Mass trapping <sup>a</sup>	Anti-aggregation, repellents	Attract and kill	Mating disruption
Consumer	detection	stink bugs; wasps	mammalian repellents	musciids; tephritids	
Pest control operators	detection	cockroach			
Turf and ornamental, Landscape	detection	Japanese beetle <sup>b</sup>	mammalian repellents; bark beetles		
Government detection and quarantine programs	detection; population delineation	management: tephritid fruit flies; eradication: boll weevil		tephritid fruit fly bait sprays	gypsy moth
Agriculture	detection; population forecasting	tropical weevils; armyworms <sup>c</sup> ; aphids; leafminers; stem borers	mammalian repellents; aphid alarm pheromones	tephritid fruit fly bait sprays; <i>Diabrotica</i> feeding stimulant bait; PBW <sup>d</sup> ; CM <sup>e</sup>	cotton; fruit and nut pests; rice; sugar cane
Animal production	detection	musciids; tabanids		screwworm; tsetse fly	
Forestry	detection; population forecasting	bark beetles	bark beetle anti-aggregation	bark beetle tree baits	defoliators; tip moths
Stored products	detection	Indian meal moth; flour beetles			Indian meal moth

<sup>a</sup> Using pheromone traps, unless specified otherwise; <sup>b</sup> pheromone/kairomone traps; <sup>c</sup> *Spodoptera* spec.; <sup>d</sup> pink bollworm, *Pectinophora gossypiella*; <sup>e</sup> codling moth, *Cydia pomonella*

### Wine - pheromone chemistry

Production of a pheromone product requires pairing chemical synthesis with controlled release development. Solutions need to be found in the following areas: Synthesis; stabilization and longevity (pheromone molecules are environmentally labile and need to be stabilized from photochemical, thermal, oxidative and hydrolytic de-

gradation, and from isomerization and racemization); blend quality (purity; composition).

#### Skin - the controlled release substrate

The second component of pheromone product development is selection of a suitable substrate. It is important to consider the following in making this choice: Production issues (cost, manufacturing ease, consistency and reliability, quality control) and application issues (shape and flexibility in design).

#### Wine and skin - the pheromone product, a controlled release device

Pheromone products are developed through the integration of pheromone chemistry with selection of the correct polymers. Development needs to produce a device that performs according to the following biologically determined parameters: Quantity and quality of released compound(s); timing of release; location in field; temperature effect on release rate; effect of field ageing on release rate profile.

This means that the right amount of the right pheromone has to be released into the field at the right time and in the right place. Overall, it must be at the right price. In summary, semiochemical product performance is a dynamic between the choice of formulation, the age of the formulation, the rate of release and population pressure.

### **Semiochemical applications in all sectors of pest management**

Products based on semiochemical active ingredients are now available in almost all sectors of pest management. Product applications can be summarized into use patterns, and categorized by industrial sector and mode of action (Table 1). In agriculture, pheromone based traps are standard population detection and monitoring tools. The most outstanding applications of pheromone trapping can be summarized as follows.

#### Largest pheromone trapping programs, 1996

The bollweevil eradication foundation in the southern United States used approximately 12.5 million bollweevil lures. The 1996 national gypsy moth monitoring program used over 300 000 lures. Pheromone traps and lures form the basis of pest management monitoring programs throughout California tree fruit, vine and nut production.

#### Commercial applications of mating disruption technology

Mating disruption technology has also gained commercial acceptance against a wide range of pests in many different crops and regions. While the technology has inherent limitations, it is a very effective pest management tool when cautiously monitored and

carefully supervised within an overall integrated pest management program. The most outstanding applications of mating disruption can be summarized as follows:

*Largest single pheromone sale and largest agricultural program, 1996.* Pheromone tender award for pink bollworm mating disruption in Egypt, for a total of \$US 5.3 million and a total treated area in excess of 200 000 acres.

*Major horticultural applications of mating disruption, 1996.* Codling moth management on 10 000 ha in Western USA; grape pest management on 14 000 ha in Germany; codling moth area-wide management on 3 000 ha in Italy; and rice stem borer disruption-based management on 4 000 ha in Spain (pest management for an environmentally sensitive watershed).

Global implementation of mating disruption is summarised in Table 2. A quick review indicates that over 30 target species have been controlled successfully, over 50 different products have been developed commercially, 10 different companies are active in the field and research, development and commercial implementation is underway in all geographical regions. This indicates that mating disruption is alive and well in 1996.

### **New directions and future prospects**

Ongoing efforts to expand the implementation and acceptance of semiochemicals are now starting to look at research and development of new wines and new skins. Some of these programs include:

*Attract and kill.* A new Ciba-Geigy technology has demonstrated considerable promise in the formulation of pheromones together with insecticides in the same drop-let. Excellent control has been achieved against codling moth in apples, and pink bollworm in cotton (Hofer & Brassel 1992; Charmillot & Hofer 1997).

*Other blends.* Several researchers have shown that mating disruption can be enhanced through the combination of pheromones and antagonists in the same treatment (*c.f.* Bengtsson *et al.* 1994). Further work should be undertaken in the evaluation of such novel blends.

*New formulations.* To facilitate mechanical application, several companies are developing better sprayable formulations (see also Rice *et al.* 1997). Further research is evaluating the application of very widely spaced, high-dosage dispensing devices to mist pheromone throughout the orchard (Mafra-Neto & Baker 1996; Shorey *et al.* 1996; Baker *et al.* 1997).

### **Conclusion**

At present, the semiochemical industry worldwide has sales income that is probably larger than US\$30 million. The industry continues to grow, with more product registrations and increasing adoption of already registered products. Ongoing growth will

require continued multidisciplinary attention to the behaviour of pests and molecules and a comprehensive understanding of formulation performance. Pheromone companies, and semiochemical researchers should look at all combinations of wine and skins, and rewards and breakthroughs will be found with the infusion of new ideas.

**Table 2** Mating disruption - types of formulation by region and company

	Hand-applied formulations	Sprayable formulations
Australia and New Zealand	OFM <sup>a</sup> , CM <sup>b</sup> , LBAM <sup>c</sup> , leafrollers, currant borer <sup>d</sup> (Shin-Etsu)	
Chile and Argentina	CM <sup>b</sup> , OFM <sup>a</sup> (Shin-Etsu, Hercon) pine shoot moth <sup>e</sup> (Consep, Biosys, 3M)	
Egypt	PBW <sup>f</sup> (Biosys, Shin-Etsu, Troy Bioscience, Consep, Ciba-Geigy)	
Japan	tea tortrix <sup>g</sup> (Shin-Etsu, Nitto Denko) cherry tree borer <sup>h</sup> , diamondback moth <sup>i</sup> , beet armyworm <sup>k</sup> (Shin-Etsu)	
South Africa	OFM <sup>a</sup> , CM <sup>b</sup> (Shin-Etsu, Consep, Hercon, Ecogen)	
USA	OFM <sup>a</sup> , CM <sup>b</sup> (Shin-Etsu, Consep, Hercon) peach twig borer <sup>l</sup> (Consep, Hercon) tufted apple budmoth <sup>m</sup> , omnivorous leaf roller <sup>n</sup> (Ecogen) grape berry moth <sup>o</sup> , peach tree borer <sup>p</sup> , diamondback moth <sup>i</sup> (Shin-Etsu) artichoke plume moth <sup>q</sup> (Ecogen)	PBW <sup>f</sup> (Ecogen, Consep, Biosys) tomato pinworm <sup>r</sup> (Ecogen, Consep) gypsy moth <sup>s</sup> (Hercon, Biosys) forest defoliators (three species) (Hercon) Mexican rice stem borer <sup>t</sup> (Hercon)
Western Europe	OFM <sup>a</sup> (Shin-Etsu, BASF, Consep) CM <sup>b</sup> (Shin-Etsu, BASF, Isagro) peach twig borer <sup>l</sup> (BASF, Consep) summerfruit tortrix <sup>u</sup> (BASF, Isagro) apple clearwing <sup>v</sup> , European grape berry moths <sup>w</sup> (BASF) rice stem borer <sup>x</sup> (Biosys)	

<sup>a</sup> Oriental fruit moth, *Grapholita molesta*; <sup>b</sup> codling moth, *Cydia pomonella*; <sup>c</sup> light brown apple moth, *Epiphyas postvittana*; <sup>d</sup> *Synanthedon tipuliformis*; <sup>e</sup> *Eucosma sonomana*; <sup>f</sup> pink bollworm, *Pectinophora gossypiella*; <sup>g</sup> *Adoxophyes* spec.; <sup>h</sup> *Synanthedon Hector*; <sup>i</sup> *Plutella xylostella*; <sup>k</sup> *Spodoptera exigua*; <sup>l</sup> *Anarsia lineatella*; <sup>m</sup> *Platynota idaeusalis*; <sup>n</sup> *P. stultana*; <sup>o</sup> *Endopiza viteana*; <sup>p</sup> *Synanthedon exitiosa*; <sup>q</sup> *Platyptilia carduidactyla*; <sup>r</sup> *Keiferia lycopersicella*; <sup>s</sup> *Lymantria dispar*; <sup>t</sup> *Eoreuma loftini*; <sup>u</sup> *Adoxophyes orana*; <sup>v</sup> *Synanthedon myopaeformis*; <sup>w</sup> *Eupoecilia ambiguella*, *Lobesia botrana*; <sup>x</sup> *Chilo suppressalis*

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*Addenda to the list of sex pheromones of  
Lepidoptera and related attractants,  
1992 - 1996*

## Addenda to the list of sex pheromones of Lepidoptera and related attractants, 1992 - 1996

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†Ernst Priesner died of an accident during a field trip in the Bavarian Alps in July 1994.

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For further updates and the meanings of abbreviations, please consult the Internet editions:

<http://nysaes.cornell.edu/pheronet/>

<http://quasimodo.versailles.inra.fr/pherolist/pherolist.html>

<http://mpi.seewiesen.mpg.de/pheronet/pherolist.html>

## Eriocraniidae

<i>Eriocrania cicatricella</i>	P	2R-Z4-hepten-2-ol 2R-heptan-2-ol Z4-7-2Kt	126 23 1.1	Zhu JCEc 21:29 1995 n	1
<i>Eriocrania sangii</i>	P	2S-Z6-nonen-2-ol a 2R-Z6-nonen-2-ol a Z6-9-2Kt 9-2Kt	a a	Kozlov JCEc 22:431 1996	2
<i>Eriocrania semipurpurella</i>	P	2S-Z6-nonen-2-ol a 2R-Z6-nonen-2-ol a Z6-9-2Kt 9-2Kt	a a	2 Kozlov JCEc 22:431 1 1996	3
<i>Eriocrania sparrmannella</i>	A	2R-Z4-hepten-2-ol 2R-heptan-2-ol		Zhu JCEc 21:29 1995	4

## Nepticulidae

<i>Stigmella crataegella</i>	A	S-E6,8-nonadien-2-ol S-Z6,8-nonadien-2-ol	1 1	Tóth JCEc 21:13 1995	5
<i>Stigmella malella</i>	P	S-E6,8-nonadien-2-ol S-Z6,8-nonadien-2-ol	10 3	Tóth JCEc 21:13 1995 L	6
<i>Trifurcula melanoptera</i>	A	R-Z6,8-nonadien-2-ol R-E6,8-nonadien-2-ol	1 1	Tóth JCEc 21:13 1995	7

## Incurvariidae

<i>Lampronia capitella</i>	I	Z11-14Ac		Löfstedt InCEcTábor 1990	57 8
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## Heliozelidae

<i>Antispila treitschkiella</i>	A	Z7-14Al		Tóth JApEn 113:342 1992	9
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## Tineidae

<i>Archimeessia sp. n.</i>	A	E3Z13-18OH E2Z13-18Ac	1 1	Buda JCEc 19:799 1993	10
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<i>Celestica</i>	A	E3Z13-18Ac	1	Buda JCEc 19:799	
<i>angustipennis</i>		E3Z13-18OH	1	1993	11
<i>Infurcitinea</i>	O	Z11-16OH	1	Tóth JApEn 113:342	
<i>albicomella</i>		Z11-16Al	1	1992	12
<i>Infurcitinea</i>	A	Z11-16OH		Tóth JApEn 113:342	
<i>finalis</i>				1992	13
<i>Nemapogon</i>	A	Z3Z13-18Ac		Buda JCEc 19:799	
<i>flavifrons</i>		Z3Z13-18OH		1993	14
<i>Nemapogon</i>	P	Z3Z13-18Ac	0.5	Oshima JPscSc 18:163	
<i>granelus</i>				1993 n	15
<i>Nemaxera</i>	A	E2Z13-18Ac		Buda JCEc 19:799	
<i>betulinella</i>				1993	16
<i>Tineola</i>	I	E2Z13-18Al	0.5	Yamaoka MassSpec 33:189	
<i>bisselliella</i>		E2-18Al	0.25	1985 n	17
<i>Triaxomera</i>	A	Z3Z13-18Ac		Buda JCEc 19:799	
<i>fulvimitrella</i>				1993	18

## Psychidae

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<i>Oiketicus</i>	P	2R-pentyl octanoate a	1	Rhains JCEc 20:3083	
<i>kirbyi</i>		2R-pentyl nonanoate a	1	1994 L	19
		2R-pentyl decanoate a	1		
		2-hexyl decanoate			
		2R-pentyl dodecanoate a			
<i>Thyridopteryx</i>	A	2R-hexyl decanoate		Warthen JCEc 22:1315	
<i>ephemeraeformis</i>				1996	20

## Gracillariidae, Lithocolletinae

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<i>Phyllonorycter</i>	A	E4E10-12Ac		Gries JCEc 19:1789	
<i>blancardella</i>				1993	21
<i>Phyllonorycter</i>	A	E9-14Ac	10	Mozuraitis ISCEPraha 163	
<i>coryli</i>		Z10-14Ac	1	1996	22
<i>Phyllonorycter</i>	A	E10-12Ac		Mozuraitis ISCEPraha 163	
<i>cydoniella</i>				1996	23

<i>Phyllonorycter elmaella</i>	A	E10-12Ac		Shearer JECnEn 87:1450 1994 24
<i>Phyllonorycter harrisella</i>	A	E9-14Ac Z10-14Ac	10 1	Mozuraitis ISCEPrah 163 1996 25
<i>Phyllonorycter junoniella</i>	A	Z10-12Ac		Mozuraitis ISCEPrah 163 1996 26
<i>Phyllonorycter mespilella</i>	P	E4E10-12Ac a E10-12Ac a	tr <2	Gries JCEc 19:1789 1993 n 27
<i>Phyllonorycter oxyacanthae</i>	A	E10-12Ac E10-12OH	10 1	Mozuraitis ISCEPrah 163 1996 28
<i>Phyllonorycter ringoniella</i>	P	E4Z10-14Ac Z10-14Ac	6 4	Boo ISCEPrah 145 1996 L 29
<i>Phyllonorycter sorbi</i>	A	E10-12OH		Mozuraitis ISCEPrah 163 1996 30
<i>Phyllonorycter sylvella</i>	A	E9-14Ac Z10-14Ac	10 1	Mozuraitis ISCEPrah 163 1996 31

## Oecophoridae

<i>Agonopterix liturella</i>	A	Z7-12Ac Z7-12OH Z9-14Ac	1 1 1	Tóth JApEn 113:342 1992 32
<i>Depressaria depressana</i>	A	Z3-10Ac		Sziráki APhpEnHu 26:497 1991 33

## Elachistidae

<i>Stenoma cecropia</i>	P	Z9E11,13-14Al a Z9E11-14Al a Z9E11,13-14Ac Z9E11-14Ac	76 11 16.5 5.5	Zagatti JCEc 22:1103 1996 34
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## Coleophoridae

<i>Augasma aeratellum</i>	A	Z7-12Ac		Tóth JApEn 113:342 1992 35
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<i>Coleophora coracipemella</i>	A	Z5-10Ac Z5-10OH Z7-12Ac	1 1 1	Tóth JApEn 113:342 1992 36
<i>Coleophora deauratella</i>	A	Z5-10Ac Z5-10Al Z7-12Ac	1 1 1	Tóth JApEn 113:342 1992 37
<i>Coleophora hemerobiella</i>	A	Z5-10OH Z7-12OH	20 1	Tóth JApEn 113:342 1992 38
<i>Coleophora sinensis</i>	A	Z5-10OH		Zhang JApEn 116:516 1993 39
<i>Coleophora trigeminella</i>	A	Z7-12Ac Z7-12OH Z5-10Ac	20 20 1	Tóth JApEn 113:342 1992 40
<i>Coleophora unipunctella</i>	A	Z7-12Ac Z7-12OH	1 1	Tóth JApEn 113:342 1992 41

## Cosmopterigidae

<i>Limnaecia phragmitella</i>	P	E11-14Ac a Z11-14Ac a	95 5	Bestmann ZNf 48c:515 1993 L 42
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## Gelechiidae, Anomologinae

<i>Isophrictis sp.</i>	A	Z5-12Ac		Tóth JApEn 113:342 1992 43
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## Gelechiidae, Gelechiinae

<i>Aproaerema modicella</i>	P	Z7,9-10Ac a E7-10Ac a Z7-10Ac	100 20 14	Hall BIOILB 16(10):1 1993 44
<i>Bryotropha dryadella</i>	A	Z11-14Ac		Tóth JApEn 113:342 1992 45
<i>Caryocolum alsinellum</i>	A	Z5-12Ac		Sziráki APhpEnHu 26:497 1991 46
<i>Caryocolum marmoreum</i>	A	Z5-12Ac		Tóth JApEn 113:342 1992 47

<i>Caryocolum proximum</i>	A	Z5-12Ac Z7-12Ac	1 1	Tóth JApEn 113:342 1992 48
<i>Recurvaria leucatella</i>	A	E3Z5-14Ac		Tóth JCEc 18:1093 1992 49
<i>Recurvaria nanella</i>	A	E3-14Ac		Tóth JCEc 18:1093 1992 50
<i>Scrobipalpa atriplicella</i>	A	Z5-12Ac		Sziráki APhpEnHu 26:497 1991 51
<i>Scrobipalpuloides absoluta</i>	P	E3Z8Z11-14Ac	1-5	Attygalle TtLt 36:5471 1995 n 52
<i>Scrobipalpuloides absoluta</i>	P	E3Z8Z11-14Ac E3Z8-14Ac	92 8	Griepink TtLt 37:411 1996 53
<i>Scrobipalpuloides absoluta</i>	O	E3Z8Z11-14Ac E3Z8-14Ac	10 1	Svatos JCEc 22:787 1996 54

## Gelechiidae, Chelariinae

<i>Anarsia lineatella</i>	P	E5-10Ac a E5-10OH a 10Ac E4-10Ac Z4-10Ac E3E5-10Ac Z3E5-10Ac	273 10 4 1 1 2 1	Millar JEcnEn 85:1709 1992 55
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## Gelechiidae, Pexicopiinae

<i>Pectinophora gossypiella</i> India	O	Z7Z11-16Ac Z7E11-16Ac		Tamhankar IntJPsMg 39:111 1993 56
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## Gelechiidae incertae sedis

<i>Symmetrischema tangolias</i>	P	E3Z7-14Ac a E3-14Ac a Z7-14Ac Z5-14Ac	62 31 6 1	Griepink JCEc 21:2003 1995 57
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## Carposinidae

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<i>Carposina sasakii</i> Korea	P	Z7-20-11Kt a Z7-19-11Kt	Boo IntSymInPsCtr 87 1996 58
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## Plutellidae

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<i>Plutella xylostella</i>	O	Z11-16Al Z11-16Ac	5 Koshihara ApEnZo 13:138 5 1978 59
<i>Plutella xylostella</i> Indonesia	O	Z11-16Al Z11-16Ac	4 Zilahi-Balogh IntJPsMg 41:201 6 1995 60
<i>Ypsolopha lucella</i>	A	Z11-16Ac Z11-16Al	20 Tóth JApEn 113:342 1 1992 61

## Argyresthiidae

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<i>Argyresthia albistria</i>	A	Z7-12Ac Z7-12OH Z9-14Ac	20 Tóth JApEn 113:342 1 1992 62 1
<i>Argyresthia retinella</i>	A	Z11-16Al	Tóth JApEn 113:342 1992 63
<i>Argyresthia semifusca</i>	A	Z7-12Ac	Tóth JApEn 113:342 1992 64
<i>Argyresthia sorbiella</i>	A	Z7-12Ac Z9-12Ac	20 Tóth JApEn 113:342 1 1992 65

## Acrolepiidae

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<i>Acrolepiopsis assectella</i>	O	Z11-16Al Z11-16Ac	10 Minks JApEn 117:243 1 1994 66
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## Bucculatricidae

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<i>Bucculatrix thurberiella</i>	P	Z9-14-nitrate Z9-13-nitrate	50 Hall TtLt 23:4811 1 1992 L 67
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## Cossidae

<i>Cossus</i> <i>cossus</i>	O	Z3-10Ac	25	Sziráki AnPhpEnHu 26:497
		Z5-12Ac	100	1991 68
		Z5-14Ac	25	
<i>Zeuzera</i> <i>pyrina</i>	A	E2Z13-18Ac	95	Pasqualini BIIEnBo 46:101
		E3Z13-18Ac	5	1992 69
<i>Zeuzera</i> <i>pyrina</i>	A	E2Z13-18Ac	95	Pasqualini BIIEnBo 47:169
		Z2Z13-18Ac	5	1993 70
		E3Z13-18Ac	5	
<i>Zeuzera</i> <i>pyrina</i>	I	E2Z13-18Ac	78	Malosse JHRChr 16:123
		E3Z13-18Ac	4	1993 71
		Z13-18Ac	18	

## Tortricidae, Tortricinae, Archipini

<i>Adoxophyes</i> <i>orana</i> Romania	O	Z9-14Ac	80	Ghizdavu RvRmBiAni 32:23
		Z11-14Ac	20	1987 72
<i>Adoxophyes</i> <i>sp. (smaller tea tortrix)</i> 8h into photophase	P	Z9-14Ac	14.8	Kou JCEc 18:855
		Z11-14Ac	9.0	1992 n 73
<i>Adoxophyes</i> <i>sp. (smaller tea tortrix)</i> 8h into scotophase	P	Z9-14Ac	94.0	Kou JCEc 18:855
		Z11-14Ac	43.3	1992 n 74
<i>Adoxophyes</i> <i>sp. (smaller tea tortrix)</i> China	O	Z9-14Ac	47	Hsiao ChJEn 10:443
		Z11-14Ac	50	1990 75
		E11-14Ac	1	
		10me-12Ac	2	
<i>Archips</i> <i>argyrospila</i> Western Canada	P	Z11-14Ac	100	Deland JCEc 19:2855
		E11-14Ac	64	1993 L 76
		Z9-14Ac	2	
		14Ac		
		12Ac	1	
		Z11-14OH	4	
		E11-14OH		
Z9-14OH				

<i>Choristoneura</i> <i>sp. n. CPG=Prince George</i>	P	E11-14Ac Z11-14Ac	13 7	Gray JEnSoBC 90:13 1993 L 77
<i>Choristoneura</i> <i>rosaceana</i>	O	Z11-14Ac E11-14Ac Z11-14OH	96.5 2.0 1.5	Delisle EnvEn 21:1007 1992 78
<i>Ctenopseustis</i> <i>herana</i>	A	Z5-14Ac		McLaren NZJCpHoSc 21:25 1993 79
<i>Merophyas</i> <i>leucaniana</i>	P	12Ac Z9-14Ac Z11-14Ac a E11-14Ac a 14Ac a Z11-14OH a 14OH	0.6 4.0 100 5.0 9.5 16 0.8	Foster BiCSyEc 16:227 1988 80

## Tortricidae, Tortricinae, Sparganothini

<i>Amorbia</i> <i>cuneana</i>	P	E10Z12-14Ac E10E12-14Ac	54.8 45.2	Hoffmann EnvEn 12:1387 1983 81
<i>Amorbia</i> <i>cuneana</i>	O	E10Z12-14Ac E10E12-14Ac	53 47	Hoffmann EnvEn 12:1387 1983 82

## Tortricidae, Tortricinae, Cnephasiini

<i>Cnephasia</i> <i>cupressivorana</i>	C	E9-12Ac		Tóth APhpEnHu 29:155 1994 83
<i>Cnephasia</i> <i>ecullyana</i>	A	Z9-12Ac Z9-12OH	1 1	Tóth JApEn 113:342 1992 84
<i>Cnephasia</i> <i>incertana</i>	A	Z10-12Ac		Witzgall CEC 7:13 1996 85
<i>Cnephasia</i> <i>jactatana</i>	P	Z11-14Ac	1.8	Foster NZJZo 20:81 1993 n 86
<i>Cnephasia</i> <i>pasiuana</i>	A	Z5-10Ac Z7-12Ac	1 1	Tóth JCEC 18:1337 1992 87
<i>Cnephasia</i> <i>stephensiana</i>	A	E8-12Ac Z8-12Ac	1 1	Witzgall CEC 7:13 1996 88

<i>Neosphaleroptera nubilana</i>	A	Z5-10Ac	Tóth JApEn 113:342 1992 89
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## Tortricidae, Tortricinae, Tortricini

<i>Acleris chalybeana</i>	C	E11-14Al Z11-14Al	95 Sanders CnEn 125:1067 5 1993 90
<i>Acleris maccana</i>	C	E11-14Al Z11-14Al	95 Sanders CnEn 125:1067 5 1993 91
<i>Acleris maximana</i>	C	E11-14Al Z11-14Al	Sanders CnEn 125:1067 1993 92
<i>Acleris obtusana fuscana</i>	C	E11-14Al Z11-14Al	95 Sanders CnEn 125:1067 5 1993 93
<i>Acleris variana</i>	P	E11,13-14Al a	Gries JCEc 20:1 1994 94

## Tortricidae, Tortricinae, Cochylini

<i>Bonagota cranaodes</i>	P	10Ac 12Ac E3Z5-12Ac a 14Ac 16Ac Z9-16Ac 18Ac methyl hexadecanoate methyl hexadecenoate methyl octadecanoate methyl octadecenoate methyl octadecadienoate	Unelius TtLt 37:1505 1996 95
<i>Cochylis posterana</i>	A	Z11-14Ac Z9-12Ac	10 Tóth JApEn 113:342 1 1992 96
<i>Phtheochroa cranaodes</i>	see <i>Bonagota cranaodes</i>		97

## Tortricidae, Olethreutinae, Olethreutini

<i>Apotomis moestana</i>	C	Z8-12Ac	Linnaluoto AnEnFn 49:64 1983 98
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<i>Celypha purpurana</i>	A	Z8-12OH	Tóth JApEn 113:342 1992	99
<i>Celypha rosaceana</i>	see also <i>Celypha purpurana</i>			100
<i>Celypha nurestrana</i>	A	Z8-12OH	Tóth JApEn 113:342 1992	101
<i>Cymolomia hartigiana</i>	C	Z10-12Ac	Witzgall CEc 7:13 1996	102
<i>Hedya pruniana</i>	C	Z8E10-12Ac	Witzgall CEc 7:13 1996	103
<i>Lobesia bicincta</i>	A	Z9-12Ac	Tóth JApEn 113:342 1992	104
<i>Metendothenia atropunctana</i>	A	Z10-12Ac	Witzgall CEc 7:13 1996	105
<i>Pseudosciaphila brandneriana</i>	A	Z8E10-12Ac	Witzgall CEc 7:13 1996	106

## Tortricidae, Olethreutinae, Eucosmini

<i>Cryptophlebia illepada</i>	A	Z8-12Ac	93	Chang IntJPsMg 41:104 1995	107
		E8-12Ac	6		
		Z8-12OH	1		
<i>Cryptophlebia leucotreta</i>	O	E8-12Ac	9	Newton EnExAp 66:75 1993	108
		Z8-12Ac	1		
<i>Cryptophlebia ombrodelta</i>	A	Z8-12Ac	93	Chang IntJPsMg 41:104 1995	109
		E8-12Ac	6		
		Z8-12OH	1		
<i>Cryptophlebia peltastica</i>	A	Z8-12Ac	Newton EnExAp 66:75 1993	110	
<i>Epiblema confusana</i>	A	E8Z10-12Ac	1	Witzgall CEc 7:13 1996	111
		Z8Z10-12Ac	1		
<i>Epiblema farfarae</i>	C	E8E10-12Ac	1	Witzgall CEc 7:13 1996	112
		E8E10-12OH	1		

<i>Epiblema foenella</i> Sweden	P	Z8Z10-12Ac a E8E10-12Ac a Z8E10-12Ac E8Z10-12Ac Z8-12Ac E8-12Ac 12Ac	11.0 1.4 2.0 0.9 1.8 0.4 7.7	Witzgall CEC 7:13 1996 n 113
<i>Epiblema fuchsiana</i>	C	Z8Z10-12Ac		Witzgall CEC 7:13 1996 114
<i>Epiblema scutulana</i>	A	Z8-12Ac		Tóth JApEn 113:342 1992 115
<i>Epiblema scutulana</i>	C	Z8Z10-12Ac		Witzgall CEC 7:13 1996 116
<i>Epinotia rubiginosana</i>	C	Z8-12Ac Z10-12Ac	1 1	Witzgall CEC 7:13 1996 117
<i>Epinotia tenerana</i>	C	E10E12-14Ac Z10Z12-14Ac	1 1	Witzgall CEC 7:13 1996 118
<i>Eucosma aemulana</i>	A	Z8Z10-12Ac E8E10-12Ac	5 1	Witzgall CEC 7:13 1996 119
<i>Eucosma agnotana</i>	C	Z8Z10-12Ac		Witzgall CEC 7:13 1996 120
<i>Eucosma cana</i>	A	Z8E10-12Ac		Witzgall CEC 7:13 1996 121
<i>Eucosma hohenwartiana</i>	A	E8Z10-12Ac Z8Z10-12Ac	1 1	Witzgall CEC 7:13 1996 122
<i>Eucosma notanthes</i>	A	Z8-12Ac		Hwang PtPrBI 36:31 1994 123
<i>Eucosma scorzonera</i>	C	Z8Z10-12Ac		Witzgall CEC 7:13 1996 124
<i>Eucosma scutana</i>	C	Z8Z10-12Ac		Witzgall CEC 7:13 1996 125
<i>Eucosma urbana</i>	C	Z8Z10-12Ac		Witzgall CEC 7:13 1996 126

<i>Gretchena semialba</i>	C	E11-14A1 Z11-14A1	95 5	Sanders CnEn 125:1067 1993 127
<i>Notocelia cynosbatella</i>	A	Z10E12-14Ac		Witzgall CEc 7:13 1996 128
<i>Notocelia incarnatana</i>	A	E10E12-14Ac E10Z12-14Ac		Witzgall CEc 7:13 1996 129
<i>Notocelia roborana</i>	P	Z8E10-12Ac a Z10-14Ac a E10-14Ac 14Ac 16Ac	5 19 3 12 3	Witzgall CEc 7:13 1996 n 130
<i>Notocelia rosaecolana</i>	A	E8Z10-12Ac Z8E10-12Ac	1 1	Witzgall CEc 7:13 1996 131
<i>Notocelia trimaculana</i>	A	Z10Z12-14Ac		Witzgall CEc 7:13 1996 132
<i>Rhopobota naevana</i>	O	Z11-14Ac Z11-14OH Z9-12Ac	69 23 8	Fitzpatrick JEcnEn 85:947 1992 133
<i>Rhyacionia neomexicana</i>	P	E9-12Ac a Z9-12Ac a E9-12OH Z9-12OH	1 0.2 0.5 0.1	Niwa EnvEn 21:1410 1992 134
<i>Spilonota ocellana</i>	P	Z8-14Ac a Z8-14OH a	100 1-5	McBrien CnEn 124:1391 1991 135
<i>Spilonota ocellana</i>	O	Z8-14Ac Z8-14OH	99 1	McBrien JCEc 20:625 1994 136

## Tortricidae, Olethreutinae, Grapholitini

<i>Cydia blackmoreana</i>	A	E8E10-12Ac		Witzgall CEc 7:13 1996 137
<i>Cydia coniferana</i>	A	E8-12Ac		Witzgall CEc 7:13 1996 138
<i>Cydia cosmophorana</i>	C	E9-12Ac		Tóth APhpEnHu 29:155 1994 139

<i>Cydia duplicana</i>	A	E8E10-12Ac		Witzgall CEC 7:13 1996	140
<i>Cydia exquisitana</i>	C	E8E10-12Ac E8Z10-12Ac	1 1	Witzgall CEC 7:13 1996	141
<i>Cydia fagiglandana</i>	P	E8E10-12Ac E8E10-12OH 12Ac	a 0.01 0.01 0.01	Witzgall CEC 7:13 1996	142
<i>Cydia ilipulana</i>	C	E8E10-12Ac		Witzgall CEC 7:13 1996	143
<i>Cydia illutana</i>	A	E8E10-12Ac		Witzgall CEC 7:13 1996	144
<i>Cydia indivisa</i>	A	E8-12Ac Z8-12Ac	1 1	Witzgall CEC 7:13 1996	145
<i>Cydia intexta</i>	A	E8E10-12Ac		Witzgall CEC 7:13 1996	146
<i>Cydia leucostoma</i>	A	Z8E10-12Ac		Witzgall CEC 7:13 1996	147
<i>Cydia milleniana</i>	A	E8E10-12Ac Z8Z10-12Ac		Witzgall CEC 7:13 1996	148
<i>Cydia nigricana</i>	P	12Ac E9-12Ac E8E10-12Ac E8Z10-12Ac Z8E10-12Ac Z8Z10-12Ac 14Ac Z5-14Ac Z7-14Ac 16Ac 18Ac	2.5 0.1 a 0.8 <.004 <.004 <.004 1.7 0.1 0.1 1.5 0.3	Witzgall JCEC 19:1917 1993 n	149
<i>Cydia oxytropidis</i>	C	E8E10-12Ac		Witzgall CEC 7:13 1996	150
<i>Cydia pactolana</i>	C	E8E10-12Ac E8Z10-12Ac	1 1	Witzgall CEC 7:13 1996	151

<i>Cydia pomonella</i>	O	E8E10-12OH		McDonough JCEc 19:1737 1993 152
<i>Cydia pomonella</i>	A	11C1-E8E10-11OH • or • 10,11-difluoro-E8E10-12OH • or • 8,9-difluoro-E8E10-12OH • or • 8,9,10,11-tetrafluoro-E8E10-12OH		Lucas JCEc 20:489 1994 153
<i>Cydia pomonella</i>	O	E8E10-12OH		McDonough JCEc 21:1065 1995 154
<i>Cydia pyrivora</i>	P	E8E10-12Ac a 12Ac 14Ac 16Ac	0.5 0.2 0.2 0.2	Witzgall CEC 7:13 1996 n 155
<i>Cydia servillana</i>	A	E8E10-12Ac		Witzgall CEC 7:13 1996 156
<i>Cydia splendana</i>	P	E8E10-12Ac E8E10-12A1	9 1	Frérot CrAcScPa 318:447 1995 L 157
<i>Cydia splendana</i>	P	E8Z10-12Ac a E8E10-12Ac a E8Z10-12OH E9-12Ac 12Ac 12OH 14Ac 16Ac 18Ac	0.08 0.01 0.01 0.01 0.14 0.01 0.07 0.01 0.04	Witzgall CEC 7:13 1996 n 158
<i>Cydia strobilella</i>	A	E8Z10-12Ac E8E10-12Ac	1 1	Witzgall CEC 7:13 1996 159
<i>Cydia succedana</i>	A	E8Z10-12Ac E8E10-12Ac	1 1	Witzgall CEC 7:13 1996 160
<i>Cydia turciana</i>	A	E8E10-12Ac E8Z10-12Ac	1 1	Chambon BISOEnFr 98:181 1993 161
<i>Dichrorampha acuminatana</i>	C	E9,11-12Ac Z9,11-12Ac	4 1	Ulenberg ProcExApEn 4:211 1993 162

<i>Dichrorampha gueneeana</i>	C	E9,11-12Ac Z9,11-12Ac	4 1	Ulenberg PrcExApEn 4:211 1993 163
<i>Enarmonia formosana</i>	P	Z9-12Ac E9-12Ac Z7-10Ac	60 40 1	McNair ISCEPraha 149 1996 L 164
<i>Grapholita compositella</i>	A	E8E10-12Ac		Witzgall CEC 7:13 1996 165
<i>Grapholita gemmiferana</i>	A	E8E10-12Ac		Tóth JApEn 113:342 1992 166
<i>Grapholita gemmiferana</i>	A	E8E10-12Ac		Witzgall CEC 7:13 1996 167
<i>Grapholita jungiella</i>	A	E8Z10-12Ac Z8E10-12Ac	1 1	Witzgall CEC 7:13 1996 168
<i>Grapholita molesta</i> Korea	O	Z8-12Ac E8-12Ac Z8-12OH	95 5 1	Boo IntSymInPsCtr 87 1996 169
<i>Grapholita prunivorana</i>		see <i>G. tobarzewskii</i>		170
<i>Grapholita tenebrosana</i>	A	Z8-12Ac		Witzgall CEC 7:13 1996 171
<i>Gypsonoma dealbana</i>	A	E8-12Ac Z10-12Ac	1 1	Witzgall CEC 7:13 1996 172
<i>Latronympha strigana</i>	A	Z8Z10-12Ac E8E10-12Ac	10 1	Witzgall CEC 7:13 1996 173
<i>Pammene albuginana</i>	A	Z8-12Ac		Witzgall CEC 7:13 1996 174
<i>Pammene aurana</i>		see also <i>Cydia aurana</i>		175
<i>Pammene aurana</i>	A	Z8-12Ac Z10-12Ac	1 1	Witzgall CEC 7:13 1996 176
<i>Pammene argyrana</i>	A	Z8-12Ac		Witzgall CEC 7:13 1996 177

<i>Pammene</i>	A	E8-12Ac	1	Witzgall CEC 7:13
<i>aurantiana</i>		Z8-12Ac	1	1996 178
<i>Pammene</i>	O	Z8-12Ac	3	Rotundo BILbEnAgFS 48:89
<i>fasciana</i>		Z8-12OH	1	1991 179
<i>Pammene</i>	see also <i>Cydia gallicana</i>			180
<i>Pammene</i>	A	E8-12Ac	1	Witzgall CEC 7:13
<i>gallicana</i>		Z8-12Ac	1	1996 181
<i>Pammene</i>	A	Z8-12Ac		Witzgall CEC 7:13
<i>regiana</i>				1996 182
<i>Pammene</i>	A	Z8-12Ac	1	Witzgall CEC 7:13
<i>spiniana</i>		E10-12Ac	1	1996 183
<i>Strophedra</i>	A	E8-12Ac		Witzgall CEC 7:13
<i>weirana</i>				1996 184

## Sesiidae, Tinthiinae, Tinthiini

<i>Paradoxecia</i>	P	E3Z13-18Ac	a 250	Tan JCEC 18:419
<i>pieli</i>		E3Z13-18OH	30	1992 n 185
<i>Tinthia</i>	A	E3Z13-18Ac		Buda JCEC 19:799
<i>brodifformis</i>				1993 186
<i>Tinthia</i>	C	E3Z13-18Ac	1	Buda JCEC 19:799
<i>hoplisiformis</i>		E2Z13-18Ac	1	1993 187

## Sesiidae, Tinthiinae, Similipepsini

<i>Similipepsis</i>	A	E3Z13-18OH		Buda JCEC 19:799
<i>takizawai</i>				1993 188

## Sesiidae, Sesiinae, Sesiini

<i>Sesia</i>	A	Z3Z13-18Ac		Buda JCEC 19:799
<i>yezoensis</i>				1993 189

## Sesiidae, Sesiinae, Paranthrenini

<i>Paranthrene</i>	P	E3Z13-18OH	4	Cowles EnvEn 25:109
<i>robiniae</i>		Z3Z13-18OH	1	1996 190

<i>Paranthrene</i> <i>tabaniformis</i>	A	E3Z13-18OH	Du CtShInEn 2:15 1981 191
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## Sesiidae, Sesiinae, Synanthedonini

<i>Bembecia</i> <i>chalciformis</i>	C	Z3Z13-18OH Z3Z13-18Ac • or • Z3Z13-18OH E3Z13-18Ac	Buda JCEc 19:799 1993 192
<i>Bembecia</i> <i>parthica</i>	C	Z3Z13-18Ac Z3Z13-18OH	Buda JCEc 19:799 1993 193
<i>Bembecia</i> <i>puella</i>	A	Z3Z13-18Ac E2Z13-18OH	Buda JCEc 19:799 1993 194
<i>Bembecia</i> <i>romanovi</i>	A	Z3Z13-18Ac	Buda JCEc 19:799 1993 195
<i>Bembecia</i> <i>scopigera</i>	A	Z3Z13-18OH	Buda JCEc 19:799 1993 196
<i>Bembecia</i> <i>scopigera</i>	A	Z3Z13-18Ac	Buda JCEc 19:799 1993 197
<i>Bembecia</i> <i>zuvandica</i>	A	Z3Z13-18Ac	Buda JCEc 19:799 1993 198
<i>Chamaesphacia</i> <i>sp.</i>	C	E3Z13-18Ac E2Z13-18Ac	1 Buda JCEc 19:799 1 1993 199
<i>Paradoxes</i> <i>prelle</i>	P	E3Z13-18Ac a E3Z13-18OH	250 Lin PApC 65:1233 30 1993 200
<i>Pyropteron</i> <i>sp. n.</i>	A	Z3Z13-18Ac Z3Z13-18OH	Buda JCEc 19:799 1993 201
<i>Synansphacia</i> <i>affinis</i>	C	E3Z13-18Ac Z13-18OH	1 Buda JCEc 19:799 1 1993 202
<i>Synansphacia</i> <i>triannuliformis</i>	A	Z3Z13-18OH	Buda JCEc 19:799 1993 203
<i>Synanthedon</i> <i>castanerora</i>	A	E3Z13-18OH	Lin PApC 65:1233 1993 204

<i>Synanthedon caucasicum</i>	A	E3Z13-18Ac		Buda JCEc 19:799 1993 205
<i>Synanthedon conopiformis</i>	A	E3Z13-18OH		Buda JCEc 19:799 1993 206
<i>Synanthedon culiciformis</i>	A	Z3Z13-18Ac		Buda JCEc 19:799 1993 207
<i>Synanthedon scoiiaeformis</i>	A	E2Z13-18Ac		Buda JCEc 19:799 1993 208
<i>Synanthedon soffneri</i>	O	E2Z13-18Ac Z3Z13-18Ac	10 1	Priesner NbBaEn 42:97 1993 209

## Choreutidae

<i>Prochoreutis shestediana</i>	A	E2Z13-18OH E3Z13-18Ac		Buda JCEc 19:799 1993 210
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## Lasiocampidae

<i>Dendrolimus punctatus</i>	P	Z5E7-12Ac	6.5	Zhao AEnSi 36:247
		Z5E7-12propionate	2.4	1993 211
		Z5E7-12OH	7.9	
		Z5-12Ac	1.0	
		Z5-12OH	2.6	
<i>Gastropacha quercifolia</i>	I	Z5-12Al	5	Bestmann InBiCMIB 23:793
		Z5-12OH	1	1993 212
		Z5-dodecenyl Z5-dodecenoate		

## Bombycidae

<i>Andraca bipunctata</i>	P	E11E14-18Al a	55	Ho JCEc 22:271
		E14-18Al	19	1996 213
		E11-18Al	7	
		18Al	19	
<i>Bombyx mandarina</i>	P	E3Z13-18OH		Gao JZhejiangAgUn 7:11 1981 214

## Sphingidae

<i>Agrius convolvuli</i>	P	E11E13-16Al		Wakamura ApEnZo 31:171 1996 215
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<i>Deilephila elpenor</i>	I	E11-16Al E10E12-16Al		Bestmann Ex 48:610 1992 216
<i>Hippotion celerio</i>	I	E10E12-16Al		Bestmann Ex 48:610 1992 217
<i>Manduca sexta</i>	O	E10E12Z14-16Al E10E12E14-16Al E10Z12-16Al E10E12-16Al Z11-16Al E11-16Al Z9-16Al 16Al	14.7 1.6 31.0 5.1 17.4 8.8 1.0 20.4	Tumlinson JCEc 20:579 1994 218

## Pterophoridae

<i>Emmelina monodactyla</i>	A	Z9-12Al Z9-12OH	20 1	Tóth JApEn 113:342 1992 219
<i>Oidaematophorus monodactylus</i>	see also <i>Emmelina monodactyla</i>			220
<i>Oxyptilus tristis</i>	A	Z7-12Ac Z7-12OH	1 1	Tóth JApEn 113:342 1992 221
<i>Stenoptilia paludicola</i>	A	Z9-14OH Z9-14Al	1 1	Tóth JApEn 113:342 1992 222
<i>Trichoptilus lobidactylus</i>	C	E11-14Ac Z11-14Ac	95 5	Sanders CnEn 125:1067 1993 223

## Pyalidae, Crambinae

<i>Chilo hyrax</i>	A	Z9-16Al		Wu APhlacSi 20:229 1993 224
<i>Chilo partellus</i>	I	Z11-16Al Z11-16OH Z9-16Al Z10-15Al Z11-14Al Z9-14Al		Hansson JInPhy 41:171 1995 225
<i>Chrysoteuchia topiaria</i>	A	Z11-16nitrile Z9-16nitrile	1 0.03	Ujváry ArcInBiCPhy 22:393 1993 226

## Pyralidae, Evergestiinae

<i>Evergestis aenealis</i>	A	Z7-12Ac Z9-14Ac	1 1	Tóth APhpEnHu 29:155 1994 227
<i>Evergestis extimalis</i>	O	Z9-14Ac Z7-12Ac	1 1	Tóth APhpEnHu 29:155 1994 228
<i>Evergestis frumentalis</i>	A	Z11-16Al Z9-14Al	20 1	Tóth APhpEnHu 29:155 1994 229

## Pyralidae, Pyraustinae

<i>Anania verbascalis</i>	A	Z9E12-14Ac Z9E12-14OH	100 1	Tóth JApEn 113:342 1992 230
<i>Cnaphalocrocis medinalis</i>	O	Z13-18Ac Z11-16Ac	10 1	Raina IndJPtPr 21:228 1993 231
<i>Cnaphalocrocis medinalis</i>	O	Z13-18Ac Z11-16Ac	10 1	Rao CuSc 65:355 1993 232
<i>Cnaphalocrocis medinalis</i>	P	Z13-18Ac a Z11-16Ac a Z13-18OH 18Ac	1 < 0.1 < 0.1 tr	Rao EnExAp 74:195 1995 n 233
<i>Notarcha basipunctalis</i> Chinese bottle tree	P	E10Z12-16Al E10E12-16Al	77.0 23.0	Honda ApEnZo 29:323 1994 234
<i>Notarcha basipunctalis</i> linden tree	P	E10Z12-16Al E10E12-16Al	78.5 21.5	Honda ApEnZo 29:323 1994 235
<i>Notarcha derogata</i>	P	E10Z12-16Al a E10E12-16Al a E10Z12-16OH E10E12-16OH 16Al 22Hy 23Hy 24Hy 25Hy	7 3	Himeno ApEnZo 27:507 1992 236

<i>Notarcha derogata</i> cotton-rosemallow	P	E10Z12-16Al		Honda ApEnZo 29:323 1994 237
<i>Notarcha derogata</i> hibiscus	P	E10Z12-16Al		Honda ApEnZo 29:323 1994 238
<i>Ostrinia furnacalis</i>	P	Z12-14Ac a E12-14Ac a 14Ac	6.6 5.8 1.7	Kou JCEc 18:833 1992 n 239
<i>Ostrinia nubilalis</i> Moravia, Slovakia	P	Z11-14Ac a E11-14Ac a 14Ac	99 1 14	Kalinová EuJEn 91:197 1994 240
<i>Ostrinia nubilalis</i>	A	14-fluoro-Z11- 14Ac 14-fluoro-E11-14Ac • or • 14,14,14-trifluoro-Z11-14Ac 14,14,14-trifluoro-E11-14Ac	95 5 95 5	Klun JCEc 20:2705 1994 241
<i>Palpita unionalis</i>	P	E11-16Ac E11-16Al	7 3	Mazomenos JCEc 20:745 1994 242
<i>Sitochroa verticalis</i>	A	E9-12Ac		Tóth APhpEnHu 29:155 1994 243

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 Pyralidae, Phycitinae

<i>Acrobasis nuxvorella</i>	P	E9Z11-16Al	>0.001	Millar BiOgMdC 4:331 1996 n 244
<i>Acrobasis nuxvorella</i>	A	E9Z11-15Al		Millar BiOgMdC 4:331 1996 245

<i>Acrobasis vaccinii</i>	P	E8Z10-15Ac	0.5	McDonough	JCEc	20:3269
		E8E10-15Ac	0.06	1994	n	246
		Z8Z10-15Ac	0.01			
		Z8E10-15Ac	0.005			
		Δ-12Ac				
		Z8-15Ac				
		Z9-15Ac				
		E9-15Ac				
		Δ-17Ac				
		14Ac				
		15Ac				
		16Ac				
		17Ac				
		12OH				
14OH						
16OH						
<i>Cadra calidella</i>	C	Z9E12-14Ac		Boughdad	IntCgEnFir	1966 247
<i>Cadra cautella</i>	O	Z9E12-14Ac	2	Quarley	EnExAp	66:237
		Z9-14Ac	1	1993		248
<i>Cryptoblabes gnidiella</i>	O	Z11-16Al	1	Anshelevich	Ppr	21:189
		Z13-18Al	1	1993		249
<i>Dioryctria resinosella</i>	O	Z9-14Ac	30	Grant	EnvEn	22:154
		E9-14Ac	1.5	1993		250
		Z9-14OH	5			
		Z9-12Ac	10			
<i>Dioryctria resinosella</i>	P	Z9-14Ac a	0.8	Grant	EnvEn	22:154
		Z9-14OH	0.2	1993	n	251
<i>Dioryctria nubella</i>	O	Z9E11-14Ac	2	Wu	ScSilSi	22:368
		Z11-16Ac	1	1986		252
		Z11-16Al	7			
<i>Ectomyelois ceratoniae</i>	A	Z7E9,11-12formate		Todd	JCEc	18:2331 1992 253
<i>Etiella zinckenella</i>	O	Z11-14Ac	100	Tóth	BiOgMdC	4:495
		Z9-14Ac	3	1996		254
<i>Euzophera punicaella</i>	I	Z9E12-14OH	4	Bestmann	ZNf	48c:110
		Z9E12-14Ac	1	1993		255

<i>Euzophera semifuneralis</i>	P	Z9-14Al	1	Ujváry ArcInBiCPhy 22:393
		Z9E12-14Al	2	1993 256
		Z9-14OH	1	
		Z9E12-14OH	2	
<i>Glyptoteles leucacrinella</i>	A	Z9-14Al	20	Tóth JApEn 113:342
		Z9-14OH	1	1992 257
<i>Hulstia undulatella</i>	P	Z9-14Ac a	8.5	Davis JCEc 19:433
		Z9-14OH a	0.3	1993 n 258
		Zi1-16Ac a	1.6	
<i>Myelopsis tetricella</i>	A	Z9E11-14Ac		Tóth JApEn 113:342 1992 259
<i>Plodia interpunctella</i> extract	P	Z9E12-14Ac	19.4	Teal JCEc 21:787
		Z9E12-14OH	4.0	1995 n 260
		Z9E12-14Al	1.4	
<i>Plodia interpunctella</i> volatiles	P	Z9E12-14Ac		Teal JCEc 21:787
		Z9E12-14OH		1995 261
		Z9E12-14Al		

## Pyralidae incertae sedis

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<i>Coniesta ignefuscalis</i>	A	Z7-12OH	500	Youm JEcnEn 88:66
		Z5-10OH	25	1995 262
		Z7-12Al	16.7	

## Geometridae, Oenochrominae

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<i>Alsophila japonensis</i>	A	Z3Z6Z9-19Hy		Ando JCEc 19:787
				1993 263
<i>Inurois fumosa</i>	A	Z6Z9-3,4epo-21Hy		Ando JCEc 19:787
				1993 264
<i>Inurois membranaria</i>	A	Z6Z9-3,4epo-21Hy		Ando JCEc 19:787
				1993 265

## Geometridae, Geometrinae

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<i>Agathia carissima</i>	A	Z3Z6Z9-20Hy		Ando JCEc 19:787
				1993 266

<i>Agathia</i> <i>visenda visenda</i>	A	Z6Z9-20Hy		Ando JCEc 19:787 1993	267
<i>Pachyodes</i> <i>superans</i>	A	Z3Z6Z9-20Hy		Ando JCEc 19:787 1993	268

## Geometridae, Sterrhinae

<i>Idaea</i> <i>aversata</i>	A	Z9Z11-14Ac E9Z11-14Ac Z7Z9-12Ac	10 0.7 1	Zhu JCEc 22:1505 1996	269
<i>Idaea</i> <i>aversata</i>	P	Z9Z11-14Ac Z7Z9-12Ac		Zhu JCEc 22:1505 1996	270
<i>Idaea</i> <i>biselata</i>	P	Z7Z9-12Ac		Zhu JCEc 22:1505 1996	271
<i>Idaea</i> <i>emarginata</i>	A	Z9Z11-14Ac Z7Z9-12Ac Z7E9-12Ac	10 10 1	Zhu JCEc 22:1505 1996	272
<i>Idaea</i> <i>straminata</i>	A	Z9Z11-14Ac Z7Z9-12Ac		Zhu JCEc 22:1505 1996	273
<i>Idaea</i> <i>straminata</i>	I	Z7Z9-12Ac E7Z9-12Ac Z7E9-12Ac		Zhu JCEc 22:1505 1996	274

## Geometridae, Larentiinae

<i>Anticlea</i> <i>vasiliata</i>	A	Z3Z6Z9-21Hy		Millar EnvEn 20:450 1991	275
<i>Epirrita</i> <i>autumnata</i>	P	1Z3Z6Z9-21Hy		Zhu EnExAp 75:159 1995	276
<i>Epirrita</i> <i>viridipurpurescens</i>	A	Z3Z6Z9-21Hy		Ando JCEc 19:787 1993	277
<i>Epirrhoe</i> <i>sperryi</i>	P	Z3Z6Z9-19Hy Z3Z6Z9-18Hy Z6Z9-19Hy	98 1 1	Millar JCEc 18:1057 1992	278
<i>Esakiopteryx</i> <i>volitans</i>	A	Z3Z6Z9-19Hy		Ando JCEc 19:787 1993	279

<i>Lobophora nivigerata</i>	P	Z3Z6Z9-21Hy Z6Z9-21Hy	Millar JCEc 18:1057 1992 280
<i>Mesoleuca ruficillata</i>	A	Z3Z6Z9-20Hy • or • Z3Z6Z9-21Hy	Millar EnvEn 20:450 1991 281
<i>Operophtera relegata</i>	A	Z3Z9-6,7epo-19Hy • or • Z3Z6-9,10epo-19Hy • or • Z6Z9-3,4epo-19Hy	Ando JCEc 19:787 1993 282
<i>Sibatania mactata</i>	A	Z3Z6Z9-21Hy	Ando JCEc 19:787 1993 283
<i>Xanthorhoe abrasaria aquilonaria</i>	A	Z3Z9-6S7Repo-21Hy Z3Z6-9S10Repo-21Hy	10 Millar EnvEn 20:450 1 1991 284
<i>Xanthorhoe ferrugata</i>	A	Z3Z9-6S7Repo-21Hy	Millar EnvEn 20:450 1991 285
<i>Xanthorhoe munitata</i>	A	Z3Z9-6S7Repo-21Hy Z3Z6Z9-21Hy	1 Millar EnvEn 20:450 10 1991 286

## Geometridae, Ennominae

<i>Abraxas grossulariata</i>	A	Z6Z9-3S4Repo-17Hy	Tóth ZNf 49:516 1994 287
<i>Abraxas nipponibia</i>	A	Z6Z9-3,4epo-18Hy	Ando JCEc 21:299 1995 288
<i>Abraxas sylvata</i>	A	Z6Z9-3R4Sepo-17Hy	Tóth ZNf 49:516 1994 289
<i>Agriopis aurantiaria</i>	P	Z6Z9-3S4Repo-19Hy	Szöcs JCEc 19:2721 1993 290
<i>Agriopis bajaria</i>	P	Z3Z6Z9-18Hy Z3Z6Z9-19Hy	Szöcs ISCEPraha 178 1996 291
<i>Agriopis leucophearia</i>	A	Z6Z9-3S4Repo-19Hy	Szöcs JCEc 19:2721 1993 292
<i>Agriopis marginaria</i>	O	Z3Z9-6S7Repo-19Hy Z3Z6Z9-19Hy	10 Szöcs JCEc 19:2721 3 1993 293

<i>Alcis</i> <i>angulifera</i>	A	Z6Z9-3,4epo-19Hy		Ando JCEc 19:787 1993	294
<i>Ascotis</i> <i>selenaria cretacea</i>	A	Z6Z9-3,4epo-19Hy		Ando JCEc 19:787 1993	295
<i>Colotois</i> <i>pennaria</i>	P	Z3Z9-6R7Sepo-19Hy a Z3Z6Z9-19Hy a Z3Z6Z9-21Hy a Z3Z9-cis6,7epo-21Hy Z3Z6-cis9,10epo-21Hy	2 1 0.1 tr tr	Szöcs JCEc 19:2721 1993	296
<i>Colotois</i> <i>pennaria ussuriensis</i>	A	Z6Z9-3,4epo-20Hy		Ando JCEc 19:787 1993	297
<i>Ectropis</i> <i>excellens</i>	A	Z9-6,7epo-18Hy • or • Z3Z9-6,7epo-18Hy • or • Z6-9,10epo-18Hy		Ando JCEc 21:299 1995	298
<i>Erannis</i> <i>defoliaria</i>	P	Z3Z9-6S7Repo-19Hy a Z3Z6Z9-19Hy a	3 1	Szöcs JCEc 19:2721 1993	299
<i>Erannis</i> <i>golda</i>	A	Z3Z9-6,7epo-18Hy		Ando JCEc 21:299 1995	300
<i>Lambdina</i> <i>athasaria</i>	P	7me-17Hy a 7me11me-17Hy a 5me11me-17Hy		Gries JCEc 20:2501 1994	301
<i>Lambdina</i> <i>fiscellaria fiscellaria</i>	O	5Rme11Sme-17Hy		Li JCEc 19:1057 1993	302
<i>Lambdina</i> <i>fiscellaria fiscellaria</i>	P	5Rme11Sme-17Hy 2me5Rme-17Hy		Silk EnTrAgSc 1:85 1993	303
<i>Lambdina</i> <i>fiscellaria lugubrosa</i>	P	5me11me-17Hy a 2me5me-17Hy a 7me-17Hy a		Gries JCEc 19:1009 1993	304
<i>Lambdina</i> <i>fiscellaria lugubrosa</i>	O	5Rme11Sme-17Hy		Li JCEc 19:1057 1993	305
<i>Lambdina</i> <i>fiscellaria lugubrosa</i>	P	7Sme-17Hy 2me5Sme-17Hy		Li JCEc 19:2547 1993	306

<i>Lambdina</i>	O	5me11me-17Hy	10	Evenden EnvEn 24:924
<i>fiscellaria lugubrosa</i>		2me5me-17Hy	10	1995 307
<i>Menophra</i>	A	Z9-6,7epo-19Hy		Ando JCEc 21:299
<i>senilis</i>				1995 308
<i>Pachyligia</i>	A	Z3Z6-9,10epo-21Hy		Ando JCEc 19:787
<i>dolosa</i>		° or • Z3Z9-6,7epo-21Hy		1993 309
<i>Plagodis</i>	A	Z3Z9-6R7Sepo-20Hy	1	Miillar EnvEn 20:450
<i>alcoolaria</i>		Z3Z6Z9-20Hy	1	1991 310
<i>Rhynchobapta</i>	A	Z6Z9-17Hy		Ando JCEc 21:299
<i>cervinaria bilineata</i>				1995 311
<i>Semiothisa</i>	P	Z6Z9-3R4Sepo-17Hy a		Tóth ZNf 49:516
<i>clathrata</i>		Z3Z6Z9-17Hy a		1994 312
<i>Semiothisa</i>	A	Z6Z9-3R4Sepo-17Hy	1	Gries JCEc 19:843
<i>marmorata</i>		Z3Z6Z9-17Hy	1	1993 313
<i>Semiothisa</i>	A	Z6Z9-3R4Sepo-17Hy	3	Gries JCEc 19:843
<i>neptaria</i>		Z6Z9-3S4Repo-17Hy	1	1993 314
<i>Semiothisa</i>	P	Z6Z9-cis3,4epo-17Hy		Gries JCEc 19:843
<i>sexmaculata</i>		Z3Z6Z9-17Hy	a	1993 315
<i>Synegia</i>	A	Z6Z9-21Hy		Ando JCEc 21:299
<i>esther</i>				1995 316
<i>Tephrina</i>	A	Z6Z9-3S4Repo-17Hy		Tóth ZNf 49:516
<i>arenacearia</i>		Z3Z9-cis6,7epo-17Hy		1994 317
<i>Theria</i>	I	Z6Z9-20Hy		Szöcs ISCEPrahá 178
<i>rupicapraría</i>		Z6Z9-21Hy		1996 318
		Z3Z6-20Hy	tr	
		Z3Z6-21Hy	tr	

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 Geometridae incertae sedis
 

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<i>Anisodes</i>	C	E11-16Ac	7	Mazomenos JCEc 20:745
<i>sp.</i>		E11-16Al	3	1994 319
<i>Nepytia</i>	P	3me13me-17Hy		Gries JCEc 19:1501
<i>freemani</i>				1993 320

<i>Nepytia freemani</i>	A	3me13me-16Hy	Gries JCEc 19:1501 1993 321
<i>Nepytia freemani</i>	P	3Sme13Rme-17Hy	King JCEc 21:2027 1995 322

## Notodontidae

<i>Notodonta dromedarius</i>	I	Z11Z13-16Al Z11E13-16Al E11Z13-16Al E11E13-16Al Z11Z13-16OH Z11E13-16OH E11Z13-16OH E11E13-16OH Z11Z13-16Ac Z11E13-16Ac E11Z13-16Ac E11E13-16Ac	Bestmann Nw 80:271 1993 323
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## Thaumetopoeidae

<i>Thaumetopoea jordana</i>	I	Y11Z13-16OH Y11Z13-16Al Y11Z13-16Ac	5 Frérot BIZoAgBa II/25:33 4 1993 324 1
<i>Thaumetopoea pinivora</i>	I	Y11Z13-16OH Z11Z13-16Al	4 Frérot BIZoAgBa II/25:33 1 1993 325
<i>Thaumetopoea processionea</i>	I	Z11Z13-16Ac Z11Z13-16OH	19 Frérot BIZoAgBa II/25:33 1 1993 326
<i>Thaumetopoea solitaria</i>	I	Z13Z15-18Al	Frérot BIZoAgBa II/25:33 1993 327
<i>Thaumetopoea wilkinsoni</i>	P	Y11Z13-16Ac	Frérot BIZoAgBa II/25:33 1993 328

## Lymantriidae

<i>Euproctis pseudoconspersa</i>	P	10me14me- 15isobutyrate	Wakamura ApEnZo 29:403 1994 329
<i>Euproctis pseudoconspersa</i>	P	10Rme14me- 15isobutyrate	Ichikawa JCEc 21:627 1995 330

<i>Euproctis similis</i>	P	Z7-18methylbutyrate	a	10	Yasuda ApEnZo 29:21	
		Z7-18isovalerate	a	6		1994 331
		Z9-18methylbutyrate		0.75		
		Z9-18isovalerate		0.9		
		Z7-18isobutyrate	a	0.5		
		Z7-18butyrate	a	0.5		
<i>Euproctis taiwana</i>	P	Z9-16me-17isobutyrate		22.2	Yasuda JCEc 21:1813	
		16me-17isobutyrate		7.5		1995 n 332
<i>Lymantria mathura</i>	C	cis7,8epo-2me-18Hy			Odell JCEc 18:2153 1992 333	
<i>Lymantria monacha</i>	P	Z7-18Hy			Gries Nw 83:382	
		2meZ7-18Hy		1		1996 L 334
		7R8Sepo-2me-18Hy		10		
		7R8Sepo18Hy		10		

## Arctiidae

<i>Arctia caja</i>	I	Z3Z6Z9-21Hy		1	Bestmann ZNf 47c:132	
		Z3Z6-cis9,10epo-21Hy		1		1992 335
		Z3Z6-cis9,10epo-20Hy		10		
<i>Pareuchates pseudoinsulata</i>	I	17Hy			Schneider JApEn 113:280 1992 336	
		$\Delta$ -17Hy				
		2me-17Hy				
		5me-17Hy				
		19Hy				
		Z9-19Hy				
		Z6Z9-19Hy				
		$\Delta$ -21Hy				
		Z6Z9-21Hy				
		Z3Z6Z9-21Hy				
<i>Pareuchates pseudoinsulata</i>	I	Z9-19Hy		2.3	Frérot CrAcScPa 317/2:1045 1993 337	
		Z6Z9-19Hy		0.6		
		Z9-20Hy		0.3		
		Z6Z9-20Hy		0.1		
		Z3Z6Z9-20Hy		0.4		
		1Z3Z6Z9-20Hy		1.8		
		Z9-21Hy		1.8		
		Z6Z9-21Hy		4.2		
		Z3Z6Z9-21Hy		13.2		
1Z3Z6Z9-21Hy		75.3				

<i>Tyria jacobaeae</i>	P	1Z3Z6Z9-21Hy		Bestmann ZNf 49c:276 1994	338
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## Noctuidae, Herminiinae

<i>Adrapsa notigera</i>	A	Z3Z9-6,7epo-23Hy • or • Z3Z9-6,7epo-22Hy		Ando JCEc 21:299 1995	339
<i>Bleptina caraadrialis</i>	A	Z3Z9-6R7Sepo-20Hy Z3Z9-6S7Repo-20Hy • or • Z3Z9-6R7Sepo-21Hy Z3Z9-6S7Repo-21Hy	1 1 4 1	Millar EnvEn 20:450 1991	340
<i>Idia americalis</i>	A	Z3Z9-6S7Repo-20Hy Z3Z9-6S7Repo-21Hy	1 1	Millar EnvEn 20:450 1991	341
<i>Paracolax pryeri</i>	A	Z3Z9-6,7epo-20Hy		Ando JCEc 19:787 1993	342
<i>Tetanolita mynesalis</i>	P	Z3Z9-6S7Repo-21Hy Z3Z6Z9-21Hy	0.56 0.23	Haynes JCEc 22:75 1996 n	343

## Noctuidae, Rivulinae

<i>Hypenomoprpha calamina</i>	A	Z6-9,10epo-20Hy		Ando JCEc 21:299 1995	344
<i>Neachrostia bipuncta</i>	A	Z3Z6Z9-18Hy		Ando JCEc 21:299 1995	345
<i>Pangrapta trimantesalis</i>	A	Z3Z6-9,10epo-23Hy		Ando JCEc 21:299 1995	346
<i>Paragabra flavomacula</i>	A	Z3Z9-6,7epo-19Hy		Ando JCEc 19:787 1993	347
<i>Rivula leucanioides</i>	A	Z3Z9-6,7epo-18Hy		Ando JCEc 21:299 1995	348
<i>Rivula sasaphila</i>	A	Z3Z9-6,7epo-19Hy		Ando JCEc 19:787 1993	349
<i>Rivula sericealis</i>	A	Z3Z9-6,7epo-21Hy		Ando JCEc 19:787 1993	350

## Noctuidae, Plusiinae

<i>Autographa gamma</i>	P	Z7-12Ac Z7-12OH	a <3.4 <0.5	Mazor JCEc 18:2373 1992 n 351
<i>Chrysodeixis acuta</i>	A	Z5-10Ac Z7-12Ac	1 1	Tóth JCEc 18:1337 1992 352
<i>Ctenoplusia albostrata</i>	I	Z9E12-14Ac		Boughdad IntCgEnFir 1996 353
<i>Diachrysis chrysitis</i>	P	Z5-10Ac Z7-10Ac	> 75 < 25	Löfstedt JCEc 20:91 1994 354
<i>Diachrysis tutti</i>	P	Z5-10Ac Z7-10Ac	< 20 > 80	Löfstedt JCEc 20:91 1994 355
<i>Plusia festucae</i>	P	Z5-12Ac Z5-12OH Z7-14Ac Z7-14OH	a 100 a 6 a 15 1	Ando JCEc 21:1181 1995 356
<i>Trichoplusia ni</i> Israel,gland	P	Z7-12Ac Z7-12OH 12Ac Z5-12Ac 11-12Ac Z7-14Ac Z9-14Ac	100 1 3.6 3.4 2.9 0.5 0.5	Dunkelblum ArcInBiCPhy 22:413 1993 357
<i>Trichoplusia ni</i> Israel, volatiles	P	Z7-12Ac Z7-12OH 12Ac Z5-12Ac 11-12Ac Z7-14Ac Z9-14Ac	100 1.2 3.6 4.7 2.2 0.5 0.5	Dunkelblum ArcInBiCPhy 22:413 1993 358

## Noctuidae, Amphipyriinae

<i>Dypterygia scabriuscula</i>	A	Z11-16Ac Z9-14Al		Stan SoLpRomBul 10:10 1992 359
<i>Gortyna flavago</i>	C	Z11-16Al Z9-16Al	100 8	Subchev CrAcBgSc 47:101 1994 360

<i>Hoplodrina octogenera</i>	C	Z11-16Ac		Stan SoLpRomBul 10:10 1992 361
<i>Oria musculosa</i>	A	Z11-16Ac		Tóth JApEn 113:509 1992 362
<i>Sesamia grisescens</i>	P	Z11-16Ac a Z11-16OH a E11-16Ac E11-16OH Z9-16Ac Z9-16OH 16Ac 16OH	1000 953 9 1 6 19 206 122	Whittle JCEc 21:1409 1995 363
<i>Sesamia grisescens</i>	O	Z11-16Ac Z11-16OH	3 2	Whittle JCEc 21:1409 1995 364
<i>Spodoptera depravata</i>	P	Z9E12-14Ac a Z9-14Ac		Kurihara JpJApEnZo 35:323 1991 365
<i>Spodoptera descoinsi</i>	P	Z9-14Ac a Z11-14Ac Z9E12-14Ac a E9E12-14Ac Z9E11-14Ac Z11-16Ac Z9-14A1	41 < 1 42 3 1 11 2	Monti JCEc 21:641 1995 366
<i>Spodoptera eridania</i>	A	Z9-14Ac Z9E12-14Ac Z9E11-14Ac Z11-16Ac	55.8 21.2 8.7 14.3	Mitchell FIEn 77:237 1994 367
<i>Spodoptera exigua</i> Japan	P	Z9E12-14Ac Z9-14OH Z9E12-14OH Z9-14Ac	98 100 83 60	Mochizuki ApEnZo 28:489 1993 368
<i>Spodoptera exigua</i>	A	Z9E12-14Ac Z9-14OH Z11-16Ac	87.2 2.5 10.3	Mitchell FIEn 77:237 1994 369

<i>Spodoptera exigua</i> Japan	O	Z9E12-14Ac	7	Mochizuki ApEnZo 29:436
		Z9-14OH	3	
		• or •		
		Z9E12-14Ac	1	
		Z9E12-14OH	1	
<i>Spodoptera latifascia</i>	P	Z9-14Ac a	75	Monti JCEc 21:641
		Z11-14Ac	< 1	
		Z9E12-14Ac a	7	
		E9E12-14Ac	1	
		Z9E11-14Ac	1	
		Z11-16Ac	8	
		Z9-14Al	8	
<i>Trachea atriplicis</i>	A	Z11-16Ac	10	Subchev CrAcBgSc 45:121
		Z11-16OH	1	
		Z11-16Al	1	

## Noctuidae, Cuculliinae

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<i>Agrochola circellaris</i>	A	Z11-16Ac		Stan SoLpRomBul 10:10
				1992 373
<i>Agrochola humilis</i>	A	Z11-16Al	100	Subchev CrAcBgSc 47:101
		Z9-14Al	3	
<i>Agrochola litura</i>	A	Z9-14Al		Subchev CrAcBgSc 47:101
				1994 375
<i>Agrochola tychnidis</i>	A	Z9-14Al		Subchev CrAcBgSc 47:101
				1994 376
<i>Atethmia ambusta</i>	A	Z7-14Ac	10	Subchev JApEn 119:207
		Z5-14Ac	1	

## Noctuidae, Hadeninae

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<i>Graphania bromias</i>	P	Z9-14Ac a		Frérot NZJZo 20:71
		Z9-14OH a		
		Z7-14Ac		
<i>Graphania insignis</i>	P	Z7-12OH a		Frérot NZJZo 20:71
		Z9-14OH a		

<i>Graphania plena</i>	P	Z5-12Ac Z7-12Ac a Z7-14Ac Z9-14Ac a Z9-14OH		Frérot NZJZo 20:71 1993 380
<i>Graphania ustistriga</i>	I	Z7-12Ac Z7-12OH		Frérot NZJZo 20:71 1993 381
<i>Leucania separata</i>	P	Z11-16Ac a Z11-16OH a 16Ac 16OH	60.0 29.3 9.0 9.3	Kou BllsZoAcSi 31:246 1992 382
<i>Mamestra suasa</i>	O	Z11-16Ac Z11-16Al Z7-12Ac	100 1 100	Subchev NICfEnSofia 1:300 1993 383
<i>Orthosia gothica</i>	A	Z9-14Ac Z9-14OH Z5-14Ac Z11-14Ac	10 10 0.5 0.5	Tóth ZNf 47c:613 1992 384
<i>Orthosia munda</i>	O	Z11-16OH Z11-16Al	20 1	Tóth JApEn 115:342 1993 385
<i>Orthosia stabilis</i>	O	Z11-16Al Z9-14Al	100 1-3	Tóth JApEn 115:342 1993 386
<i>Pseudaletia separata</i> Taiwan	I	Z11-16Ac Z11-16OH 16OH 16Ac	60.0 29.3 9.3 9.0	Kou BllsZoAcSi 31:246 1992 387

## Noctuidae, Noctuinae

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<i>Agrotis clavis</i>	A	Z5-10Ac		Tóth JCEc 18:1337 1992 388
<i>Agrotis segetum</i>	A	Z7-12OH Z5-10OH • or • Z7-12OH Z5-10Ac	1 1  100 0.01	Tóth JApEn 112:202 1991 389

<i>Agrotis</i>	O	Z5-10Ac	1	Tóth JCEc 18:1337	
<i>segetum</i>		Z7-12Ac	1	1992	390
Eight other countries		Z9-14Ac	8		
<i>Agrotis</i>	O	Z5-10Ac		Tóth JCEc 18:1337	
<i>segetum</i>				1992	391
Zimbabwe, South Africa					
<i>Agrotis</i>	O	Z5-10Ac	3	Wu PhyEn 20:81	
<i>segetum</i>		Z5-12Ac	0.3	1995	392
Sweden		Z7-12Ac	15		
		Z9-14Ac	7.5		
<i>Noctua</i>	A	Z7-12Ac		Coroiu SoLpRomBul 10:16	
<i>orbona</i>				1992	393

## Noctuidae, Heliiothinae

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<i>Helicoverpa</i>	O	Z9-16Al	100	Cai SiZo 9:11	
<i>assulta</i>		Z11-16Al	9.5	1992	394
China					
<i>Helicoverpa</i>	O	Z9-16Al	20-25	Park KorJApEn 33:26	
<i>assulta</i>		Z11-16Al	1	1994	395
Korea					
<i>Helicoverpa</i>	P	Z9-16Al	189	Park JCEc 22:1201	
<i>assulta</i>		Z9-16Ac	23	1996	n 396
2 h in		Z11-16Al	70		
scotophase		Z11-16Ac	6		
<i>Helicoverpa</i>	P	Z9-16Al	260	Park JCEc 22:1201	
<i>assulta</i>		Z9-16Ac	30	1996	n 397
4 h in		Z11-16Al	122		
scotophase		Z11-16Ac	9		
<i>Helicoverpa</i>	P	Z9-16Al	238	Park JCEc 22:1201	
<i>assulta</i>		Z9-16Ac	30	1996	n 398
6 h in		Z11-16Al	344		
scotophase		Z11-16Ac	25		
<i>Helicoverpa</i>	P	Z9-16Al	261	Park JCEc 22:1201	
<i>assulta</i>		Z9-16Ac	21	1996	n 399
8 h in		Z11-16Al	614		
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<i>Helicoverpa assulta</i> 2 h after scotophase	P	Z9-16Al	57	Park JCEc 22:1201
		Z9-16Ac	4	1996 n 400
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<i>Heliothis maritima</i>	I	Z11-16Al		Kovalev KhPriSoy 4:574
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		Z11-16OH		
		16Al		
<i>Heliothis maritima</i>	P	Z11-16Al a	88.0	Szöcs EnExAp 66:247
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		16Al	4.8	
<i>Heliothis zea</i>	O	Z11-16Al	99	Halfhill StwEn 10:176
		Z9-16Al	1	1985 403
<i>Heliothis zea</i>	O	Z11-16Al	87	Lopez StwEn 15:1
		Z9-16Al	3	1985 404
		Z7-16Al	2	
		16Al	8	
<i>Pyrrhia umbra</i>	A	Z11-16Ac		Stan SoLpRomBul 10:10 1992 405

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<i>Alabama argillacea</i>	P	9Sme-19Hy a	10	Hall BIOILB 16(10):1
		Z6Z9-21Hy	0.5	1993 406
		Z6Z9-23Hy	1	
<i>Anomis texana</i>	P	7Sme-17Hy a	4	Hall BIOILB 16(10):1
		Z6Z9-23Hy	1	1993 407

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