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Pheromones and other Biological Techniques for Insect Control in Orchards and Vineyards

editors:

Peter Witzgall, Basilis Mazomenos &
Maria Konstantopoulou

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

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

Pheromones and Other Biological Techniques for Insect Control in Orchards and Vineyards

Proceedings of the Working Group Meeting

Samos, Greece
September 25-29, 2000

Edited by Peter Witzgall, Basilis Mazomenos and Maria Konstantopoulou

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Preface

Pheromones and plant volatiles are most promising tools to replace conventional insecticides, for sustainable control of herbivorous insects. Key insecticides are being deregulated all over, due to their detrimental environmental effects. This accelerates the evolution of insect resistance against the remaining compounds and further emphasizes that new methods need to be made available.

Current applications of sex pheromones for insect control are based on the pheromone identifications and first field studies done at academic institutions, during the seventies and eighties. This laid the ground for further work achieved by industries and extension organizations, including large-scale synthesis, economic dispenser materials and field implementation.

Much remains to be done. The issues are further improvement and implementation of pheromone-based control methods, development of methods aiming at ovipositing females and more reliable population monitoring. This concerns key species from the Northern hemisphere, as well as many new species from developing countries.

The biology and chemistry of behaviourally active compounds of plant and insect origin remains a fascinating research field. The question we pose today is whether the academic circuit or the industry will invest a sufficient effort to enable widespread practical use of semiochemicals for environmentally safe insect control.

This meeting was to celebrate the 25th Anniversary of our Working Group. The beauty of the island of Samos helped to make it a memorable event.

Alnarp, December 2002

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Regulatory issues in the commercial development of pheromones and other semiochemicals

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Abstract: The regulatory issues in the commercialization of semiochemicals are addressed in the light of political and economic factors which impact the criteria on which regulatory decisions are based. Other issues affecting international harmonization of registration requirements are also discussed.

Key words: pheromones, semiochemicals, regulation, registration, lepidoptera, pesticides.

Introduction

The successful development and commercialization of any type of pest control product involves various participants, collaborators and cooperators including scientists, regulators, marketers and of course the end-user or customer. Even when all of the participants are involved from earliest stages in the development process there are still many issues to be resolved; often the scientists do not fully appreciate the regulators and the marketers, the marketers can be at odds with the regulators and the scientists, and the end-users are often perplexed by what they perceive to be weaknesses and failures in the eventual product.
Consideration of this situation leads to the realization that in semiochemical commercialization there are opportunities and constraints; these are best illustrated in a table.
Of the three main contributing factors (Table 1) only the political and "business" factors will be discussed here as they pertain to the regulatory issues which include policies and economics.

Regulatory issues

In the U.S. the decision to approve a pest control product is based on the acceptability of data demonstrating a benefit to man and the environment while ensuring that the product use will not cause unreasonable effects on human health or the environment. In most other jurisdictions, approval is granted once the registrant has demonstrated that the product is safe to humans and the environment, and is efficacious. At the crux of the regulatory decision is the risk to human health and the environment, such risks are dependent on two parameters [a] inherent toxicity of the substance or product, and [b] the exposure to the substance or product.

Table 1. Semiochemical commercialization - opportunities and constraints

Factor	Opportunity	Constraint
Political	Environmental awareness	Lack of knowledge by policy makers, politicians, etc.
	Safer pesticide policies	*Regulatory policies*
	Mandated pesticide use reductions	Private sector/public sector cooperation
	Non-toxic mode of action	
	Private/public sector cooperation	
Technical	Target specificity	Target specificity
	Low application rates	Raw material sourcing
	Enhancement of biological control	User education
	User education	Lower efficacy
	Increasing pesticide resistance	Shorter product shelf life
	Creation of secondary pests	High product quality demands
	Compatibility with both biological and insecticide control strategies	
Business	*Regulatory policies*	Lower application rates
	Costs	Proprietary rights
	Marketing methods	Costs
		Market size
		Lack of economic incentives
		Lack of user confidence in the technology

Amongst the factors affecting the exposure to pest control products are global/individual usage (insecticide sales, currently registered active ingredients/products, properties of the active ingredient) and type of usage (in traps [releaser type, amount of active ingredient]; area-wide uses [formulation type, amount of substance]). A comparison of the insecticide sales in the years 1991, 1996 and 1997 is given in Table 2.

Table 2. Insecticide and semiochemical sales in 1991, 1996 and 1997

	1991	1996	1997
World insecticide		\$8.65 billion	\$9.1 billion
U.S.	\$1.20 billion	\$2.68 billion	\$3.6 billion
Western Europe		\$2.28 billion	
Rest of the world		\$3.69 billion	
Pheromones/semio-chemicals	\$7 - \$8 million[a]	\$19.8 million [a,b]	

[a] Estimated

[b] The \$19.8 million value is the estimated sales in 1999 for disruption products only

The figures for pheromones/semiochemicals are estimated for 1991 and 1996, the $19.8 million in 1999 is for disruption products only, sales for all semiochemical products is estimated at about $200 million. Note that U.S. insecticide sales increased three times in the six years and the increase in disruption product sales increased accordingly. The biopesticide market segment that contains pheromones is estimated to increase 10.6% between 1999 and 2004, finishing with a value of $208 million.
Another way of comparing exposure is to consider the number of registered products for several active ingredients; this was done for several U.S. EPA registered pesticides and pheromone products and is illustrated in Table 3.

Table 3. Number of U.S. EPA approved labels for various pesticide active ingredients

Material	Active ingredient	#Approved labels
Pesticide	Permethrin	730
	Lambdacyhalothrin	28
	Glyphosate	87
	Phosmet	17
	B.t. var.Kurstaki	74
Pheromones	Gossyplure	13
	Codlemone	14
	OFM pheromone	13
	Tomato pinworm pheromone	13
	Muscalure	12

From the data in Tables 2 and 3 it can be seen that the exposure to the pheromone active ingredients and products is very much smaller than to the other types of pest control agents.

A comparison of registered "biochemical" pest control agents in the U.S. and in the European Community is given in Table 4, and illustrates both a more advanced understanding of these types of product by the U.S. regulatory community and a greater cooperation between the public and private sectors.
As of February 1999 the pheromone/attractant authorizations in the European Community member states were 27 in Spain, 18 in Greece, 5 in France, 2 in Germany and Austria, and one each in Netherlands, Luxembourg, Poland and Italy. Note that the following member states have no pheromone/attractant approvals, Finland, Sweden, Denmark, Ireland, Belgium and the United Kingdom. The high number of approvals in Greece and Spain reflect the need to register monitoring in addition to area-wide disruption products.

A review of several semiochemical databases [including those of the Pherolist and Tohoku University] presents information that there are over 500 known pheromones and attractants for lepidopteran insects and that there are 193 semiochemicals belonging to 11 orders which begin with the letter 'D' (*eg.* from danaidal and (2,6-Z,E)-7-methyl-3-propyl-2,6-decadien-1-ol to (Z)-3-dodecenolide and dopamine). The number of arthropod pheromones and attractants whose structures are known is staggering but it must be noted that only an extremely small percentage of these materials will ever be considered for commercialization, and only a small percentage of those will be used in area wide control products, most being incorporated into monitoring and other trapping products.

Table 4. Registered "biochemical pest control agents" in the U.S. and European Community [active ingredients/products]

U.S. EPA	
Biochemical active ingredients	
Pheromones	[31/157]
Floral attractants/plant volatiles	[18/74]
Repellents	[18/64]
Miscellaneous (from plant and insect sources)	[6/11]
European Community	
Attractants [includes hydrolyzed protein]	[41]
Repellents	[8]

Another aspect of exposure relates to properties of the semiochemical such as ubiquity, non-semiochemical uses, stability, non-toxic mode of action, *etc.* For example geraniol occurs in over 250 essential oils in addition to having been isolated from 14 insect species from seven families in five orders. It is commonly used in the fragrance industry and as a flavoring for alcoholic and non-alcoholic beverages, ice cream, candies and baked good, *etc.* Annually, over 800,00 lbs are used in cosmetics, soaps and detergents. In the U.S. geraniol is classified by the Food and Drug Administration as being GRAS [Generally Regarded As Safe] and may be added directly to food for human consumption as a flavoring, adjuvant or additive. It is also well known that many of the lepidopteran pheromones, especially those containing multiple double bonds and/or other labile functional groups such as the aldehydic group are very susceptible to UV catalyzed oxidative degradation.

Table 5. Acute toxicity of several semiochemicals

Compound	Oral LD_{50}	Dermal LD_{50}	Inhalation LC_{50}
Acetate[a]	>5 - 34.6 g/kg [b] [10]	>2 - 20.25 g/kg [b] [9]	>3.3 - 32 mg/l [b] [5]
Alcohol[a]	>3 - >50 g/kg[b] [7]	>2 - 5 g/kg [b] [4]	5.26 mg/l [b] [1]
Aldehyde[a]	> 5 mg/kg [b] [3]	>2 - >5 g/kg [b] [3]	>5 - 16.8 mg/l [b][3]
Muscalure	> 5 mg/kg [b]	> 2 mg/kg [b]	>5.0 g/ m^3 [b]
Citronellol	3.45 g/kg	2.65 g/kg	
Geraniol	3.6 - 4.8 g/kg	>5 g/kg [b]	
Farnesol	>5 g/kg [b]	>2.01 g/kg [b]	0.917 mg/kg
Hexyl butyrate	>5g/kg [b]	>5g/kg [b]	
Linalool	2.79 g/kg	5.61 g/kg	

[a] denotes saturated and unsaturated compounds of 11 to 18 carbons chain length. The numbers in parenthesis after the values for these compounds represents the number of compound included in these data.

[b] denotes limit testing, the guidelines of the U.S. EPA for acute toxicity testing contain "principles which provide that if no mortality is produced by administration of a specified dose level, no further testing is required".

Consideration of the various ways in which pheromones are used, and of the formulations used leads to the conclusion that while area-wide use of semiochemical products would generally lead to a greater exposure than use in traps, such exposure is likely to be insignificant. In the U.S. the regulatory agency believes that with the majority of lepidopteran pheromones there is no evidence of risk when the use does not exceed 150 grams of active ingredient per acre per year. In reality, this level is rarely reached when pheromones are used commercially. It is of interest to note that no residues of lepidopteran pheromones have been detected on fruit [grapes, apples and peaches] from the use of pheromones on fruit from distinct point source formulations at application rates from 5.0 to 126.4 grams per acre. In the case of tomato pinworm pheromone, residues on unwashed fruit ranged from 21 - 72 ppm on the day of application, and 0.29 - 1.2 ppm on day 30 however washing the tomatoes at anytime brought the residues below the level of detection.

Focusing on the second component of the risk equation, namely inherent toxicity of the semiochemical, some acute toxicity data are reviewed in Tables 5 and 6.

Table 6. Toxicity of acetate and aldehyde lepidopteran pheromones to non-target avian, fish and aquatic invertebrate organisms

Toxicology Test	Acetate	Aldehyde
Avian acute oral [mallard]	LD_{50} >2 - >10 g/kg [3]	LD_{50} > 2 g/kg [1]
Avian acute oral [quail]	LD_{50} >2 - >2.25 g/k [2]	LD_{50} > 2 g/kg [1]
Fish toxicity [bluegill sunfish]	LC_{50} >100 - 540 ppm [4]	
Fish toxicity [rainbow trout]	LC_{50} >100 - 270 ppm [3]	LC_{50} 320 ppm [1]
Aquatic invertebrate toxicity [*Daphnia magna*]	LC_{50} 1.30 - 6.80 ppm [3]	LC_{50} 0.45 - 2.23 ppm [2]

For avian acute toxicity studies U.S. EPA guidelines state that "satisfactory data should establish that the avian single dose oral LD_{50} is greater than 2 g/kg". For fish and aquatic invertebrate toxicity, satisfactory data must establish an LC_{50} greater than 100 ppm or >100,000 times the maximum expected environmental concentration or estimated environmental concentration when the end-use product containing the active ingredient is used as directed.

Discussion

From the information presented above, the rational conclusions which may be drawn are: (1) the use of semiochemicals in pest management presents far less risk to humans, other mammals, non-target organisms and the environment than conventional chemical insecticides; (2) the burden of demonstrating that use is safe or will not cause unreasonable effects of health and the environment should be significantly less than for conventional chemical insecticides; (3) semiochemical products are capable of surviving in markets where both the size and profitability does not interest the major pesticide manufacturers. In this regard the need for, and the cost of expanded requirements is not likely to be offset by larger, global markets; (4) in the U.S., agriculture has benefitted from the evolution of a regulatory process which first recognized the differences between chemical pesticides and biopesticides

[including pheromones and semiochemicals], and then the responsible agency [U.S. EPA] worked in concert with stakeholders to develop a reasonable and reduced set of data requirements for the registration of such materials.

Amongst the countries which have adopted regulatory policies for semiochemical pest management products, the most progressive is in the U.S. The current status of semiochemical regulation is illustrated in Tables 7 and 8.

Table 7. Regulatory/Registration status of semiochemicals in U.S. based on formulation and use

Monitoring/Survey	Arthropod pheromones, attractants and minimum risk pesticides can be used and be *exempt* from registration
Mass trapping	Arthropod pheromones can be used for pest control and be *exempt* from registration
Kairomonal use	of a semiochemical in pest control strategies is *exempt*
Disruption	All pheromones, irrespective of formulation require to be registered, but when used on food and feed crops certain lepidopteran pheromones are *exempt* from the requirement of a tolerance
Attract & kill	Pheromones used in conjunction with an insecticide in a formulated product require to be *registered*, and although the pheromone may be exempt from tolerance for food and feed crop use the insecticide and inerts must either have a tolerance or an exemption from tolerance

Although the EPA has stated that at this time it does not intend to grant any further regulatory relief to pheromones and other semiochemicals it will [a] on a case by case basis consider any reasonable proposal an applicant/registrant wishes to make regarding registration requirements and work with the applicant/registrant, and [b] as the database of information on other types of pheromones and semiochemicals grows it will then consider further regulatory relief.

In order to facilitate development, registration and the use of semiochemicals in other areas of the world the OECD through its Working Group on Pesticides is committed to harmonizing the regulation of semiochemicals.

Table 8. Regulatory/Registration status of semiochemicals in the U.S. based on chemical type

Arthropod pheromones	Lepidopteran pheromones	Arthropod semicochemicals
Exempt - sole active ingredient when used in a trap for monitoring or control	Certain lepidopteran pheromones[a] are *exempt* from the requirement of a tolerance, irrespective of formulation provided the use rate does not exceed 150 g/ai/acre/year	Attractants, certain miscellaneous semiochemicals when used only to attract pests, and labeled accordingly are *exempt* from regulation under FIFRA[b] "not deemed to be used for pesticidal purposes"
Exempt - when acting as a kairomone for a beneficial arthropod	Certain lepidopteran pheromones[a] are *exempt* from the requirement of an experimental use permit for testing on acreage up to 100 hectares [250 acres]	Semiochemicals which are used to behaviorally manipulate beneficial arthropods *eg.* Honey bees are *exempt* from regulation under FIFRA[b]
Exempt - when used in "retrievably sized polymeric matrix dispensers" when applied to growing crops only at a rate not exceeding 150 g/ai/acre/year		
Registration - products used in area-wide pest control strategies		

[a] "certain lepidopteran pheromones" is an EPA designation for straight chain aliphatic (9 - 18 carbons) alcohols, aldehydes or acetates with up to three double bonds.

[b] FIFRA is the acronym for Federal Insecticide, Fungicide and Rodenticide Act.

As a first step OECD is attempting to establish a common core of data requirements for pheromones and other semiochemicals and most recently a PMRA sponsored workshop held in Ottawa in September 1999 made recommendations to the OECD Secretariat concerning core requirements. A review of the recommendations, which propose separate data requirements for straight chain lepidopteran pheromones and other semiochemicals, give the impression that there is a common core of data requirements with a high degree of commonality and there could be multi-countries data reviews with exchange of information and data. At the OECD Biopesticides Steering Group in February of this year, although the name of the Ottawa document was changed to "Guidance for registration requirements for pheromones and semiochemicals" there was general agreement regarding the content of the document. There was also agreement that no further work on the harmonization would take place for three years until the member countries gain experience in the registration process and worksharing.

At the same time that the Workshop was being held in Ottawa, the AgNet list server of the University of Guelph carried a statement on pesticides, by Dr. Bruce Ames, Professor of

Biochemistry & Molecular Biology, and Director of the National Institute of Environmental Health Sciences Center at the University of California at Berkeley, in which he said "Scares about tiny traces of synthetic chemicals, such as pesticides, are a distraction from important risks. The amount of pesticide residues ingested are so small, relative to levels that have been shown to have toxicological effects, they are toxicologically implausible as health risks".

On the other hand, there is the alarming trend of almost universal adoption of the Precautionary Principle in Europe in regard to the use of any new technology, process, chemical or any new activity whatsoever. The differences in these two philosophies illustrates why harmonization although desirable will be most difficult to achieve, proponents of the approach represented by the views of Dr Ames will continue to want semiochemicals regulated on presumption of negligible risk while the adherents of the Precautionary Principle will maintain that an examination of the full range of alternatives must be examined, including the alternative of doing nothing, and that the registrant must accept the "burden of proof of harmlessness".

It took seventeen years from the proof of the structural elucidation of the first pheromone [bombykol (1961)] to the granting of the first registration of a pheromone for broadcast use in crop protection [gossyplure (1978)], hopefully there will not be such a time lag in expediting the introduction of semiochemicals in to the global market-place through regulatory harmonization.

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We wish to thank the following persons for supplying data or helpful discussions, Jack Jenkins, Myles Stewart-Hesketh, Charles Descoins, Lise Lange, Charles Doane and Albert Minks.

Note added in proof

In late 2001 the Environment Directorate of the OECD published as No. 12 in their series on pesticides a document entitled Guidance for Registration Requirements for Pheromones and Other Semiochemicals Used for Arthropod Pest Control. this document may be downloaded from the OECD World Wide Web site at http://www.oecd.org/ehs

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Mating disruption

Use of pheromones and other semiochemicals in integrated production
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Mating disruption of codling moth: a perspective from the Western United States

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Abstract: An historical perspective is provided of pest management programs in western apple orchards using the Washington situation as an example. The adoption of mating disruption as a key pest management tactic in western pome fruit orchards has resulted from the availability of new technology, a changing regulatory environment, concerns about food safety, and large-scale demonstration projects. Organization of the Codling moth Areawide Management Project (CAMP) is discussed. Results from a specific case study of one CAMP site, Howard Flat, are discussed in detail with comparisons made to other CAMP sites. The future of pheromone-based pest management programs is discussed relative to their long-term stability, research needs, and continuing changes in the US pesticide regulatory environment.

Key words: mating disruption, codling moth, codling moth areawide management project

Historical perspective

History can be a great teacher. By understanding the past we may be better able to understand the present and anticipate the future. A brief history of Pacific Northwest apple pest control is presented in order to place in perspective recent changes in programs. This history provides the foundation on which the current program has been built and now becomes the new basis for assessing future change.

In the 1960s, a crisis was developing in Washington's apple production system. Reliance on chlorinated hydrocarbon insecticides for control of the region's key pest, the codling moth, *Cydia pomonella* L., resulted in increased problems with spider mites, specifically the McDaniel spider mite, *Tetranychus mcdanieli* McGregor, and European red mite, *Panonychus ulmi* (Koch). Specific miticides were employed to control spider mites, but resistance to these products developed rapidly. It was common in late summer for apple orchards to take on a brownish cast due to injury by spider mites, even after several miticides had been applied. Growers were facing a crisis and, as so often happens, this was the environment in which a radical shift in pest control could occur. Dr. Stan Hoyt (WSU-TFREC) observed that in some orchards where lower rates of organophosphate (OP) insecticides were used spider mites were less of a problem. His detailed research in integrated mite management culminated in what is still recognized as a breakthrough in pest management (Hoyt 1969). He showed that the western predatory mite, *Galandromus occidentalis* (Nesbitt), could tolerate low rates of certain OP insecticides and provide

adequate control of the key pest, codling moth. Growers rapidly adopted the principles of integrated mite management, and by the end of the 1960s most Washington growers had stopped applying specific miticides in apple orchards.

The apple pest management program was stable throughout the 1970s. Codling moth was controlled using below maximum label rates of OP insecticides, with an average of about two applications per hectare per year. Biological control suppressed spider mites below damaging levels in most orchards. Resistance to OP insecticides began to appear in some secondary pest insects such as the white apple leafhopper, *Typhlocyba pomaria* (McAtee), and apple aphid, *Aphis pomi* (De Geer); however, these pests could be controlled with selective insecticides at relatively low rates in a manner that did not disrupt biological control of spider mites.

In the 1980s, there was an erosion in stability of the apple pest management program. Leafrollers, *Pandemis pyrusana* Kearfott and *Choristoneura rosaceana* (Harris), appeared as serious problems in some orchards (Brunner 1984). The increased problems with leafrollers were tied to reduced efficacy of certain OP insecticides, especially chlorpyrifos (Brunner 1991). A new pest appeared, the western tentiform leafminer (WTLM), *Phyllonorycter elmaella* Doganlar & Mutuura. The increase in pest status of the leafminer was thought to be associated with the development of resistance to OP and most carbamate insecticides. The only effective insecticide against WTLM was oxamyl, a carbamate insecticide that was highly toxic to the western predatory mite. Thus, the WTLM problem caused an erosion in integrated mite management programs in some orchards. Some stability was returned to the apple pest management system when research showed that a small parasitoid, *Pnigalio flavipes* (Ashmead), was an effective biological control of WTLM (Barrett and Brunner 1990). Codling moth control using OP insecticides was still effective; however, by the end of the 1980s the average number of insecticide applications for this pest had risen to almost three per year (Table 1). There was interest in introducing synthetic pyrethroids into the apple pest management system during the 1980s, but recognition of the detrimental impact on integrated mite management (Croft et al. 1987), and pest management in general, resulted in growers rejecting use of these products for pest control.

In the early 1990s, growers were facing increasing difficulties controlling codling moth, and resistance to certain OP insecticides, especially azinphosmethyl, was reported (Dunley and Welter 2000, Knight et al. 1994, Varela et al. 1993). In Washington, the increased problem controlling codling moth were reflected in the gradual increase in the average number of applications per hectare per year (Table 1). Leafrollers problems occurred in more orchards (Brunner 1994b), and growers were seeking ways to control this pest without disrupting biological control of other pests (Brunner 1994a), especially spider mites and WTLM. The concern about the impact of agricultural chemicals on the environment and residues on food fueled public debate and scientific inquiry (NAS 1993). Research on mating disruption as a viable alternative for controlling pests in fruit crops was stimulated by success against the oriental fruit moth, *Grapholitha molesta* (Busck), (Rothschild 1975, Weakley et al. 1987) and promising evaluations against the codling moth (Knight 1996, Gut and Brunner 1998).

Table 1. Average number of insecticides applied per acre and the percent of acres treated at least one time to apple in Washington from 1989 to 1999.

Insecticide treatment	Average number of applications per acre (% total acres treated)					
	1989[a]	1991[b]	1993[b]	1995[b]	1997[b]	1999[b]
azinphosmethyl	2.9 (98)	2.8 (90)	3.3 (81)	3.3 (94)	2.9 (91)	2.3 (78)
phosmet	2.4 (4)	2.1 (9)	1.1 (19)	2.4 (2)	1.2 (<1)	2.0 (7)
methyl parathion	1.1 (17)	1.5 (28)	1.2 (24)	1.2 (19)	2.0 (33)	1.1 (5)
ethyl parathion	1.2 (42)	1.0 (32)	1.0 (8)	nr [c]	nr	nr
chlorpyrifos	1.3 (56)	1.4 (65)	1.3 (85)	1.3 (80)	1.4 (91)	1.3 (78)
Bacillus thuringiensis	-	-	1.9 (24)	2.2 (21)	1.5 (26)	2.0 (19)
esfenvalerate	-	-	-	-	-	-
spinosad	nr	Nr	nr	nr	nr	1.4 (39)

a Survey of pesticide use in Washington – (Beers and Brunner 1991)
b NASS (National Agriculture Statistics Service) survey of pesticide use in tree fruit.
c not registered

Changing regulatory environment

The Food Quality Protection Act of 1996 (FQPA) set the stage for radical change in pest management programs in US agriculture. This law established a new standard for determining risk of a pesticide, that there would be "a reasonable certainty that no harm will result from aggregate exposure." This law required all pesticides to be evaluated within 10 years starting with those deemed of greatest risk to humans, i.e. the OP insecticides. The result of FQPA has been elimination or restriction in the use of most broad-spectrum pesticides, especially on crops that are important foods for infants and children. Apple, pear and peach are all important foods for infants and children in the United States. While there remains a great deal of uncertainty about the final effect of FQPA on tree fruit production, it is not too difficult to realize that yesterday's pest management programs will look much different than tomorrow's.

Codling moth mating disruption

Codling moth is the "key" pest in western apple and pear orchards. It is primarily controlled by summer applications of three to four broad-spectrum insecticides per year (Beers and Brunner 1991, NASS 1994, 1998, 2000). Insecticides used to control codling moth constitute over half of all insecticides applied to apple during the summer months. Toxicity of these broad-spectrum insecticides to most natural enemies has severely limited opportunities for biological control of many pests in western apple orchards.

The use of sex pheromones has been investigated as a selective control for codling moth since the early 1980s, but it was not until the early 1990s that reliable commercial products were available to growers in the United States. Initial studies demonstrated the potential of using pheromones to control codling moth (Knight 1996, Gut and Brunner 1998), but they also pointed to areas of concern. For example, under conditions of high codling moth

densities, mating disruption was not an adequate "stand-alone" tactic. In addition, orchard borders had consistently higher levels of fruit injury than orchard interiors, and leafrollers increased as a problem where mating disruption was used for codling moth and broad-spectrum insecticide use was reduced. Even with these limitations, growers began adopting codling moth mating disruption (CM-MD), and by 1994 an estimated 4,800 hectares in Washington were being treated (Figure 1).

Codling moth areawide management

In 1994 a proposal was submitted to federal agencies for funding of a Codling Moth Areawide Management Project (CAMP). The goals of CAMP were: (1) to control codling moth using mating disruption as the primary tactic over large contiguous areas; (2) to reduce the use of broad-spectrum insecticides by 80% over five years; (3) to assess changes in pests and natural enemies in "areawide" sites over time; and (4) to enhance the biological control of secondary pests.

CAMP was funded by a special allocation from the Agricultural Research Service of the United States Department of Agriculture (USDA-ARS) for the implementation of an areawide pheromone-based codling moth management program in the western United States. The project involved scientists at the USDA-ARS laboratory in Wapato, Washington; Washington State University; Oregon State University; and the University of California, Berkeley. Five CAMP sites were selected, three in Washington and one each in Oregon and California. Each CAMP site had its own unique character representing different combinations of fruit crops, farming practices, codling moth populations, and resistance levels, and ecological settings (Calkins 1998).

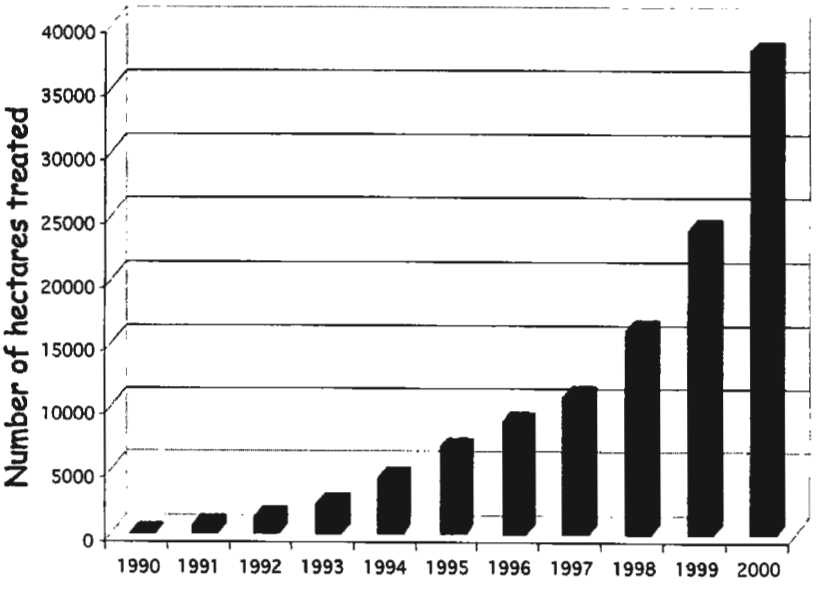

Figure 1. Area treated with mating disruption products for management of codling moth in Washington State from 1990 to 2000.

Pear was the predominant crop grown at the two CAMP sites in Oregon and California. The Randall Island CAMP site (300 hectares) in California had actually been initiated in 1993 in response to high levels of codling moth resistance to OP insecticides. In this crisis situation, CM-MD was seen as an alternative that could be used to reduce the number of insecticide sprays and as a possible resistance management tactic. The Carpenter Hill CAMP site located in southern Oregon near Medford (180 hectares) used CM-MD to reduce broad-spectrum insecticide use in summer as a strategy to encourage biological control of another serious pest, pear psylla, *Cacopsylla pyricola* (Foerster).

The three CAMP sites in Washington represented a mix of orchard production practices found throughout the state. The Parker Heights CAMP site near Yakima was a mixture of apple, pear and stone fruits (160 hectares). The Lake Osoyoos CAMP site was located in the Okanogan valley and bordered Canada. This site was primarily apple (160 hectares) and used a novel approach of combining CM-MD, reduced insecticide applications and release of sterilized codling moth. Sterile codling moths were provided by a Canadian program as part of a cooperative effort to seek new technologies for pest control in orchards. The Howard Flat CAMP site was primarily an apple production area (440 hectares) located near Chelan.

To encourage participation in CAMP and to reduce the economic risk to growers of using CM-MD, CAMP provided $125/hectare ($50/acre) to growers as a means of subsidizing the cost of the pheromone that at full rate was $275/hectare ($110/acre). This subsidy was only provided for the first three years of the project, and in the last two years growers paid all the pheromone costs. Growers at most sites decided to use the Isomate-C mating disruption product (Pacific Biocontrol) but at some sites, at least in the initial years, growers also used the CheckMate-CM product (Consep, Inc.).

Standard protocols were adopted for monitoring codling moth and other pests across all CAMP sites as a means of comparing project results. Codling moth were monitored using "high-load" (10 mg codlemone) lures changed every three weeks in the spring and every two weeks in the summer. Wing-type traps were used and these were placed in the upper 1/3 of the canopy or just below the placement of the pheromone. In addition, orchards outside the CAMP sites within each region were monitored for pest and natural enemy populations and crop damage using the same methods employed in CAMP sites. These orchards used conventional methods of pest control and provided a means of contrasting results with CAMP sites.

Howard Flat CAMP site - a case study

A detailed discussion of the Howard Flat CAMP site provides an insight into the experiences encountered in CAMP. Thirty-six growers produced fruit at the Howard Flat site, and in various ways these growers received guidance from 16 crop consultants in the production of their crop. Growers and crop consultants met to elect a Management Board consisting of three growers and five crop consultants. The Management Board hired a Project Coordinator who was responsible for the intensive monitoring of codling moth and leafrollers. Crop consultants or growers were responsible for monitoring other pests. Information collected by the Project Coordinator was shared weekly with all participants through a newsletter and by posting the data on a bulletin board located within the project boundaries. Decisions to apply pest controls were the responsibility of the grower based on recommendations of crop consultants. Scientists associated with CAMP provided technical support to the Howard Flat Management Board, assisting them in making decisions and setting direction for the project.

Data from 1994 on pesticide use, codling moth damage and moth captures in pheromone traps were obtained as a baseline for the Howard Flat CAMP site. An average of 2.9

insecticides per hectare was applied for codling moth control in 1994 with an average crop loss of 0.8%. In the spring of 1995, CM-MD products were applied to orchards of 34 growers at Howard Flat. Two growers had decided not to participate in the project, underscoring the point that participation was totally voluntary. In the first three weeks of codling moth flight over 3,000 moths were captured in 450 traps. However, following application of the first insecticide, moth captures declined to low levels where they remained the remainder of the summer. In the second codling moth flight, pheromone trap capture was 80% lower compared to the first flight. Fruit damage at harvest averaged 0.55% for the entire project, and the average number of control sprays applied for codling moth declined slightly more than one per hectare, from 2.9 (1994) to 1.7. Growers and crop consultants considered the first year of CAMP a success. Damage by codling moth had been reduced in most orchards and no serious problems with secondary pests were observed. One large grower who decided not to join CAMP in 1995 applied four insecticides against codling moth and still suffered more than 2.5% damage. He decided to join CAMP in 1996 after seeing the results his neighbors had achieved.

In 1996, the average number of codling moths captured in pheromone traps over the entire season was 83% lower than in 1995, 1.5 moths/trap versus 8.8 moths/trap. Growers responded to the reduced moth captures by reducing the number of supplemental insecticide applications. An average of only 1.1applications were made for codling moth control in 1996, and fruit damage at harvest was only 0.2%. Most (80%) of the damage experienced by growers in Howard Flat occurred in only five blocks of fruit surrounding the one grower who did not participate in CAMP both years. Unfortunately, this grower did not follow good pest control practices, and codling moth damage in his orchard (3 hectares) was so high (estimated to be at least 25% damage) that it could not be harvested for fresh market. This experience demonstrates the vulnerability of an areawide CM-MD management project to growers who do not follow good pest control practices. Growers at Howard Flat solved their own problem when they convinced the one grower not participating in CAMP to lease his orchard to another CAMP participant, and in 1997 the orchard was not a contamination source for neighboring growers.

From 1997 through 1999, codling moth captures in pheromone traps at Howard Flat remained low, with 80-90% of the traps catching no moths the entire season. The average number of insecticides applied to control codling moth continued to decline from 0.7 (1997) to less than 0.5 (1999) applications per hectare, and crop damage at harvest ranged from 0.01 to 0.03%. As growers at Howard Flat saw codling moth pressure decline, they reduced the number of mating disruption dispensers from 1000 (Isomate-C plus) to an average of 560 per hectare.

Other CAMP sites

A story similar to Howard Flat could be told for each of the other CAMP sites. At all CAMP sites codling moth populations were reduced, and damage associated with codling moth declined over the duration of the project. In years four and five of CAMP, fruit injury levels were 60 to 97% lower than in the first year of the project at all but one site. At the Parker CAMP site codling moth injury in the fourth year was equal to damage in the first year of the project (0.18%) and was substantially higher in the final year (0.68%) of the project compared to the first. The increased fruit injury by codling moth at Parker was most likely the result of a too aggressive reduction in number of dispensers per hectare, 1000 to 500, coupled with a reduction in the number of supplemental insecticide sprays, an average of 1.0 to 0.2 per hectare. Although the CAMP project did not reach the 80% reduction target for broad-

spectrum insecticides three of the five sites showed a significant reduction over time. However, by the end of the project CAMP sites were applying about 75% fewer broad-spectrum insecticides relative to the comparison orchards or to other conventionally managed orchards.

Other pests and natural enemies

During the five years of CAMP no consistent or unexpected pest problems arose in apple orchards. Leafrollers were present in most CAMP sites after year one, but this was anticipated and growers employed control programs that maintained them at acceptable levels without disrupting biological controls for other pests. Actually, leafroller densities were consistently higher in comparison orchards than in CAMP site orchards, and fruit damage was about two fold higher. Damage from true bugs, Pentatomidae or Miridae, tended to be slightly higher in CAMP site orchards than in comparison orchards although in the final year of the project this trend was reversed. There were no outbreaks of secondary pests due to reductions in broad-spectrum insecticide use in CAMP site orchards.

Populations of some secondary pests were lower in CAMP site orchards than in comparison orchards, and this was associated with higher densities of the natural enemies of these pests. For example, densities of the western tentiform leafminer were consistently higher in comparison orchards and parasitism, at least in the second generation, lower than in the CAMP site orchards over the duration of the project. Spider mites were not a serious problem in CAMP site or comparison orchards, but their densities were consistently higher and predatory mite densities consistently lower in the latter. Densities of white apple leafhopper nymphs were similar in CAMP site orchards and comparison orchards, but parasitism of overwintering leafhopper eggs was consistently higher in the CAMP site orchards. Aphid densities, primarily *Aphis pomi*, were slightly higher in CAMP site than in comparison orchards; however, the density of general predators associated with the aphids was similar in these orchards (Beers et al. 1999).

The key secondary pest in pear orchards was the pear psylla. In CAMP site orchards pear psylla densities and fruit injury were substantially lower than in comparison orchards in most years of the study. Spider mites were consistently higher in comparison than in CAMP site orchards, but predatory mite densities were low in both. Leafrollers, which are not considered a serious problem in pear, caused higher fruit injury in CAMP site orchards than comparison orchards.

Adoption of mating disruption

The adoption of CM-MD was influenced strongly by CAMP in two ways. First, CAMP sites offered demonstration and educational opportunities for growers, helping them to understand and consider implementing CM-MD as an integral part of their orchard management program. Second, the CAMP supported the establishment of new areawide projects with one-year grants at several locations from 1997 through 1999. In Washington 1995, approximately 8,800 hectares of pome fruit were treated with mating disruption products and the three CAMP sites accounted for less than 10% of this acreage (Figure 1). The In 1999, there were 24,000 hectares of orchards treated in Washington, or about 30% of apple and pear acreage. In 2000, the area treated with CM-MD continued to increase to an estimated 38,000 hectares (Figure 1).

Pheromone-based pest management: what is the future?

It seems likely that apple and pear growers will continue to seize the opportunity to move towards a pheromone-based pest management system in their orchards. Factors that will continue to promote the adoption of mating disruption include long-term benefits associated with increased biological control of secondary pests, threats of losing traditional broad-spectrum pesticides because of FQPA implementation, and continued concern about safe food by the public. Factors that are most likely to slow adoption of mating disruption include low prices growers are receiving for apples and pears, the relatively high cost of mating disruption and a perception of higher risk of crop loss compared to a conventional pest control approach.

While CAMP was a highly successful project it exposed weakness in the pheromone-based pest management approach that must be dealt with to promote long-term stability. Pheromone-based monitoring systems are not optimal for use in pheromone treated orchards. Non-pheromone monitoring systems, especially for Lepidoptera, would provide a better means of estimating pest densities in mating disruption treated orchards and reduce the incidence of unanticipated crop loss. Use of OP insecticides as supplemental controls for CM-MD limits the full expression of biological controls in many orchards. Adopting the use of more selective insecticides to supplement control of codling moth, as well as for other pests such as leafrollers, in CM-MD orchards would enhance biological control of many secondary pests. However, a more complete understanding of the impact of new insecticides on biological control agents is required.

Federal dollars have recently been provided through two grants supporting projects that address many of the issues discussed above. These grants fall under the umbrella title of "Building a multi-tactic pheromone-based pest management system in western orchards" and keeps together the team of scientists that had been involved in CAMP.

Objectives of these projects are to: (1) promote the adoption of CM-MD through development of new pheromone delivery technology, mating disruption approaches for other Lepidoptera, non-pheromone monitoring systems, and feeding stimulants or baits to enhance selective insecticides; (2) double the impact of biological control agents in orchards through the use of selective control tactics; (3) stabilize the management of specific pest populations through manipulation of orchard ecosystems, including groundcovers and surrounding habitats; (4) create an integrated educational plan to support the implementation and sustainability of a pheromone-based IPM system for western orchards.

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Use of pheromones and other semiochemicals in integrated production
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Use of mating disruption in cotton in North and South America

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Abstract: The pink bollworm, *Pectinophora gossypiella* (Saunders), is one of the most serious pests of cotton worldwide causing losses in both yield and quality. Control with conventional insecticides is difficult because the pest is well protected within cotton squares and bolls. Furthermore insecticide costs are high and applications of broad-spectrum materials have contributed to outbreaks of secondary pests. Mating disruption has provided a viable alternative for pink bollworm control. Development of mating disruption for the pink bollworm began 25 years ago and the first EPA registration of a pheromone formulation for mating disruption was issued for the pink bollworm in 1978. Since that time many formulations have been developed and commercialized. These materials represent various use methods and modes-of-action. The effectiveness of these formulations has been demonstrated in several areawide programs. These programs integrate IPM techniques conducive to mating disruption and avoid some of the factors detrimental to the technique.

Key words: pink bollworm, *Pectinophora gossypiella*, mating disruption, pheromone, gossyplure

Cotton pest pheromones

Insect pheromones have been identified for most of the important cotton pests in North America and commercial synthetic formulations have been developed for management of many of these pests either for monitoring or control. Economic thresholds have been established for trap and pheromone lure systems for some pests; however, except for the boll weevil (*Anthonomus grandis*) and the pink bollworm (*Pectinophora gossypiella* Saunders) trapping systems are not widely used for monitoring cotton pests chiefly due to expense and labor.

Sex pheromone formulations have also been developed for the direct control of several potential cotton pests either by classical mating disruption, attracticide or bioirritation. In the 1980's pheromone systems were developed in the USA for management of Tetranychus mites, Heliothis/Helicoverpa and the boll weevil. These products did not survive due to low efficacy and availability of cheaper insecticide alternatives. Currently the omnivorous leafroller (*Platynota stultana*), an occasional pest of cotton has several mating disruption formulations registered in the USA but not for use in cotton. A hand-applied formulation is now registered with the US-EPA for mating disruption of the beet armyworm (*Spodoptera exigua*) in cotton but because this pest is sporadic and difficult to predict in cotton, control by mating disruption will be limited.

Pink bollworm mating disruption

The most successful development of mating disruption for cotton pests has been with the pink bollworm (PBW). Part of this success is a result of the pest's biology. PBW has only one important host – cotton, it is widely spread throughout the world, and where it occurs it is generally a serious problem. Yield reductions of 30% or higher can result from PBW

feeding on seeds. It is well protected from traditional chemotherapy. Insecticides applications aimed at adult moths can be expensive and difficult to time. The pheromone of PBW, gossyplure, is relatively cheap to produce and stable in the environment (2). It is commercially available from a number of sources.

In 1978, the first mating-disruption product was registered with the US-EPA for PBW control (3). Development of pheromone delivery systems and application technology progressed as the use of mating disruption increased. The first innovation in mating disruption of PBW came early in 1980 when observations led to the attracticide technique for this pest. Male moths may approach and contact synthetic pheromone dispensers (4). Furthermore, male encounters with pheromone dispensers could be quantified by looking at moth scales associated with point sources. Studies showed small amounts of insecticide incorporated into the pheromone dispenser had no negative impact on beneficial insects (5) but could result in a more robust system than conventional mating disruption (6). Several commercial formulations adopted this approach.

PBW mating disruption formulations can be divided into several categories based on dispenser type and application technique (Table 1): (1) Reservoir, high rate systems that must be hand applied; (2) female*23*systems; (3) female equivalent, low rate hand-applied systems; (3) microdispersible, low rate systems that are sprayable.

In the high rate, reservoir systems the number of dispensers per hectare is relatively low (250-1000). The advantage is long field life. Generally only a single application is needed and gaps between applications are eliminated. The disadvantage is labor cost for hand application - a negative in some markets such as the USA but an advantage in others where cheap labor is abundant.

The second and third groupings are female equivalents, low rate systems that are either sprayable with specialized equipment or hand applied. Dispenser numbers vary between hundreds and thousands per hectare. Longevity is short: 7 - 28 days depending on temperatures. Mode-of-action is either mating disruption or attracticide. Most Female Equivalent formulations can be used as attracticides and some actually have insecticides premixed into the formulation.

The last category is microdispersible, low rate, sprayables. These formulations have the advantage of application via conventional equipment. Many can be tank mixed with insecticides and applied simultaneously in attempt to achieve bioirritation. Most of these materials are shorter-lived and rain wash-off is a concern. There are tens-of thousands of points sources or, in the case of the microcaps, a fog rather than a distinct point site.

Table 1. Commercial PPW mating disruption formulations

Reservoir, high-rate, hand-applied

Producer	Product Trade Name	Dispenser Type	Points per ha	Field Life (days)	Mode of Action*
Shin-Etsu	PB-ROPE L®	Plastic tube	250 - 500	90	MD
Biosys	Frustrate Band®	Plastic band	250	90	MD
Scentry	NoMate PBW Spiral®	Plastictube	500-1000	60	MD

Female equivalent, low rate

Producer	Product Trade Name	Dispenser Type	Points per ha	Field Life (days)	Mode of Action*
Sprayable (special equipment)					
Scentry	NoMate PBW Fiber®	Hollow Fiber	5000-12,500	7-21	MD, A&K
Hercon	Disrupt PBW®	Laminate Flake	12,000-32,000	7-21	MD, A&K
Hand-applied					
Troy	Last Flight®	Liquid polymer	750	28	MD, A&K
Novarits	Last Call®	Viscous paste	5,500	28	MD, A&K

Microdispersible, low rate, sprayable

Producer	Product Trade Name	Dispenser Type	Points per ha	Field Life (days)	Mode of Action*
ICI	Pectone®	microcap	fog	10-30	MD, BI
Fermone	Stirrup-PBW®	Na	fog	na	MD, BI
Agrisense	Decoy Beads®	macrocap	fog	10-28	MD, BI
Scentry	NoMate PBW MEC®	microcap	fog	7-21	MD, BI
Consep	Checkmate PBW®	macrocap	50,000	14-28	MD, BI
Consep	Checkmate PBW-F	microcap	fog	7-21	MD, BI

* MD – classical mating disruption (false trail following, camouflage, habituation, etc.)
* A&K – attract and Kill (attracticide)
* BI – bioirritation

PBW areawide management

PBW has been used in several areawide programs in North and South America (1, 7, 8, 9). One of the more successful examples of PBW mating disruption was the Parker Valley Program (10). This effort first began as a boll weevil eradication program and grew to include PBW and whitefly management on as many as 700 fields and more than 10,000 hectares. This

areawide program was supervised by the Arizona Cotton Research and Protection Council and incorporated many of the factors that contribute to the efficacy of mating disruption: early application (at pinsquare), economic thresholds (1 moth/trap/day), careful monitoring (weekly readings, 1 trap/ha), reduction of immigration from outside sources. This program had excellent results through 5 years with a gradual reduction in control costs (Table 2). However, in 1996, PBW infestations increased significantly, as a result of mild winter and because a late harvest of neighboring wheat fields left a source of PBW infestation that grew undetected until midseason. The result was higher than normal infestation and increase control cost (11). This unfortunately led to the demise of the project in 1997 as growers switched to Bt-cotton.

Table 2. PBW infestation and control cost -Parker Valley Project, AZ

Year	Bolls Inspected	infested (%)	Cost per ha ($ USD)
1989	26,879	23.35	-
1990	34,726	9.91	107.50
1991	35,477	1.42	107.50
1992	30,064	0.86	137.50
1993	25,200	0.00	56.25
1994	16,109	0.02	72.00
1995	16,520	0.38	81.90
1996	45,597	2.63	127.28

Conclusion

Management of PBW by mating disruption has been one of the most successful examples of pheromone use in the USA, and in the world. Since the first commercial formulation entered the marketplace more than 20 years ago, millions of hectares of cotton have been treated with several distinct formulations. However, the use of this technique for PBW control began to decrease in the mid-90's as cotton acreage decreased in the desert southwest of North America and the use of transgenic Bt-cotton increased. An estimated 21,300 hectares of cotton were treated PBW mating disruption products during 1999, down from 46,800 in 1997 (Table 3).

Table 3. Estimated area treated with PBW mating disruption (ha)

Country	1997	1998	1999
USA	41,600	20,500	18,000
Mexico	5,200	4000	3,200
Total	46,800	24,500	21,200

Mating disruption has not been widely adopted in cotton in South America. Poor economies and decreasing cotton acres have limited market development. Currently only Peru has significant use of mating disruption with approximately 5,000 hectares treated for PBW. Still, this represents only approximately 10% of the total cotton acres.

Bt-cotton is very effective in controlling PBW. It is easier to use and cost competitive.

Approximately 20% of cotton in Arizona was transgenic in 1996, 50% in 1997, 60% in 1998, and 42% in 1999 (L. Antilla, personal communication). Mating disruption has been relegated to decreasing areas of non-Bt cotton such as Pima cotton or to refugia zones.

There are now several powerful weapons to combat the PBW including transgenic cotton, mating disruption and sterile moth technique. Utilization of these techniques can provide a strong, diversified integrated management program for PBW. The USDA and CDFA tested this approach in the Imperial Valley of California during the late 1990's with good results. A larger program is now planned for the El Paso/trans-Pecos area of Texas. If successful, this program could spread to New Mexico, Arizona and the Mexican states of Chihuahua and Baja California Norte. The goal is eradication of PBW from the desert southwest.

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Area-wide mating disruption for improved control of Oriental fruit moth *Grapholita molesta* in Victoria, Australia

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Abstract: Area-wide mating disruption had been applied to improve the protection of orchards against migration, edge damage and outbreaks of oriental fruit moth. Experiment started in the 1997-98 season, when the area over 800 hectares of 18 orchards in northern Victoria was treated with mating disruption and continued in the 1998-99 season in the extended area over 1,100 hectares of 40 orchards in the same region. Mating disruption dispensers were applied to all fruit trees including not only peaches and nectarines treated before, but also pears, apples, apricots and plums where growers did not apply mating disruption normally. Detailed monitoring of oriental fruit moth population and regular shoot tip and fruit damage assessments were conducted. The results indicated that application of area-wide mating disruption during 2 consecutive seasons demonstrated an outstanding success in control of oriental fruit moth. The area-wide mating disruption application in the first year helped growers to reduce the number of insecticide sprays by half and in the second year, most of the growers did not spray against oriental fruit moth at all.

Key words: area-wide mating disruption, *Grapholita molesta,* oriental fruit moth, sex pheromone, stone and pome fruit orchard.

Introduction

Oriental fruit moth *Grapholita molesta* Busck, (Lepidoptera: Tortricidae) (OFM) is one of the most important pests of commercial orchards in the northern Victoria, Australia. OFM is able to severe damage, not only peaches and nectarines, but also pears, apples, apricots and plums.

Identification of the OFM female sex pheromone structure (Carde et al., 1979) has made new methods for control of this pest possible. The method based on the release of large amounts of sex pheromone, restricting the ability of males to locate virgin females, was called mating disruption (MD) (Rothschild, 1975). Rothschild (1979) also demonstrated that MD treatments could be as effective in controlling OFM as insecticides. Later research (Vickers et al., 1985) suggested that MD may become even more effective, when all orchards in a district are treated, so as to reduce the likelihood of mated OFM females migrating from untreated areas.

First area-wide MD treatment for OFM control was initiated during 1991-92 in the Tulbagh Valley in South Africa (Barnes and Blomefield, 1997). MD treatment in this experiment was only applied as five-row borders in adjacent fruit around 1200 hectares (ha) of peaches and nectarines previously treated with MD. This project had an outstanding success after two seasons, but later when MD applications were stopped, OFM population quickly increased and started to cause severe damage again. This experiment suggested that the success of area-wide MD treatment depended on effective management of borders of MD treated orchards and blocks.

Mating disruption is now a corner stone of Integrated Pest Management in Australian orchards. OFM has been successfully controlled by MD for many years in Victoria, but recently some farmers reported that shoot tip and fruit damage has occurred on the border of MD peach blocks adjacent to fruit blocks under insecticide treatments (Sexton and Il'ichev, 2000). Studies of OFM movement have indicated that most adults do not disperse over distances greater than 200 m, although a few individuals may cover distances exceeding 1 km (Rothschild and Vickers, 1991). Later observations (Il'ichev et al., 1998) indicated that migration of mated OFM females from pear blocks under insecticide treatment to adjacent peach MD blocks resulted in damage at the edge of the peach blocks.

Our experiment with area-wide MD treatment in Victoria (Il'ichev et al., 1999a) aimed to demonstrate that the OFM population would be reduced with the use of MD on the whole orchards in the designated area. The expectation was that this approach would be more reliable and cost effective than combination of MD and insecticide treatments. The significant reduction of insecticide use will be an environmental benefit from this project. This paper reports the initial results of the area-wide MD experiment in northern Victoria, Australia.

Materials and methods

Description of the study area and monitoring technique.

The orchard area located on south of Cobram, northern Victoria, was chosen to conduct area-wide MD experiment because most of the growers have had experience in managing MD. The most severe OFM damage in this area was typically found at the edge of peach blocks under MD, adjacent to pear blocks under insecticide treatments. This pattern of damage is known as an 'edge effect'. When the severe damage spread around MD treated stone fruit blocks to adjacent pome fruits and the outbreak of higher level of OFM population was recorded in the entire area, such area was called "hot spot".

Our 1996-99 experiments investigated whether applying MD to all orchards in an area-wide basis would improve the effectiveness of MD in the OFM "hot spots" and "edge effects" area.

Food traps were used to monitor the population of both male and female of OFM in fruit blocks under MD. These food traps are not specific to OFM, but capable to indicate the level of OFM population. Each trap (Efecto-fly trap, Avond Pty.Ltd., Western Australia) was filled with 1 litre of 10% brown sugar solution and 12 drops of terpinyl acetate solution (48.5 mL of terpinyl acetate with 1.5 mL of non-ionic wetting agent and 50 mL of warm water). The food traps were monitored weekly by collecting moths and changing the sugar and terpinyl acetate solutions.

To monitor the whole area-wide MD experiment at least one food trap was placed in all blocks of each fruit variety inside each orchard that was part of the area-wide MD. Additional food traps were placed into blocks larger than 3 ha and overall the average trap density was one trap per 4 ha.

The identified hot spot in property 2 was monitored with 21 food traps distributed in 3 lines of 7 traps through the interface of pears and peaches. The hot spot in property 1 was much bigger than in property 2 and had additional set of 21 food traps to cover whole area infested. There was a distance of 5 trees between traps. The weekly monitoring of area-wide MD experiment and hot spots each season usually started in August, before the first flight of OFM after winter and was continued until two weeks after the last OFM flight finished in April.

Application of the area-wide MD treatment and monitoring the OFM population (1996 - 97 season).

This season, before the start of area-wide MD experiment, approximately 550 ha of separated peach and nectarine blocks were treated with MD. The Cobram growers treated such stone fruit blocks inside of the orchards with 4 dispensers of 'Isomate OFM Plus' (Shin-Etsu Chemical Co. Ltd., Japan for Biocontrol Ltd., Australia) per tree or 1000 dispensers per ha. 'Isomate OFM Plus' is a controlled release formulation of OFM sex pheromone that contains Z-8-dodecenyl acetate (130.3 mg/dispenser), E-8-dodecenyl acetate (8.4 mg/dispenser) and Z-8-dodecenol (1.3 mg/dispenser). The blocks of pears, apples, plums and apricots in these orchards were under the insecticide treatment with spray application of parathion-methyl and/or azinphos-methyl. MD in stone fruits was usually applied before the end of September and insecticide treatments were applied about 7-14 times during the season. Food traps were placed in the stone fruits under MD. The monitoring data was used to identify the initial level of OFM in properties with edge damage effect and hot spots before the area-wide MD application. Such preliminary information was very important in the future experimental planning and design process.

1997 - 98 season.

The area-wide MD experiment was established in September-October, in over 800 ha on 18 orchards to the south of Cobram region. The area included 550 ha of peaches and nectarines, which had been treated with MD in previous season. The balance of 250 ha under area-wide MD included pears, apples, plums and apricots that had not been treated with MD previously. Area-wide MD was achieved by treating every tree in the entire experimental area with OFM pheromone at the recommended rate of 4 dispensers of 'Isomate OFM Plus' per tree or 1000 dispensers per ha. The whole area was considered to be extensive enough to ensure that, within it, any edge effects and OFM migration problems in hot spots would be greatly reduced.

More than 230 food traps were placed in the area for monitoring. Three sites without MD treatment were designated as control. Two were separate orchards, adjacent to the north and south borders of the area-wide MD experiment and one was a block of pears left under insecticide treatment opposite to peaches under MD within the area-wide MD.

1998 - 99 season.

The area-wide MD experiment was continued over a larger area of more than 1,100 ha on 40 orchards. All fruit trees were treated with 'Isomate OFM Rosso' (Shin-Etsu Chemical Co. Ltd., Japan for Biocontrol Ltd., Australia) at the recommended rate of 2 dispensers per tree or 500 dispensers per ha. 'Isomate OFM Rosso' is a controlled release formulation of OFM sex pheromone that contains Z-8-dodecenyl acetate (223 mg/dispenser), E-8-dodecenyl acetate (14.5 mg/dispenser) and Z-8-dodecenol (2.5 mg/dispenser). The new 'Isomate OFM Rosso' dispensers replaced 'Isomate OFM Plus' because of the longer life time and lower application rate (Sexton and Il'ichev, 2001).

More than 280 food traps for OFM monitoring and mapping of the hot spots were placed in area-wide MD. Weekly monitoring of the area-wide MD experiment in this particular season started early in the middle of August 1998, before the start of the first flight of OFM after winter and continued longer than two weeks after the last OFM flight finished in April 1999. The long period of monitoring was useful for better analysis of the monitoring data across two seasons.

Five control sites were designated this season. Four were separate orchards without MD, adjacent to the north, west and south borders of the area-wide MD experiment and one was a

property on the western part of experimental area, inside the expanded area-wide MD and surrounded by orchards under MD.

Shoot tip and fruit damage assessments.

The OFM larvae damage actively growing shoot tips of peach trees by tunneling into the shoot for 8-10 cm. This causes the tip to die or wilt. Larvae can also damage developing peach fruits, causing them to exude gum. Larvae enter the fruit and burrows to the stone, filling the tunnel with brown particles of excreta. But unlike the damage caused by codling moth (*Cydia pomonella* L.) in apples and pears, this excreta is not usually conspicuous on the outside of the peach. OFM damage to pome fruit looks like codling moth damage but when the fruit is cut open the damage does not usually include the pips.

The detailed shoot tip damage assessments were carried out in the hot spot of the property 1 on the following dates: 16.12.96, 27.02.97, 16.12.97, 21.01.98, 3.01.99 and 24.02.99. Fifty shoot tips (twenty-five at random on both the east and west sides of the canopy) were counted on each of the 21 peach tree in the 6 rows where food traps had been placed across the pear and peach interface. Property 2 had no visible tip damage after the 1-st year of area-wide MD experiment and no assessments were taken.

Fruit damage assessments were also carried out only in the property 1. There was no damaged fruit in property 2 after the 1-st year of area-wide MD experiment. The assessments were made prior to the first colour picking on 24.02.97, 28.01.98 and 3.03.99 and at the time of harvest on 3.04.97, 17.03.98 and 22.03.99. A random sample of 25 peaches was taken from the same trees that were assessed for shoot tip damage.

Shoot tip and fruit damage assessments were taken to investigate the distribution of the damage throughout the interface of the MD peach block adjacent to the pear block under 3 different treatments. First was MD on the whole pear block called 'Pear MD', second was MD on a 10 pear tree barrier adjacent to the peach block called 'Pear Barrier', and third was pear block under insecticide chemical spray program called 'Pear Chem'. Shoot tips and fruits were assessed from each of 21 peach trees making up the 6 rows where food traps had been placed in the hot spot.

Management and analysis of data.

The number of damaged shoot tips and fruits was recorded and the percentage of damaged shoot tip and fruit in each tree was calculated. The percentage of shoot tip and fruit damage down 6 rows of peach trees in each experiment was analysed for the trends and autocorrelation using ASREML (NSW Agriculture) and GENSTAT 5. Release 4.1 (Lawes Agricultural Trust, Rothamsted Experimental Station).

A geographic information system (GIS) was used for the management, visualisation and analysis of the monitoring and damage assessment data from the area-wide MD experiment. The location of orchard blocks and traps were entered into the GIS using sketch maps of each property in conjunction with satellite imagery and digital cadastral information for the area-wide MD experiment. Once all of the monitoring data had been entered, a desktop GIS package *ArcView 3.1* (ESRI Inc. USA) was used to locate and interpret the data with respect to cadastre of the Cobram area and the road network. The zooming functionality of *ArcView 3.1* enabled the mapping scale of the data to be easily manipulated, and could range from the entire study area to individual orchards. The GIS data base was also used to provide all participating growers with weekly reports of OFM numbers monitored on their property and regularly inform them about the situation with outbreaks and hot spots on the whole experimental area.

Results

Monitoring the OFM population in the area-wide MD experiment (1996 - 97 season). According to our observations of shoot tip damage distribution and monitoring data 4 properties with edge damage effect were identified at the end of the 1996-97 season. Also during this season 2 properties in the experimental area were designated as hot spots according to our monitoring data. In these areas, shoot tip and fruit damage was also high and more than 10-15 OFM in average during the season were caught per week in food traps.

The average weekly catch of OFM per trap from the food traps showed three distinct peaks of OFM flights (first, second and fourth generations), especially in the pear blocks under insecticide treatments, where the peak of the first generation reached average of 35 OFM per week per trap (OFM w/t) (Figure 1). The OFM numbers decreased after the first flight in all peach MD blocks. The population of OFM in the Pears MD barrier remained low after second flight whereas, in the rest of pear block under insecticide treatments, the population showed two distinct peaks at the second and fourth generations. The peak of the second generation flight was higher under insecticide treatment and reached average of 50 OFM w/t. These results indicated that the MD worked effectively in almost all peach blocks, decreasing the initial level of OFM population compared with pears under insecticide treatments (Pear Chem.) (Figure 1).

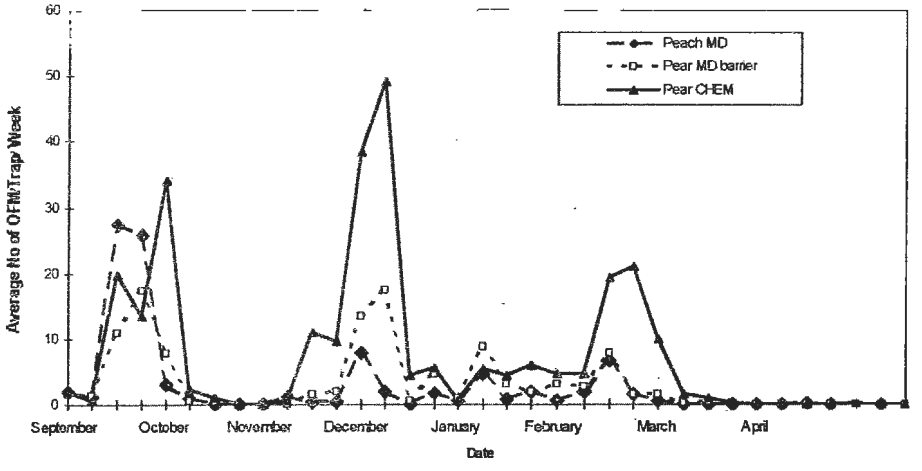

Figure 1. Average number of OFM in pear and peach blocks under different treatments. Monitoring by food traps was conducted during the 1996-97 season in the hot spot area of property 1 in Cobram before application of area-wide MD.

There were two main hot spots found in two properties within the experimental area in the 1996-97 season. The average trap catch in the whole area designated for area-wide MD experiment during this season was between 5-10 OFM w/t, but in hot spots it was much higher between 20-30 OFM w/t. MD was applied only on peaches in these hot spots, and severe damage occurred on the edges of MD peach blocks adjacent to pear blocks under insecticide treatments.

1997 - 98 season.

Monitoring data of the first OFM flight in 1997-98 from the food traps placed on 18 properties under area-wide MD confirmed two distinct hot spots in properties 1 and 2.

In the hot spot at property 1, the initial population level on peaches was higher and the peak of the first generation flight was about 85 OFM w/t in 1996-97, when adjacent pears were treated with insecticides, and 45 OFM w/t in 1997-98, when adjacent pears were treated with MD (Figure 2). In 1997-98 under area-wide MD treatment, the OFM numbers dropped during the second generation and did not show any increase up to the end of the season, although the numbers continued at a level of 10-20 OFM w/t.

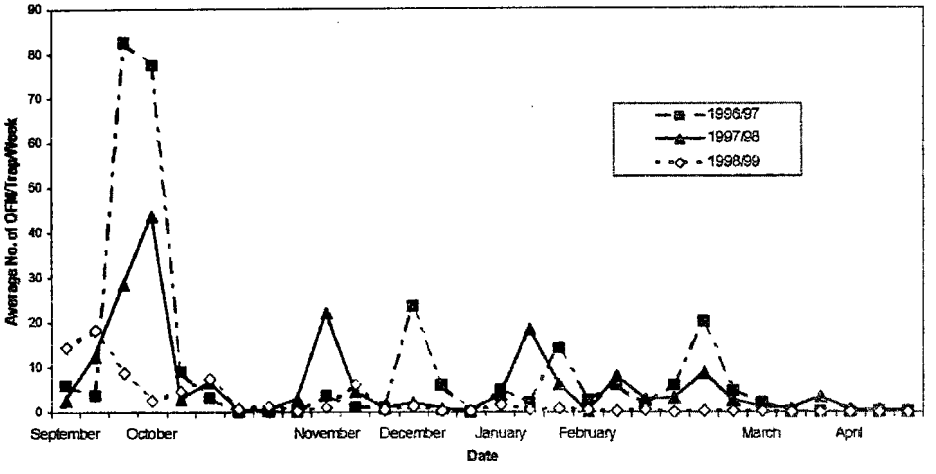

Figure 2. Average number of OFM in peach block under MD in the hot spot area of property 1. Monitoring by food traps was conducted during the 1996-97, 1997-98 and 1998-99 seasons.

In the hot spot at property 2, after area-wide MD application, the OFM population was greatly reduced and it continued to be low till the end of the season. The initial level of the first OFM generation in property 2 was much lower (10-15 OFM w/t) than in property 1 (about 85 OFM w/t). In subsequent generations, the OFM number became insignificant in property 2 and there was no visible shoot tip and fruit damage in this property. Therefore the data from the hot spot in the property 2 was not presented. This result indicates that one season of area-wide MD application was able to control the hot spot with a medium level of OFM population (10-15 OFM w/t). The area-wide MD experiment in the first year of application helped growers to reduce the number of insecticide sprays against OFM by half.

1998 - 99 season.

This season the new dispensers of Isomate OFM Rosso for area-wide MD experiment were used. The new dispensers have longer lifetime and lower rate of application (500 dispensers per ha) compare to Isomate OFM Plus. This helps to make the area-wide MD approach more cost effective for growers because of the reduced labor cost.

The monitoring results of this season indicated that area-wide MD experiment during the second year successfully reduced the OFM in the hot spot in property 1. The initial population level in this hot spot in the 1996-97 season was about 85 OFM w/t. After 2

consecutive seasons of area-wide MD application, the peak of the first generation in 1998-99 was about 20 OFM w/t and in second and following generations it was low (0-3 OFM w/t) (Figure 2). During the second year of area-wide MD experiment most of the growers did not spray insecticides against oriental fruit moth at all.

Shoot tip and fruit damage assessments.

Figure 3 shows the percentage of shoot tip damage in peaches under MD in the hot spot at property 1. Shoot tip damage assessments were usually carried out after second OFM flight during 3 consecutive seasons. The graphic lines in Figure 3 demonstrated the distribution of the average percentage of shoot tip damage from 3 lines of 21 peach trees adjacent to the pear block in the hot spot at property 1. Statistical analysis of the results indicated that shoot tip damage was significantly higher in the first 4 rows of trees in the 1997-98 season. There was a decline in shoot tip damage from tree 1 to tree 4 ($p<0.01$), thereafter, there was no change in shoot tip damage down the rows ($p=0.86$). The correlation in shoot tip damage between neighboring trees from tree 5 to tree 21 down the rows was recorded ($p<0.01$).

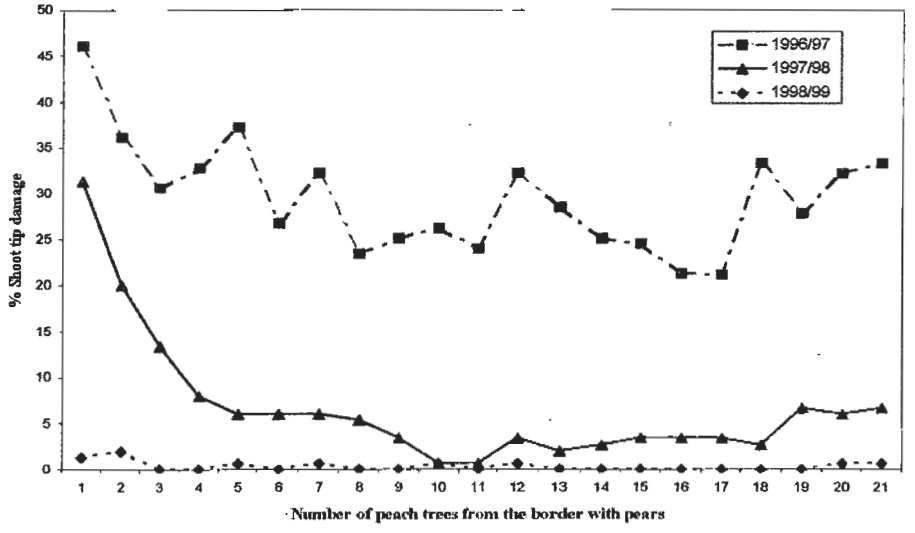

Figure 3. Shoot tip damage assessments for the 1996 - 97, 1997 - 98 and 1998 - 99 seasons. The average percentage of shoot tip damage from 3 lines of peach trees adjacent to pear block in the hot spot at property 1.

The results of fruit damage assessments and the distribution of damage throughout the interface of peach MD block (Figure 4) statistically confirmed the higher level of fruit damage in the border of peach MD blocks next to the pear blocks. The statistical analysis of the fruit damage distribution indicated that in the peach MD block there was a reduction in fruit damage to tree 4 ($p<0.04$), and a further reduction from tree 4 to tree 21 ($p=0.05$) in the 1996-97 season. There was no correlation in fruit damage between neighboring trees from tree 5 to tree 21 down the rows ($p=1$). Log damage was a reasonable negative linear fit to tree numbers (adjusted $R^2=23\%$; $p<0.001$), thus indicating that decline in fruit damage was greatest nearer tree 1. During the next 1997-98 season the similar pattern in fruit damage distribution was

recorded with significant reduction in fruit damage from tree 1 to tree 3 ($p<0.04$), but there was no further reduction from tree 4 to tree 21 ($p>0.05$). Log damage was a reasonable negative linear fit to tree numbers (adjusted $R^2=25\%$; $p<0.001$), thus indicating that decline in fruit damage was greatest nearer tree 1 (Figure 4).

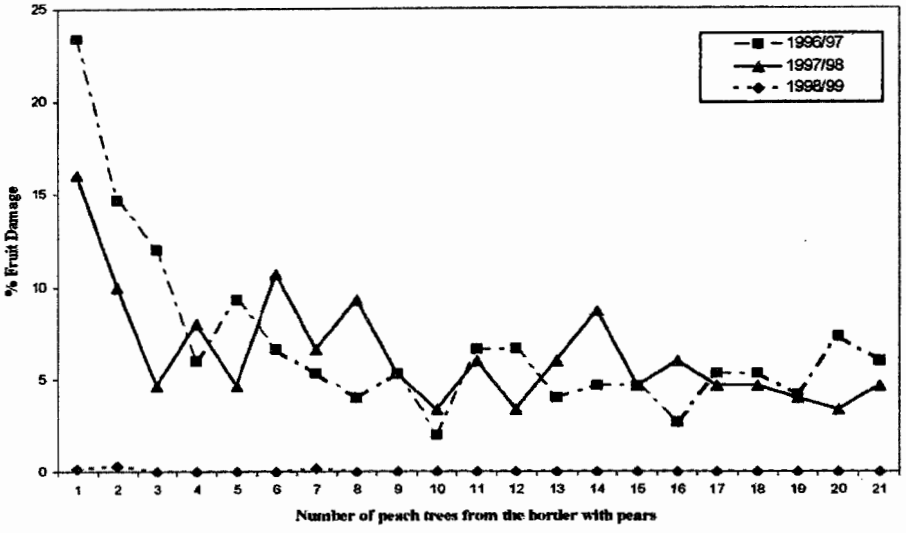

Figure 4. Fruit damage assessments for the 1996 - 97, 1997 - 98 and 1998 - 99 seasons. The average percentage of fruit damage from 3 lines of peach trees adjacent to pear block in the hot spot at property 1.

Discussion

The concentration of the higher level of shoot tip and fruit damage near the border (edge effect) of the peach MD block with adjacent pear block without MD treatment may indicate that OFM population in pears contributed to the infestation of neighboring peach trees treated with MD. Our further observations indicated that higher damage at the edge of the peach blocks may be due to migration of mated OFM females from pear blocks under insecticide treatment to adjacent peach MD blocks (Il'ichev et al., 1998).

Peach shoot tips and fruit could attract mated females for oviposition from adjacent pear blocks, where they have developed a high population. The occurrence of shoot tip and fruit damage mostly on the borders of MD peach blocks may also indicate a behavioural avoidance of unmated females to MD. The females may have responded to a large amounts of synthetic sex pheromone by moving outside of the MD area to mate and then return for oviposition in response to the food attractant from shoot tip or ripening peach fruit (Il'ichev et al., 1999b). Although there is no direct evidence that OFM females can detect their own sex pheromone, such a response has been reported in the females of some tortricid species (Den Otter et al., 1996; Palaniswamy and Seabrook, 1978).

It is known that MD works most effectively in the presence of a low OFM population, and always required a sufficient amount of pheromone to be present in the atmosphere. Through the use of food traps it has been shown that mated females, albeit in small numbers, can be present even within large peach orchards where MD is applied. This situation indicates

the possibility that mechanisms other than pheromone calling may lead to mating. In practice however, it is not possible to reliably estimate the population density of OFM, or to measure the amount of sex pheromone present in the air within the orchard (Cravedi, 1992). The use of portable electroantennogram recording equipment in the orchard allows measurement of the airborne pheromone concentrations (Rumbo et al., 1995). Electroantennogram measurements demonstrated that concentration of pheromone for MD can fluctuate due to air movement and leaf absorption in an orchard (Suckling and Angerilli, 1996).

The South African experiment (Barnes and Blomefield, 1997) suggested that the success of area-wide MD depended on effective management of borders of orchards and blocks treated with MD. Two factors are relevant: the decrease in the concentration of pheromone from the MD dispensers at the edges of MD blocks due to wind (Suckling and Karg, 1997), and the migration of mated females of OFM from non mating disruption blocks into adjacent MD areas (Barnes and Blomefield, 1996).

The results reported in this paper also supported the view that the migration of mated OFM females from pears to peaches can lead to the breakdown of MD in the edge of peaches in this situation. The results of pear fruit damage assessment indicated higher level of OFM larva infestation and also suggested that OFM can concentrate in pear blocks under insecticide treatment and may provide a pest reservoir for further infestation of adjacent peaches under MD.

Comparison of the shoot tip damage level before area-wide MD experiment and two years later after area-wide MD treatment, demonstrated the significant reduction of damage in peach MD blocks. Figure 3 shows that the shoot tip damage was greatly reduced in the peach MD block within area-wide MD application in the 1997-98 compared to that of the 1996-97, and fell to almost zero during the 1998-99 season in the hot spot at the property 1. The hot spot with initially high number of OFM such as in property 1 was eliminated by area-wide MD application in the second year (Figure 2). The hot spot with initially lower level of OFM in property 2 was controlled after 1 year of area-wide MD application.

Initial damage to peach fruits in the hot spot in property 1 (1996-97) was very high (about 25%) before the area-wide MD experiment. After the first year of area-wide MD application, the damage was reduced to 15% and after the second year of the experiment, damage was almost zero throughout whole peach block (Figure 4). Therefore, the OFM numbers (Figure 2), shoot tip (Figure 3) and fruit damage (Figure 4) were greatly reduced over 2 years of area-wide MD application. This indicates that the area-wide MD approach worked effectively.

Monitoring data from 4 places with edge damage effects (in 1996-97 during the first generation counts were about 4-8 OFM w/t) demonstrated a reduction in OFM population during the 1997-98 season (during second and following OFM generations were about 0-1 OFM w/t) after area-wide MD application. There was no detectable shoot tip and fruit damage found in these places at the end of the 1997-98 season. The situation was similar in all 4 locations. According to the OFM monitoring results and examinations of shoot tip and fruit damage all 4 places with edge damage effects were successfully controlled by the end of the 1997-98 season due to area-wide MD application in the whole territory.

The analysis of the monitoring data indicated that one season of area-wide MD application was able to control the hot spot with a medium level of OFM population (10-15 OFM w/t). Two consecutive seasons of area-wide MD application were able to control the higher level (up to 85 OFM w/t) of OFM hot spot.

The results of the OFM flight monitoring and assessment of damage distribution from shoot tip and fruit also supported that OFM can concentrate in pear blocks under insecticide treatments and may provide a pest reservoir for further infestation of adjacent peaches under MD. This also supports our view that the migration of mated females from pears to peaches

can be one of the possible reasons leading to the breakdown of MD in the edge of peach blocks in this situation.

Our results indicated that the area-wide MD approach worked effectively and was able to control high level of OFM hot spots successfully. The area-wide MD experiment shows that the OFM population in the hot spots can be gradually reduced and that migration of mated females in the hot spots and any edge damage effects can be reduced. The area-wide MD application in the first year helped growers to reduce the number of insecticide sprays against OFM by half and in the second year, most of the growers did not spray against oriental fruit moth at all.

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Use of pheromones and other semiochemicals in integrated production
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Evaluation of the mating disruption method for the control of the pink bollworm *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae) and comparison of this method with insecticidal treatments

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Abstract: In a cotton field in Central Greece, the mating disruption method was applied aiming to the control of the pink bollworm *Pectinophora gossypiella*. For this reason, two commercially available slow release pheromone formulations, PB-Rope and SELIBATE, were used. The effectiveness of the method was compared to the infestation levels recorded at a neighboring cotton field of equal size, where three insecticidal treatments had taken place. Every field was divided in six blocks of equal size and two pheromone traps were placed in each of these blocks. The seed used belonged to Zeta-2 and Allegria cotton varieties. The traps were checked daily while 100 fruiting bodies and 20 leaves per block were randomly collected every week. In two of the blocks of the pheromone-treated field an insecticidal spray was applied in mid-August. The infestation rates in these two blocks were not significantly different compared to the rates recorded in the untreated blocks. At the same time, treatments resulted in an abrupt increase of the population of leaf feeding insects, mainly of aphids. Moth catches in the pheromone-treated field were recorded after the first week of September while in the insecticide-treated field the number of moths caught was very high, even between treatments. No significant difference was noticed between the two methods; also, no significant differences were found in the infestation levels between the two cotton varieties used. A considerably greater number of larvae were found in pheromone-treated than in insecticide-treated blocks, in unharvested bolls which were collected in mid-October.

Key words: *Pectinophora gossypiella*, gossyplure, mating disruption

Introduction

The pink bollworm, *Pectinophora gossypiella* is one of the most threatening pests of cotton in Greece (Davaris et al. 1992, Buchelos et al. 1999). It can cause very important quantitative losses and qualitative degradations, while at the same time high population densities can be built up very quickly (Henneberry and Clayton 1980, Henneberry 1986, Hutchinson et al. 1991). This fact, forces growers in Greece, in order to control this pest, to conduct sprays with several insecticides; usually 3 to 5 at 10-15 day intervals. One of the most common side effects of these applications is the concomitant increase of the populations of several other cotton pests (such as aphids and mites). The most common decision criterion is practically the larval infestation in the plant's fruiting bodies (mainly bolls). Many critical values for the infestation in bolls have been suggested so far; however, the proposed threshold varies from 5-10 % (Henneberry and Clayton 1982, Hutchison et al. 1988, Buchelos et al. 1999). The

validation of larval infestation meets several difficulties, because a) in middle and late season, eggs and larvae are usually protected from direct contact with insecticides b) most eggs are laid under calyces and bracts of bolls c) young larvae usually enter bolls within 20-30 min upon hatching and d) females can lay up to 85 % of their total egg complement in 7-10 days, and as a result, larval infestation can become well established between applications (Hutchison et al. 1988, 1991).

During the last two decades gossyplure, the sex pheromone of the pink bollworm, is widely used for monitoring and timing of the insecticidal applications. However, due to several reasons, trap catches do not always correspond to actual changes in infestation levels (Henneberry and Clayton 1982, Flint et al. 1993, Beasley and Adams 1994, Buchelos et al. 1999). On the other hand, the use of gossyplure for mating disruption has been proposed from several researchers, as an alternative for the control of *P. gossypiella* (Staten et al. 1987, Flint et al. 1993, Cardé and Minks 1995, Cardé et al. 1998). The application of this method has given very promising results so far (Flint and Merkle 1983, Chamberlain et al. 1994, Cardé et al. 1998). Nevertheless, its evaluation still has several implications, mainly because the immigration of mated females for neighboring crops can not be totally avoided (Jones 1998). In Greece, mating disruption has been tested successfully, during the last few years (Kyriakidou and Recca 1991, Yamvrias and Foundoulakis 1995). However, insecticidal sprays practically remain the main control measure for the control of the pink bollworm in Greece (Davaris et al. 1992).

Materials and Methods

The experiment was carried out in the region of Farsala (Thessaly, Central Greece), during the 1998 growing season. In this region, cotton is the main cultivation and *P. gossypiella* is the main cotton pest. Two adjacent rectangular cotton fields, 120000 m^2 each, were used for experimentation. These two fields were surrounded by beets, wheat, industrial tomato and wheat fields, from East, West, North and South, respectively.

Each field was divided into 6 blocks of equal size. On the first field, three insecticidal sprays took place a) bifenthrin (28 July) b) alpha cypermethrin (12 August) and c) permethrin (26 August). On the second field, two commercially available slow release disruptant formulations of technical gossyplure (Z,Z- and Z,E-7,11-hexadecadienyl acetate) were applied (3 blocks each): a) PBW rope at a rate of 120 dispensers/1000 m^2 (Shin-Etsu, Japan) and b) SELIBATE EXTRA at a rate of 40 dispensers/1000 m^2 (Agrisense BCS, UK). These dispensers were hand-adapted around the main stem of the cotton plants, on the 29th of June. Two varieties were used on each field (3 blocks per field) a) Zeta 2, which is a Greek variety of Acala-type (KESPY, Greece) and b) Allegria (Stoneville, USA). The seeding was carried out on 20 and 21 of April 1998 (approx. 15 plants per meter on the row, 1 m between rows), followed by standard cultivation care. In addition, two adhesive pheromone traps (Delta trap, Agrisense BCS, UK) were placed in each block on 20/5. Counting of adults captured was conducted daily, while the replacement of the pheromone was taking place every 20 days. On the 16th of August, two blocks (one block per variety) of the second (untreated) field were sprayed with bifenthrin, in order to determine the influence of a single insecticidal application late in the season combined with the method's application (for preventing boll infestations late in the season). No chemical defoliation or plant growth regulators were applied late in the sampling period.

Each week, from late May until early October, 100 fruiting bodies were collected randomly from each block. Due to flower absence, during the first two weeks of sampling, pinhead squares were collected; during the following two weeks, flowers and during the last

ten, bolls. These fruiting bodies were checked in the laboratory for infestation by *P. gossypiella* larvae, and the number of larvae per boll was recorded. Additionally, during the same period, 20 leaves were collected randomly from each block, at weekly intervals (one leaf per plant). Each leaf was placed separately in a plastic bag. These bags were then brought to the laboratory, and checked for insects, in order to determine differences among treatments. Aphid mummies, attached on a leaf piece were placed in small plastic boxes at 22° C and 65 % rh, in order to collect the parasitoids (Kavallieratos and Lykouressis 1999). The seed cotton was harvested mechanically on 14th of October. Finally, on the 20th of October, from each block 100 uncollected bolls were examined, and the presence of *P. gossypiella* larvae (usually internally of the cottonseed) was recorded.

Leaf counts were expressed as mean number of individuals per leaf (adults and immature stages combined). Before the analysis of variance, data were transformed to standardize means and homogenize variances (Little and Hills 1978). For means' comparison, the Duncan's multiple range test was used (Duncan 1955). The statistical package JMP (SAS 1989) was used, at a significance level of p=0.05.

Results

a. Trap catches.

Early in the season captures were higher than 20 males per trap (Fig. 1.). In the insecticide-treated field trap catches were notably high, regardless of the sprays' application. Two additional peaks were observed: in late July (>35 males/trap) and early in September (>20 males per trap). One the other hand, in the pheromone-treated field, captures were totally suppressed, from the day of dispenser placement and on. In this field catches were gradually increased early in September, but numbers of captured males continued to be lower than the ones in the insecticide-treated field. No significant differences were noted on the trap catches between cotton varieties (df=1.190, F=0.017, P=0.893).

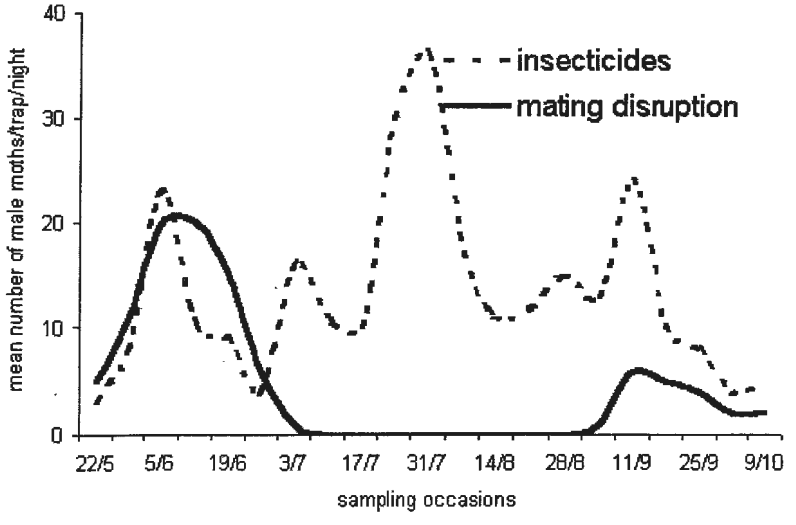

Figure 1. Captures of *P. gossypiella* male adults in gossyplure-baited sticky traps, during the sampling period, in the pheromone-treated and the insecticide-treated blocks.

b. Infestation level.

During the entire sampling period, infestation level was below 10 % (Fig. 2). During the first weeks, relatively higher percentage of larval infestation was noted in the insecticide-treated field. This fact is reversed during the last sampling occasions. Despite this, no significant differences were recorded between the two fields (df=1.30, F=0.046, P=0.830). Additionally, no significant differences were noted between the insecticide treated and the insecticide free blocks of the pheromone-treated field, from the date of the insecticidal application and on (df=1.14, F=0.287, P=0.601). As infestation level increased, the mean number of larvae per boll was exponentially increased (Fig. 3). Additionally, the rate of this increase was more intense in the insecticide treated field. However, more than one larvae per boll were common only in high infestation levels (>20 %). Significant differences were recorded among treatments (pheromone, insecticide, pheromone + insecticide), in the number of *P. gossypiella* larvae found in uncollected bolls (df=2. 297; F=3.25; P=0.0401). Nevertheless, although significantly higher numbers of larvae were noted in the pheromone-treated blocks, the percentage of larval presence at this stage was rather low (< 0,1 larvae per boll).

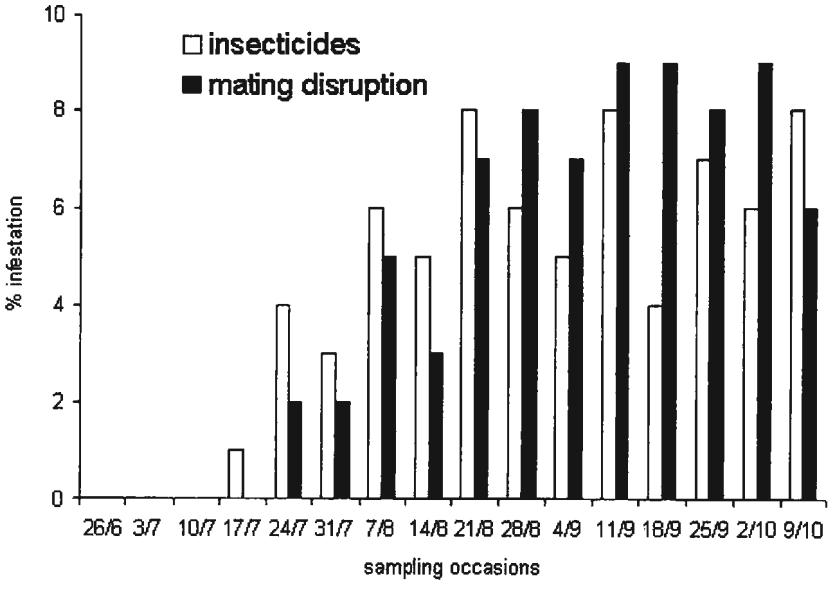

Figure 2. Mean percentage (%) of infested fruiting bodies by *P. gossypiella* larvae, during the sampling period, in the pheromone-treated and the insecticide-treated blocks.

c. Differences among varieties and formulations.

The mean percentage of infested bolls by P. gossypiella larvae was relatively higher in the blocks with Zeta-2 than those with Allegria in the insecticide-treated field (6.74 % against 6.07 %) as well as in the pheromone-treated field (5.90 % against 5.53 %). However, no significant differences were observed between the two varieties used (df=1.190, F=0.510, P=0.476 for the insecticide-treated blocks and df=1.190, F=0.132, P=0.715 for the pheromone-treated blocks). Moreover, no significant differences were observed on the

infestation level between the two pheromone formulations used (df=1.94, F=1.001, P=0.319).

d. Other insects.

More than forty insect species were found on the cotton leaves examined (including Collembola, Diptera and others); the most abundant are presented on Table 1. Most of them are cotton pests; yet a considerable number of beneficial insects were found. In general, predators and parasitoids were found mainly in the insecticide-free blocks.

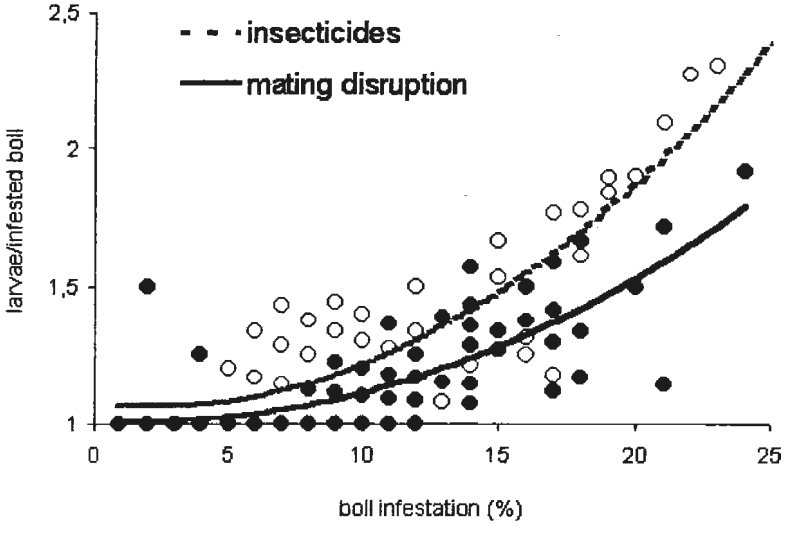

Figure 3. Relationship between boll infestation (%) and mean number of *P. gossypiella* larvae per boll, in the pheromone-treated and the insecticide-treated blocks.

Aphis gossypii was the most abundant aphid species during the entire sampling period. On the other hand, most of *Myzus persicae* individuals were found early in the season (first four sampling occasions), while *A. fabae* was found mainly during August. Significantly higher numbers of *A. gossypii* individuals were found in the insecticide-treated blocks, while sprays did not manage to suppress aphid population (Fig 4). Furthermore, in the sprayed pheromone-treated blocks aphids were found in high numbers, from mid-August (date of the bifenthrin application) and on.

The most numerous thrip species were *Thrips tabaci* and *Frankliniella occidentalis*. However, *F. intonsa*, *F. tritici* and *Aeolothrips* sp. were found in small numbers during the sampling season. *T. tabaci* fluctuation results were similar to that of *A. gossypii* (Fig. 5); after the insecticidal treatments population density was increased rapidly. On the other hand, in the insecticide-free blocks *T. tabaci* presence was constantly reduced (<1 ind. per leaf).

On the other hand, most *E. decedens* individuals were found in the insecticide-free blocks (Fig. 6). Hence, in the insecticide-treated blocks, *Empoasca decedence* presence was low, mainly between applications.

Table 1. Mean number of individuals per leaf for each species found, in the pheromone-treated, insecticide-treated and the sprayed pheromone-treated blocks. Means on the same row followed by the same letter, are not significantly different (Duncan's multiple range test at a=0.05). Only the species with more than 0.01 individuals per leaf were statistically compared.

Species	Pheromone	Insecticide	Pheromone+Insecticide
HEMIPTERA			
Aphididae			
Aphis fabae Scopoli	<0.001	<0.001	<0.001
Aphis gossypii Glover	1.92a	8.14c	3.89b
Myzus persicae (Sulzer)	0.012a	0.010a	0.017a
Cicadellidae			
Empoasca decedens Paoli	1.09a	0.73a	0.98a
Empoasca decipiens Paoli	<0.001	<0.001	<0.001
Nabidae			
Nabis ferus (L.)	0.008	<0.001	0.002
Miridae			
Lygus sp.	<0.001	<0.001	<0.001
Macrolophus sp.	<0.001	<0.001	<0.001
Anthocoridae			
Anthocoris nemorum L.	0.011a	<0.001b	0.006a
Orius laevigatus Fieber	0.013a	<0.001c	0.003b
Orius niger Wolff	<0.001	<0.001	<0.001
Aleurodidae			
Bemisia tabaci (Gennadius)	3.21a	8.89b	4.76a
NEUROPTERA			
Chrysopidae			
Chrysoperla sp.	<0.001	<0.001	<0.001
THYSANOPTERA			
Aeolothripidae			
Aeolothrips sp.	0.05a	0.06a	0.04a
Thripidae			
Frankliniella intonsa (Trybom)	0.07a	0.12a	0.13a
Frankliniella occidentalis Pergande	0.13a	1.44c	0.35b
Frankliniella tritici (Fitch)	<0.001	<0.001	<0.001
Thrips tabaci Lindeman	0.25a	1.17b	0.44a
COLEOPTERA			
Coccinellidae			
Donia variegata (Goeze)	<0.001	<0.001	<0.001
Coccinella septempunctata L.	0.004	0.002	0.004
Hippodamia undecimnotata (Schn.)	<0.001	<0.001	<0.001
Scymnus sp.	<0.001	<0.001	<0.001
HYMENOPTERA			
Aphelinidae			
Aphelinus sp.	<0.001	-	-

Aphidiidae			
Aphidius colemani Viereck	0.004	0.003	0.003
Lysiphlebus fabarum (Marshall)	0.085a	0.011b	0.050a

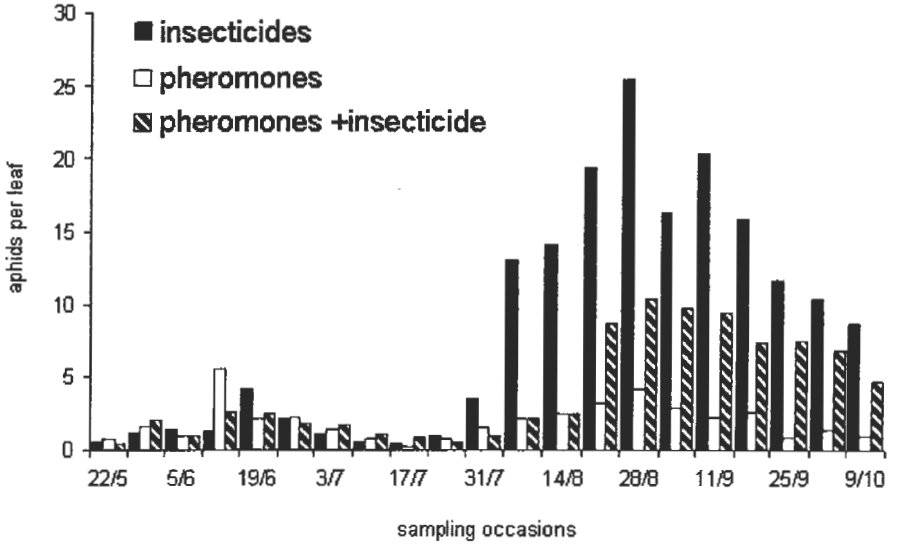

Figure 4. Mean number of *A. gossypii* individuals per leaf, during the sampling period, in the pheromone-treated, insecticide-treated and sprayed pheromone-treated blocks.

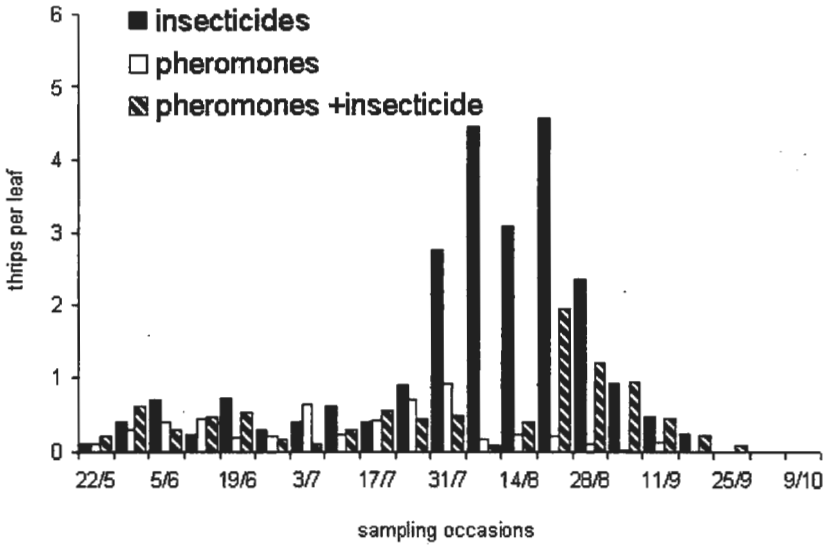

Figure 5. Mean number of *T. tabaci* individuals per leaf, during the sampling period, in the pheromone-treated, insecticide-treated and sprayed pheromone-treated blocks

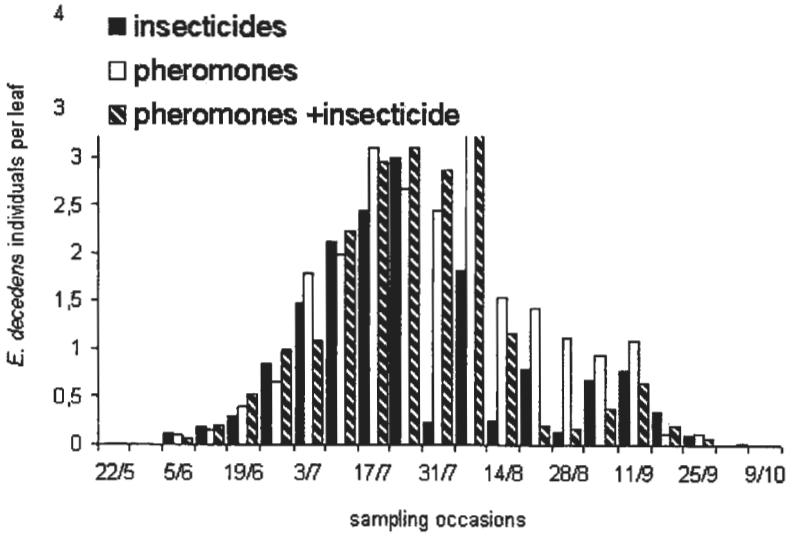

Figure 6. Mean number of *E. decedens* individuals per leaf, during the sampling period, in the pheromone-treated, insecticide-treated and sprayed pheromone-treated blocks.

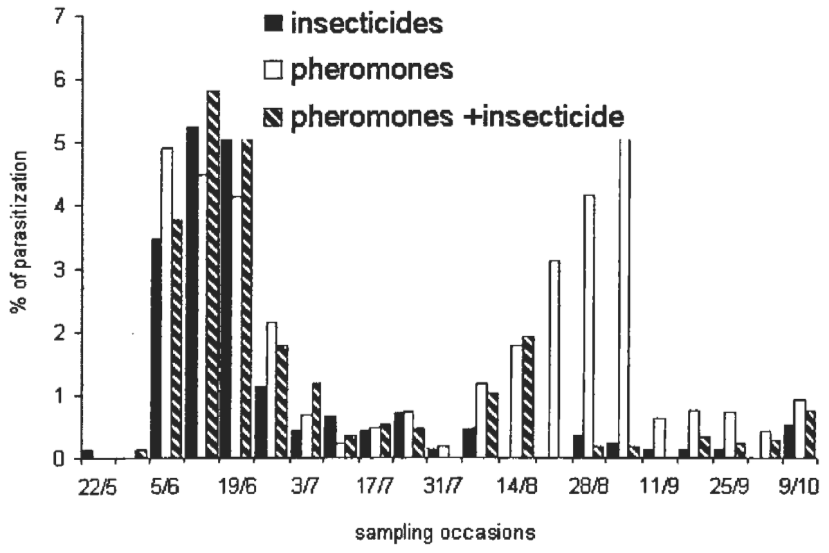

Figure 7. Percentage (%) of *A. gossypii* individuals parasitized by *L. fabarum*, during the sampling period, in the pheromone-treated, insecticide-treated and sprayed pheromone-treated blocks.

Discussion

The nihilism of the captures in the pheromone-treated blocks, was indicative of the reduced rate of mating occurred in this field. Even in cases of non complete suppression of the captures, mating disruption works, provided that the suppression percentage is high, usually more than 95 % (Jones 1998). However, this suppression does not establish zero (or at least reduced) infestation level, because the immigration of mated females from vicinal fields still remains the main drawback of the method (Cardé and Minks 1995, Jones 1998). Even in an insecticide-treated field is well known that numbers of adults in traps between applications are not reliable, and as a result any use of these figures for prediction may be inaccurate (Henneberry and Clayton 1982, Hutchison et al. 1991, Buchelos et al. 1999). On the other hand, the absence of adults in the traps (due to suppression in the case of mating disruption) is only an indication, and does not reflect the infestation level. Hence, for prediction, even when mating disruption is applied, it is necessary to combine trap inspections with frequent samplings in the fruiting bodies (mainly bolls). In our case, infestation level among treatments was similar, but in must be noted that % boll infestation in the insecticide-free blocks tended to increase late in the season, which could justify the need of a late season (late August, early September) insecticidal application. However, the infestation level in the sprayed pheromone-treated blocks, which was similar to that of the other treatments, indicated that there is no warrant for this assumption. Moreover, the infestation level has different meaning when is examined according to sampling occasion, because late bolls usually contribute less in yield. Thus, it is expected that late in the season infestation level, as well as number of larvae per boll, increases (Henneberry and Clayton 1982, Hutchison et al. 1988). The results of the present study indicate that mating disruption can reduce the number of eggs laid in the same

boll, and consequently the number of larvae per boll, because in an insecticide-treated field, infestation level is likely to be increased, due to oviposition between applications (Hutchison et al. 1991). However, more larvae are present after harvest in the pheromone-treated filed, because in this case, the reduced release of the disruptant can allow mating and oviposition. On the other hand, a late season insecticidal application is likely to protect non-harvested bolls. Nevertheless, larval survival is influenced by several factors; hence, the presence of larvae late in the season (after harvest) does not constitute a reliable indicator for the upcoming infestation level (Watson 1980, Henneberry 1986).

Our results indicate that the cotton variety is not the "key" factor for the determination of the infestation level by *P. gossypiella* larvae. Chu et al. (1991) also noted that although differences can be observed between varieties other factors and cultivation strategies (including irrigation termination, plant growth regulators and chemical defoliation) are more determinative for the infestation level. The combination of these factors with the appropriate cultivation technique is the best way to utilize the possible natural host-plant resistance (Henneberry 1986, Chu et al. 1991).

Apart from the aforementioned drawbacks, a single insecticidal application late in the season (in order to provide a preventive treatment due to the upcoming reduction in the disruptant's release) in a pheromone-treated field does not provide further protection and thus, is unnecessary. On the other hand, a single application in this case at any rate leads aphid populations to increase rapidly. Same holds for other serious pests, such as thrips and *B. tabaci*, while the only pests found to be controlled by insecticides (at least between applications) were *Empoasca* spp. It must be noted that for these species, no natural enemies were found in Greek cotton so far (Kyriakidou and Drossopoulos 1993, Kyriakidou, personal communication). Apparently, a single application can eliminate the populations of the predators and the parasitoids. In a previous study, Yamvrias and Foundoulakis (1995) also noted that beneficial insects are more numerous in pheromone-treated blocks, but the authors did not estimate pest populations. In the present study, the presence of almost all pests that were found in cotton leaves were low, as compared to the insecticide-treated blocks. This must be seriously taken into account, because the control of population outbursts for certain species (aphids, mites etc.) often following chemical control, is very difficult and often more important than pink bollworm infestation.

In conclusion, mating disruption method is reliable, easy to use and effective. Apart from all factors that were analyzed in this paper and the generally accepted disadvantages for its application (Staten et al. 1987, Cardé and Minks 1995, Jones 1998), this method is the most suitable for area-wide pest management, under the IPM principles. Moreover, it is absolutely essential to examine other factors rendering the requirement of co-operation of groups of farmers and the related social constraints, and hence, further research is required by social scientists (Lyon 1994).

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Control of *Sparganothis pilleriana* Schiff. and *Lobesia botrana* Den. & Schiff. in German vineyards using sex pheromone-mediated mating disruption

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Abstract: Investigations were conducted from 1996 to 2000 to determine the feasibility of controlling the tortricid pest *Sparganothis pilleriana* Schiff. using its sex pheromone. Intitially, different pheromone blends were tested in the field and the results have been published in previous papers. In 2000, after treating three generations of the pest with pheromone, the larval infestation was finally evaluated. The pheromone treatment reached a very good efficacy in reducing the larvae. Evaluation of larval density in 2000 after using mating disruption to control three generations of the pest revealed that pheromone could provide economic control. Investigations on the use of the mating disruption technique against another very important tortricid in German viticulture, *Lobesia botrana* Den. & Schiff., showed the control of this pest with pheromones was very effective. Most importantly, mating disruption was effective against an occasional third generation of this pest that cannot be controlled using insecticide. The use of pheromone and insecticide could reduce the density of *L. botrana* to levels where pheromone alone could be used to achieve economic control.

Key words: viticulture, *Sparganothis pilleriana*, *Lobesia botrana*, pheromones, mating disruption, third generation

Introduction

Sparganothis pilleriana Schiff. (Lepidoptera, Tortricidae) is a tortricid native to Germany that causes severe damage in several German and European vine growing regions. Since 1990 in some viticultural regions of Germany, for example in the southern palatinate, the population density of this tortricid has significantly increased and remained at a very high level. The insecticides registered for use in Germany until 1999 were in most cases less successful in reducing the larval infestation below the damage threshold. Based on these problems and the severe economic damage caused by the pest in 1996 a research project was launched with the objective of developing an environmental friendly method to control *S. pilleriana*.

The grape berry moths *Lobesia botrana* Den. & Schiff. and *Eupoecilia ambiguella* Hbn. are the most important insect pests in German viticulture. The mating disruption technique is registered for both species since 1986 (Eupoecilia) and 1994 (Lobesia). Depending on climatic and other conditions in some viticultural areas both species occur sympatrically whereas in other areas only one species is known to occur. If both tortricids occur together, often one is predominate. In many regions of the palatinate the dominant species is *L. botrana*. There is also a distinct shift: In some areas *Lobesia* populations increase and *Eupoecilia* populations decrease at the same time. A change in dominance has been observed in some areas.

Feldhege (1993) showed that the efficacy of mating disruption of *L. botrana* declined if

moth density exceeded approximately 4000 females and males per hectare This is one of the reasons that it is generally recommended not to use pheromone in areas with very high tortricid populations. A high density of *L botrana* is one of the reasons the use of the mating disruption technique did not increase in viticultural practice the last few years. At present in Germany about 20 percent (20.000 ha) of the total viticultural area is treated with the mating disruption technique to control *L. botrana* and *E. ambiguella.*

Mating disruption was observed beginning in 1992 for control of *L. botrana.* In a second project initiated in 1999 the main goal was to determine if it would be possible to combine the use of mating disruption and insecticide to reduce *L. botrana* density to a level where pheromone alone could be used to achieve economic control. In the past two to three years in several German vine growing regions a third generation of *L. botrana* developed. Insecticides cannot be used against this generation due to the short time before harvest. Therefore, an important question to answer was if pheromones could have an impact on the development of the third generation of *L. botrana.*

Materials and methods

Sparganothis pilleriana

Over a period of four years (three pest generations) mating disruption tests on different sizes plots (1 – 5 ha) were conducted in the South Palatinate. A mixture of different components of the sex pheromone of *S. pilleriana* (blend 1: *E*/*Z*9-12:Ac; *Z*11-14:Ac; *E*/*Z*9-12:OH) was used. The experimental design used during 1996 to 1999 have been described in previous IOBC Bulletins (IOBC Dachau, Hohenheim, Schmidt-Tiedemann et al. 1999, in press). In spring 2000 the number of larvae per vine was examined within the same small check-plots not treated with insecticides in which the number of eggmasses was evaluated in 1999.

Lobesia botrana

In one 60 ha test area (site 1) having intermediate to low population pressure RAK 1+2 (BASF, 500 dispensers per ha) was used to control *L. botrana* (Louis and Schirra 2001, in press). Evaluations were conducted in this site beginning in 1992 to examine the long term effect of the mating disruption over a period of years.

A second test area (site 2) was located 200 m from site 1. This site had a very high population density of *L. botrana* and was used until 1998 as the untreated control for calculating the degree of effectiveness of the mating disruption in site 1. In 1999 in site 2 a two-year project was begun to evaluate the combined use of mating disruption and insecticide for controlling *L botrana.* The main goal of this project was to examine if by using insecticides together with pheromones the individual density of the tortricids could be reduced to a level low enough for exclusive use of mating disruption technique in future seasons. To get to know something about the efficacy of the pheromone treatment control plots not treated with insecticides were established within site 2.

In 2000 another project to assess the effect of pheromones on the third generation of *L. botrana* was begun. The flight activity of the third moth generation in untreated control plots was compared with the flight activity in site 2 and in a 10 ha-area within site 2, where in August 2000 new RAK 1+2 dispensers were applied. For montoring the flight activity pheromone traps were used.

Results and discussion

Sparganothis pilleriana

In 2000 the pheromone treatment reduced larval density to 86 % at site 1 and 79 % at site 2 (Table. 1). A comparison with the results of 1998 and 1999 using blend 1 (80 % to 98 %) showed that the effectiveness of using sex pheromone is in most cases better than the effectiveness of insecticides. In addition to its effectiveness, the use of mating disruption has further advantages. The long period of emergence of the larvae out of their overwintering sites in spring makes it extremely difficult to determine the right date for the application of insecticides. Moreover the larvae live in leaf shelters that give them protection against insecticides. The investigations also showed that the pheromone treatment is successful in small plots (5 - 11,5 ha) in contrast to the experiences with *E. ambiguella* and *L. botrana* where a plot size of at least 20 ha is required for successful use of mating disruption.

In this project an environmental friendly way was developed to control *S. pilleriana* by use of sex pheromone in mating disruption technique (sex pheromone-mediated mating disruption) with very high effectiveness. This method is not registered yet. One reason for this might be due to the relatively small areas in Europe infested by *S. pilleriana*.

Table 1. Effectiveness of blend 1 (*E*/*Z*9-12:Ac; *Z*11-14:Ac; *E*/*Z*9-12:OH) in mating disruption of *S. pilleriana* in grape vine

	Treatments 1999/2000			
	Site 1		Site 2	
	Blend 1	Control	Blend 1	Control
Plot size [ha]	8,5		11,5	
No. pheromone traps	15	9	18	12
Average catch (± SD) per pheromone trap	1,1±1,22	35,6±13,07	0,8±2,15	151,3±170,0
No. of vines examined for eggmasses	100	60	120	80
Average no. of eggmasses per vine	0,1±0,33	1,1±1,22	0,5±0,75	3,0±4,20
No. of vines examined for larvae	100	60	120	80
Average no. of larvae per vine	1,0±1,5	7,15±5,85	2,9±2,97	11,3±8,68

SD = Standard Deviation

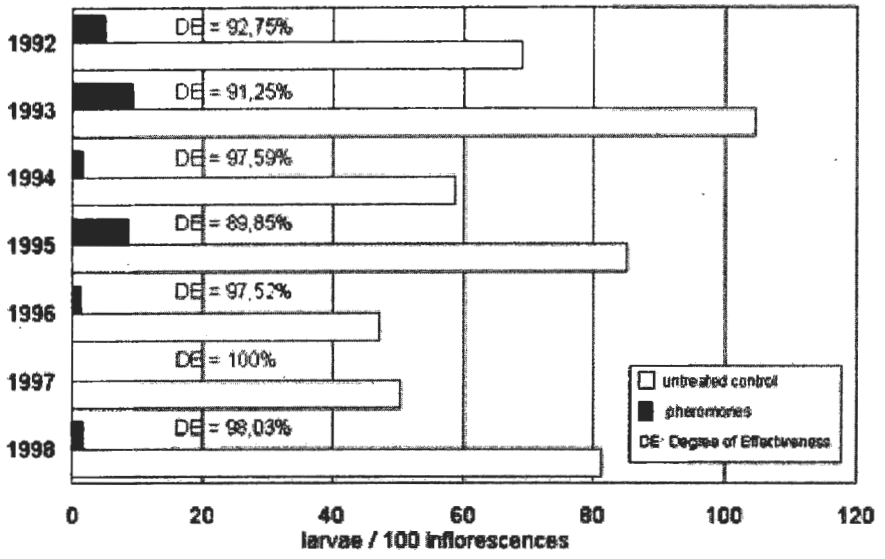

Figure 1. Effect of pheromone treatment (RAK 1+2) on the first generation of *Lobesia botrana*, 1992 - 1998, Neustadt - Haardt

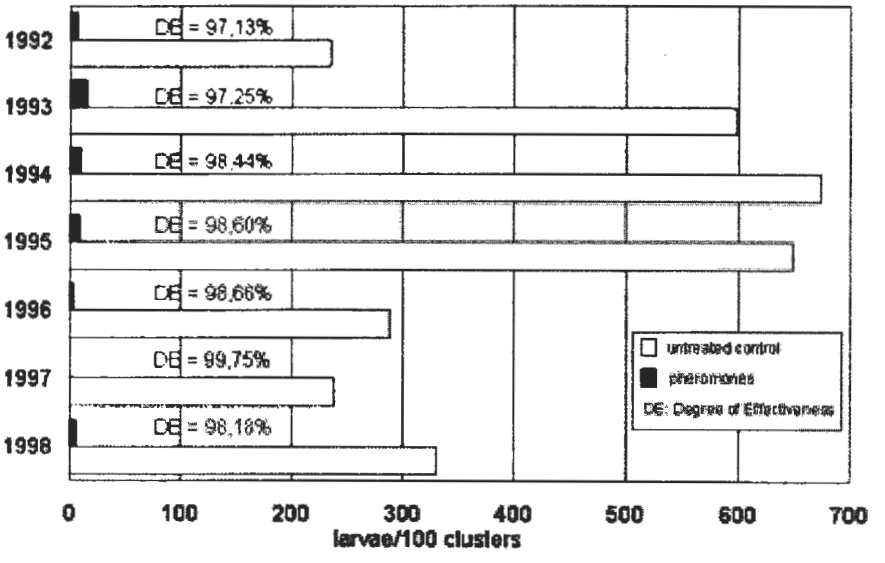

Figure 2. Effect of pheromone treatment (RAK 1+2) on the second generation of *Lobesia botrana*, 1992 - 1998, Neustadt - Haardt

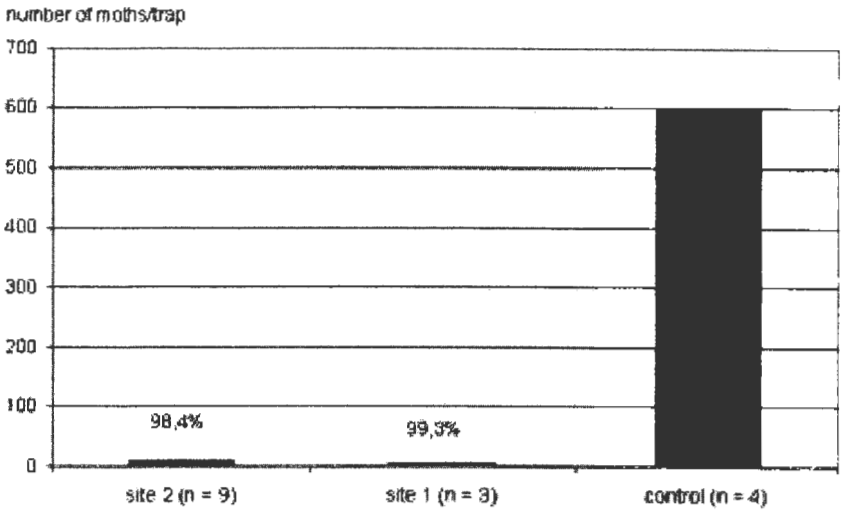

Figure 3. Effect of pheromone treatment on the capture of *Lobesia botrana* – moths in pheromone traps, first generation 2000, Neustadt – Haardt

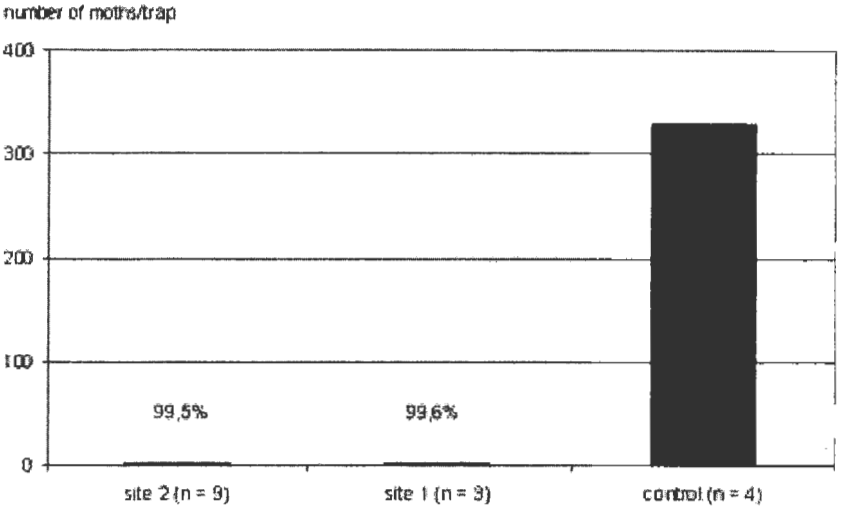

Figure 4. Effect of pheromone treatment on the capture of *Lobesia botrana* – moths in pheromone traps, second generation 2000, Neustadt – Haardt

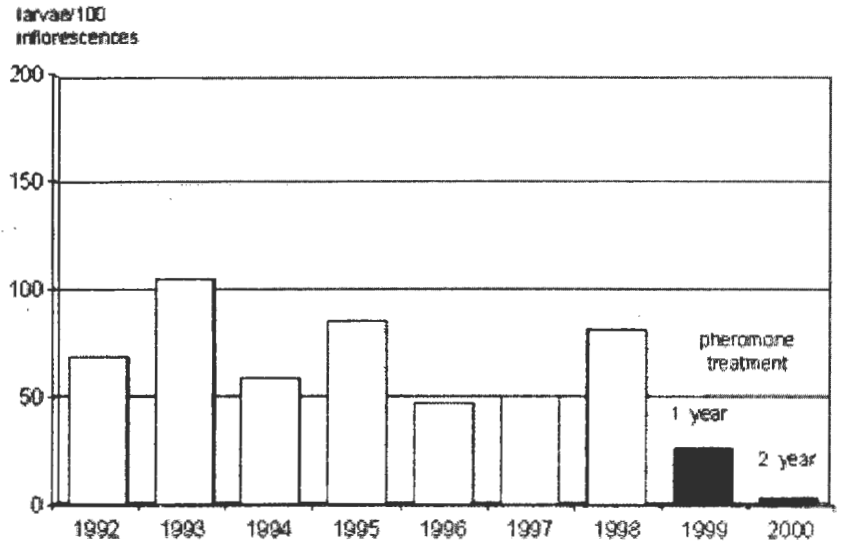

Figure 5. Infestation rates of *Lobesia botrana* larvae, first generation in site 2 (1992 – 2000), Neustadt - Haardt

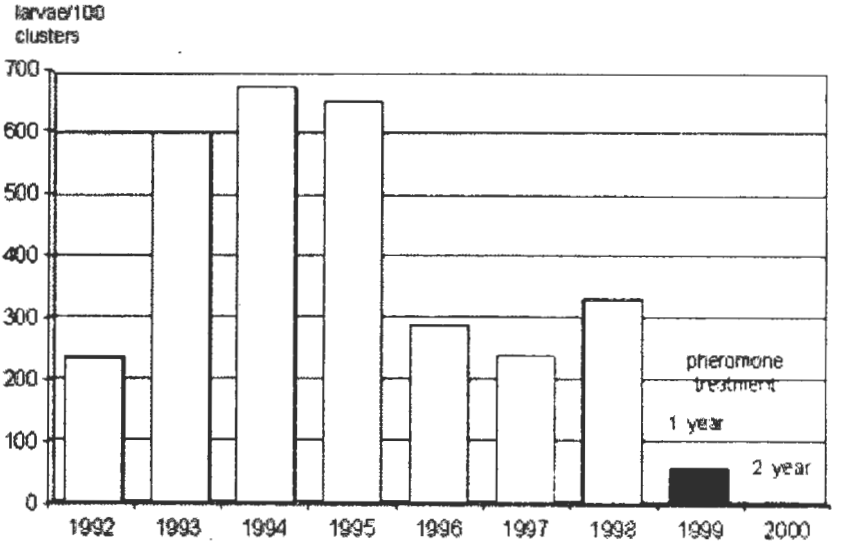

Figure 6. Infestation rates of *Lobesia botrana* – larvae, second generation in site 2 (1992 – 2000), Neustadt - Haardt

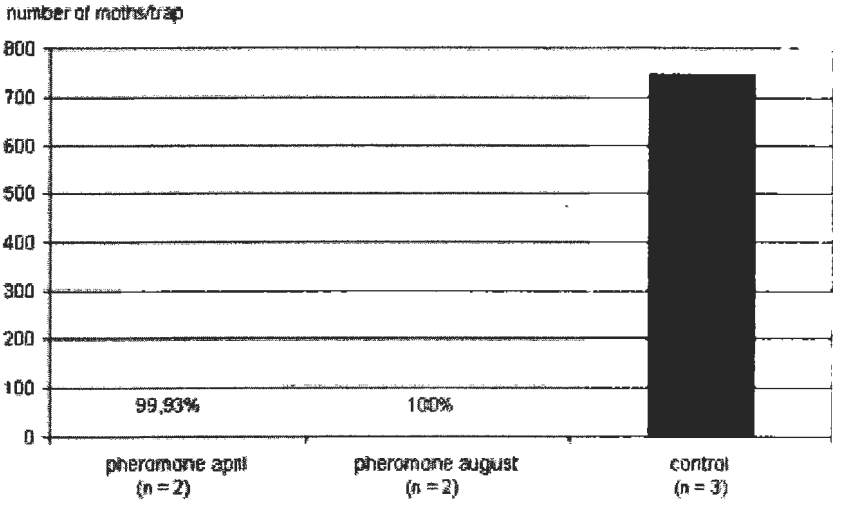

Figure 7. Effect of pheromone treatment on the capture of *Lobesia botrana* males in pheromone traps, third generation 2000, Neustadt - Haardt

Lobesia botrana

Between 1992 and 1998 the mating disruption technique used in site 1 was very successful. In most cases the degree of effectiveness was higher than 90 % for the first larval generation (Figure 1) and more than 97 % for the second larval generation (Figure 2). In the untreated control plots for the first generation the average rates of infestation ranged between 50 and 100 larvae per 100 inflorescences. For the second generation approximately 200 to more than 600 larvae per 100 clusters could be found in the vegetation periods investigated.

In 1999 in site 2 within the first year of the combined treatment with pheromones and insecticides the effect of disorientation reached 94,3 % for the first generation of *L. botrana* and 92,1 % for the second moth generation. Compared to the results obtained in site 1 (99,3 % for both generations), these low efficacy rates could be expected in the first year. In 2000 the disorientation rates in site 2 were significantly higher reaching 98,4 % in the first moth generation (Figure 3) and 99,5 % in the second generation (Figure 4). The results obtained for the second moth generation were almost as good as those obtained in site 1 (99,6 percent).

The infestation rates at site 2 before the beginning of the combined treatment, 1992–1998, were evaluated within untreated control plots (control plots for pheromone site 1), where the infestation was extremely. The results obtained using the combined treatment in 1999 and 2000 were evaluated in small control plots without inseciticide application to determine the exclusive effect of the mating disruption technique. In 2000, the infestation rates within these pheromone plots were significantly lower than in the years 1992 – 1998 (Figures 5 and 6).

These results provide evidence that the combined use of insecticide and mating disruption can be used to reduce population density of *L. botrana* to a level where mating disruption alone can be used to control this pest.

The results also demonstrate that pheromone applied before the onset of flight of the first generation in April remained highly effective until the end of the third moth flight in October. In vineyards in which new pheromone dispensers were applied shortly before the beginning of the third flight in August a disorientation rate of 100 % was achieved (Figure 7).

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Effects of mating disruption against the Mediterranean corn borer, *Sesamia nonagrioides*, on the European corn borer *Ostrinia nubilalis*

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Abstract: In many Mediterranean areas, two stalk borers, *Sesamia nonagrioides* and *Ostrinia nubilalis* affect maize yield. The efficacy of mating disruption techniques against the first corn borer has been evaluated in recent years. In the present paper the effect of such a control method on populations densities of *O. nubilalis* is reported. Densities of *O. nubilalis* in fields treated with *S. nonagrioides* pheromone released from PVC dispensers were always lower than in untreated fields. However, *O. nubilalis* populations were not lower than in untreated fields when the pheromone blend was applied with a liquid formulation and was composed of two components. The influence of using a liquid formulation, which has higher release rates, may explain the different effects. Additionally, trap catches of *O. nubilalis* were reduced when the pheromone of *S. nonagrioides* was added to the pheromone of *O. nubilalis* in traps and, inversely, catches of *S. nonagrioides* were reduced when the pheromone of *O. nubilalis* was added to traps baited with the pheromone of *S. nonagrioides*.

Key words: Maize, pheromone, mating disruption, *Sesamia nonagrioides, Ostrinia nubilalis*.

Introduction

The Mediterranean corn borer (MCB), *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) and the European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), are two sympatric corn borers that cause major losses in maize in the Mediterranean Basin. In northeast Spain the insects go through two complete broods and a partial third one. Whereas the first adult flight of MCB occurs earlier (May) than that of ECB (June), the second flight is concurrently observed in late July or early August (Alfaro, 1972; Riba et al., 1992). Larval and pupal development is mostly completed inside the stem or the ear, so they are poorly sensitive to insecticides, particularly the MCB, and alternative control methods have been tested.

Among non-chemical control tactics, synthetic pheromone sprays have been tested to disrupt the mating of MCB with good results. Sex pheromone of MCB was first identified as composed of two components, Z11-16:Ac and Z11-16: OH, by Sreng et al. (1985). Later Mazomenos (1989) completed the composition with two additional components, Z11-16:Ald and 12:Ac and determined the optimal component ratio to be 69:8:8:15. More recently, Sans et al. (1997) determined the proportion 77:8:10:5 as optimal for an MCB population of northeast Spain in EAG, wind tunnel and field studies. Sex pheromone of ECB is a blend of two isomers, Z11-14:Ac and E11-14:Ac (Klun et al., 1973; Klun & Cooperators, 1975) in a ratio that varies according to geographic areas. In the study area, Sans et al. (1993) observed the so-called Z strain of ECB (97Z: 3E) to be predominant. The respective pheromone blends are routinely used to monitor the flight of the two corn borers.

The effects of releasing a high amount of pheromone on the non-target biotic environment have rarely been studied. Some studies have investigated the effects of releasing pheromones in the environment for mating disruption purposes on insects taxonomically close

to the target species (Johnson et al., 1991; Ferrao et al., 1998) and also on its parasitoid complex (Niwa & Daterman, 1989). However, there are no studies to assess the impact of the mating disruption technique on non-target species that share food resources with the targeted species, as in the case of ECB and MCB in maize.

In fact, the coexistence of more than one species that concurrently bore the stem of the same host species (e.g. maize, rice, or sugarcane) is a common feature in many areas of the world and leads to interspecific competition for food. Some of the species usually belong to Pyralidae or Crambidae (e.g. *O. nubilalis*, *Chilo* spp., *Diatraea* spp., *Scirpophaga* spp.), whereas sympatrically occurring stem borers belong to Noctuidae (e.g. *Sesamia* spp. and *Busseola* spp.). Several of the cited stem borers are targeted in mating disruption programmes and the effect that such a control method may have on the potential competitors of the target pest should be known in order to better understand the impact of releasing pheromones on the crop pest complex. Potential interference between sympatrically occurring species that share at least one pheromone component has been reported by several authors (e.g. Deland et al., 1994). Allomonal effects of pheromones on competing species are far less documented in the literature. Among bark beetles, aggregative pheromones are used to inhibit the attraction of competing species to the host (Blum, 1996).

The work reported here aimed to investigate the effects of releasing MCB pheromone blend for mating purposes on its competitor, the ECB.

Materials and Methods

Application of the pheromone blend.

Several field trials were carried out from 1990 to 1999 to evaluate the effectiveness of the mating disruption technique against MCB. For this, maize fields variably sized between 4 and 12 ha were treated with MCB pheromone blend or left untreated as control. The number of pheromone components, the formulation and the doses applied varied during the experimental period to improve the efficacy of the method according to the results obtained in the previous years and those obtained in behavioural studies (Sans et al., 1997; Lopez et al., 1999). Pheromone blend composition, component ratio, formulation, doses and the generations targeted were modified as summarised in Table 1.

Field trials may be divided into three categories according to the blend composition and formulation. In the first three experimental years, the 69:8:8:15 proportion and PVC dispensers were used. In the following two years, the proportion was modified to 77:8:10:5 as determined by Sans et al. (1997) for a local population, but the same PVC dispensers were used. Finally, in last three years, two liquid formulations with two components were sprayed to make the application easier.

Effects on ECB population density.

ECB population densities were estimated by dissecting 180 maize plants (9 sets of 20 plants each) and recording the number of larvae per plant in each treated or control field after the end of the second flight (late August-early September) and before harvesting (early October).

Pheromone trap catches.

Funnel pheromone traps located at least 50 m apart were placed in a maize field from June 5 to October 15. The traps were baited with one of the three treatments: (i) MCB pheromone (200 µg of the 69:8:8:15 blend) (ii) ECB pheromone (100 µg of the 97:3 blend, Z11-14:Ac, E11-14:Ac), and (iii) MCB and ECB pheromones. Polyethylene vials and red rubber septa were used for the MCB and ECB pheromone blends respectively. Each treatment was

replicated 3 times. The number of MCB and ECB males caught in each trap was recorded weekly. Additionally, catches of *Mythimna unipuncta* (a species that shares the major pheromone component with MCB) were recorded.

Monitoring of pheromone release rate.

In the years 1990-1996, the amount of pheromone was calculated in the PVC dispensers. Samplings were made at 0, 10, 30 and 60 days. In each sampling 3 dispensers were collected from the maize field. Each dispenser was extracted with hexane in ultrasounds for 30 min (3 x 30 ml). The extract was analysed by GC using a packed column (OV-101, 2 m x 2 mm i.d.). The amount of Z11-16:Ac was quantified using an internal standard (tridecyl acetate).

In 1998 and 1999 the amount of pheromone in the maize leaves was evaluated. Samplings were made every 2 days from the day of application until the 10th day after treatment. In each sampling 40 maize leaves were picked randomly in the field. Only leaves among the top five were collected. In the laboratory the leaves were separated into groups of 10 leaves. From each leaf a square of 10 cm^2 was cut, and the total of 100 cm^2 for the 10 leaves was extracted with 50 ml of pentane in ultrasounds for 30 min. Thus, four replicates were made in each field on every sampling date. The extract was concentrated to 1 ml and analysed by GC using a capillary column (SP-2330, 30 m x 0.25 mm x 0.2 μm). The amount of Z11-16:Ac was calculated using an internal standard (tridecyl acetate).

Statistical analyses.

The data of ECB larvae per plant and the percentage of attacked plants were submitted to a one-way ANOVA. Trap catches were analysed by a two-way (week and type of bait) ANOVA. When needed, means were compared using Duncan's Multiple Range Test ($P<0.05$).

Table 1. Conditions in which field trials of mating disruption against MCB were carried out.

Year	Pheromone blend		Formulation	Doses gr/ha[a]	Commercial supplier	Generations targeted
	Components	Ratio				
1990	Z11-16:Ac Z11-16:OH Z11-16:Ald 12:Ac	69:8:8:15	PVC	49(100)	Agrisense	1st & 2nd
1993	as in 1990	69:8:8:15	PVC	80(100)	SEDQ	1st & 2nd
1994	as in 1990	69:8:8:15	PVC	80(100)	SEDQ	2nd
1995	as in 1990	77:8:10:5	PVC	80(100)	SEDQ	2nd
1996	as in 1990	77:8:10:5	PVC	80(100)	SEDQ	2nd
1997	Z11-16:Ac Z11-16:OH	90:10	Liquid	80(100)	SEDQ/NPP[b]	2nd
1998	as in 1997	90:10	Liquid Liquid	80(100)	SEDQ/NPP[b] SEDQ/TNO[b]	2nd
1999	as in 1997	90:10	Liquid Liquid	80(100)	SEDQ/NPP[b] SEDQ/TNO[b]	2nd

[a]Values in brackets show the number of dispensers per ha, [b]Pheromone components were synthesised by the first firm and formulated by the second firm.Results

ECB density in fields treated with MCB pheromone (1990, 1993, and 1994).
The number of ECB larvae per plant was always lower in fields treated with MCB pheromone than in untreated fields within each year and sampling date (Table 2).

ECB density in fields treated with MCB pheromone (1995 and 1996).
As in the previously cited years, ECB populations were lower in fields treated with MCB pheromone, though only the second generation of MCB was targeted in 1995 and 1996 (Table 3).

ECB density in fields treated with MCB pheromone (1997, 1998, and 1999).
When liquid formulation with the two major pheromone components was sprayed to disrupt mating of MCB, ECB densities were never significantly ($P<0.05$) lower in treated than in untreated fields (Table 4).

Table 2. Mean (±s.e.) number of larvae of ECB per plant in fields treated with the MCB pheromone and untreated fields. Within each year and sampling date, means followed by a different letter are significantly different ($P<0.05$).

Year	Sampling date	Larvae/plant	
		Treated	Untreated
1990	September	0.6±0.5 b	1.9±1.0 a
	October	0.6±0.4 b	0.9±0.5 a
1993	September	0.8±0.8 b	2.4±1.9 a
	October	0.9±1.0 b	2.5±1.9 a
1994	September	0.5±0.5 b	1.0±0.6 a
	October	0.5±0.4 b	1.5±0.7 a

Table 3. Mean (±s.e.) number of larvae of ECB per plant in fields treated with the MCB pheromone and untreated fields. Within each year and sampling date, means followed by a different letter are significantly different ($P<0.05$).

Year	Sampling date	Larvae/plant	
		Treated	Untreated
1995	September	0.6±0.5 b	1.9±1.0 a
	October	0.6±0.4 b	0.9±0.5 a
1996	September	0.8±0.8 b	2.4±1.9 a
	October	0.9±1.0 b	2.5±1.9 a

Table 4. Mean (±s.e.) number of larvae of ECB per plant in fields treated with the MCB pheromone and untreated fields. Within each year and sampling date, means followed by a different letter were significantly different (P<0.05).

Year	Sampling date	Larvae/plant	
		Treated	Untreated
1997	September	0.5±0.8	0.6±1.0
	October	0.5±0.9	0.5±1.0
1998	September	2.8±2.9	2.4±3.4
	October	2.3±1.9	2.6±2.4
1999	September	1.4±0.9 a	0.6±0.4 b
	October	1.4±0.7 a	0.6±0.6 b

Pheromone trap catches.

Catches of MCB and ECB males in traps baited with MCB or ECB or both MCB and ECB pheromone blends are shown in Figures 1 and 2. As expected, the highest number of ECB and MCB males were caught in traps baited with their own pheromone, whereas practically no catches were recorded in traps baited with the pheromone of the other species. The addition in the same trap of the pheromone of the other species caused the number of catches to decrease significantly (P<0.05). For *M. unipuncta* catches, a pattern similar to that of MCB was observed (Fig. 2).

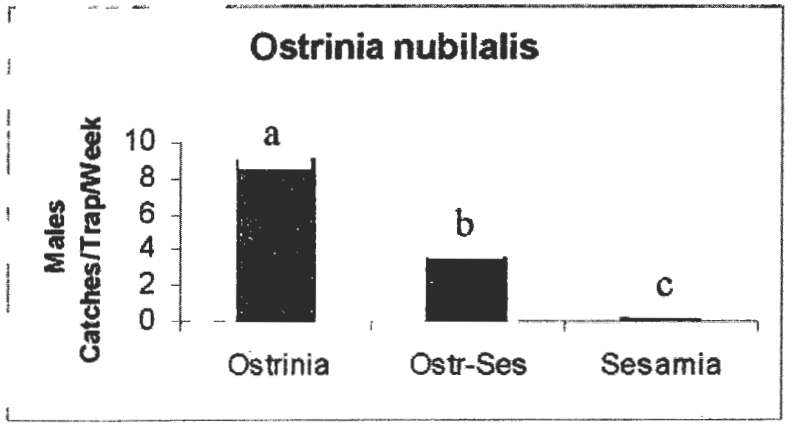

Figure 1. Mean number of *O. nubilalis* males caught in traps baited with the pheromone of *O. nubilalis*, the pheromone of *S. nonagrioides* or with the two pheromone blends. Traps were placed in maize fields from June 5 to October 15. The three means shown were significantly different (P<0.05) from each other.

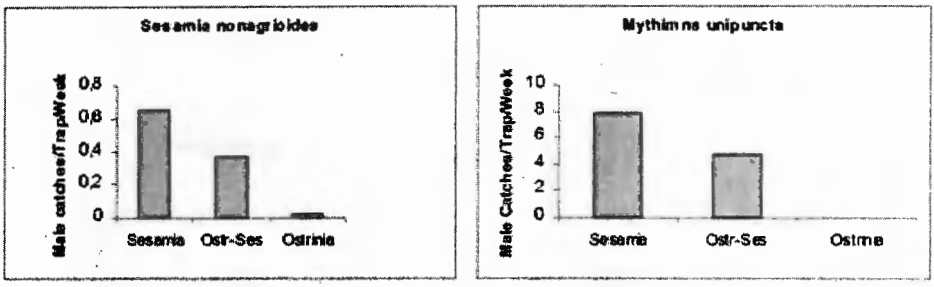

Figure 2. Mean number of *S. nonagrioides* and *M. unipuncta* males caught in traps baited with; the pheromone of *O. nubilalis*, the pheromone of *S. nonagrioides* or with the two pheromones blends. Traps were placed in maize fields from June 5 to October 15. The three means shown in each figure were significantly different ($P<0.05$) from each other.

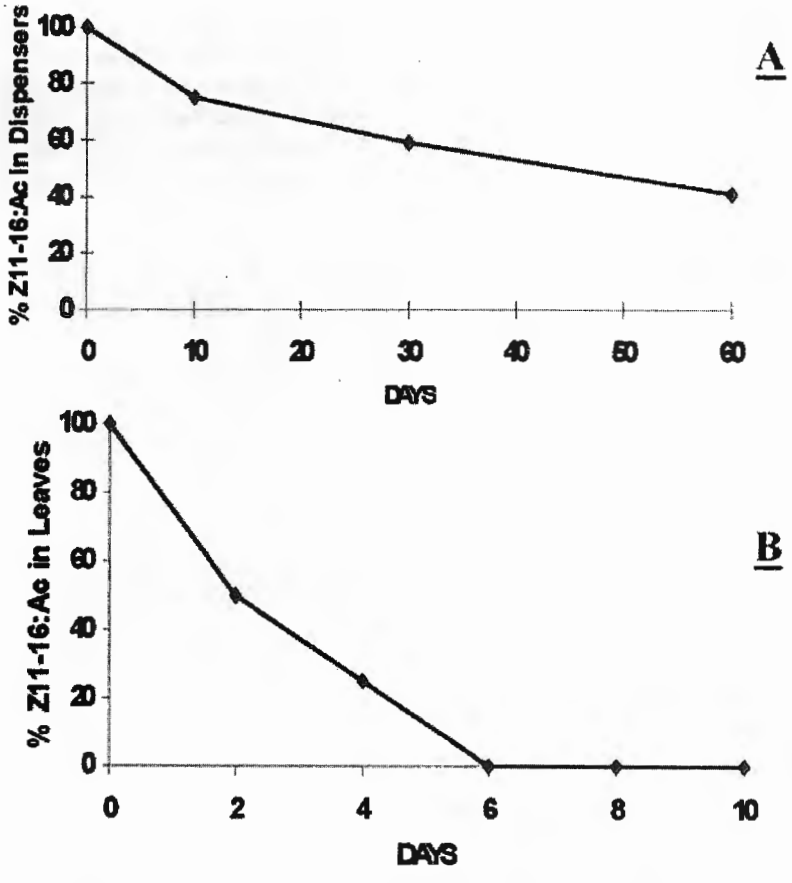

Figure 3. Percentage of Z11-16:Ac, major component of *S. nonagrioides* pheromone blend, that remained in PVC dispensers in 1994 (A) or in leaves in 1998 (B) in the days following the establishment of dispensers in the field or leaf spraying respectively.

Pheromone release rate.
Release rate of the major pheromone component Z11-16:Ac was far lower when the pheromone was applied in a solid dispenser than when it was sprayed with a liquid formulation. Figure 3 shows values of the release rate for two years. In the first case, 40 % of the pheromone component remained in the dispensers 60 days after they were placed in the field, whereas in the case of liquid formulation practically 100% of the component sprayed had been released only six days after spraying.

Discussion

The application of pheromone to disrupt MCB mating decreased ECB population densities in the first 5 years of the experimental period when solid PVC dispensers were used, whereas it had no effect in the last three years in which the pheromone blend was sprayed as a liquid formulation. These results are similar to those obtained when the efficacy of mating disruption against MCB was evaluated in the same field trials (author's unpublished results). The liquid formulations showed a lower pheromone persistence than the solid dispensers, which could explain the lower effectiveness in the control of MCB and also the lower effect of MCB pheromone on ECB populations. Also, the liquid formulations were composed of only the two major components of MCB pheromone and this could also contribute to their lack of effects on ECB density. The results of trap catches confirm the mutual interference of MCB and ECB when the complete sex pheromone of the other species is added to their own pheromone. The decrease in the number of catches of *M. unipuncta* when ECB pheromone is added to MCB pheromone in traps would further confirm such a hypothesis. It should be noted that *M. unipuncta* shares the major component with MCB and that it is usually caught in traps baited with MCB virgin females (Albajes et al., 1985).

Allomonal effects of aggregative and epideictic pheromones on competing species have been observed in bark beetles (Prokopy, 1981; Birch, 1984; Blum, 1996). Observations on interspecific interactions of sex pheromones have been restricted to species that share one or more pheromone components. The results reported here suggest that sex pheromones can also play a role in the competition of herbivores even if they do not share components in their pheromones, as is the case of *S. nonagrioides* and *O. nubilalis*. Research should be done to determine whether only males are sensitive to the pheromone of the competing species, as shown in the trap experiment, or the females may also respond to the allomonal effects of the pheromone of competitors. Note that Palanaswamy & Seabrook (1978) showed that, at a high concentration in the environment, the sex pheromone of *Choristoneura fumiferana* may act as an epideictic pheromone that enhances the dispersal of mated females in overcrowded areas.

In summary, the results of this work suggest that the sex pheromone of a stem borer may have allomonal effects on concurrent stem borer species. Further research is needed to determine which mechanisms and pheromone components are involved in this interaction; this is currently being investigated in our laboratory. This knowledge may be useful for controlling the maize pest complex by mating disruption techniques and also for predicting the potential impact of mass use of pheromones in maize fields.

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Use of pheromones and other semiochemicals in integrated production
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Mating disruption field trials to control the currant clearwing moth, *Synanthedon tipuliformis* Clerck: a three-year study

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Abstract: Mating disruption to control the currant clearwing moth, *Synanthedon tipuliformis* Clerck (Lepidoptera, Sesiidae) was applied for three consecutive years in a red currant plantation in Trentino, alpine Italian region. The major sex pheromone component (E,Z)-2,octadecadien-1-ol acetate was formulated in polyvinyl chloride (PVC) strings. Pheromone traps catches were reduced by 100% in the treated plot. The average number of larvae per metre decreased from 0.90 (prior to the treatment) to 0.25. Extensive sampling of pruned branches showed that a parasitoid wasp, *Macrocentrus marginator* Nees (Hymenoptera, Braconidae) killed from 38.8 to 55% of the larvae in the oldest wood.

Key words: mating disruption, *Synanthedon tipuliformis* (Lepidoptera, Sesiidae=Aegeriidae), sex pheromone (E,Z)-2,octadecadien-1-ol acetate, parasitisation, *Macrocentrus marginator* (Hymenoptera, Braconidae), red currant (*Ribes rubrum*).

Introduction

The Currant clearwing moth *Synanthedon tipuliformis* Clerck is a serious pest of both red and black currant. It is widely dispersed in Eurasia, where it is considered the key pest on Ribes crops. There is one generation per year. Adults emerge in May in the valleys and in June in uplands. Egg laying starts 10-15 days after emergence. Females lay eggs (35-50/female) on buds, stumps or bark wounds; two weeks later the larvae bore into the cane, where they feed and complete their development until the following spring.

Larvae complete their development in a tunnel within the pith, prior to creating 'exit windows' for the escape of adult moths. Pupation takes place within a loose silken cocoon in the cane near the 'exit window'. Feeding and tunnelling by larvae causes a depletion of the plant food reserves, weakening and breakage of canes, shoot dieback and uneven bud break in the damaged sections of canes. Indirect fruit yield losses accrue from larval feeding activity within canes.

Biological features and life behaviour make this pest difficult to control. The efficacy of chemical pesticides is limited by the short period when the larvae are not protected within the canes. Moreover, the emergence peak often coincides with the harvest period, when pesticide use is prohibited. Pesticide effectiveness is also limited by the scarcity of active ingredients registered for currant/gooseberry crops in Europe (Jörg, 1998).

Alternative control methods are hence necessary. 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 three consecutive years. The effectiveness of mating disruption was assessed by male captures in pheromone traps and by measuring wood infestation levels before and after dispenser application.

Materials and methods

Plot selection and description.
The trial was organised in a field (see map) of about 2000 sqm, selected in a typical productive area, at about 1000 m ASL. Red currant plants (cultivar Rovada) were 7 years old and trained in the linear system. Before application, more than 3 branches/plant were trained, but at the time of the first application, the grower started a rejuvenation plan, with the aim of reducing the size of the bushes to only 3 young branches/plant. This allowed us to collect and inspect a large number of pruned branches. The plantation was surrounded by grassland; the nearest trees and Ribes orchard were more than 300 m away. This area is particularly windy during the summer (prevalent north-north/east) direction, along the rows) and this could affect the efficacy of mating disruption. No insecticides were applied during the trial. Fungicides (copper oxychloride, dichlofluanide and copper sulphate) were applied 2-3 times each year.

Pheromone formulations.
The major sex pheromone component (E,Z)-2,octadecadien-1-ol acetate, [(E,Z)-2,13-18:Ac] was formulated in dispenser white polyvinyl chloride (PVC) tubular strings (Isomate CCM), supplied by Shin-Etsu Chemicals Co., Ltd, Tokyo, Japan. Catching traps were baited with a mixture of (E,Z)-2,13-18:Ac in proportion 100:3 with the sex attractant synergist (E,Z)-3,13-octadecadien-1-ol acetate [(E,Z)-3,13-18:Ac] as suggested by Szöcs *et al* (1991). Isagro Ricerche, Novara, Italy, supplied the mixture in small plastic vials.

Mating disruption.
The pheromone dispensers were applied in May, before the emergence of adult moths. The distance between dispenser placement along each row was determined following the New Zealand formula (A+B)/(C*D), where A= length of row*number of rows (metres), B= length of block boundary (shelter) in metres, C= size of the block (ha), D= desired dispenser rate/ha. D ranges between 250 (maintenance levels), to 500 (high infestations). Due to the high pest infestation and to the negative features of the area, a range of rates from 599 (1996) to 406 (1998) dispenser/ha was used. Strings (20 cm long) were hung 1.6 m high on the plant. More strings were applied on the perimeter of the field. The pheromone release rate during the first year was empirically assessed in a sample of ten dispensers by recording the changing weight of the dispenser, and by measuring the length of the air bubble left after the diffusion of the volatile.

Table 1. Pheromone release rate from 10 dispensers during spring-summer 1996. Average pheromone content of about 37 mg/dispenser

Control date	mean dispenser weight (mg)	mean air content (cm)	n° days from previous control	daily weight decrease (mg)	daily air increase (cm)
02.05.96	815.6	2.74	-	-	-
21.05.96	814.8	3.56	19	0.04	0.043
04.07.96	808.8	6.28	45	0.13	0.060
17.07.96	805.9	7.15	13	0.22	0.067
31.07.96	804	8.02	14	0.13	0.062
21.08.96	798.9	9.09	21	0.24	0.051

Effectiveness assessment.
Effectiveness of the technique on overwintering larval populations was assessed by sampling branches, and inspection before and after each application of dispensers. Pruned branches were collected in April and separated directly in the field into 3 classes: class A -the basal portion of the branch and connection portion with lateral canes (wood more than 3 years old); class B - the intermediate wood (2-3 years old), and class C - wood grown in the previous season.

Wood was measured; wood of class A was placed in emergence boxes in a warm room (25 °C - 70% R.H. – 17:7 LD photoperiod) to assess the number of adult moths and parasitoids emerging. After 5-6 months, rearing boxes were opened to collect adults that could not exit. Wood of classes B and C was opened in laboratory to extract the larvae.

Flight of male adult moths in the field was assessed using pheromonal traps; 1 trap was placed in the middle of the treated field. Two additional traps were set in the nearest red currant orchard (treated with malathion and carbaryl against *Synanthedon tipuliformis*).

Results and discussion

Trap catch
The total number of *Synanthedon tipuliformis* males captured in 1994 to 1997 in the traps placed outside the trial field is presented in figure 1. Data for the first two years precede the mating disruption trials, and data for 1998 are not available.

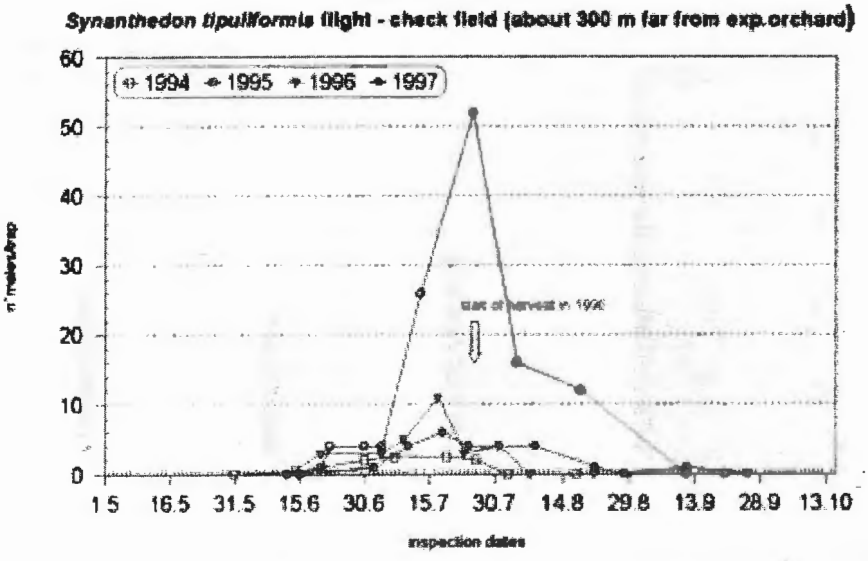

Figure 1. *Synathedon tipuliformis* flight in the nearest red currant orchard

The pattern of flight in the four years indicates a peak of emergence in July. The size of the population in 1995 is much bigger in comparison with the other years. Since there were no particular differences in chemical treatments, this increase may be explained by the biology of the insect. According to several authors (Zuccherelli, 1970; La_t_vka, 1983), clearwing moths may have different lengths of development depending on climatic and particularly on temperature conditions: 1-year development in warm, but 2-years development in cold conditions. Since the 1993-94 season was much colder than the following one, we can suppose that in 1995 our traps monitored the emergence of 1993 and 1994 larvae. On the other hand, the 1995 increase may be related to a cyclical fluctuation of *Synanthedon tipuliformis* populations.

No males were caught during the three years when a trap was placed in the experimental orchard. This indicates a good overall effect of mating disruption.

Mating disruption and parasitisation

The number of larvae/m of wood was drastically reduced during the three years of the mating disruption experiment (figure 2). The lowest infestation was reached after two years of application, although an increase was recorded after the third year. This increase may be the result of fewer dispensers being used in 1998. The trial site was particularly windy. In view of this, and the size of the plantation, a higher rate of dispensers would have been appropriate.

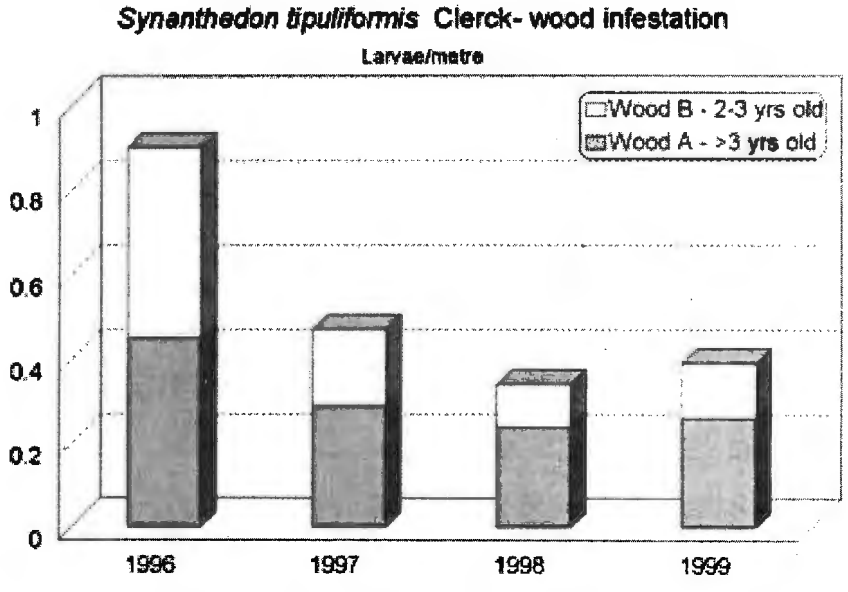

Figure 2. Larvae/m of wood

Interestingly, the highest percentage of decrease in the numbers of larvae was observed in wood of class B. It is possible that the covering effect of the pheromone was better on the youngest wood, since dispensers were hung on this portion of the branches. Moreover, wood of class A is more susceptible than the youngest wood to larval infestation.

The percentage of larvae infesting class A wood that were parasitised by Braconid wasps varied from 38.8% to 55% (Table 2). Larvae infesting wood of class B were also parasitised, but data have not been included, since many larvae on this wood were killed during the extraction.

Parasitoids, identified as *Macrocentrus marginator* Nees (Hymenoptera, Braconidae), started to emerge a few days after wood collection and storage in the warm room (Fig. 3).

The first parasitoid pupae were observed in mid-April on wood of class B. This suggests that they probably overwinter at the larval stage inside their host. Pupation occurs early in spring, when they kill their host emergence. Males emerged before females. Most of the adults collected were females.In the warm room, *Macrocentrus marginator* started to emerge about 17 days before *S. tipuliformis*. This indicates that the parasitoids are probably already active when *S. tipuliformis* starts to fly in the field.

Figure 3 shows two clear and separate peaks of emergence, probably depending on the larval stage of *S. tipuliformis* when parasitised. Adult wasps emerging in June parasitised the last larvae of the previous season. Most of parasitisation probably occurs on the first *S. tipuliformis* larvae of the next generation.

Table 2. Summary data of *Synanthedon tipuliformis* mating disruption trial

	1st sampling	2nd sampling	3rd sampling	4th sampling
n° of old branches inspected (min. 3 ages of wood present)	325	302	309	214
% of old branches inspected on the total branches in the field	13%	12,30%	11,25%	8,30%
total length of wood of class A inspected	219,1	221,7	219,95	181,01
total length of wood of class B inspected	650,2	912,85	249	0
total length of wood of class C inspected	685,85	799,55	869,92	682,18
mean n° of larvae/metre of wood of class A [a]	0,45	0,29	0,24	0,26
mean n° of larvae/metre of wood of class B	0	0	0	0
mean n° of larvae/metre of wood of class C [b]	0,45	0,18	0,1	0,13
% of larvae parasitised on wood of class A	55%	39,30%	38,80%	47,90%

[a] Adults of *S.tipuliformis* emerged in warm room + parasites larvae (1 parasite means 1 S.tipuliformis larvae)

[b] Larvae of *S.tipuliformis* alive + larvae killed during extraction + parasitised larvae + parasites larvae or cocoons

This was confirmed by observations made during wood of class B inspections too. The major part of parasitoid pupae was found inside short tunnels, close to *S. tipuliformis* entrance holes below pruning cuts of the previous year. This suggest that parasitisation probably occurs early

in the spring, when larvae are still young. Parasitised larvae continue to live and feed inside the wood, but always with less intensity and they remain alive over the winter.

Pheromone release from dispensers

The approximate empirical measurement method indicated that pheromone emission in the 1996 trial conditions was slow and prolonged (Table 1). About 20 mg of pheromone/dispenser (54% of its content) were still available on 21 August, after more than 3 months exposure.

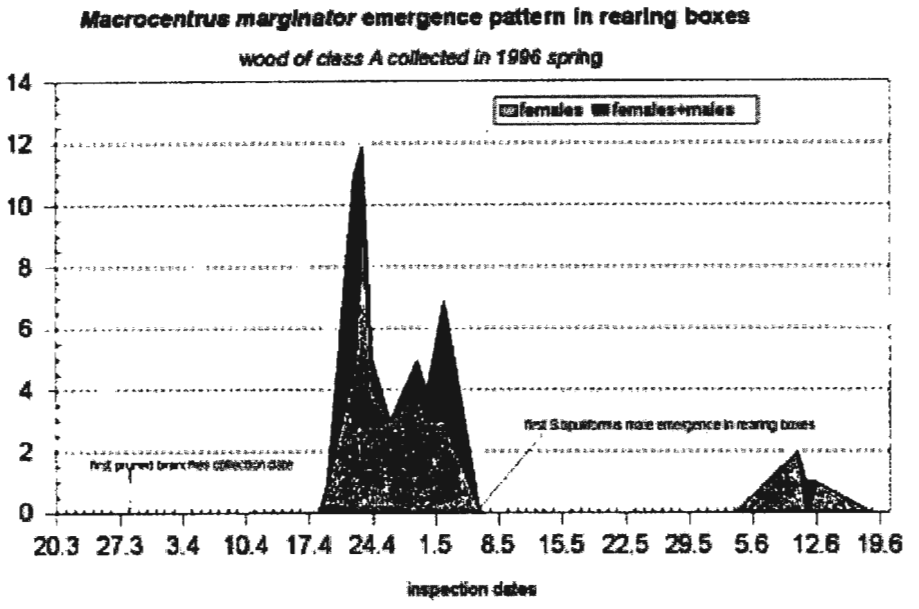

Figure 3. *Macrocentrus marginator* emergence pattern in rearing boxes

Maximum daily pheromone release rate from dispensers (0.24 mg) occurred during August, which is usually the hottest month of the summer in the region. It is equivalent to a theoretical release rate of 0.014 mg/sqm/day. Minimum threshold for *Synanthedon tipuliformis* mating disruption, fixed by Shin-Etsu to 0.2 µg/sqm, is supplied even when there is the minimum daily decrease of 0.04 mg during May, when pest flights have not yet started (0.04 mg/dispenser correspond to 0.23 µg/sqm/day).

Data suggest that a single application of dispensers theoretically guarantees an adequate covering during the whole *S. tipuliformis* flight period. If we assume that the larval infestation recorded after mating disruption applications results from mating between couples of *S. tipuliformis* inside the trial field, this probably occurred during windy days when the pheromone cloud was dispersed. In summer in this area, the wind blows with a speed ranging between 5 and 25 m/sec. (data collected by IASMA meteo-station placed at 1 Km from the trial field).

Conclusions

Results of this trial can be considered satisfactory. Larval infestation has been progressively reduced to tolerable levels by three consecutive years of mating disruption application. Parasitisation by *Macrocentrus marginator*, not disturbed by insecticide sprays, completed the control action.

Results are particularly interesting considering the main features of the trial site, which are typical of our region; small fields, windy sites and, normally, severe larval infestations. In addition, there are difficulties with chemical control, such as the availability of inadequate active materials (malathion, carbaryl, lambda-cyhalothrin and mineral oil), and the inability to apply sprays during the peak of adult emergence because it coincides with the fruit harvest period.

Larval infestations increased in the last year of application, when the number of dispensers was reduced to 406/ha: this suggests that a minimal application rate of 500-600 dispensers/ha should be used every year.

This trial indicates that mating disruption may be considered as an alternative low impact control method to reduce pest populations progressively in red currant fields. It should be integrated with monitoring, cultural practices, preventive measures application and *Macrocentrus marginator* exploitation (avoiding harmful treatments).

Uptake of the mating disruption method to control *Synanthedon tipuliformis* on red currant depends on the registration of Isomate CCM dispensers in Italy. Preliminary toxicological and eco-toxicological studies are not available for this product, and the costs of such studies could not be justified, given the limited potential acreage in Trentino. Use of data for registration of other similar dispensers and pheromones could be the only way to get an easier and faster registration.

Acknowledgements

We wish to thank APA S.Orsola Cooperative, that supported by grants this trial, Mr. Cimadom Enrico and Mrs.Rodler Celestina, who permitted to test this method supplying us their plantation, Mr Veronelli Vittorio (CBC Europe) for his precious help for contacts with Shin Etsu company and dispensers supplying. A special thank to Dr. Ing. C. van Achterberg for the identification of the parassitoids (Dr. Ing. C. van Achterberg – Curator of Hymenoptera and Diptera, National Natuurhistorisch Museum, Postbus 9517, 2300 RA Leiden, Netherlands). Many thanks to Dr Trefor Woodford (SCRI Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA UK) for the language revision.

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Mating disruption - methods

Use of pheromones and other semiochemicals in integrated production
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Can wind tunnels help improve the efficacy of mating disruption?

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Abstract: Wind tunnels can provide useful information for improving mating disruption in the field. However, certain precautions must be taken. Levels of disruption determined in the tunnel may be an overestimate unless allowances are made for males remaining active for several days and locating females in subsequent flights. On the other hand disruption might be underestimated because many males that locate females in the tunnel may not be able to sustain orientated flight long enough in the field to reach calling females. Levels of disruption for spruce budworm and Oriental fruit moth were similar at similar concentrations of atmospheric pheromone with a significant level at 20-30ng synthetic pheromone/m^3. However, disruption in the spruce budworm was primarily due to males flying to the wrong source, but in the Oriental fruit moth disruption was primarily due to sensory fatigue. This suggests that different mechanisms of disruption will occur for different species and that different formulations may be appropriate for different species.

Key words: mating disruption, wind tunnel, behaviour, spruce budworm, *Choristoneura fumiferana*, Oriental fruit moth, *Grapholita molesta*, Tortricidae, Lepidoptera

Introduction

The year 2000 marks the jubilee, not only of the IOBC-WPRS Working Group "Use of Pheromones and Other Semiochemicals in Integrated Control", but also the discovery in 1975 that the sex pheromone of the spruce budworm, that had been previously identified as E-11-tetradecenal, consisted in fact of a blend, the E-11-tetradecenal plus the isomer, Z-11-tetradecenal, in a ratio of 97E:3Z (Sanders and Weatherston 1976). That discovery marked the start of work on the application of the pheromone in the integrated control of spruce budworm.

Research covered two fronts, the use of pheromone-baited traps for monitoring population densities, and the use of the pheromone for controlling populations by mating disruption. The first application, population monitoring, is now in operational use throughout North America, and recent developments in this program are presented later in this symposium (Lyons et al.). In addition to the trapping program, research was also carried out to assess the potential of controlling budworm populations by using synthetic pheromone components or their analogues for mating disruption.

Small-scale field trials showed promise, and trials were therefore scaled up to the semi-operational stage with the aerial application of pheromone in various formulations. However, these all showed limited success, possibly due to shortcomings in the formulations or to the ever-present possibility of female moth migration into the treated plots. Such field trials are costly, and, because spruce budworm are univoltine, only one trial can be carried out each year, limiting the rate of progress. Therefore attention was turned to the possibility of using a wind tunnel to refine the technology.

The characteristics of the tunnel have been described elsewhere (Sanders et al. 1981). It differs from most other tunnels used in pheromone research in that all the surfaces are glass, for easy cleaning, and the moving background pattern that can be used to prolong moth flight

is in the ceiling and not the floor. The standard protocol used in all experiments was to house virgin female moths in small cages at the upwind end of the tunnel and then to release male moths at the downwind end of the tunnel and monitor their responses. With appropriate conditioning at least 90% of the male moths regularly took flight, locked on to the pheromone plume from the females, and flew upwind to the caged females. To assess the impact of synthetic pheromone components and pheromone analogues on male behaviour and their success in locating the female moths, the candidate chemicals were incorporated into rubber septa which were then arranged in a 3-dimensional array surrounding the caged females. Using this technique it was simple to show that pheromone homologues, the 14-C acetates or alcohols which are components of a sibling species, the jack pine budworm, had no effect as disruptants. Furthermore it was established that disruption of the males was greatest with pheromone closest to the natural E:Z ratio of around 95:5 (Sanders 1995). Therefore all subsequent experiments concentrated on this blend.

One of the key questions in field trials, not only for the spruce budworm, but for mating disruption of most pests, is what concentration of pheromone is necessary to achieve a given level of disruption. Accordingly, the candidate chemical that was being tested for disruption was loaded into rubber septa, and these were then pinned to a wire frame in 4 rows. The first and third rows contained 6 evenly spaced septa, the second and fourth had 2 septa positioned in the gaps of the other two rows. Each septum was backed by a 2.5 x 2.5 cm tape to create turbulence. The result was a 3-dimensional array of 16 septa around the caged-females. In order to approximate conditions in the field, where males would be exposed to the ambient pheromone during the day before becoming active in the evening, the male moths were left in the ambient pheromone conditions to acclimatize for several hours. Their behaviour was then compared to that of moths kept in clean air. To achieve this, the tunnel was divided horizontally into two halves, with clean air in the bottom half and the pheromone treatment in the top half. This ensured that the two groups of males were subjected to the same conditions of airflow and light intensity. Each day the male moths for assay were put individually into small cages, and one half were kept in the top half, one in the bottom. They were then moved into position, one at a time, directly down-wind of the caged females and the lid of the cage was removed. In the following presentation the results are summarised under 4 categories: a) flying to the caged females; b) flying to a septum; c) arrestmant (ceasing upwind flight and veering off to the surface of the tunnel); d) inactivity (not taking flight although shown to be capable of it).

Results

As the loading of pheromone in the septa increased so the number of males reaching the females decreased and the number reaching the septa instead increased (Figure 1). An important point to note here is that there was virtually no arrestment of activity among the naïve males (those that had been kept in clean air with no previous experience of pheromone). Among the experienced males (those that had been held for 3 hours in the ambient pheromone) the percentage of males reaching the females or the septa was very similar to that for the naïve males, but a significant number of males became incapacitated, presumably because they were much more active while kept in the ambient pheromone conditions which resulted in damage to their wings. Again, as with the naïve males, there was very little arrestment of activity. This implies very little sensory fatigue (adaptation or habituation). This same pattern was found even when males were held in the ambient pheromone for four days (Sanders 1996).

It can argued that demonstrating disruption with rubber septa in a wind tunnel has little

relevance to field conditions where different types of formulation are used. One way of making the data more relevant is to determine the atmospheric concentration of pheromone necessary for disruption. Once the atmospheric concentration necessary for a satisfactory level of disruption has been determined in the tunnel, then formulations can be designed to achieve this concentration in the field. Ideally atmospheric concentrations of pheromone should be determined by direct measurement. However, so far attempts to do this have failed due to lack of sensitivity of the techniques at the lowest concentrations. Possibly this could be achieved by using the technique described by Koch later in this symposium. In the absence of a direct method, estimates of ambient concentration were made by using the formulae of (Butler and McDonough, 1979) and adjusting these for wind speed and the cross section of the tunnel. There is no clear threshold figure above which disruption is successful, however, the calculations suggest that an atmospheric concentration of ca 20 ng/m^3 is necessary to produce a significant amount of disruption (Figure 1).

A weakness of these experiments is that observations on each male were terminated when the males reached a pheromone source or when they stopped flying, whereas in the field they remain available and may make repeated attempts to locate the pheromone source. The importance of this can be assessed by determining the behaviour of males subsequent to the end of normal observations. Do they remain quiet or do they become active again and resume the search? To answer this the activity of males was recorded over a 24 hour period using a video-recorder. The numbers of re-visits by the males to the pheromone sources was then recorded by replaying the video tapes. The results (Sanders 1995) showed that from groups of 12 males left in the tunnel there were on average over 40 subsequent visits to the caged females with a background of septa loaded with 1μg of synthetic pheromone and 15 with a loading of 10μg.

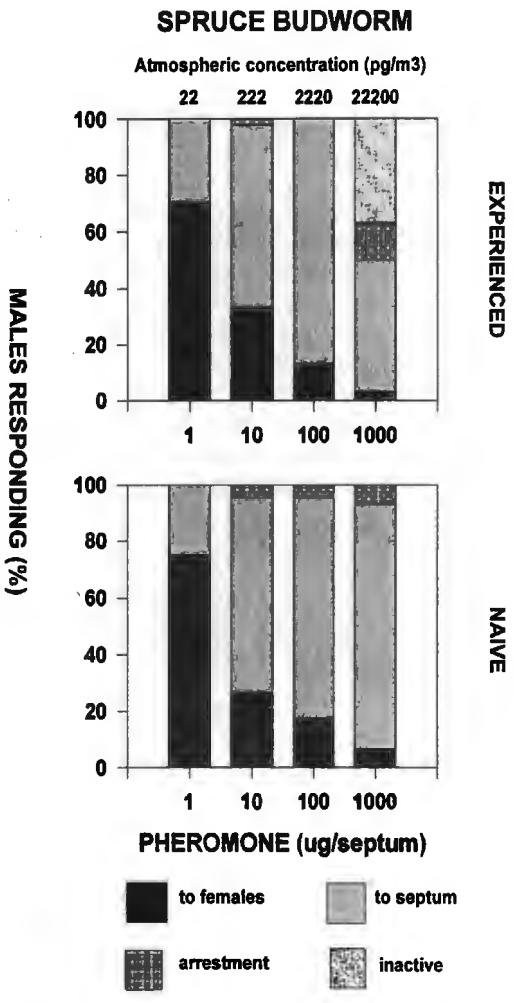

Figure 1. Responses of male spruce budworm to caged virgin females surrounded by septa loaded with synthetic pheromone. From Sanders and Lucuik (1996)

Another shortcoming of the tunnel is that the distance males had to fly before they reached the females or a septum was less than 2 m and took only a few seconds.

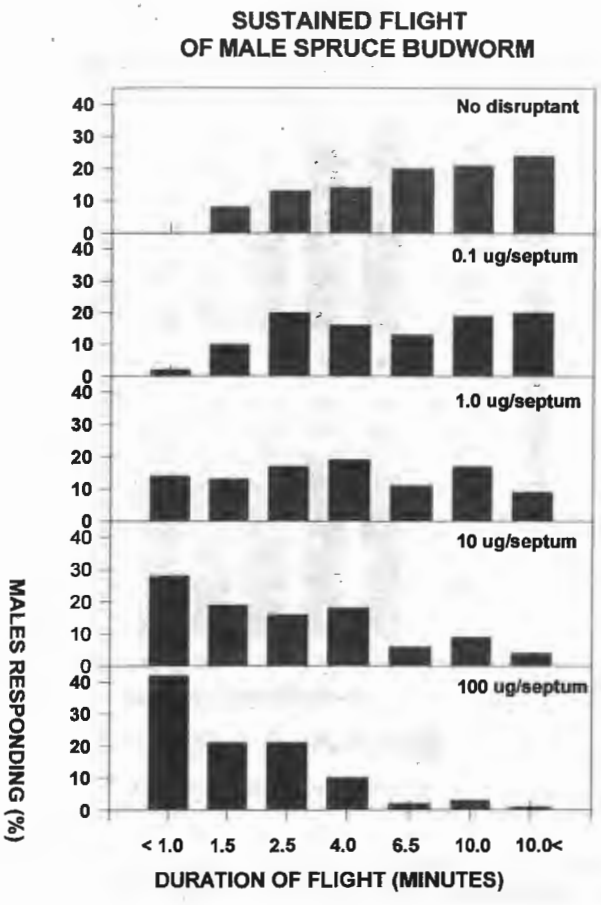

Figure 2. Duration of sustained flights of male spruce budworm to caged females surrounded by septa loaded with synthetic pheromone in a wind tunnel. Flights sustained by a moving ceiling pattern. From Sanders (1998)

In the field they may have to fly many metres, and flights may last for a minute or more. To determine the significance of this, the moving ceiling pattern of the wind tunnel was utilised. Males were released as before in different concentrations of ambient pheromone, but when the moths showed orientated flight (zig-zagging) the ceiling was set in motion and the duration of sustained flight before the moths landed on the tunnel surfaces was recorded.

In clean air (no septa present) males flew for several minutes with many flights longer than 10 min, and one 65 min (Figure 2). As the concentrations of synthetic pheromone released into the air space increased so the duration of flights decreased (Figure 2), i.e. arrestment occurred much sooner. Therefore, in addition to disorientation, the chances of many males that lock-on and reach the females in the wind tunnel may be reduced under field conditions because of sensory fatigue during the longer flight times.

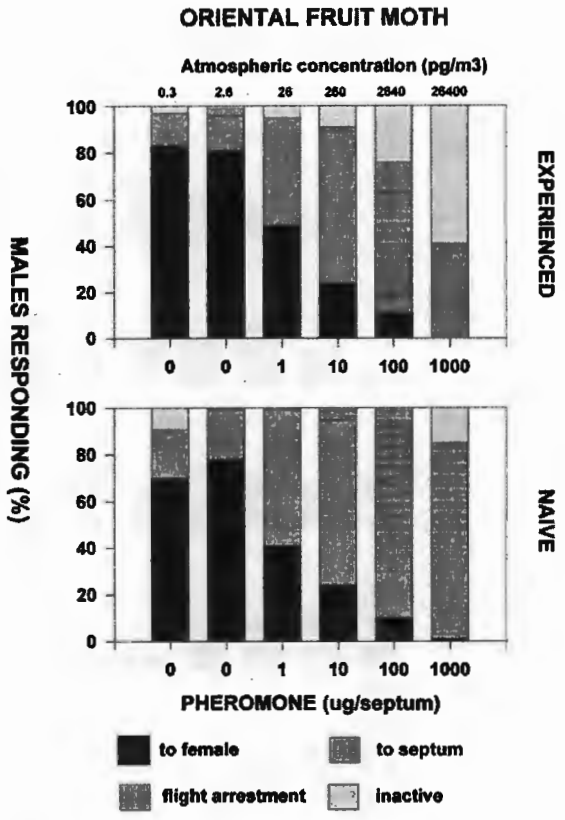

Figure 3. Responses of male Oriental fruit moth to caged virgin females surrounded by septa loaded with synthetic pheromone. From Sanders and Lucuik (1996)

A further question is how do these results compare to those that might occur with other species. Therefore for comparative purposes, similar experiments were carried out with the Oriental fruit moth. The insects for these experiments were kindly provided by R.M. Trimble, AgCanada, Vineland, ON. They were treated in a similar manner to that described above for the spruce budworm. The results (Figure 3) show a similar level of disruption to that found for the spruce budworm, as measured by the numbers of males successfully locating the caged females. However, when comparisons of the mechanisms of disruption, it can be seen that disruption of Oriental fruit moth was due to much higher levels of inactivity and flight arrestment than were found in the spruce budworm (Figure 4).

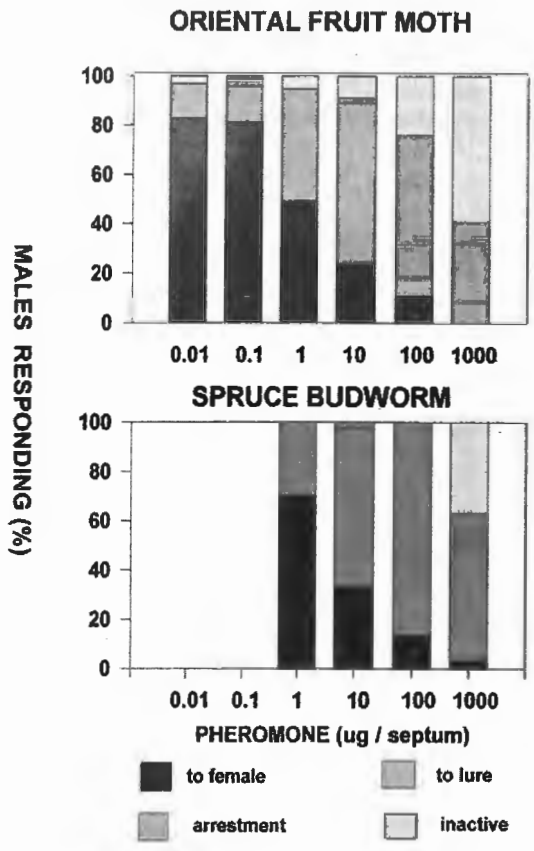

Figure 4. Comparison of male spruce budworm and Oriental fruit moth responses in a wind tunnel, taken from 'experienced' males from Figures 1 and 2

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Use of pheromones and other semiochemicals in integrated production
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Field EAG measurements of sprayable pheromone for mating disruption of *Sesamia nonagrioides*

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Abstract: Results of measurements of airborne pheromone concentrations in maize fields in southern France treated for mating disruption of *Sesamia nonagrioides Lef.* with a sprayable formulation are reported for the period from 1997 to 1999. In order to obtain short sampling times, results without delay and limited equipment cost, a portable field EAG measurement system was used. The concentration measurements were made from the day of spray treatment over a period of up to 10 days in intervals of 1 to 3 days in different fields. The results show a consistent picture of the gradual decay of the pheromone concentration over time.

Key words: pheromone, mating disruption, sprayable formulation, field EAG, portable electroantennogram, pheromone concentration, time dependence, concentration decay

Introduction

In the development of a sprayable pheromone formulation, one of the key questions is to assess the time span over which the sprayed product is actively suppressing mating activity in the target pest. Apart from observations of trap shutdown, one would like to have a reliable measurement of pheromone concentration in the field. The standard analytical method (Flint, 1993) consists of sampling large amounts of air, trapping the pheromone on an adsorbing agent and analyzing the eluate by GC-MS. This method yields absolute pheromone concentration values, but sample processing is time consuming and results are often available only after considerable delay.

The field EAG measurement system developed in our lab is capable of providing reliable pheromone concentration measurements, yielding results within an hour after the measurement. It has been used to measure pheromone concentrations in vine yards (Milli, 1990; Sauer, 1991; Karg, 1992; Färbert, 1992, 1995; Termer, 1992; Karg *et al.*, 1990, 1995; Karg and Sauer, 1995; Koch *et al.*, 1992, 1995), in cotton fields (Cardé *et al.*, 1993; Färbert and Koch, 1993; Färbert, 1995; Färbert *et al.*, 1996, 1997), in pea fields (Bengtsson *et al.*, 1994), in apple orchards (Milli, 1993, 1994; Suckling et al., 1994; Milli et al., 1997; Koch et al., 1997; Witzgall et al., 1999), in forests attacked by gypsy moths (Thorpe et al, 1999 unpublished), in cranberry bogs (Polavarapu et al, 1999 unpublished), and in alpha alpha fields (Cardé et al, 2000, unpublished). Since 1993, our system makes use of a sophisticated calibration system, and a special signal superposition technique to suppress the influence of plant odors and other non-pheromone airborne stimuli on the pheromone concentration measurements. The fast measurement cycle and short evaluation time permit to follow up on the development of the pheromone concentration on a day by day basis, to construct a decay-curve and even signal the moment when retreatment would be advisable, in accordance with trap shutdown data.

Methods

The field EAG system used in these experiments has been described in detail in Färbert et al. (1996, 1997). It consists of an excised antenna of *Sesamia nonagrioides* placed in a special antenna holder. The holder is mounted in an antenna chamber 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. The antennal responses to activation of the calibration syringes with pheromone concentrations in three decade 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. Therefore, it cannot be used as a reliable measure for pheromone concentration. While the filter remains open, additional calibration pulses are released. The additional responses of the EAG signal to the superimposed calibration puffs are used to calculate the airborne pheromone concentration using a mathematical model of the antenna and the data from the dose-response curve (calibration). The repetition of the calibration every 50 seconds avoids errors which could arise from the continuous change of the antenna's sensitivity.

An important parameter is the threshold of a given antenna. This is the pheromone concentration at which the antenna ceases to yield clear responses. Pheromone concentrations below this threshold cannot be detected by the field EAG measurement system. Antenna thresholds vary from one male to another and vary also over the time of the antenna's use. Some correlations between the quality of antennae and the general fitness of the male have been observed, but clear effects of changes in the rearing method on antenna quality have not been established.

The relative pheromone concentration units used in 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 1 part of pheromone. This concentration value has been measured to be in the order of 1 ng/m^3 in the case of *Cydia pomonella*. 1 ng/m^3 is a concentration value usually found in successful mating disruption experiments involving standard dispensers.

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 positioned on a pole between 0.3 and 2.8 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 2.8 m height.

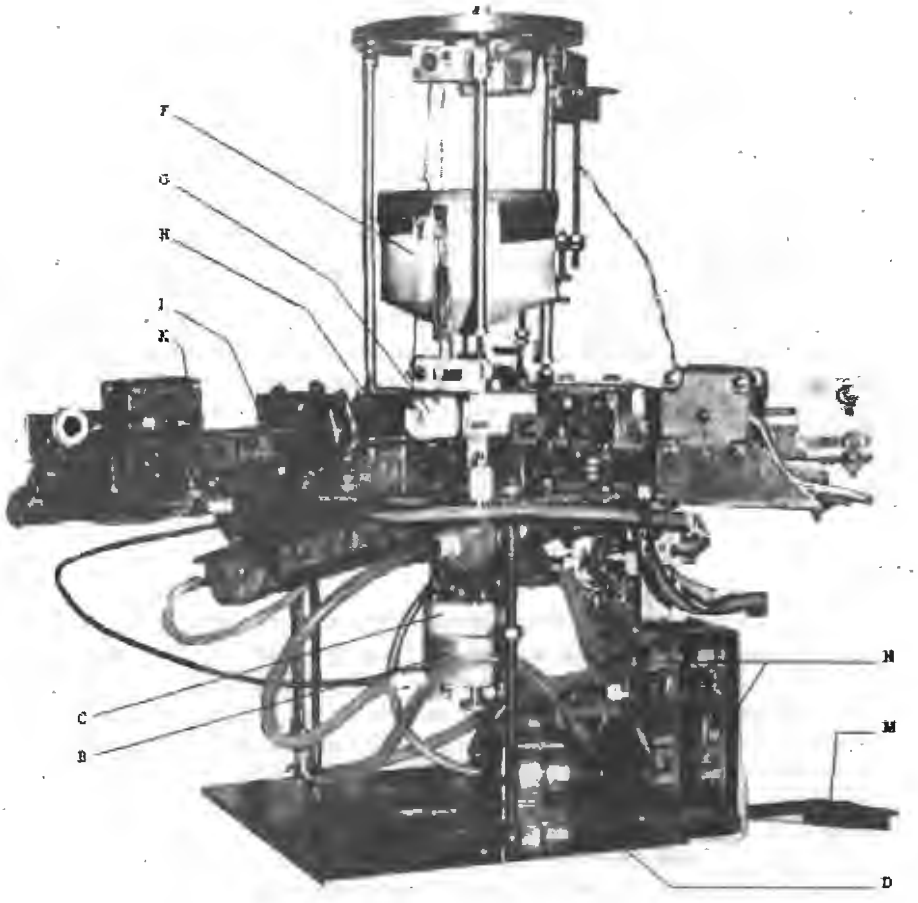

Figure 1. View of the EAG measurement probe: B: antenna chamber, lower part; C: antenna chamber, upper part; D: suction pump; F: charcoal filter; G: teflon vial with mixture of pheromone and oil; H: calibration syringe; I: piston rod; K: step motor; M: main cable; N: flow regulator

Measurement sites and conditions for EAG measurements

In 1997, one field (St.Avit) in the area of Revel was measured on the day of the treatment and during the following 3 consecutive days.

In 1998, three fields (UE 2, NPP 2, and NPP 5) in the Revel area were routinely measured to follow the development of the pheromone concentration over time: Each field was measured on 5 to 7 individual days in a time span of 0 to 9 days after treatment

In 1999, we measured pheromone concentrations in 4 different fields (Peyre, Lassentiat, Caucou and Miracle) in the area of Saverdun (Ariège). In order to track the development of the pheromone concentration in each field, we took measurements on three or four days, most often with one or two days of pause. Apart from days with batches of rainfall, the most stringent reason for the pauses was the extensive irrigation.

The measurements were always made in the early morning hours (6:00 to 9:00) in order to record the pheromone levels in wheather conditions close to the ones existing at the flight time of *Sesamia.* Care was taken to measure always at a position in the field where the wind was coming from a major part of the treated field in order to load up the air with a representative amount of pheromone. In order to establish a vertical concentration profile, three probe heights were routinely used: 30 cm (ground level), 150 resp. 100 cm (within the maximum foliage) and 270 cm (above the canopy). The plant height was around 220 cm in 1998 and 200 cm in 1999.

One important disturbance in the measurements was the irrigation. Measurements could not be made during or within 6 hours after irrigation. In the Revel area (1997 & 1998), this posed not a major difficulty, since irrigation was scheduled once a week or once every 4 to 5 days. In the department of Ariège, irrigation turned out to be a problem. Since the type of maize in the Ariège fields was for seeding purposes, farmers were maintaining a high rate of irrigation to keep the maize turgid, since this is an advantage in the different castration operations. As a consequence, irrigation was present almost every other day. In one case, we measured directly after a rainfall and were not able to detect any pheromone. The pheromone signal reappeared the next day, however. Since then we tried to schedule our 1999 measurements in such a way that they were not influenced by the irrigation.

Results

Figure 2 shows a representative measurement result from the measurement site NPP2 (1998). The graph shows a clear pheromone signal in the range of $0.9*10^{-6}$ to $2.0*10^{-6}$ relative units, well about the individual antenna threshold levels. The concentration is very similar near the ground (30 cm) and in a medium height (150 cm) near the maximal density of the foliage. In all cases, there is a clear drop of the concentration at 270 cm probe height, which is somewhat above the canopy height of ca. 200 cm. This indicates that within the foliage, the pheromone is evenly distributed from the ground to the canopy. Above the canopy, the wind mixes the pheromone with fresh air and thus the pheromone content is diluted.

In order to gain an overview over the performance of the spray formulations at each measurement location, the measurement results from probe heights 30 and 100 cm of each measurement day were averaged. These averages were plotted versus time after the treatment. Figs. 3, 4 and 5 show the results from NPP2, NPP5 and UE2 as examples.The figures show a continuous decay of pheromone concentration over time.

Considering the different averaged concentration curves from the individual fields, several common characteristics can be discerned. The high values found shortly after treatment go down to the threshold level and seem to stay there. This does not mean, however, that the pheromone level stays constant at this value. Rather, we must assume that the pheromone concentration decays further. But whenever the antenna threshold is reached, the decay cannot be tracked any more, since the measurement system cannot distinguish between „pheromone" and „no pheromone" below the threshold. Any hypothesis about the dynamics of the decay therefore can only be tested in the range between initial concentration and threshold.

For all EAG graphs, we chose a logarithmic concentration scale. In such a diagram, a decay following an exponential law must appear as a straight line with a negative slope. An exponential decay can be a valuable first approximation to the time course of the pheromone concentration decay process since it is the adequate description in a system where the rate of evaporation is proportional to the amount of substance remaining.

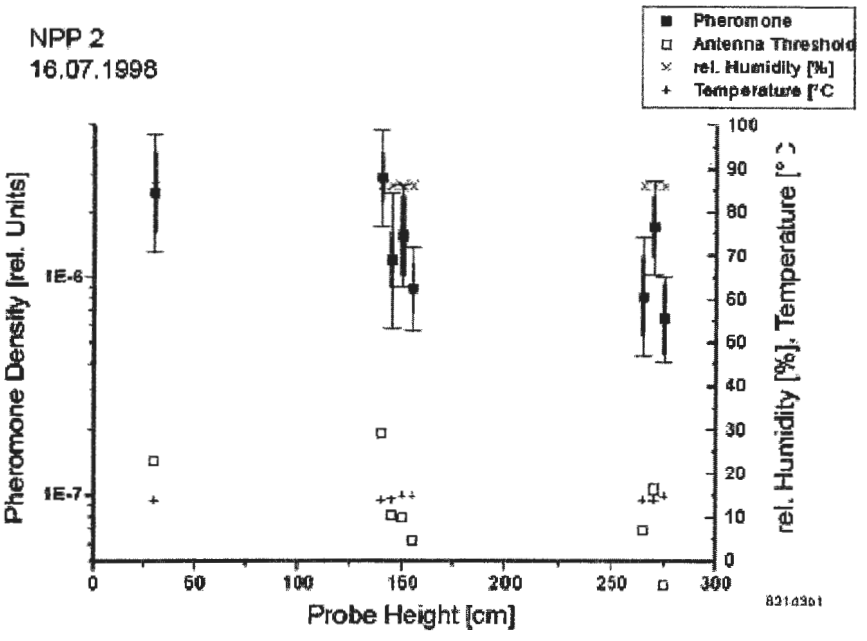

Figure 2. Typical measurement result from a field treated with spray formulation. The pheromone concentration is plotted versus probe height. The thin error bars with caps indicate the confidence interval of each individual measurement. The thick black error bars (without caps) represent the confidence interval of the mean of several measurements as an indicator of the variability of the individual pheromone density readings.

Assuming that this decay dynamics is valid, we can fit straight lines into the time course graphs recorded from all the different fields and years. The slope of these graphs yield a time constant, i.e. the time it takes for the concentration to decay to 1/e of ist initial value.The results of these fits plotted in Fig 6 show a slight increase in the time constant between 1998 and 1999, indicating that the sprayed product showed a slightly longer lifetime in 1999. However, the very large error bars have to be considered, so that a significant time constant difference between 1998 and 1999 can hardly be established. Apart from differences in antenna quality, the larger errors in 1999 stem mainly from the restrictions in possible measurements imposed by irrigation and rain.

The results of the EAG measurements 1997-1999 show the the pheromone concentration decays exponentially with time constants of 5 to 8 days. A slight tendency for longer time constants seems to be visible for the 1999 NPP formulation. The TNO formulation performs as well or better than the NPP, however the precision of these results should be improved. Strong reductions of the pheromone concentration were observed in 1999 immediately after irrigation. A return to previous concentration values was found when the maize leaves had dried. This effect might reduce the efficacy of mating disruption in regions with very frequent irrigation or rainfall.

A spayable formulation capable to evaporate the pheromone more evenly over time would be advantageous for further improvement of mating disruption in maize fields.

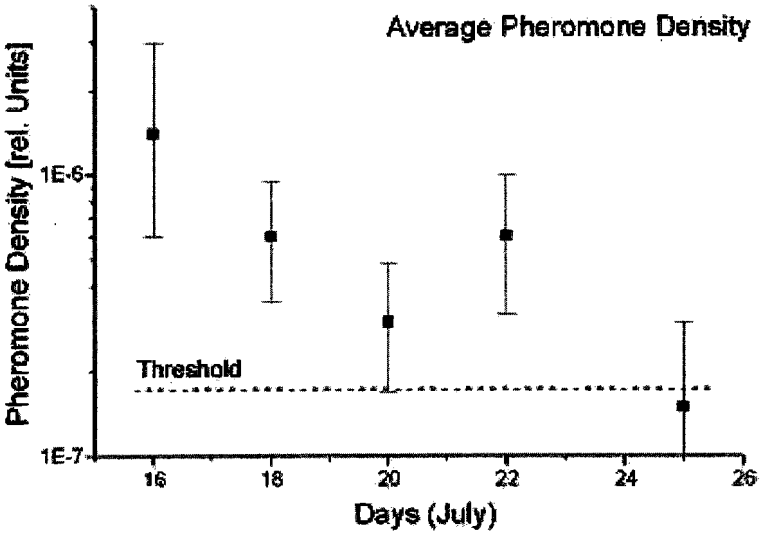

Figure 3. Development of average pheromone concentration over time after spray treatment on July 16 1998 at NPP2. After about 8 days, the pheromone concentration had decayed tothe theshold, which was about 10 times below initial concentration.

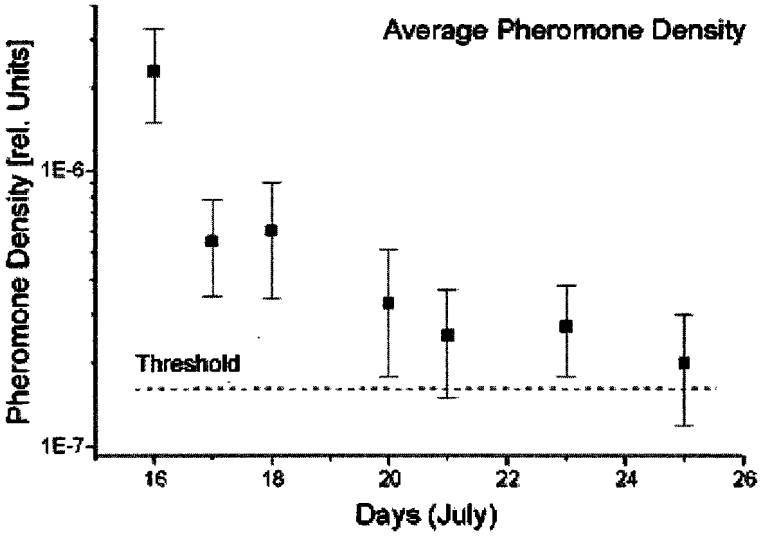

Figure 4. Development of average pheromone concentration over time after spray treatment on July 16 1998 at UE2. Note the very high initial concentration. After about 8 days, the pheromone concentration had decayed to a level about 10 times below initial concentrations

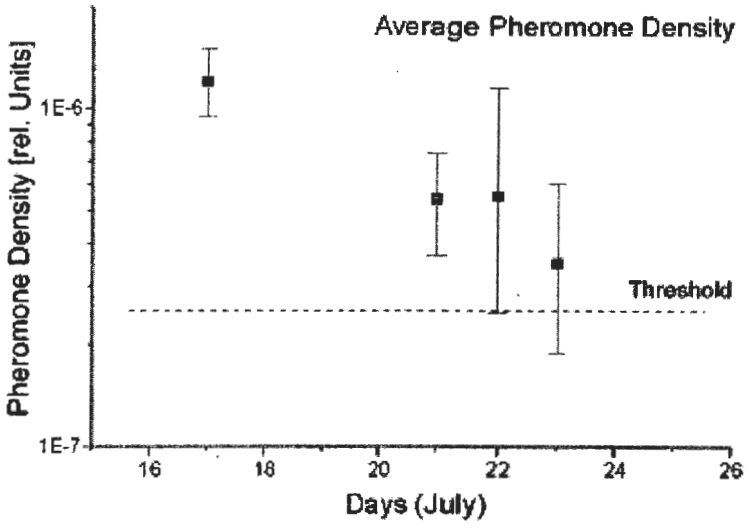

Figure 5. Development of average pheromone concentration over time after treatment at NPP5. After ca. 7 d, the pheromone concentration has reached threshold and cannot be tracked further.

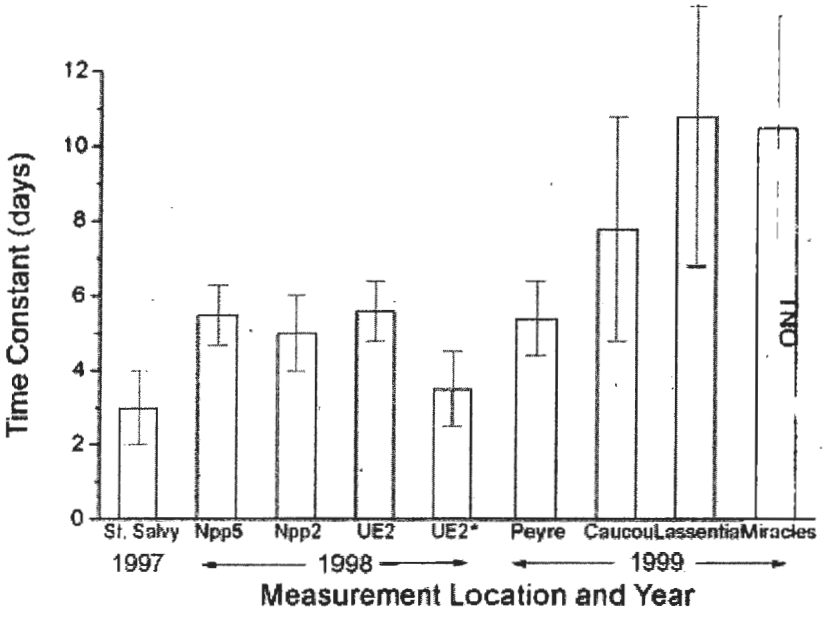

Figure 6. Time constants of pheromone concentration decay in the fields measured in 1997-1999. A slight trend to longer time constants can be observed from 1998 to 1999. The larger error bars in 1999 are mainly due to the restrictions in measurement schedule imposed by irrigation and rainy weather. The value of UE2* stems from an alternate evaluation including the very first data point.

Acknowledgements

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Use of pheromones and other semiochemicals in integrated production
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Methods for reliable measurement of pheromone dispenser performance

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Abstract: The standard methods for measuring dispenser release rate – weighing the dispensers between periods of field exposure or measuring the pheromone load remaining in the dispensers - suffer from a variety of systematical errors. We have developed a standardized method to measure the bulk release rate of dispensers within a few days with high resolution and reproducibility. For dispensers releasing more than one pheromone, we have substantially improved existing gas chromatographic methods to measure the release rates of the individual components. Since our methods are non destructive, they can be applied repeatedly over the lifetime of individual dispensers.

Key words: mating disruption, pheromone, pheromone dispenser, release rate, dispenser standard, precision weighing, GC analysis

Introduction

Up to now, the bulk release rate of pheromone dispensers was usually determined in the following way: Individual dispensers were taken from the field, weighed and then put back in the field in the same position, and this procedure was repeated in e.g. 7 day intervals. This procedure is subject to several systematical errors: 1) Since the release rate depends on wind and temperature, this type of procedure inseparably measures both the weather effects and the ability of the dispensers to release pheromones; 2) differences in weight may result from changes in the water content of the dispenser material as caused by changes in relative humidity and rainfall; 3) differences in weight may also occur when dust particles stick to the dispenser surface. We have developed a method for measuring dispenser release rate which avoids the above mentioned systematical errors.

Methods and results

The dispensers are held in a specially developed wind tunnel in which wind speed, temperature and relative humidity are kept constant (Fig. 1). The dispensers are weighed every 12 hours using a scale with a resolution of 0.01 mg. Usually, a period of 4 days is sufficient to determine the release rate with an error of less than 0.1 mg/day (Fig. 3, 4). Depending on the type of dispenser, an accomodation period of 1 to 3 days is necessary to obtain a straight line in the weight decay curve, indicating a stable release rate (Fig. 2).

When dispensers contain several active ingredients, the measurement of bulk release rate as described above cannot yield information about the relative contribution of each ingredient to the bulk release rate. Up to now, the usual method for measuring the release rate in this case was to retrieve dispensers from the field, extract all ingredients remaining in the dispenser, measure their quantity by gas chromatography and repeat this procedure in 2-4 week intervals. Beside the interference of weather effects as discussed above, this method suffers from the fact that for the determination of release rates, a difference between two measurements of active ingredient content from different samples is needed. The relative

error of such a difference measurement is quite high, unless a large number of specimen is tested. This, however, is prohibited by the relatively high costs of GC-analysis

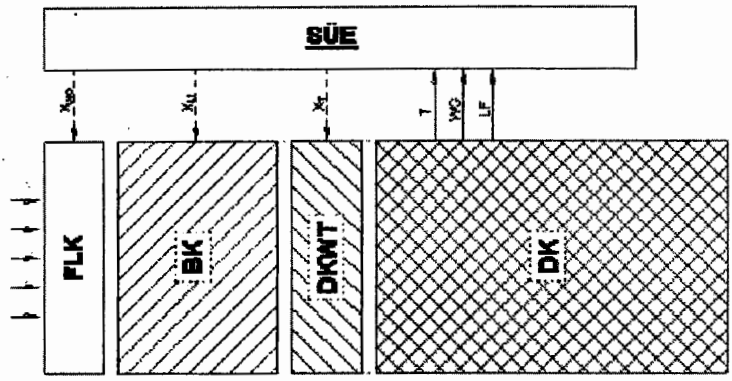

Figure 1. Schematic diagram of wind tunnel for measurements of dispenser bulk release rate. Wind speed (WG), temperature (T), and humidity (LF) are measured at the dispenser chamber (DK) and transferred to the central control unit (SÜE) which in turn varies ventilators (FLK), humidity unit (BK) and heat exchanger (DKWT) to keep these variables constant with high precision. Weight is measured by a precision scale (resolution 0.01 mg) connected to a computer for fast and reliable evaluation.

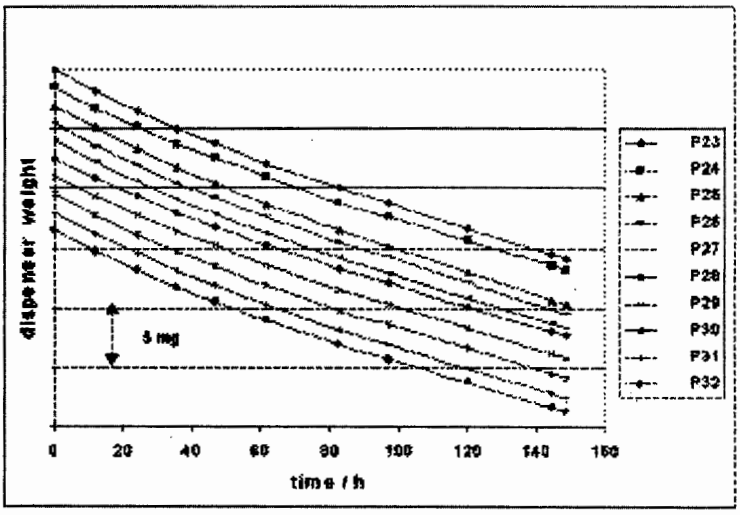

Figure 2. Weight of dispensers as determined with high precision scale plotted versus time (the individual weight of each dispenser at t = 0 was subtracted to yield equally spaced starting points). Note the initial curvature of the graphs which indicates that the dispensers had not yet reached equilibrium values of release rate.

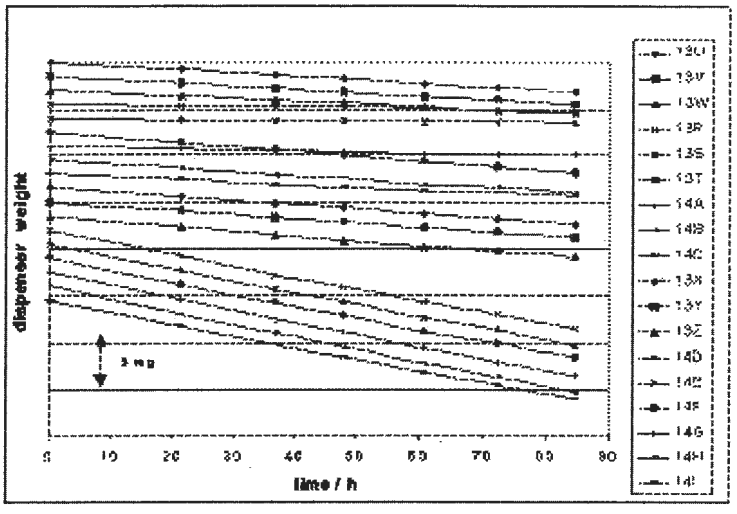

Figure 3. Weight changes of different dispenser types in a routine wind tunnel measurement. Note that dispensers 13R and 13S show extremely small but measurable weight changes.

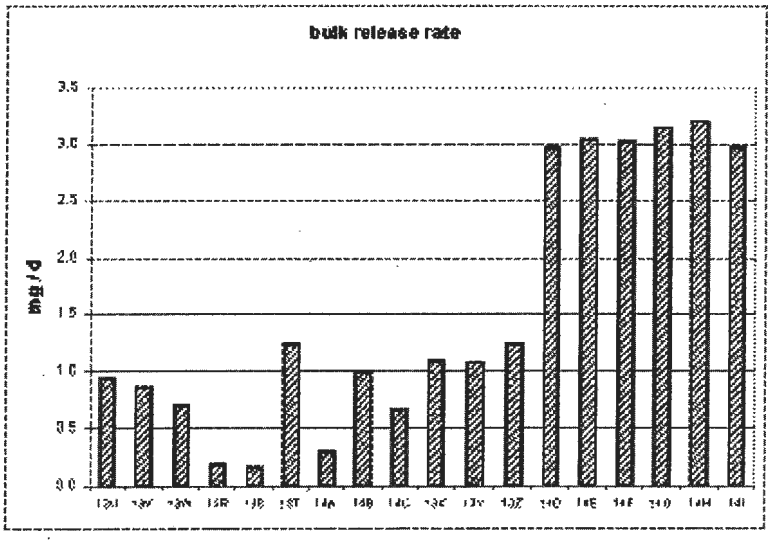

Figure 4. Bulk release rates of dispensers displayed in Figure 3, as calculated from straight line fits to the data of Figure 3. Note that the smallest release rate measured here was 0.2 mg/d (13R & 13S).

Based on a method published by Arn et al.(1997), we have developed a procedure to measure release rates of individual active ingredients in a short time without affecting the dispenser and its properties. The dispenser is mounted in a tube inside a temperature controlled air space (Fig. 5). A stream of air with constant velocity is drawn through the tube by means of a suction pump. After passing the tube containing the dispenser, the whole air stream is drawn through a filter cartridge containing an adsorbent material (Fig. 5). After 1-4 hours of sampling, the cartridge is washed with a solvent which in turn is analyzed for its content of active ingredients by GC analysis.

This type of measurement can yield useful data only if the dispenser is at an equilibrium state, i.e. a condititon in which the release rate has become constant under wind tunnel conditions as stated above. Thus, several dispensers are first observed in a standard bulk release rate measurement. Then, a typical and an extreme specimen are chosen and their release rate of individual active ingredients is measured in the adsorption device. Under normal conditions, the bulk release rate and the sum of the release rates for the individual components match within a 20% error margin.

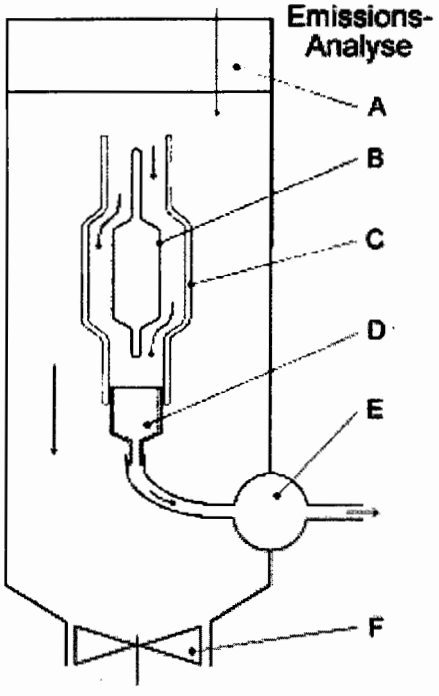

Figure 5. Schematic drawing of method for measurement of release rate of individual components. A fan (F) moves air through the chamber, entering through a heat exchanger (A) driven by a water thermostate. The dispenser under test (B) is mounted in an airflow chamber (C) modelled to maintain constant air speed around the dispenser. A suction pump (E) moves air through the adsorption cartridge (D) which retains all active substances to be later analyzed by GC.

Discussion

We have presented an improved method for the measurement of dispenser bulk release rate. This method measures the release rate independent of weather effects and therefore can be used to compare dispenser performance over different periods of use, climate conditions or production batches.

Together with a specification of appropriate temperature, wind speed and relative humidity in the wind tunnel, it can be used to specify dispenser release rate and its persistence over time as the decisive criterion for comparing dispenser performance between different products.

The use of the method for measurements of release rates of individual components is essential whenever information about the function of multi-component dispensers is needed. Our method offers the possibility to measure the behavior of one individual dispenser repeatedly over time and enables developers and users of dispensers to gain information fast and reliably.

In several cases, we found dispensers which, after a certain time of use, released one main active ingredient normally but were unable to release the other ingredient. In these cases, the bulk release rate measurement yielded „normal" results, and the analysis of dispenser content showed that „enough" oft the critical ingredient was still inside the dispenser. Only the individual component analysis showed that it did not come out any more.

We would like to mention that the facilities at the University of Kaiserslautern are available for measurements of dispenser properties such as bulk release rate, release rate of individual components and contents of remaining active ingredients. We welcome inquiries about measurements in small or large quantities for individuals or commercial enterprises.

Acknowledgements

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Development of aerosol devices for management of codling moth and leafrollers.

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Abstract: The effectiveness of battery-powered aerosol devices (Paramount Aerosol Pheromone Dispenser; aka - puffers) emitting sex pheromones were evaluated for the management of both *Cydia pomonella* and *Choristoneura rosaceana* in replicated field trials during 1999-2000 in 16-ha apple orchards near Brewster, WA, U.S.A. The array of puffers in all orchards was similar: units were placed 50 m from the orchard's edge and spaced 100 m apart. In addition, a 10-m band around the perimeter of each orchard was treated with hand-applied Isomate dispensers at a rate of 1,000 per ha. During 1999 four orchards were treated with puffers releasing 7.5 mg of (*E,E*)-8-10-dodecadienol (codlemone) and 7.5 mg (*Z*)-11-tetradecenyl acetate (Z11-14:AC). Moth catch of released sterile *C. pomonella* and native *C. rosaceana* were compared with similar orchards treated with either 500 or 1,000 Isomate CM/LR dispensers per ha. Four three-week tests were conducted to evaluate the performance of puffers releasing pheromone every 15 min for 24 h, 30 min for 24 h or 15 min for 12 h daily versus the hand-applied dispensers. No differences in moth catch for *C. pomonella* was found among pheromone treatments in any of the four tests. However, significant differences in moth catch of *C. rosaceana* occurred among treatments during three of the four tests. Mean moth catch was higher in the puffer-treated plots than in plots treated with either dispenser rate during June. Conversely, moth catch was lower in the puffer-treated plots than in those treated with the low rate of Isomate CM/LR during the August and September trials. During 2000 the effectiveness of puffers for *C. pomonella* alone or for both species was evaluated in sixteen orchards. Puffers released either 7.5 mg puffs of codlemone, 7.5 mg puffs of both codlemone and Z11-14:AC (dual high), or 5.0 mg codlemone and 7.5 mg Z11-14:AC (dual low) every 15 minutes for 12 hours per day (1500 – 0300 hours). Fruit injury was measured prior to harvest and results were compared with paired orchards treated with Isomate-C+ dispensers at a rate of 500 dispensers per ha. Fruit injury from *C. pomonella* and *C. rosaceana* ranged from 0.0 to 3.1% and 0.0 to 0.9%, respectively among the puffer-treated orchards. The mean *C. pomonella* injury in the puffer-treated orchards was not significantly different than in the Isomate-C+ comparison blocks. Fruit injury by *C. rosaceana*, however, was significantly lower in the dual puffer-treated versus the orchards not treated with leafroller pheromone.

Key words: Sex pheromone, apple, *Cydia pomonella, Choristoneura rosaceana*, mating disruption, sex pheromone, pest management.

Introduction

The technology of emitting the sex pheromone of codling moth *(Cydia pomonella* L.) from a high density array of plastic dispensers has been adopted in over one half of the apple and pear acreage (40,000 hectares) in Washington State in just ten years. During this rapid transition in orchard pest management Washington growers have reduced the density of dispensers applied and have supplemented dispensers with limited use of organophosphate insecticides over the majority of this acreage. Today the average codling moth management program entails the use of 500 dispensers per hectare and one cover spray (Alway 1997). The

incentives that led to this design were both the high cost of using sex pheromones and their perceived moderate efficacy.

The effectiveness of disrupting codling moth mating in orchards treated with sex pheromone has been questioned by the consistent body of data generated with light traps, passive interception traps, and more recently with traps baited with a bisexual kairomone lure showing that 50 – 100% of the female moths trapped in pheromone-treated orchards are mated (Howell 1992, Knight 2000, Knight and Light 2000). The complementary hypothesis that a delay in mating by female codling moths may also contribute to the observed population reduction in pheromone-treated orchards has been supported with experimental studies (Knight 1997). In addition, the removal of organophosphate insecticide sprays can enhance the role of biological control of eggs and neonate codling moth (Knight et al. 1997). Nevertheless, sole reliance on the use of sex pheromones to manage codling moth is rarely undertaken unless the population density of this pest has been strongly suppressed by other factors, i.e. chemical control, orchard isolation, or area wide management (Calkins 1998).

The successful use and rapid adoption of sex pheromones for codling moth is now threatened by the current poor economics in tree fruit production in the United States. Growers have abandoned orchards and reduced their general use of pesticides. It appears that the regional density of codling moth in Washington's major fruit growing areas is increasing and this likely portends increasing difficulties in managing its population without insecticides. The loss of registrations for organophosphate insecticides coupled with the moderate effectiveness of the newer, more selective insecticides creates an important challenge for tree fruit pest management in the first decade of the new millennium. In addition, the emerging importance of other pest species such as tortricid leafrollers and pentatomid bugs requires the development of new integrated management programs. To address a portion of these dynamic issues we have conducted studies to develop a lower cost and more effective sex pheromone-based management program for codling moth. Concurrently, we have examined the potential for managing leafroller populations with sex pheromones (Knight et al. 1998, Knight and Turner 1999).

Farkas et al. (1974) first envisioned the idea of using widely spaced high emission sex pheromone emitters to achieve mating disruption. Shorey and co-workers formulated a working hypothesis that the effectiveness of any sex pheromone-based mating disruption system is controlled by the mean airborne concentration of pheromone and is thus regulated by the interaction of point source emission rate and the spacing of emitters (Shorey et al. 1996). The development of a mechanical device (puffer) allowed them to test this hypothesis for several lepidopteran pest species (Shorey et al. 1996, Shorey and Gerber 1996a, b, c). Puffers are battery-powered devices that release pheromone from pressurized aerosol cans. Three advantages of puffers were perceived to be their flexibility in allowing users to release pheromone only during pre-selected time periods, protection of the sex pheromones from environmental degradation, and maintaining a uniform release rate independent of ambient temperature. Shorey and Gerber (1996b) evaluated puffers for codling moth by ringing the perimeter of walnut orchards with puffers spaced 44 m apart to create a density of 2.5 puffers per hectare. This approach appeared promising and orchard trials in California were conducted in apple, pear, and walnut with puffer densities between 2.5 and 5.0 units per hectare. My preliminary studies with puffers in Washington State followed the work by Dr. Shorey and focused on two species of tortricid leafrollers, but following Dr. Shorey's death in 1998 the study was expanded to include codling moth.

Two major changes were made to Dr. Shorey's original approach with puffers to make their use competitive with the cost of the most common usage of hand-applied dispensers for codling moth in Washington State (500 Isomate-C+ dispensers/ha). Based on current retail

prices of dispensers and the wholesale cost of sex pheromone, I estimated that deploying one puffer per hectare would be somewhat less expensive than the current program. Furthermore, I assumed that the spacing between units required to treat only the perimeter of the orchard with this low density of puffers would be too great to be effective and that puffers placed on the downwind side of the orchard would be the least effective. Subsequent studies with codling moth demonstrated the sphere of influence of puffers on lure-baited traps to be < 10 m into the orchard when placed on the downwind edge versus > 50 m when placed on the upwind edge or in the center of the orchard (unpubl. data). Instead, I designed a program in 1998 based on the use of an internal grid of puffers. Puffers were placed 50 m from the edges of the orchard and 100 m apart (1 puffer/ha). In addition, the perimeter of the orchard (a 10 – 20 m wide border) was treated with hand-applied dispensers at the full, labelled rate (1,000 Isomate dispensers per hectare). This idea was inspired by the earlier work of Charmillot et al. (1995) with *Lobesia botrana* in grape where they treated the borders of vineyards with a high density of dispensers and used a large spaced grid of dispensers inside.

Herein, I report the results of studies conducted in apple during 1999 and 2000 to evaluate the success of this program coined I. - H.E.L.P. (Integrated High Emission Low Point) for both codling moth and oblique banded leafroller, *Choristoneura rosaceana* (Harris).

Materials and Methods

All studies were conducted during 1999-2000 in 16-hectare apple orchards situated near Brewster, WA. Orchards were either plantings of 'Red Delicious', 'Fuji', or 'Granny Smith'. During 1999, twelve orchards were selected and pheromone treatments were randomly assigned to create four replicates of three pheromone treatments: I. - H.E.L.P. with a 10 m-wide border application of 1,600 Isomate CM/LR dispensers, 500 Isomate CM/LR dispensers per hectare, and 1,000 Isomate CM/LR dispensers per hectare. Pheromone treatments were applied during the first week of May. Four separate tests were conducted to evaluate several puffer emission rates. Each test lasted three weeks and puffers were removed from orchards for one week between tests. Puffers were programmed to release 7.5 mg of both (*E,E*)-8-10-dodecadienol (codlemone) and 7.5 mg (*Z*)-11-tetradecenyl acetate (Z11-14:AC). Test 1 was conducted from 8 June to 1 July and puffers emitted pheromone every 30 minutes for 24 hours each day. Test 2 was conducted from 7 - 27 July and puffers released pheromone every 15 minutes for 24 hours each day. Test 3 was run from 3 – 25 August and puffers released pheromone every 15 minutes for 12 hours each day (1500 – 0300 hours). Test 4 was run from 1 – 22 September and puffers released pheromone every 30 minutes for 24 hours each day. Orchards were monitored with 12 traps baited with lures for both species (Trece Inc., Salinas, CA). During tests 1 and 2 codling moth was monitored with 10 mg red septa and with both 1 and 10 mg lures during tests 3 and 4 placed in separate traps approximately 25 m apart. Traps in all tests were placed in the upper third of the canopy. Lures for *C. rosaceana* were always placed in the same trap with the 10 mg codling moth lure. Chilled codling moth adults sterilized with radiation in Canada and marked with an internal dye were transported to orchards each week and 8,000 moths were released from eight pre-assigned sites. Mean moth catch per test for each lure type was first transformed (square root [x]) and then compared among treatments for each test with analysis of variance. Significant means were separated with Fisher's LSD test.

During the 2000 season, sixteen apple orchards were treated with the I. - H.E.L.P. program. Orchards were treated daily from May 1 to September 25 with sex pheromone puffs released every 15 min from 1500 – 0300 hours. Three types of puffers were used: five

orchards were treated with 7.5 mg puffs of codlemone and Z11-14:AC (High Dual), four orchards were treated with 5.0 mg and 7.5 mg puffs of codlemone and Z11-14:AC, respectively (Low Dual), and seven orchards were treated with only 7.5 mg puffs of codlemone (CM only). Orchards treated with dual puffers were ringed with 1,600 Isomate CM/LR dispensers, while orchards treated with CM only puffers were ringed with the same number of Isomate-C+ dispensers. Eight traps were deployed around the perimeter of each orchard and baited with the lures of both species. Fruit injury was assessed in each orchard prior to harvest by inspecting 600 fruit per orchard quadrant (30 fruit from 10 trees situated in the center and on the edge of the quadrant). Each puffer-treated orchard was paired with a similar orchard treated with Isomate C+ based on an assessment of their initial pest pressure, similar management staff, insecticide usage, cultivar, and location (> 400 m but < 1,600 m distant from each other). Fruit injury in these paired orchards was compared using the non-parametric Wilcoxin Matched Pairs statistical test.

Results

Recapture of sterile codling moths in 1999 was remarkably uniform among traps within each orchard and no significant differences were found in moth catch by either 1 mg or 10 mg-baited trap among treatments during any of the four tests conducted (Table 1). Moth catches were six- and three-fold lower in the 1 mg versus the 10 mg-baited traps in the third and fourth test across all treatments, respectively. Few wild codling moths were caught in any of the 12 apple orchards (a range of 4.17 to 4.43 wild moths per trap per season among the three treatments).

Table 1. Mean (SE) recapture of sterile, male codling moths in pheromone-baited traps loaded with 1 mg and 10 mg codlemone lures in replicated 16 hectare apple orchards (N = 4) treated either with the I. – H.E.L.P. arrangement of Paramount puffers or 500 or 1,000 Isomate-CM/LR dispensers per hectare. Puffers released 7.5 mg codlemone per puff.

	Dates of Test Daily Cycle / Puffing Frequency			
Pheromone treatment	6/11 - 7/1 24 h - 30 min 10 mg lure	7/7 - 7/27 24 h - 15 min 10 mg lure	8/3 - 8/25 12 h - 15 min 1 mg / 10 mg lure	9/01 - 9/22 24 h - 30 min 1 mg / 10 mg lure
Isomate CM/LR 500/ha	31.4 (2.3)	30.4 (1.2)	4.2 (0.5) / 30.3 (2.0)	3.7 (0.3) / 11.1 (1.0)
Isomate CM/LR 1,000/ha	26.6 (4.5)	33.6 (2.7)	4.4 (0.6) / 25.2 (1.7)	3.2 (0.1) / 10.4 (0.6)
Paramount PufferI. – H.E.L.P.	30.5 (3.3)	35.1 (1.6)	4.2 (0.3) / 25.7 (1.5)	3.3 (0.3) / 10.2 (0.2)

No significant differences were found among column means, ANOVA, $P > 0.05$.

Catches of male obliquebanded leafroller varied among treatments in three of the four tests (Table 2). Mean moth catch was significantly higher in puffer-treated blocks (30 minute puffs for 24 hours per day) than Isomate CM/LR-treated orchards during the first test, $F = 6.32$; df = 2, 9; $P < 0.05$). However, in the second test when puffers released pheromone every 15 min for 24 hours per day no significant differences among treatments were found ($P = 0.47$). Mean moth catch was significantly lower in the puffer-treated orchards than in orchards treated with 500 Isomate CM/LR dispensers per hectare during the third test, $F = 4.50$; df = 2, 9; $P < 0.05$. The puffer setting of 30 minute puffs for 24 hours per day was repeated in the fourth test and the results differed from test 1 (Table 2). During September moth catch per trap in the puffer treatment was significantly lower than in orchards treated with 500 Isomate CM/LR dispensers per hectare, $F = 6.85$; df = 2, 9; $P < 0.05$ (Table 2).

Table 2. Mean (SE) capture of wild, male oblique banded leafrollers in pheromone-baited traps in replicated 16 hectare apple orchards (N = 4) treated either with the I. – H.E.L.P. arrangement of Paramount puffers or 500 or 1,000 Isomate-CM/LR dispensers per hectare. Puffers released 7.5 mg (Z)-11-tetradecenyl acetate per puff.

	Dates of Test Daily Cycle / Puffing Frequency			
Pheromone treatment	6/11 - 7/1 24 h - 30 min	7/7 - 7/27 24 h - 15 min	8/3 - 8/25 12 h - 15 min	9/01 - 9/22 24 h - 30 min
Isomate CM/LR 500 per hectare	2.1 (0.6)b	5.4 (0.5)a	3.3 (0.4)a	5.1 (0.7)a
Isomate CM/LR 1,000 per hectare	1.4 (0.5)b	5.8 (0.3)a	2.5 (0.4)ab	4.0 (0.3)ab
Paramount Puffer I. – H.E.L.P.	4.6 (0.8)a	5.1 (0.5)a	1.8 (0.2)b	2.8 (0.1)b

Column means followed by a different letter were significantly different, ANOVA, $P < 0.05$, Fishers LSD.

During 2000, no significant differences were found in codling moth fruit injury between orchards treated with any of the three puffer types (Dual Low, Dual High, and CM-only) versus orchards treated with Isomate C+ dispensers (Table 3). Injury levels > 1.0 % occurred in only two puffer-treated orchards. Moth catch per trap in both of these orchards exceeded the threshold established for codling moth in pheromone-treated orchards during the first flight (> 4.0 moths per trap [Gut and Brunner 1996]), but they were not supplemented with insecticides until later in the summer. Similarly, the Isomate C+-treated orchards paired with these two orchards were also not sprayed during the first moth generation and suffered high levels of fruit injury.

Fruit injury by the oblique banded leafroller across all nine orchards treated with the Dual puffer ranged from 0.0 to 0.9% and was significantly lower ($Z = 2.24$, $P < 0.05$) than the paired orchards not treated with any sex pheromone for leafrollers (Isomate C+ only) (Table 3). However, the full impact of sex pheromone in managing oblique banded leafroller was difficult to discern in this study as all but one orchard pair was treated with either spring or

summer applications of *Bacillus thuringiensis* Berliner or spinosid-based insecticides.

Table 3. Mean (SE) fruit injury from codling moth and oblique banded leafroller at harvest in 16-hectare apple orchards treated with either the Paramount puffer releasing both codlemone and (Z)-11-tetradecenyl acetate at 5.0 mg and 7.5 mg per puff, respectively (Dual Low), both pheromones at 7.5 mg per puff (Dual High), or 7.5 mg puffs of codlemone (CM only) versus paired orchards treated with Isomate-C+ dispensers (500 dispensers per hectare), Brewster, WA, 2000.

Puffer treatment	Mean (SE) % CM Injury		Mean (SE) % OBLR Injury	
	Puffer - treated orchards	Isomate C+ - treated orchards	Puffer - treated orchards	Isomate C+ - treated orchards
Dual Low n = 4	0.70 (0.49)	1.71 (0.93)	0.13 (0.09)	0.58 (0.28)
Dual High n = 5	0.66 (0.61)	0.40 (0.38)	0.25 (0.11)	0.73 (0.13)
All Dual n = 9	-	-	0.19 (0.10)	0.66 (0.14)
CM only n = 7	0.13 (0.10)	0.31 (0.21)	-	-

Mean oblique banded leafroller fruit injury between all Dual puffer-treated and their paired Isomate C+-treated orchards (n = 9) was significantly different, $P < 0.05$ (Wilcoxin Matched Pairs test).

Discussion

The use of a widely-spaced array of mechanical devices releasing high rates of pheromone has been tested for a number of lepidopteran pest species over the last eight years (Shorey et al. 1996; Shorey and Gerber 1996a,b,c; Mafra-Neto and Baker 1996, Baker et al. 1997, Shorey et al. 1998, Issacs et al. 1999). These studies have used a variety of battery-powered devices including the Technical Concepts (Chicago, IL) puffer (Shorey et al. 1996), the MSTRS device (metered semiochemical timed release system) (Mafra-Neto and Baker 1996), the Paramount Aerosol Pheromone Dispenser (Shorey et al. 1998), and the Michigan State Microsprayer (Issacs et al. 1999). Unfortunately, due the rapid development of this technology few studies have been conducted to address the effectiveness or the factors contributing to the effectiveness of this approach for any given pest species. Thus optimisation of these puffer-based pest management programs within a crop-based management system have not been completed.

The development of puffers by Shorey and co-workers for the management of codling moth in pears in California has probably been the most well studied system (Shorey et al. 1998). Their initial work used a unit designed as an indoor bathroom deodorizer. Units were wrapped in aluminium foil and sprayed with silicon to allow them to operate in orchards treated with overhead irrigation and to withstand rainfall. Originally, these puffers were placed only on the perimeter of the orchards (2.25 puffers per hectare) and released codlemone (68.5 mg per spray) onto a cloth pad every 30 min for 24 hours per day (Shorey and Gerber 1996). However, by 1998 the Paramount Aerosol Pheromone Dispenser was developed (Shorey et al. 1998) and this unit gave the user greater flexibility in specifying and adjusting the amount of pheromone released, the frequency of release, the daily period of release, and included the use of a lower temperature release threshold. Shorey's design still

placed puffers on the perimeter of the orchard but also included mid-orchard transects. Under this arrangement puffers were used at a density of up to five units per hectare. The biggest change made from Shorey's 1996 design was that the puffers were programmed to release 7.5 mg pheromone puffs every 15 minutes for 12 hours per day (Shorey et al. 1998). This represented a 75% reduction in the amount of pheromone used during the season. The Paramount Aerosol Pheromone Dispenser was registered in the U.S. in 1999 and was used effectively at a density of 3.5 to 4.0 units per hectare on over 800 hectares of pear in 2000.

The success of the I. – H.E.L.P. program in Washington apple orchards during 2000 represents a substantial reduction in the use of sex pheromone for disruption of codling moth compared with the Shorey design. Studies reported herein support use of the 15 minute / 12 hour cycle of pheromone release for both codling moth and the oblique banded leafroller. However, the success during the 2000 season of treating orchards with the Dual Low puffer releasing only 5.0 mg codlemone puffs suggests that further studies are needed to establish the minimum effective dose required for disruption of codling moth.

Integrating the internal grid of puffers with a high density border treatment of dispensers likely improves the distribution of pheromone (Milli et al. 1997) in the most sensitive portion of the orchard to codling moth attack. However, disrupting codling moth within border areas will remain problematic due to this area's greater wind speed and turbulence and higher moth density. The common practice of supplementing sex pheromones with border insecticide applications (Knight 1995) would likely be an effective management practice replacing the border dispenser application to supplement the internal grid of puffers. However, future studies will examine the integrated use of border applications of microencapsulated sprayable pheromones, attract and kill formulations, and insecticide-impregnated bait stations with the internal puffer grid.

Mechanical devices have allowed greater flexibility in the application of pheromone. However, the high cost of individual units ($25 – 40 U.S.) limits the density of units that can be deployed. In addition, a number of operational problems have occurred during our studies that limit their practical use: loss of units due to theft or vandalism (< 5%), units not functioning due to problems with the unit or batteries (10%), and the common occurrence of severe phytotoxic effects on the surrounding fruit and foliage. Puffers were monitored weekly due to the potential impact on pest management following the loss of even one puffer for a short time. This high cost of monitoring the status of the Paramount Aerosol Pheromone Dispenser is increased further by the difficulty in assessing its operational status with a remote control device. Instead we were forced to develop a rating system to judge each unit based on the physical characteristics of the cabinet and the surrounding foliage. While studies are planned to further optimise the performance of puffers for management of codling moth and leafrollers, we will also begin to develop high emission passive dispenser systems.

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Pheromone mating disruption of the pine sawfly *Neodiprion sertifer*: is the size of the treated area important?

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Abstract: Few attempts to control forest insects by means of pheromone mating disruption have been reported. The first such experiments with the European pine sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae), resulted in nearly complete trap catch reduction, but no effects on population density or sex ratio were noted. Unmated females lay eggs, which develop into males only. Therefore, if mating disruption would be successful, a more male-biased sex ratio is expected the next generation. One possible explanation for the early results is that mated females disperse into the treated area, and thus obscure the effects of the treatment. In order to reduce the effect of such immigrating females, the treated area was increased in the experiment described here from the earlier used 0.5 – 4 ha to 25 ha. The acetate of erythro-3,7-dimethyl-2-pentadecanol was used for disruption and released from dispensers every 10 m. The influence on male orientation was monitored by pheromone traps, baited with the acetate of (2*S*,3*S*,7*S*)-3,7-dimethyl-2-pentadeanol and placed at 100 m interval along two perpendicular, 1500 m lines intersecting the treated area. The trap catch reduction was near 100% during the first month, but then declined to around 90% during the second month. Mating frequencies were checked by comparing the sex ratio of the next generation from within and outside the treatment area. No effect of the treatment on the sex ratio was detected, and the frequency of mated females could be assumed to be independent of treatment. Alternative hypotheses to explain the failure of pheromone mating disruption in *N. sertifer* are discussed.

Key words: Hymenoptera, Diprionidae, pest management, sex attractant, 3,7-dimethyl-2-pentadecanol

Introduction

Many species within the sawfly family Diprionidae, pine or conifer sawflies, cause severe defoliation of pine (*Pinus* spp.) forests over large areas in Europe, Asia and North America (Smith 1993, Day & Leather 1997). Outbreaks are regularly controlled by aerial application of chemical insecticides such as Dimilin and various pyrethroids. Recently, attempts have been made to use the female produced sex pheromone for controlling populations of *Neodiprion sertifer* Geoffroy, one of the most widespread and economically important diprionids. The method used has been mating disruption. In such experiments a relatively large amount of the pheromone is released in order to obstruct the mate finding behaviour of the male. Unmated females lay eggs, which develop into males only. Therefore, if mating disruption was successful, no drastic population decline would be expected but instead a more male-biased sex ratio would occur in the next generation.

In the first mating disruption experiments with *N. sertifer* a near complete trap catch reduction was obtained in small (0.5 ha) plots. However, due to a general collapse of the population in the area, no evaluation of sex ratio or density could be done of the next

generation (Anderbrant et al. 1995a). In the following experiments the treated areas were increased to around 4.5 ha. Also in this case a dramatic decline in trap catch was recorded, but no apparent effects on sex ratio, larval density or defoliation could be detected (Anderbrant et al. 1995b). These two studies used the attractive pheromone isomer, the acetate of (2*S*,3*S*,7*S*)-3,7-dimethyl-2-pentadecanol, either alone or in its *erythro*-blend, as disruption agent. In a third study, the antagonistic (2*S*,3*R*,7*R*)-isomer was used, either alone or in combination with the attractive isomer, but this failed to improve the mating disruption (Anderbrant et al. 1998).

One hypothesis that could explain these negative results is that, due to the high population density during such epidemic conditions that prevailed during these experiments, males are still able to locate females. However, observations of females either inside or outside a treated area clearly showed that only a small fraction of those inside mated compared with those outside (Östrand et al. 1999). An alternative hypothesis is that mated females disperse into the treated area, and thus obscure the effects of the treatment. In order to reduce the effect of such immigrating females, the treated area was increased in the experiment described here from earlier 0.5 – 4 ha to 25 ha. The success of the pheromone treatment was monitored by traps within and outside the treated area and by collection of next generation larval colonies for a check of the sex ratio.

Materials and methods

The study was performed in a Scots pine, *Pinus sylvestris* L., plantation near Valdmarsvik in the province of Östergötland, southeast Sweden, during 1993. The treated area consisted of several stands mainly with young pines, 2 – 5 m in height. Some stands with older trees or mixed with Norway spruce, *Picea abies* Karst., were also included.

The mating disruption dispenser consisted of a dental cotton roll (Celluron No. 2, Paul Hartmann, S.A., France) impregnated with 8 mg of the acetate of erythro-3,7-dimethyl-2-pentadecanol (Hedenström & Högberg 1994). The dispensers were hung in trees at about 2 m height under a sun- and rain-protection made from wax-impregnated cardboard. Dispensers were placed 10 m apart in a square grid 500 by 500 m on the 27 July to 1 August. Based on measurements in the laboratory and field (Anderbrant et al. 1992), the release of the active (2*S*,3*S*,7*S*)-isomer was estimated at about 180 mg ha^{-1} for the whole season (60 d).

The ability of the males to find an odour source was monitored by Lund-I sticky traps (Anderbrant et al. 1989) placed 100 m apart along two perpendicular lines intersecting each other at the centre of the treated area. Eight traps were inside the treated area, four were at the border and 20 traps were outside. The traps were placed in pines about 2 m above the ground and were loaded with 100 µg of the acetate of the attractive (2S,3S,7S)-isomer, synthesised according to Högberg et al. (1990). Sticky bottoms were replaced on 23 August and 7 September, and baits were renewed on 7 September. The trapping was finished on 6 October.

Larval density within the plantation was recorded in spring before the experiment. Two trees every 50 m along two lines were inspected and the number of colonies counted. However, because nearly all of these trees were located within an area, which later was chosen for treatment, a complementary census of the defoliation was performed. This was made according to the method described in Anderbrant et al. (1995b), using four trees every 100 m along half the length of each transect, from the centre to the outer end. In total 40 trees outside and 20 inside the treatment were inspected.

The sex ratio of the generation following the mating disruption treatment was estimated by collection of larval colonies, which were reared until cocoon formation. Rearing took place in ventilated cardboard boxes and larvae were moistened daily and fed fresh pine twigs when needed. Twentyfour colonies were collected from each of the areas inside and outside the

treatment. This was done in June 1994 and the colonies were reared individually. In addition, approximately 15 colonies were collected from each of five sites inside the treated area and four sites outside it, along one of the transects. All colonies collected from one individual site were reared together until cocoon formation. The cocoons were sex determined based on size, females being about twice as large as males.

Results and discussion

Similar to the results from previous studies, the pheromone treatment caused a nearly complete trap shut down (Figure 1): Average trap catches of male *N. sertifer* for the whole season were 612 ± 298 (SD, n=20), 180 ± 161 (n= 4) and 38 ± 47 (n=7, one trap had fallen down and was excluded from the analysis) for traps outside, at the border and inside the treated area, respectively. The catches, transformed to log(catch+1), inside and outside were significantly different (P=0.002, t-test, unequal variances). The trap catch reduction was complete during the first three weeks, but during the last month of trapping it was only just over 90 % (Figure 2).

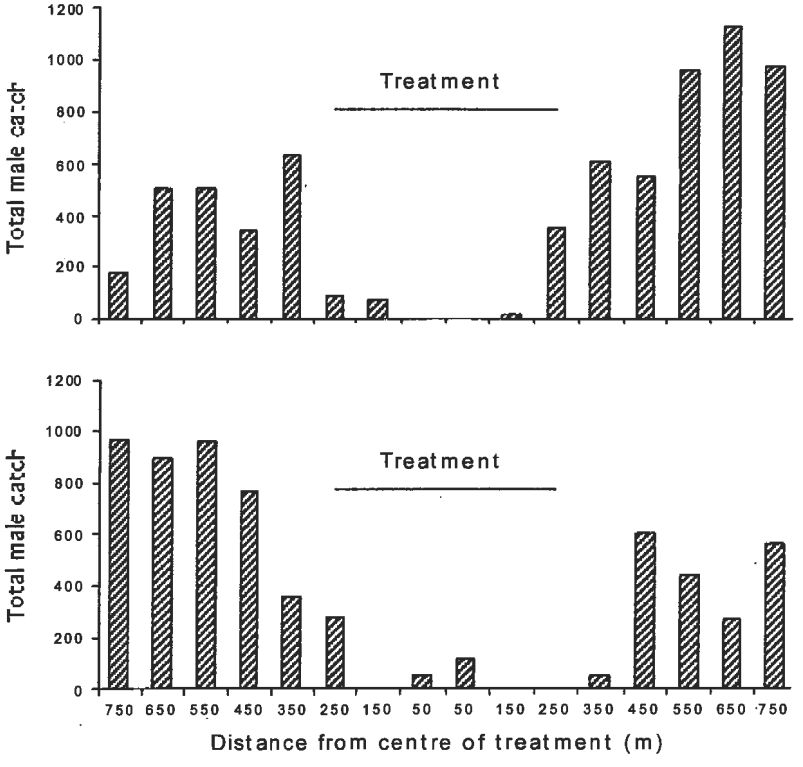

Figure 1. Total catch in pheromone traps along the two perpendicular tansects through the mating disruption treatment.

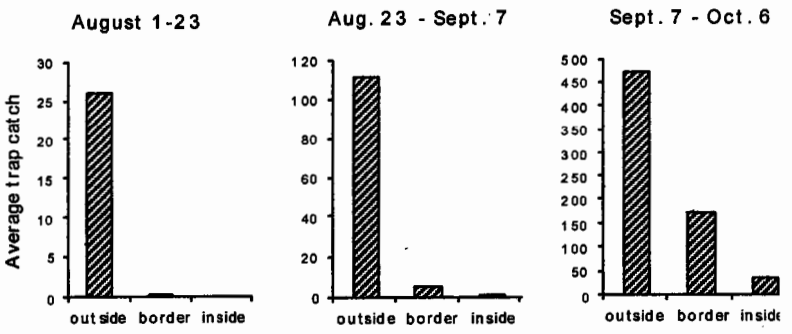

Figure 2. Catch in pheromone traps positioned along the transects outside, inside or at the border of the mating disruption treated area during different periods since start of the experiment.

On the average 7.4 larval colonies per tree (n=48) were recorded in the plantation before the treatment. Considering that most trees were small (mean 2.4 m in height) this represents a high population level and some of the trees were completely defoliated. This was confirmed by the defoliation census performed in the autumn: The proportion of inspected shoots that was consumed by the larvae inside the treated area was 0.37 ± 0.36 (n=20) compared with 0.73 ± 0.32 (n=34, six trees were omitted from the analysis as their height made the inspection unreliable) outside it. Although this difference was statistically significant, $P<0.05$ (t-test of arcsine square-root transformed proportions), the population densities before the application of mating disruption were at the same high level inside and outside the treated area.

The survival rate was rather low of the larvae collected for determination of sex ratios from the generation following the disruption experiment. In many of the individually reared colonies less than 10 individuals survived to cocoon formation (Figure 3). However, it was quite obvious that in all of the colonies, with a reasonable number of survivors, both sexes were represented. The average sex ratio (proportion of males) was not significantly different outside, 0.49 ± 0.28 (n=24) compared to inside 0.46 ± 0.30 (n=24) of the treatment. The results from the rearing of groups of colonies were similar (Figure 3), with a mean sex ratio of 0.52 ± 0.16 (n=4) outside and 0.56 ± 0.05 (n=5) inside the treated area.

The results obtained in this experiment confirm those from earlier attempts to disturb the mating in *N. sertifer* (Anderbrant et al. 1995a, b, 1998); it is possible to reduce the catches to very low levels without affecting the proportion of mated females inside the treatment, as measured by the sex ratio of colonies. However, it has also been clearly shown that females inside pheromone treated areas are only rarely able to attract males (Östrand et al. 1999). Instead the females seem to disperse if they have not been able to attract males during the first days in the area. These contradicting results may be explained by a massive immigration of mated females into the treated area. Because no female attractants are known, this hypothesis is difficult to test. An alternative hypothesis is that even if the movement of females was higher inside than outside the pheromone treatment (Östrand et al. 1999), the females sooner or later mated while still inside the treatment. A test of this hypothesis requires tracking the females inside a pine plantation, and a suitable technique for doing this will certainly be difficult to develop.

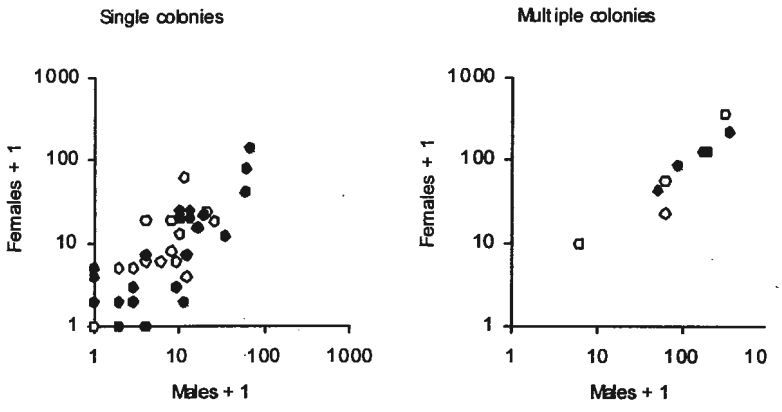

Figure 3. Number of male and female cocoons in colonies collected inside (filled) or outside the mating disruption treated area. Colonies were reared either singly or several together.

The pheromone dose used in this experiment is about 100 times lower than what is normally used in many lepidopteran mating disruption experiments (e.g. Leonhardt et al. 1996). Although the trap shut down and the reduction in mating frequency seemed to be nearly complete in the *N. sertifer* experiments, there is still a possibility that the final result would be different if higher pheromone doses were used. One could also hypothesise that the "restlessness" in females is not only a response to the lack of courting males, but also to the pheromone *per se*. A prerequisite for this is that females can perceive their own pheromone. Although this is the case for a few lepidopteran species (see e.g. Ljungberg et al. 1993), it is as yet unknown if it occurs among pine sawflies. It should also be observed that the experiments were done under epidemic conditions, while most suceessful disruption attempts involving moths have occurred when the population densities have been relatively low.

In conclusion, our results indicate that the size of the treated area, up to 25 ha, is of little importance for the success of mating disruption in *N. sertifer*. Possible improvements include use of higher dose and, maybe more important, treatment of less dense populations. Also treatment of isolated stands, where immigration is negligible, would possibly generate results that are easier to interpret.

Acknowledgements

We thank Fredrik Östrand, Rolf Wedding and Erling Jirle for their excellent help with the fieldwork. Funding was provided by Carl Trygger Foundation, C. F. Lundströms Stiftelse, Stiftelsen Futura and the Swedish Council for Forestry and Agricultural Research (SJFR).

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Other control methods

Use of pheromones and other semiochemicals in integrated production
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Control of codling moth *Cydia pomonella* by autosterilisation

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Abstract: Autosterilisation is based on the same principle as the attract and kill technique but the insecticide devoted to kill the males by contact is replaced by a product able to sterilise them, in this case by the insect growth regulator (IGR) fenoxycarb. In a small dwarf trees orchard, a trial to control codling moth *Cydia pomonella* was made from 1996 to 2000, by applying on the trees 1'540-4'400 autosterilising droplets per ha, in 2 or 3 treatments per season. In comparison with a high stem non-treated check orchard, population density of codling moth, estimated by the diapausing larvae caught in cardboard band traps, decrease strongly, suggesting that autosterilisation could be more efficient than attract and kill to control that pest.

Key words: *Cydia pomonella,* autosterilisation, sex pheromone, fenoxycarb, population dynamics

Introduction

The attract and kill method (Sirene CM®) is a novel approach using sex pheromones, recently developed by Novartis Crop Protection, to control codling moth *Cydia pomonella.* A viscous paste, containing a sex pheromone and an insecticide, is distributed as small droplets in the crop. Males are attracted by the pheromone and are killed by the insecticide (Hofer & Brassel, 1992; Charmillot et al., 1996; 1997; 2000).

Autosterilisation is based on the same principle as the attract and kill technique but the insecticide devoted to kill the males by contact is replaced by a product able to sterilise them, in this case by the insect growth regulator (IGR) fenoxycarb. Preliminary trials have been made the last 5 years to control codling moth by autosterilisation in a dwarf apple tree orchard of the Research Station of Changins.

Material and methods

Product and application technique.
Novartis formulation contained 0.16% codlemone E-8, E-10-dodecadien-1-ol (E8,E10-12:OH) to attract the males and 5% fenoxycarb to sterilize them. The paste was applied with a specially designed dose tube applicator. 50 µl droplets were dispensed on the branches, containing in average 0.08 mg codlemone and 2.5 mg fenoxycarb. Approximately one third of the droplets was applied in the lower part of the treetop and two thirds in the upper part.

Experimental plot.
The small orchard at Changins has a surface of 0.15 ha and consists of 3 rows of 11 apple trees and 2 rows of 17 pear trees. From 1996 to 1999 two applications were made, a first one about one week after the beginning of codling moth flight, generally by the second week of May, and a second application was made 5-8 weeks later, at mid to end of June. In 2000, a

third application was made at the end of July. Depending on the year, each application varied between 77-220 g of product per ha corresponding to 1'540-4'400 droplets per ha (Table 1).

Assessment of the autosterilisation treatments in the orchard.

During June, corrugated cardboard band traps were placed around tree-trunks in the experimental plot. These were collected in the autumn to estimate the hibernating population density. Corrugated cardboard band traps, placed in a non-treated high stem orchard at Genolier, a neighbouring village, served as control to evaluate the population density fluctuation from one year to the other.

Table 1. Amount of autosterilisation paste formulation applied in the experimental orchard at Changins from 1996 to 2000.

year	1st application (g/ha)	2nd application (g/ha)	3rd application (g/ha)	Total (g/ha)
1995	-	-	-	-
1996	126.3	126.3	-	252.6
1997	220	140	-	360
1998	128	77	-	205
1999	115	83	-	198
2000	154	192	180	526

Results and discussion

The autosterilising formulation almost completely eliminated the catches in the pheromone traps. Figure 1 report the evolution of population density of diapausing larvae in the non-treated high stem orchard at Genolier from 1996 to 2000 as well as from 1995 to 2000 in the experimental orchard were autosterilisation was tested from 1996 onwards. In the high stem check orchard, population density varied from 31 to 76 diapausing larvae per tree from 1996 to 1999 depending on the climatic conditions. However, due to a very precocious and favourable season in 2000, it exploded to 173 larvae per tree.

In the experimental orchard, without any treatment, population reached 26.4 larvae per apple tree in 1995, than it decreased progressively to 1.56 larvae per tree in 1997 after two years of control by autosterilisation. The small increase of population density from 1997 to 1999 can be attributed to a too low amount of applied product corresponding approximately to 200 g per ha and season (Table. 1). Indeed, the increase of population in apple trees can be attributed at different factors. The almost 3-fold increase of population in the untreated control orchard demonstrates that year 2000 was exceptionally favourable for codling moth development. However, the three applications of droplets in the experimental orchard, instead of two applications, and the much higher amount of product, couldn't avoid an increase of population to 7.48 larvae per apple tree. In pear trees, where cardboard band traps were placed from 1996 on, population dynamics was quite similar as in apple trees, but at a lower level, except in 2000 when the population did not increase.

This trial demonstrates that autosterilisation could be a new method of codling moth control, but it does not bring prove that it's efficiency is higher than attract and kill technique.

However, a simulation study on codling moth dynamics under different pheromone based control techniques suggests that autosterilisation should be about twice as effective as attract and kill (Potting at al., 2001).

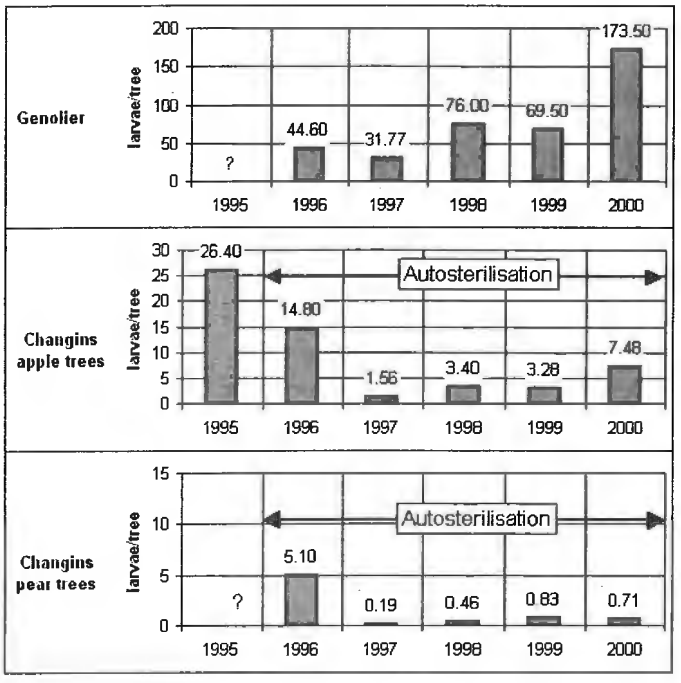

Figure 1. Evolution of larval diapausing population of codling moth in a high stem non-treated orchard at Genolier and in a dwarf trees orchard in Changins where the pest was controlled by autosterilisation from 1996 to 2000.

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The control of *Cydia molesta* in stone- and pome-fruit orchards by false-trail following

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Abstract: The defence of stone- and pome-fruit orchards from *Cydia molesta* by the method of false-trail following, or "disorientation", has been evaluated. Such a method consists in the setting up of several prevailing pheromone trails, released by an appropriate number of dispensers loaded with low pheromone dosage, able to compete with those of the female insect and thus disorientate males in their search for partners. The soundness of the disorientation method has been demonstrated through experimental and large-scale demonstrative trials, covering more than 200 hectares of peach orchards and 32 hectares of pome-fruit orchards located in several Italian regions in 1998-99.Specific, biodegradable, pheromone dispensers, named Ecodian, numbering 2000/ha for a total amount of 20 g active ingredient per hectare, were used for each application, which had an average duration of 45-50 days. In peach orchards, in 1998 damages lower than 5% were registered on 159 of the 186 demonstration tests (81%), against 186 of the 209 tests (89%) in 1999. Damages less than 1% were registered in 74 tests in 1998 and 119 tests in 1999. In pome-fruit orchards, the dispensers for *Cydia molesta* were applied in mid-July on fields where, for the most part, the mating disruption was practiced with Ecopom Isagro dispensers for the defence against *Cydia pomonella*. Of the 22 demonstration trials in 1998, only 1 suffered damage of 2%, while in the other 21 the control was total. An excellent control of *Cydia molesta* was also achieved in 1999, only 1 out of the 31 tests suffered damage higher than 5% (mainly due to the border effect) while in 24 tests the attack was kept within 1%.

Key words: pheromone, false-trail following, disorientation, *Cydia molesta*, stone fruit, pome fruit

Introduction

Over the last few years, the agro-industrial system has undergone substantial changes consequent to both the growing power of the large-scale retail companies, which can determine the demand of "controlled" (certified) products to promote the sales of their own brand names, and the development of European, National and Regional environment-friendly politics encouraging the use of techniques of integrated production.

These changes have resulted in the implementation of new plant protection guidelines granting a further premium on the adoption of innovative methodologies that are respectful of the environment and of consumers' health.

Within this frame, the method of false-trail following or "disorientation" for the control of *Cydia molesta* and *Anarsia lineatella* represents a valid tool, since not only it can be applied in biological control programs, but is particularly suitable for the protocols of integrated pest management (Molinari *et al.*, 2000a, b).

Such a method consists in the setting up of several prevailing pheromone trails, released by an appropriate number of dispensers loaded with low pheromone dosage, able to compete with those of the female insect and thus disorientate males in their search for partners.

The effectiveness of the method is likely to depend on its keeping males busy visiting the artificial pheromone sources most of the time rather than the females actually present in the treated area. For this purpose we optimised, by means of the laboratory and field tests here described, a new biodegradable pheromone dispenser.

Dispenser design

Isagro's Ecodian dispensers are made of Mater-Bi, a proprietary product of the company Novamont , that is a mixture of biodegradable materials, such as cornstarch and thermoplastic polymers (Bastioli *et al.*, 1992).

Various industrial grades Mater-Bi have been tested for their suitability in pheromone absorption and release, ranging from practically impermeable materials to very fast releasing ones. Mixtures of them, different thickness of dispensers, and different pheromone loadings have also been tested.

Table 1 reports the half-lives of dispensers obtained with selected materials in open field conditions.

Table 1. Half-lives of Ecodian dispensers made of different Mater-Bi

Entry	Material	Pheromone loading (% w/w)	Half-life (days)
1	ZF03U + AF05H (1:1)	1	80
2	ZF03U	1	22
3	ZF03U/A	1	25
4	ZF03U/A	0.5	23
5	ZI01U	1	18

Granules of Mater-Bi, industrial grade ZF03U/A, have been treated in an horizontal mixer with the pheromone blend for *Cydia molesta*, then moulded into a suitable shape to obtain Ecodian CM dispensers, (Figure 1). Pheromone loading was 1% w/w, i.e. 10 mg/dispenser. Ecodian AL dispensers were manufactured in the same way, using the pheromone blend of *Anarsia lineatella* (Rama *et al.*, 1999).

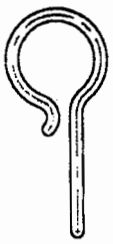

Figure 1. Ecodian dispenser

Determination of release rates were carried out by exposing the dispensers under controlled conditions (35 °C, air speed 1 m/s), as well as in the open field. Each dispenser was weighed, dissolved in 20 ml of tetrahydrofurane containing n-hexadecanol as internal standard, and analysed by gas chromatography.

Figures 2 and 3 show the release rate of Ecodian CM and AL standard- and high thickness-dispensers (3.5 mm o. d.), periodically picked up from both environments.

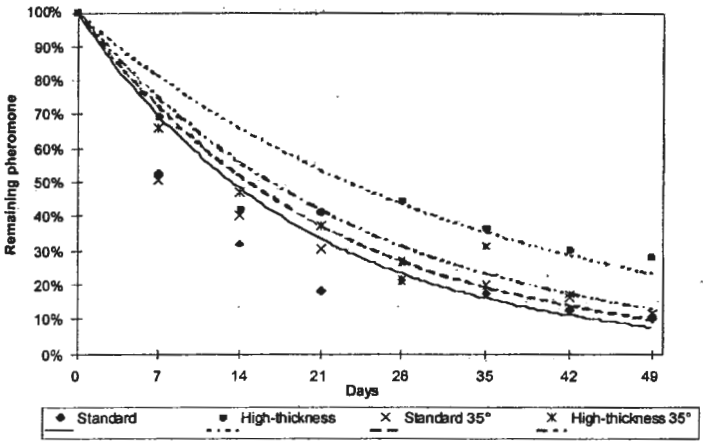

Figure 2. Release rates for *Cydia molesta* formulations

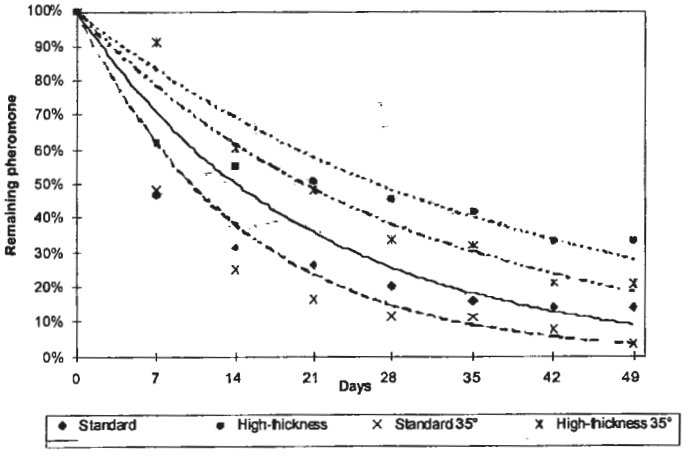

Figure 3. Release rates for *Anarsia lineatella* formulations

Field tests

The experimental trials have been conducted during a four-year period, 1996–99, while demonstrative tests were carried out on a large scale during a two-year period, 1998-99, in an area of over 200 ha in various Italian Regions. Specific biodegradable dispensers, numbering 2000/ha for a total amount of 20 g. a.i./ha, were used for each application, which had an average duration of 45-50 days. In stone-fruit orchards, two different applicable protocols were used: the first (A) consisted in the application of dispensers at the start of the flight of the first generation of *Cydia molesta*, while in the second one (B) the dispensers were applied at the start of the flight of the second generation of the pest followed by an insecticide treatment with the purpose to reduce the population.

In the case of apple and pear orchards, the experimental tests were carried out over two years, 1998-1999, and involved an area of 32 ha in the Regions of Emilia Romagna and Trentino Alto-Adige. The dispensers for *Cydia molesta* were applied in mid-July on fields where, for the most part, the mating disruption was practiced with Isagro's Ecopom dispensers for the defence against *Cydia pomonella.*

Assessment of the method efficacy included: (1) weekly controls of pheromone traps catches; (2) visual checks on 500 shoots and 300 fruits in at least five different locations in the plot for stone fruit, on at least 100 fruits in five different locations in apple orchards; (3) evaluation of total damage at harvest, splitting it into four categories, from 0 to 1%, 1 to 5%, 5 to 10%, and >10% of attacked fruits on the total of production.

Discussion

Stone fruit

In more than 95% of stone-fruit orchards involved in the trials, the contemporary presence of *C. molesta* and *A. lineatella* was observed, thus it was necessary to control both pests at the same time by applying a joint strategy through the distribution of both Ecodian CM and Ecodian AL dispensers. The results obtained, outlined in Figures 4 and 5, are hence referred to the total damage caused by both pests.

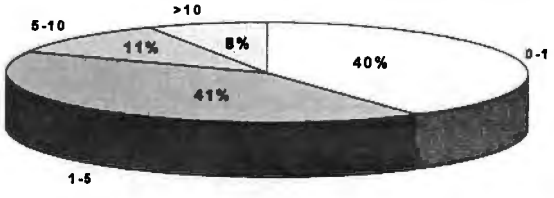

Figure 4. Stone fruit: classes of damage 1998

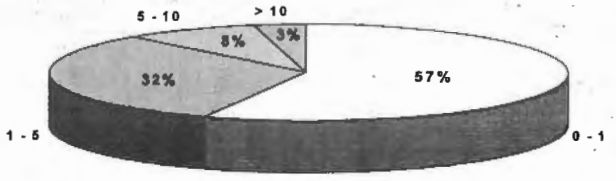

Figure 5. Stone fruit: classes of damage 1999

It is worth noting that in the second year better results were achieved, mainly because the application protocols were more precise. In 1999 mistakes such as those in the timing of application of either dispensers or insecticide planned by protocol B, were sharply reduced. Moreover, much more attention was paid in the choice of orchards, avoiding those too small or of irregular shapes and particular consideration was used in the interpretation of pheromone trap catches (see Table 2).

In both years, no further chemical treatments except those planned by protocol B were needed on almost 75% of total trials. In few situations it was necessary to stop the tests in order to avoid greater damage.

Table 2. Stone fruit: incidence of chemical treatments 1998-99

	Pesticides on support (%)		Closed trials (%)	
Motivation	1998	1999	1998	1999
Orchards not suitable	4.30	0.48	1.08	---
Wrong application	2.15	0.96	1.61	0.96
High pest population	3.23	4.78	3.76	0.48
Dispensers depletion[a]	8.06	8.13	---	---
Other pests	9.00	11.00	---	---

[a]Dispenser depletion indicates the partial efficiency loss shortly before the harvest (7-10 days before); therefore in some cases a short-efficacy insecticide was used rather than carrying out the second installation of dispensers

Pome fruit

All orchards submitted to experimentation showed the simultaneous presence of *Cydia pomonella* and *C. molesta*. Also in the case of pome fruit it was hence necessary to adopt a combined approach for pest control. Various strategies were implemented after the application of Ecodian dispensers and their distribution is summarized in Table 3.

Table 3. Control strategies against *C. pomonella*

Type of treatment	Apple (No. of trials)		Pear (No. of trials)	
	1998	1999	1998	1999
Bacillus thuringiensis	6	5	3	--
Mating disruption	6	10	3	13
Chemical control	--	3	1	--
No treatment	--	--	3	--
Total	12	18	10	13

***Combination of Ecodian and* B. t.**

Some trials have been carried out combining chemical treatments against *C. pomonella* prior of the application of Ecodian CM dispensers for *C. molesta* and continuing with *Bacillus thuringiensis* treatments, specifically aimed at controlling codling moth without interfering with the biology of *C. molesta*, since the two pests show different development stages at different times. Such a strategy proved to be very effective both on apple and pear keeping the overall damage within 1%.

Combination of Ecodian and mating disruption (Ecopom)

A good orchard protection was achieved also with the combination of the two Isagro pheromone-based products (Ecopom and Ecodian). This method was applied in about half the trials in 1998, increasing to over 60% on apple and reaching the totality on pear in 1999. Tables 4 and 5 report the damage distribution for apple and pear, respectively.

On apple, the good efficacy of Ecodian in the control of *C. molesta* was confirmed; only one of the 16 trials carried out in the two years, showed a damage of about 8%. It has to be noted, however, that this cultivar (Fuji) was harvested in October, about 80 days after the installation of the method, i.e. when the dispensers had already lost their activity.

Table 4. Apple - Damage distribution

	Cydia molesta		*Cydia pomonella*	
Damage	1998	1999	1998	1999
0 to 1%	6	8	2	2
1 to 5%	0	1	0	7
5 to 10%	0	1	3	1
> 10%	0	0	1	0

In the case of pear, the efficacy of the combination mating disruption-disorientation was also satisfying, but in 1999 greater damage by *C. molesta* was recorded on the borders of a single plot and on cv. Passacrassana, harvested in October. Even the results achieved on codling moth by mating disruption were good in both years.

Table 5. Pear - Damage distribution

	Cydia molesta		*Cydia pomonella*	
Damage	1998	1999	1998	1999
0 to 1%	3	9	2	9
1 to 5%	0	3	1	3
5 to 10%	0	1	0	1
> 10%	0	0	0	0

Combination Ecodian-insecticides

Very good results, with overall damages less than 1%, were obtained also in the trials on apple carried out in Trentino, where the classical chemical control was combined with Ecodian disorientation. The kind of treatments and their timing are reported in Table 6.

Table 6. Chemical control of *C. pomonella* and *C. molesta* in Trentino

Epoch	Chemical control	Disorientation
Beginning May	IGR	IGR
Mid May	IGR (Leafrollers)	IGR (Leafrollers)
Mid June	IGR	IGR
End July	Organophosphate	Organophosphate
End July-beginning August		Ecodian application
Mid August	Organophosphate	

Ecodian alone

In those pear trials, where no insecticides were applied after mid July, the damage by *C. pomonella* increased accordingly to the period of ripening of fruits, from less than 1% on cv. Conference (mid August) up to 6% on cv. Abate (mid September). It was hence necessary to perform a chemical spray on cv. Passacrassana (mid October). The control of *C. molesta* was instead almost complete, having registered a damage lower than 1% in three different trials while only one showed 2.3% attack.

Conclusions

The method of disorientation, or false trail following, tested on more than 200 Ha of stone-fruit orchards afforded an effective control of the populations of *Cydia molesta* and *Anarsia lineatella*, comparable to traditional insecticide treatments.

Stone-fruit orchard protection was achieved by applying the method both at the beginning of first generation of pests and later on, at the start of the second generation.

In pome-fruit orchards, the disorientation of *C. molesta* can be successfully utilized to integrate the various strategies for the control of *C. pomonella*. Of particular interest is the possibility to combine two different pheromone-based techniques, applied on diverse pests and at different times according to the real needs. In any case, since the higher risk of damage by *C. molesta* on pome-fruit is normally recorded close to the harvest, the opportunity to

control this pest without the use of insecticides makes it possible to obtain residue-free fruits.

In both cases, stone- and pome-fruit, the high versatility of the disorientation method allows its implementation in biological control programs and particularly in the protocols of integrated pest management.

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Control of codling moth by attract and kill

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Abstract: The codling moth is controlled using mating disruption on about 10% (1200 ha) of the apple growing area in Trentino. The widespread adoption of this control method has been facilitated by the financial support of the growers associations. Financial support reduced the per ha cost to growers of using mating disruption, but the technique must still be applied to relatively large, contiguous areas, and this has been a barrier to wider adoption. Another semiochemical based approach is the attract and kill strategy, involving the combination of a semichemical lure with an insecticide. The principle has been successfully used to control codling moth, although some critical parameters affecting its efficacy are still to be investigated. In this paper we report on experiments designed to know some of the limits of the method. In particular we determined: longevity of the lure (more than a month) and of the insecticide efficacy (3-5 weeks depending on the insecticide added), the influence of the infestation sources close (less than 80 m) to the orchard and the satisfactory field efficacy using a low number of droplets (less than 2000/ha) in the colder fruit growing areas where the codling moth is less abundant

Key words: sex pheromone, attract and kill, release rate, *Cydia pomonella*, Tortricidae, Lepidoptera

Introduction

The codling moth *Cydia pomonella* L., is the key pest of pome fruit in the region of Trentino, one of the major apple growing areas in Europe. An average of nearly three applications of insecticide is applied per hectare each year to menage this pest. Recently growers and crop consultants have reported an increase in damage caused by the codling moth. Currently, codling moth control relies primarily on conventional spray application, predominantly of insect growth regulators (IGR) or organophosphorus (OP) insecticides. IGR's are frequently applied in spring to control overwintering leafroller larvae and codling moth, whereas OP's are applied in summer to control the second generation of the codling moth. The increasing public awareness and changes in social attitude towards exposure to pesticides together with the development of resistance against the insecticides used for the codling moth control (Ioriatti *et al.*, 2000; Ioriatti and Bouvier, 2000) makes it necessary to introduce alternative strategies and develop novel control methods compatible with the aims of integrated pest management. The introduction of codling moth mating disruption (Ioriatti *et al.*, 1997) has resulted in about 10% (1200 ha) of the regional apple growing area using this selective method, concentrated where the pest pressure is higher. The widespread adoption of this control method has been facilitated by the financial support of the growers associations. This fact contributed to reduce only one of the problems related with the MD application, the cost of the pheromone active ingredients, but the large size of the treated plot required by the method is still a restriction for a further development in the marginal fruit-growing area.

Another semiochemical based approach is the attract and kill strategy, involving the combination of semichemical lure with an insecticidal effector. Compared with spray applications, an attracticide, as well as mating disruption, may be better accepted by consumers because its application to parts of the plant that are not harvested avoids

insecticide residues on harvested crop. Ten years have elapsed since Angst and Hofer (1990) presented the first attempt of codling moth control with this novel strategy. The principle is already successfully applied to the control of codling moth (Charmillot *et al.*, 1996, 1997; Kirsch, 1997; Trematerra *et al.*, 1999; Ebbinghaus *et al.*, 2000), and other pests (Hofer and Brassel; 1992, Suckling and Brockerhoff, 1999), although some critical parameters affecting its efficacy are still to be investigated. In this article we report on experiments designed to know some of this critical parameters.

Materials and methods

Attracticide formulation.
The attract and kill technique developed by Ciba-Geigy (now Syngenta) ha been registered in Italy this year under the name of Sirene CM®. It is formulated as a viscous paste containing 0.16% codlemone (E,E)-8,10-dodecadien-1-ol to attract males and 6% permethrin (or cypermethrine), a fast-acting pyrethroid insecticide, to kill them. Using hand applicators developed specifically for the purpose, the material is applied as 50 mg droplets as high as possible on the branches or scaffold limbs of the tree. Males contacting a drop die within some hours, with a resulting decrease in mating, egg fertility and infested fruits.

Release rate of codlemone from the droplets.
The release rate of codlemone from droplets was determined in two ways: (1) 50 droplets were applied to a glass sheet (10x15 cm); six glass sheets were prepared and exposed in an orchard under a shelter. At two week intervals a glass sheet was brought into the laboratory and washed with redistilled hexane, which was analysed by gas chromatography (GC-MS) and the remaining pheromone determined; (2) three glass sheets (2.5x7.5 cm) each bearing ten droplets were exposed in the field. The emission rate was evaluated at the time of exposure (Day 1), and 15, 30 and 45 days thereafter, suspending each glass sheet in stopped glass flask for 17 hours. After this time, the glass sheets were removed and the 3 flakes were washed with a redistilled hexane, which was analysed by GC-MS (Baker *et al.*, 1980).

Attractiveness of the droplets.
Twelve droplet (Sirene CM®) baited traps were placed in an apple orchard during the second generation of codling moth. In six of them the lure have been weekly renewed, while in the others it remains unchanged for the period of the trial. Catches were checked, moths removed, and traps rotated twice a week.

Longevity of insecticide efficacy.
Groups of 10 droplets were applied on different twigs at the beginning of June. The efficacy of the insecticides (cypermethrin and permethrin) present in the two attract & kill formulations was evaluated immediately after application, one day later and then at weekly intervals, bringing the twigs in the laboratory. Moths emerged in the laboratory from the overwintering generation, were laid on the droplet for 5 sec. using tweezers. Mortality was checked after 24 and 48 ho*143* no-treated control (Abbott, 1925).

Management of infestation sources.
In order to determine the influence of a source of infestation, overwintering larvae have been released just outside a plot treated with A&K. Even though the first moths were caught on 23 May the experimental plot was treated with lufenuron (Match® 100g/hl) on 7 May when, according to the degree day sum, the first eggs were laid. After confirming that none of the

fruit were injured by codling moth, instead of repeating the chemical treatment, Sirene CM® was applied on 7 June. Fruit damage was monitored randomly sampling 1000 fruits/block at weekly intervals during the summer and chemicals treatments sprayed when the threshold of 2% of fruit damage was passed. The efficacy of the control strategy was evaluated dividing the plot in four blocks 30 m wide located at increasing distance from the infestation source and by checking the fruit damage at the end of the two generations.

Field trials.

Field trials were conducted in two small orchards (tab.1) with different level of codling moth populations to determine the efficacy of the control method when a low density of drops (less than 2000 drops/ha per application) were used. The attracticide droplets applied in all the treated orchard averaged 57 mg (determined after deployment by weighing the remaining material). The first application took place immediately after the first capture of male moths in pheromone-baited traps; a second and a third application of droplets was made at five week intervals. Standard pheromone traps (carpotrap® – Isagro) were installed in each plot and checked once a week. The efficacy was evaluated by assessing the fruit damage on samples of 1000 apples, randomly chosen, at different time and location in the plots. Data were compared with the surrounding conventional treated orchards. The "Toss" orchard is in part a small young orchard planted with the cultivars "hapke" and the rest with "G. delicious" 15 years old and three meter high. It has been treated on 10 and 22 May with flufenoxuron (Cascade®) at the rate of 60g/hl for the leafrollers control.

Table.1.Attract and kill field trials against codling moth (*Cydia pomonella*) in Trentino.

Year	Orchard	Surface (ha)	Applications		Sirene CM		Damage at harvest (%)	Curatives treatments
			N°	Date	Drops/appl./ha	(g/ha)		
1999	Toss	0.6	3	15.6- 15.7- 17.8	1173	134	0.1	-
1999	Bleggio	0.75	3	10.6- 14.7 18.8	1550	265	1.4	-
2000	Bleggio	0.75	3	22.5 29.6 9.8	2000	342	1.5	1 O.P.

Bleggio is a 6 years old apple orchard, planted with "R. gala" and "G. delicious"; the tree height is 3 meter. An IGR (Lufenuron, Match®) was applied during May of each year for the leafrollers control.

The orchards were located in the upper and colder growing area where the codling moth is still pest that have to be sprayed, but because of the less favourable climatic condition, the level of population is normally low.

Results and discussion

Release rate of codlemone from the droplets.
The emission of pheromone from the drop is estimated to be between 75 and 152 ng/h for the first four weeks of exposure, thereafter, emission decreased rapidly and ranged from 5 – 17 ng/h (Fig.1). The average emission of codlemone from the droplets measured in steady air (Fig.2) is 3.1 ng/h when fresh and 2.2 ng/h after 15 days of weathering under field conditions, but decrease to 0.2 ng/h after 30 days.

Attractiveness of the droplets.
The 50 moths caught in the traps in the first 4 weeks were equally distributed between the traps containing droplets that were renewed and droplets that were not renewed (Fig.3).

Longevity of insecticide efficacy.
The mortality of adult moths was 100% during the first three weeks of exposure for droplets containing permethrin and during the first five weeks of exposure for droplets containing cypermethrin (Fig. 4).

Management of infestation sources.
The fruit damage on the untreated trees at the point of the release reached 49.6% at the end of the first generation. In the block adjacent to the point of release of codling moths, damage caused by first-generation codling moths averaged 3.5%. In the block 50-80 m from the source of infestation damage averaged 1%. There was no damage in the blocks located 80-110 and 110-140 m from the source of infestation. Because of the high level of fruit damage in the first generation, the two blocks closest to the source of infestation have been treated twice at 20 days interval with O.P. during the second generation. Damage caused by second-generation codling moths was 11.3% in the block adjacent to the source of infestation and 3% in the block located 50-80 m from the source of infestation. The damage increased again in the third block (7%), but was dramatically reduced in the fourth (0.5%).

Field trials.
At Toss the pheromone traps installed in the orchard did not catch any moths and fruit damage caused by first- and second-generation codling moths was 0.1%. In the surrounding orchards treated with one to two summer insecticides, the harvest damage varied between 0 and 1%. At Bleggio; although few moths were captured in the pheromone-baited traps in both the years, a chemical treatment early in August was necessary to keep the pest under control during the second year. The average fruit damage at the harvest was 1.4% in the first year and 1.5% in the second year. During both years the years the damage was primarily located along the south and west sides of the orchard adjacent to some untreated walnut trees and abandoned pear trees that probably served as a source of infestation that would have to be managed by the application of the droplets. In the conventionally treated orchard close to the experimental orchard, the average fruit damage registered at the harvest was 0.8% in 1999 with one summer insecticide, and 4.8% in 2000 with three summer insecticides.

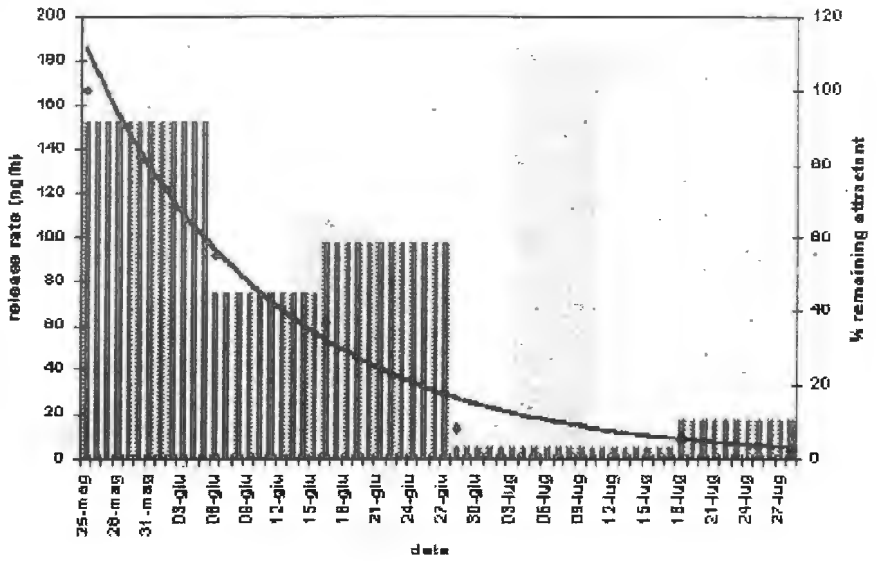

Figure 1. Release rate of codlemone (bars) from a droplet estimated according to the remaining attractant (line) determined by GC-MS.

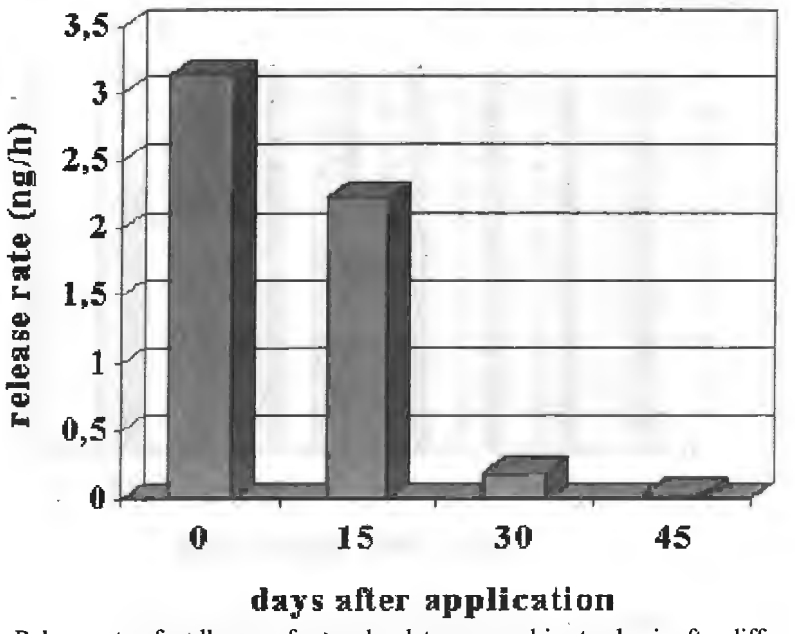

Figure 2. Release rate of codlemone from a droplet measured in steady air after different field aging periods

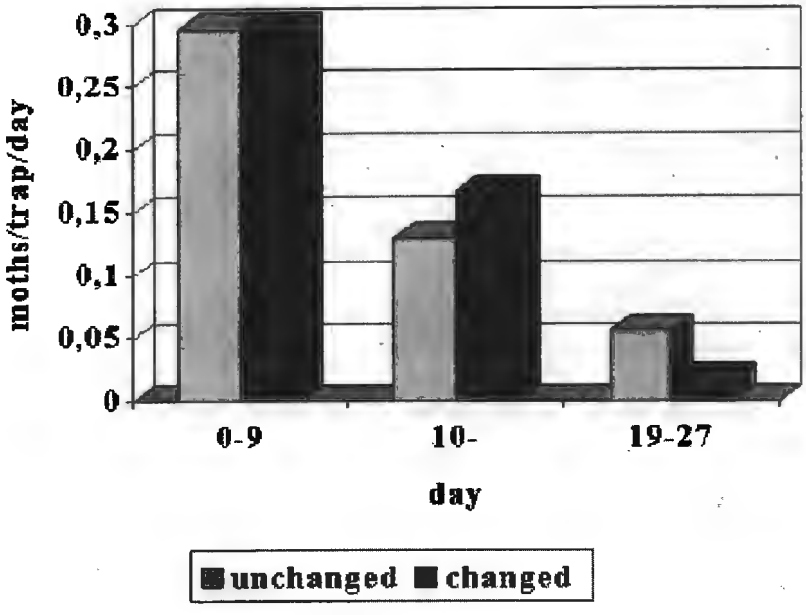

Figure 3. Averaged number of moths caught per trap and per day; gray bars = unchanged lure, black bars = renewed lure

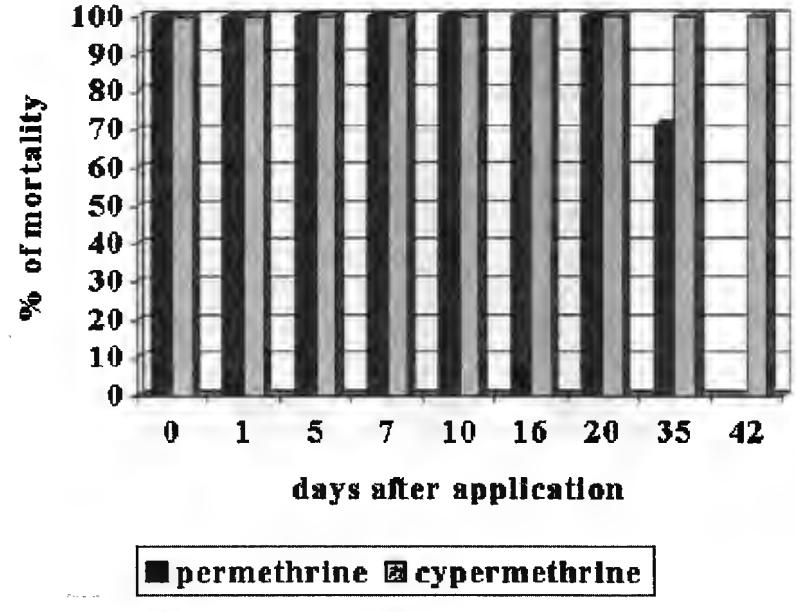

Figure 4. Longevity of the insecticide efficacy

Discussion

The use of attract and kill to control the codling moth suffers from some of the same contraints as mating disruption, including the high degree of pest selectivity, a reduction in efficacy with increasing pest density and risk of immigration of mated females. In contrast to mating disruption, the use of attract and kill removes males from the adult moth population, much lower amount of pheromone are required, and it can be applied in the small irregular and isolated orchard typical of the marginal fruit-growing areas. The results of these trials permit to better know some of the limits of the attract & kill strategy. For the formulation containing permethrin, the longevity of the insecticide efficacy more than the attractiveness of the droplets set a time-limit for the application. After 3 weeks the insecticide activity decrease even thought the droplets still remain attractive for the male moths. In our climatic conditions the formulation containing permethrin would require reapplication after three-four weeks of exposure whereas the formulation containing cypermethrin would require reapplication after five-six weeks of exposure. The different longevity in the insecticide activity of the two formulations has been confirmed by the study carried out on *Lobesia botrana* D&S (Angeli, unpublished data)

In our field experiment, we observed damage in a treated orchard up to 80 m from a source of codling moth infestation. These results suggest that sources or infestation adjacent to a Sirene CM®-treated orchard will also have to be treated with this product to avoid the immigration of mated females.

The efficacy of the control method came from two different mechanisms: mating disruption due to point source competition and attracticide (Charmillot et al., 1996; Suckling and Brockerhoff, 1999). The efficacy of both this mechanisms is reduced when the attraction of the drop is less than that of the moth. Our results demonstrate that the attractiveness of the droplets remain unchanged during a month.

Concerning the number of drop/ha, as the males are likely to be attracted to the droplets as to females, the attracticide is likely to work best with low pest population density so that the success of the treatment is likely to depend ultimately on the density of calling female moths per tree. As the release rate of a Sirene CM® droplet is similar to what is estimated released by a calling female (Bäckman, 1997), the greater the number of attract and kill droplets the smaller would be the chance of males finding a female prior to contact with an insecticide sources, there has to be a trade-off between gains in reliability and increased labour and material costs incurred (Lösel *et al.*, 2000). Our results seem to indicate that a reduced number of drop/ha (1500-2000drops/appl.) could still be effective in the higher elevation and colder fruit growing areas where the codling moth is less abundant.

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Attract and kill of the olive fruit fly *Bactrocera oleae* in Greece as a part of an integrated control system

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Abstract: The "Attract and Kill" method was evaluated for several years in two olive groves to control the olive fruit fly *Bactrocera (Dacus) oleae*. Results indicated that in isolated olive groves and in areas where the olive fruit fly develops low or medium population densities, one killing device per tree, baited with ammonium bicarbonate and pheromone, has the potential to keep the olive fruit fly population and the fruit infestation low. The level of fruit infestation was similar to that obtained in the control field, treated at least three times by ground bait spray of protein hydrolyzate-dimethoate. In non-isolated olive groves and in areas where the olive fruit fly develops high population densities, one killing device per tree provides inadequate protection and at least one treatment by ground bait spray, with protein-insecticide is needed to keep the fly population and the fruit infestation at low level. The results showed that the "Attract and Kill" method could progressively replace the use of insecticides for the control of the olive fruit fly.

Key words: *Bactrocera (Dacus) oleae*, "Attract and Kill", Integrated Pest Management, Pheromones, Food Attractants.

Introduction

The olive fruit fly, *Bactrocera (Dacus) oleae* (Gmel.) is the most serious pest of olives in the Mediterranean countries. Economic losses due to this pest have been estimated to reach up to 15% of the olive crop, in spite of the fact that, pesticide treatments are applied every year to control the fly population.

The olive fly develops two to five generations per year. The females lay eggs in the mesocarp of the olive fruits; the larva feeds in the fruit and pupates in the fruit or in the soil. Larval stages develop from midsummer to late autumn. In regions where olive fruits remain on the trees in spring, the olive fly develops one or two generations in spring. There may be a wide overlap in generations due to adult longevity and a long oviposition period.

Protein hydrolyzate mixed with organophosphorous insecticides bait sprays applied either by air or the ground, have been used for many years against the olive fly (Nadel, 1966; Manousis and Moore, 1987). Usually three to five treatments may be required, especially in years favourable to the pest.

The damage caused by the olive fly and its control measures' results in: a) reduction in yield and quality of fruit and hence of olive oil. b) use of expensive chemicals and application machinery that increases production cost. c) the use of toxic chemicals creating many environmental problems. These concerns have emphasised the need for more selective methods for the olive fly control. Accordingly, development of alternative improved management technology for the control of the olive fly has been the goal of a broad research effort since the early 1970s.

Attempts to control the olive fruit fly by luring them into killing devices were initiated

in 1960's. McPhail traps baited with a solution of protein hydrolyzate were used to lure the flies into the traps (Orphanidis *et al.*, 1958), Visual (yellow color) sticky traps have also been used to control the fly, (Economopoulos *et al.*, 1977), but as many authors have emphasised, these traps can be detrimental to beneficial insects that also respond to the lures. (Broumas *et al.*, 1983; Kapatos and Fletcher, 1983; Jones, 1987).

Since the pheromones of the olive fruit fly were identified (Baker et al., 1980; Mazomenos and Haniotakis, 1981; 1985), pheromone traps have been developed and tested as monitoring and control tools (Mazomenos *et al.*, 1983; Ramos *et al.*, 1983; Broumas and Haniotakis, 1987; Montiel-Bueno, 1987; Haniotakis *et al.*, 1987; 1991).

In this paper we report the results obtain from the evaluation of the "Attract and Kill" method integrating pheromone and ammonium bicarbonate as lures in two regions in Greece, Markopoulo and Stylis, Attikis and Phiotidos, province respectively. The tests lasted for five years with a goal to develop environmentally safe method to control the olive fruit fly

Materials and Methods

Plot Selection and Description.

The test areas were selected in Markopoulo, 30 Km from Athens and Stylis in Central Greece. The 8 ha experimental olive grove in Markopoulo was the main grove in this region surrounded by vineyards and pasture fields. The olive varieties caltivated, are Megaritiki, Manaki, Amphissis (table olives) and Koroneiki olive oil producing variety. The grove is intensively cultivated and regularly irrigated. During the previous years, the olive fruit fly population was suppressed by ground protein-organophorsporous (Dimethoate) bait sprays, and also cover spray insecticides treatments (Deltamethrin or Dimethoate) were applied to suppress the olive moth *Prays oleae* population. The olive trees were 8-12 years old, 5 m tall and 6-7 m apart; the tree density was ca 150 trees/ha. A small olive grove approximately 3 ha, 500 meter apart was treated with protein-dimethoate and was used as control. This olive grove was not irrigated; the cultivars present was more or less similar to that in the "Attract and Kill" grove the same control measures were applied during the previous years to control the olive fly and the olive moth population.

A second experimental olive grove located in Stylis central Greece was added in 1994. The reason of selecting the Stylis region to evaluate the performance of the integrated approach developed was: The caltivar that predominates in this region is the Amphissis that suffers high fruit infestation from both *P. oleae* and *B. oleae*. The results obtained in the semi-isolated grove at Markopoulo were promising and our goal was to test the efficiency of the method in a non-isolated olive orchard. The experimental 33 ha olive grove in Stylis is located within a landscape that is entirely covered with olive trees. Olive fruit fly control in the Stylis region is achieved by air spray with protein hydrolizate-dimethoate baits. Usually 3 to 5 treatments are applied every year. Also 2-3 cover spray treatments are applied to control the olive moth population. The trees were more than 50 years old approximately 10-12 meters high and 9-12 meters apart. The tree density was 100-120/ha.

Pheromone formulations used.

The major female olive fruit fly pheromone component 1,7-dioxaspiro (5,5) undecane was formulated in polyethylene vials or in _-cyclodextrin (_-CD) (Mazomenos *et al.*, 1989, Kondilis *et al.*, 1990). The _-CD-spiroacetal complex was placed in small plastic bags; eight ml of water were added to the bag to improve the release rate of spiroacetal.

Treatments.

During 1992 and 1993 in Markopoulo the killing divices used, were paper plastic bags impregnated with 10 mg of deltamethrin, 10% sugar solution and 1% glycerol (Vioryl A.E Kato Kifissia Greece). Each device was baited with seventy grams of ammonium bicarbonate placed inside the bags and a pheromone dispenser loaded with 50 mg of spiroacetal. Half of the killing devices, one every other tree were installed the third week of June. The first week of September, killing devices were added to the remaining trees. Under the condition tested the killing efficiency of these devices was limited to approximately 45 days from installation and the traps has to be replaced more than once.

During 1994, 1995 and 1996, the killing devices used were consisted of a wire cylindrical frame subtended by a cotton cloth. The cloth was dipped in a concentrated water solution of flowable Desis. It was designed to deliver on each device 40 mg of active ingredient. The attractant components of the killing devices included Ethylene Vinyl Acetate (EVA) board co-melted with ammonium bicarbonate salt (10 g) (AgriSence-BCS, Pontyprid, U.K.) and plastic bag containing a solution of water and 500 mg of -CD-spiroacetal complex (the net pheromone was 50 mg per trap). Bioassays indicated that these killing devices under natural conditions are effective and kill insects for more than 5 months. The same killing devices were also used in 1994 and 1995, to control the olive fly in the Stylis olive grove. Each year in both groves the devices were suspended to the olive trees the last week of June. Killing devices baited with ammonium bicarbonate were hung on every tree, while on every third tree a plastic bag containing the suspension of -CD-spiroacetal was added.

The insecticide treated groves used as control, were treated by ground bait sprays, using protein hydrolyzate and insecticides (dimethoate) and were followed the control programme applied every year by the ministry of agriculture.

Assessment.

The efficiency of "Attract and Kill" and insecticide treatments was assessed by comparing fly catches in McPhail and pheromone traps. Five 20 x 20 cm plywood boards coated with sticky material were used as pheromone traps. Five pheromone traps, baited with a 1 ml polyethylene vial loaded with 25 mg of spiroacetal and five McPhail traps, baited with 3% ammonium bicarbonate solution, were placed in each grove. The same number of traps was placed also in the insecticide treated groves. The traps were in operation from April to November to monitor the fly population. Traps were inspected weekly. Pheromone dispensers were replaced every three-months, while the ammonium bicarbonate solution in the McPhail traps was renewed every week.

Trap catches were transformed to log (x+1) prior to statistical analysis (ANOVA). Means' comparisons were made using the Duncan's multiple-range test.

Fruit infestation by *B. oleae* was also assessed; 10 trees in each plot were randomly selected, four twigs bearing olive fruits from each tree were collected and the number of fruits infested was recorded. Infestation data were statistically analysed using a chi-square 2 x 2 test of independence. The infestation level recorded in the Attract and Kill olive grove was compared to the infestation level recorded in the insecticide treated grove. The P=0.05 level was set for the rejection of the null hypothesis.

Results

Monitoring.

The efficiency of pheromone and McPhail traps to monitor the olive fly population throughout the entire flight season as an overall view of the combined data obtained with

McPhail and pheromone traps, from the five years studies in Markopoulo is presented in Figure 1. Trap catches indicated that the olive fly population varied through the year seasons. In spring (March - May) more flies were caught compared to the flies caught during the hot and dry summer months. In autumn the number of catches in both traps was increased. Pheromone traps were found to be more effective in trapping males during spring and autumn, (F=5.5, df=14 and F=31.8, df=26, P=0.05), while during summer McPail traps were more effective (F=24.7, df=26, P=0.05). Comparing the number of males and females caught in McPhail traps there is not significant difference in spring and autumn (F=3.2, df =14, and F=1.5, df=20, P=0.05), whilst in summer significant more females were caught (F=5.3, df=26 P=0.05). The pheromone traps caught very few females.

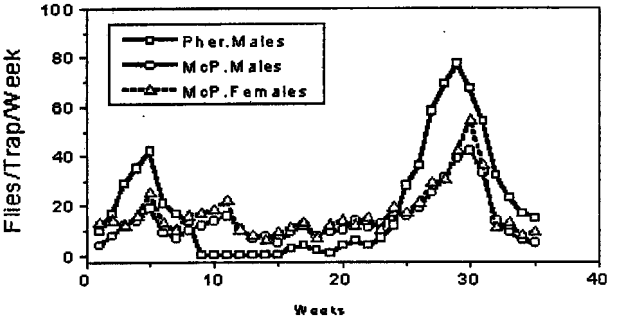

Figure 1. Number of olive fruit fly males and females caught in pheromone and Mcphail traps. Mcphail traps were baited with 3% solution of ammonium carbonate.

Attract and Kill (Markopoulo).

Trap catches in 1992 and 1993 indicated that the fly population remained low in the "Attract and Kill" olive grove, until late August, where it was slightly increased. In this region this is the date of emergence of the most damaging generation of the fly (Fig 2A, 2B). The installation of the same number of new traps to the trees that were left without traps in June resulted to the decreased of the fly population. The same development of the fly population was observed also for the insecticide treated field. This field received three bait sprays protein hydrolyzate-dimethoate treatments by ground in 1992 and two in 1993. The number of flies caught in both olive groves was not significant different in 1992 (F=0.21, df=74, P=0.05), while in 1993 significant more flies were caught in the insecticide treated grove (F=6.71, df=74, P=0.05).

In the insecticide treated grove, three ground bait spray treatments were applied in 1994 and two in 1995 and 1996. The mean number of flies caught were not significant different in 1994 (F=1.61, df=74, P=0.05) while in 1995 and 1996 more flies were caught in the insecticide treated olive grove (F=4.3, df=74 and F=5.2, df=74, P=0.05).

In 1994, 1995 and 1996, (Fig. 3A, 3B and 3C), where the new killing devices were used, after the installation of the killing devices the fly population in the "Attract and Kill" grove remained low until fruit harvesting. 80% of the olives in this grove are table olives and are harvested beginning of October (green olives).

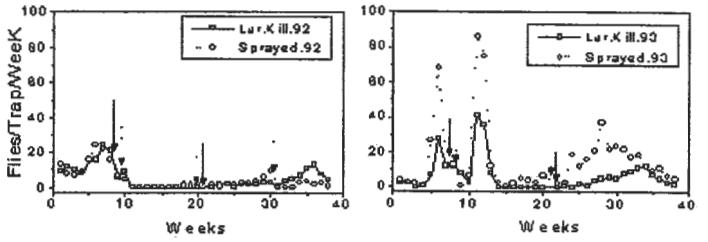

Figure 2. Mean number of *B. oleae* flies caught in McPhail traps in groves treated with the "Attract and Kill" and with bait spray insecticide. Arrows indicate the dates of treatments [Markopoulo, 1992 (A), 1993 (B)].

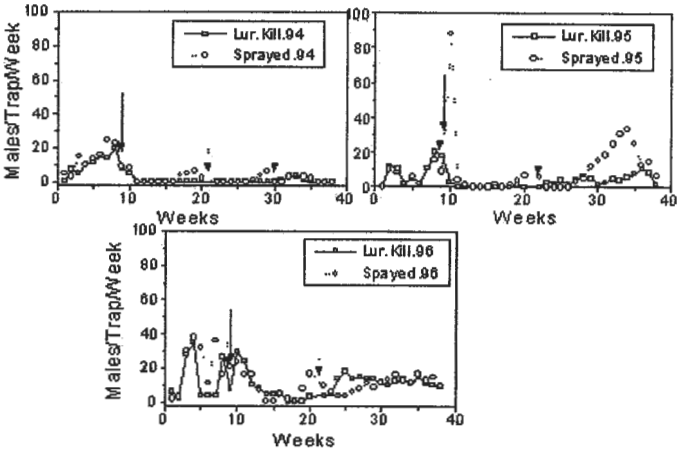

Figure 3. Mean number of *B. oleae* flies caught in Mcphail traps in olive groves treated with the "Attract and Kill" and with bait spray insecticide. Arrows indicate the dates of treatments [Markopoulo 1994 (A), 1995 (B), 1996 (C)].

Fruit infestation.

The fruit infestation in 1992 remained low, 3 to 4% of the olives harvested in October was found to be infested, whilst the level of infestation for the olive oil producing varieties, that were harvested in November was 8% not statistical different than the final infestation measured in the insecticide treated grove (Z=3.7, P=0.05) (Fig. 4). In 1993 a year of low olive fruit production the level of infestation was relatively high in both fields and significant higher in the insecticide treated grove (Z=16.8, P=0.05) (Fig. 4). In 1994 and 1996, high fruiting years the level of fruit infestation remained low and not significant different (Z=0.2 and Z=0.9, P=0.05), in both "Attract and Kill" and insecticide treated groves, while in 1995 a low fruiting year the level of infestation was higher, in the insecticide treated plot (Z=16.3, P=0.05).

Attract and Kill (Stylis).

In 1994 trap catches in spring and early summer was quite high in the experimental as well as

the insecticide treated groves (Fig 5A). At this time of year females lay infertile eggs, and its contribution to the fruit infestation is practically nil although female punching to the olive fruits of the Amphissis variety resulted in secondary fungus infestation. The damage caused by secondary infestation was ranged to 5-8%. The application of the killing devices on June 26th, followed by air bait protein hydrolyzate-dimethoate spraying on June the 28th of all the olive groves in this region reduced the fly population. McPhail trap catches were almost nil in the "Attract and Kill" olive grove from July to October, when a slight increase of flies caught occurred shortly before fruit harvesting. In the insecticide treated olive groves, the traps catches were increased late August and mid of September and two insecticide treatments were applied to keep the fly population low. The mean number of flies caught in both olive groves was not significant different (F=0.5, df=70, P=0.05)

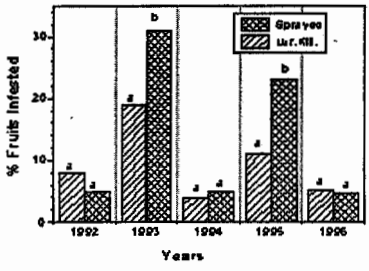

Figure 4. Percentage of olive fruits infested in olive groves treated with the "Attract and Kill" method and groves treated with bait spay insecticides. Bars with the same letter are not statistically different (Duncans multiple range test P=0.05) (Markopoulo, Attikis Greece).

In 1995, killing devises were hung on the trees on June 24th, the fly population remained low and about at the same level as that in the insecticide treated grove until the middle of September (Fig. 5B). From middle of September the olive fly developed high population in this region, due to favourable weather conditions. In the "Attract and Kill" treated grove the fly population was increased, rather due to flies migration.

To protect the olive fruits from damage we decided to spray by ground in the "Attract and Kill" grove with protein hydrolyzate-dimethoate on September 28. The control olive grove was sprayed four times to secure acceptable level of fruit damage. The mean number of flies caught was not significant different in both groves (F=0.3, df=70, P=0.05).

Fruit infestation.

The final fruit infestation in 1994, in the "Attract and Kill" and insecticide treated groves was 7% for the "Attract and Kill" and 4% for the control groves (Fig. 5). In 1995, the fruit infestation in both groves remained low until September and then started increasing. The final fruit infestation recorded was 12.3% and 11.8% for the "Attract and Kill" and insecticide treated groves respectively. (Fig 5).

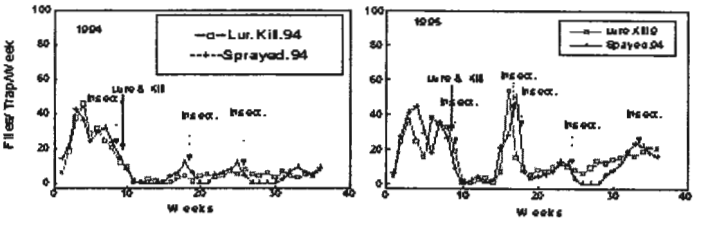

Figure 5. Mean number of *B. oleae* flies caught in McPhail traps in olive groves treated with the "Attract and Kill" and with bait spray insecticide. Arrows indicate the dates of treatments [Stylis 1994 (A), 1995 (B)].

Discussion

McPhail and pheromone trap catches indicated that the olive fly adults are active through March to December. In both type of traps the higher number of flies was caught between September-December, another peak of fly activity was also recorder every year early in spring. The fly population density decreased during May to mid August. The number of males caught into the pheromone traps was nil, whilst few males and females were caught in Mcphail traps. Pheromone traps attracted more males in spring and autumn compared to McPhail traps. In summer more males were attracted to the McPhail traps. Our results are in agreement with those reported by (Ramos and Jones 1983, Montiel-Buenos 1987). It has been reported that the olive fly adults are sexually immature early in summer and the males are not responding to the pheromone traps. Maturation of the olive fruit fly depends on weather conditions and fruit ripeness (Fletcher and Kapatos, 1983). The olive fly sexual maturation and fruit susceptibility to egg attack and larval development is synchronous (Delrio and Cavalloro, 1977; Kapatos and Fletcher, 1983). The onset of male trap catches to the pheromone traps during summer is an advantage because it provides an accurate timing to applied control measures. During this period almost all the fly population present is sexually mature and applying control measures, the possibility for the fly to build up high population density is minimised. For accurate measurements of the fly population and timing the control measures it is suggested that both types of traps are necessary to be in operation, since during the summer months, where the temperature is high, the relative humidity low and limited food recourse available, both sexes respond better to the food attractant baited McPhail traps, than to the pheromone traps, also with McPhail traps a good estimation of the female population present in the olive grove is obtained.

The "Attract and Kill" method applied for five consecutive years in Markopulo and two years in Stylis olive groves clearly indicated that this method has the potential to replace or reduce substantially the insecticide treatments for the control the olive fruit fly. In Markopoulo olive grove that is a semi-isolated grove and the olive fruit fly develops moderate population during the year, one killing device per tree placed at the end of June maintained low level of fly population throughout the entire season and keep the fruit infestation in low level, similar to those obtained in olive groves treated at least three times with insecticide. In years where the control olive grove was treated twice with insecticide the level of fruit infestation was higher than that in the "Attract and Kill" olive grove. On the other hand in the experimental olive grove where no insecticides were use for five years to control the olive moth and the olive fruit fly the number of beneficial insects was increased, improving the

olive ecosystem self defence capacity against these pests and against other pests such as the black scale *Sassetia oleae*. *S. oleae* became recently major pest, due to the use of insecticides to control the olive fruit fly and the olive moth.

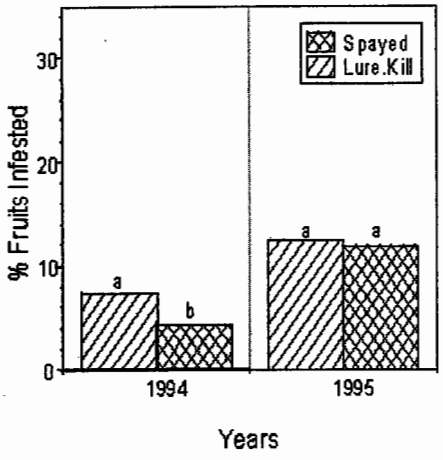

Figure 6. Percentage of olive fruits infested in olive groves treated with the "Attract and Kill" method and groves treated with bait spay insecticides. Bars with the same letter are not statistically different (Duncans multiple range test P=0.05) (Stylis, Phiotidos Greece).

The results also indicated that the fly population was higher in the insecticides treated grove in years following high fruiting years. This is rather attributed to the availability of ovipotional substrate for the spring generation to lay eggs because many fruits remained on the olive trees after harvesting.

Results obtained from Stylis olive grove that is located in the middle of a landscape that is entirely covered with olives and usually high olive fruit fly population is developed, and extensive migration of the fly occurs, indicated that the "Attract and Kill" method is not sufficient to keep the fly population and the fruit infestation in acceptable level and additional control measures are needed. However even though additional control measures are needed the number of insecticide treatments is reduced substantially. Our findings are in agreement with those reported by other workers that in isolated olive groves or in regions where the fly develops low populations per year the "Attract and Kill" method is self effective, while in regions where the fly develops high population density at least one insecticide treatment is necessary to keep the fruit infestation low (Broumas *et al.*., 1983; Haniotakis *et al.*, 1991). The problem of the fly migration can be overcome in cases where the method will be applied in the entire landscape where the presence of the killing devices through June to December will not allow the fly to build up high population density.

The "Attract and Kill" in general, presents certain advantage in controlling the olive fruit fly, since the killing devices are compatible with insecticide application. It is a simple method not requiring extensive technological background and a great amount of knowledge to be transferred to the farmers. Whether an integrated management approach will be adopted by the state authorities and the farmers, and will be used to control the olive pests and replace the insecticides, depends on the commercial availability of the killing devices, the attractants

used, and the authorities concern on the protection of the environment and the improvement of the olive products quality. The amount of information available indicates that the replacement of toxic insecticides with environmentally safe methods to control the olive pest is now possible.

Acknowledgements

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Use of pheromones and other semiochemicals in integrated production
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Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals

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Abstract: The use of semiochemicals, both sex pheromones and food attractants, in monitoring the olive fly, *Bactrocera oleae,* has become well established in most olive-growing countries of the Mediterranean basin. There is an ever-increasing degree of sophistication that is being introduced in the way trap catch data are collected and interpreted, making for a substantially more rational use of conventional insecticides.

The introduction of semiochemical-based products as control methods in IPM packages for *B.oleae* has been very successfully accomplished on a pilot scale in a number of olive growing countries. These pilot scale trials are now being extended into area-wide programmes in Spain, Italy and Greece and will, in due course, be extended to the whole of the Mediterranean basin.

Key words: sex pheromones, food attractants, monitoring, control, mass trapping, lure and kill, olive fly

Introduction

The olive tree is regarded as being of significant socio-economic importance in the Mediterranean basin which has 98% of the world's cultivated olive trees. These trees number about 800 million and occupy a surface area of approximately 10 million hectares. They produce about 1.6 million metric tonnes per annum of olive oil, in addition to 750,000 metric tonnes of table olives - about 9% of the area's production of olives.

The losses, cause in this crop by insect pests, fungi and weeds, have been quoted by some authors to be as high as 30% of production. It is reasonable to estimate therefore that the damage caused to harvested fruits by insect pests to be at least 15% of production, which equates to 800 million US dollars per annum. This comes despite the fact that olive growers spend annually more than 100 million US dollars combating these pests and of which 50% corresponds to pesticides.

The predominant method of insect pest control has been through the use of conventional pesticides. They have been very effectively used over the last forty years and have ensured yields and benefits to the olive growers. The continued use of such products has however been questioned in recent years especially by environmentalists. Residues of pesticides have been detected in olive oil and in the environment where olives are grown.

This has caused concern in most olive growing countries and has lead to a concerted effort to reduce the amount of pesticides that is being used to control pests in olive groves. As in many other agricultural crops, the concept of Integrated Pest Management (IPM) has also been developed in the olive growing industry as a means of rationalising the use of pesticides. IPM has been defined by the International Organisation of Biological Control (IOBC) as "a pest management strategy employing all methods consistent with economic, ecological and

toxicological requirements to maintain pests below economic threshold while giving priority to natural limiting factors". IPM systems are now widely accepted as the best strategies for sustainable crop protection.

Technologies and techniques which have found a useful role in IPM strategies include; the use of resistant plants varieties, cultural practices, the use of predators and parasites, microbial pesticides (entomopathogenic bacteria, viruses and fungi), botanical insecticides, insect growth regulators and semiochemicals. Many of these have been researched as constituents of an IPM strategy for olive pests. However, for the purposes of this paper, only the role of semiochemicals in the monitoring and control of *B. oleae* will be discussed.

The use of semiochemicals in olive fly management

Semiochemicals are defined as 'substances which transmit messages between living organisms, both plant and animals' (Law and Regnier, 1971). Semiochemicals which areemitted by an individual and produce a response in another individual of the same species are referred to as pheromones (Karlson & Luscher,1959). Pheromones can be classified interms of the response which they produce (Shorey, 1977) e.g., aggregation pheromones, alarm pheromones, recognition pheromones and sex pheromones.

In the case of the olive fly it has long been known that the species uses a sex pheromone as part of its mating behaviour. A sex pheromone released by virgin females attracts male *B.oleae* (Haniotakis, 1974); Haniotakis *et al.,* 1977). The principal component of this sex pheromone was identified in late 1979 as 1,7-dioxaspiro [5.5] undecane (Baker *et al.* 1980). Other components were identified from the sex pheromone by various authors but none were found in subsequent field trapping experiments to be critical in attracting male *B.oleae* to traps. It has also been shown that during periods without reproductive activity in early summer, male *B.oleae* can also produce measurable quantities of the spiroacetal major component described above and the authors concerned think that it could act as an aggregation signal during such times to bring others of the same species to food sources (Mazomenos and Pomonis, 1983).

In a pest management context, however, it is the practical use of these substances, which is of interest, and their use to date can be divided into two main categories, pest monitoring and pest control.

Monitoring of *Bactrocera oleae*

In order to monitor insect populations adequately, it is important to have systems, which record both biological and climatic data. These data need to be collected in real time and give us information about both adult and larval stages. The use of such information is only valid if it is incorporated into pest management models, which in turn allow us to foresee and prevent damage to the crop. In the case of *B.oleae*, monitoring of adults in traps and observations of larval stages in fruit samples are coupled with climatic data to make predictions of damage and take preventive measures. Such climatic data is collected from automatic agro-climatic weather stations, which are capable of recording and storing great quantities of climatic data and sending it automatically via a phone line to a central collection point.

Traditionally, water based trapping devices baited with olfactory attractants such as ammonium salts or protein hydrolysates have been used to monitor adult populations of the olive fly. Traps such as the McPhail traps are still in use today and give very useful information especially about female *B.oleae* activity, although in some quarters they may be considered as old fashioned. They do however have some disadvantages such as their low

efficacy during periods of high humi*165*riods, and their lack of specificity in that they also attract non-target insects, some of which are beneficial predators or parasites in the olive grove.

It has long been known that yellow colours are attractive to Tephritid Diptera. This has lead to the development of trapping devices consisting of plastic strips of approximately 17 x 23 cm which have the appropriate shade of yellow for maximum attraction and which is covered in a non-drying adhesive. The distance of attraction of such traps however is not very great and in general does not go further than the immediate surroundings of the tree in which it is suspended and for this reason the trap usually has a very low trapping efficiency (Delrio, 1985).

When these visual traps are baited with vials containing 25 mg of the spiroacteal major component of the sex pheromone, their efficacy and radius of attraction is significantly increased (Jones *et al.,* 1983; Delrio, *et al.*, 1983). The enhanced attraction of males to such traps when baited with pheromone was clear from the very beginning but there was also some evidence that the traps were more attractive to females (Montiel and Jones, 1989) - this fact was later to prove important in the use of this pheromone for control purposes as discussed in Section 2 of this paper.

For most of the 1980's the pheromone dispenser used consisted of a polyethylene vial which required changing every 6 to 8 weeks. However, long life lures are now available which are loaded with 80 mg of spiroacetal and which last over 6 months. These are very useful not only in monitoring traps but also in lure and kill target devices used to suppress *B. oleae* populations as is discussed later in this paper.

One disadvantage of this trap, which is based totally or partially on attraction to colour, is that it also attracts non-target insects, and at high trap densities, such traps can cause damage to beneficial insect populations (Neuenschwander, 1982). This problem can be overcome or eliminated using traps of a colour other than yellow, which is not attractive to beneficial insects (Haniotakis *et al.,* 1982), or by installing the traps in the tree in such a way that they selectively catch *B. oleae* (Jones *et al.*, 1985).

In terms of practical application in the field, it has been found in Jaén, Spain, that a combination of traditional trapping systems such as the McPhail trap and the more recent sex-pheromone baited traps gives the best population monitoring information for pest management purposes (Montiel, 1987). While the pheromone system gives the best information about pest populations and their dynamics (Montiel and Madueño, 1995a,b), the McPhail traps are used to generate extra information such as female fecundity levels, which is obtained by dissecting females caught in these traps and assessing the stage of development of the ovaries.

When used correctly this combination system can give information on (1) the presence or absence of B. oleae in the olive grove; (2) the period of sexual activity in the fly population. During early summer, when temperatures are high and the olive fruit is still not of a sufficient size for the females to oviposit, there is a cessation of sexual activity. However, as autumn approaches and the fruits mature, sexual activity is initiated once more, and the yellow traps baited with the pheromone at this point immediately start to catch a much larger number of male *B. oleae* than other yellow traps which are not baited with the pheromone. This information helps the field operator to decide on the optimum time for spray applications (Delrio, 1985; Montiel, 1987).

It can show the efficacy of treatments against the pest by measuring levels of adult captures before and after treatments. Correlations can be obtained between trap catches and infestation of fruit (Ballatori *et al.*, 1980; Montiel and Moreno, 1983; Crovetti *et al.*, 1983) so that spray thresholds can be established (Delrio, 1985; Montiel & Madueño, 1995 a,b) and

with which population models can be established which predict levels of risk of attack from the pest (Montiel and Moreno, 1983; Montiel & Madueño, 2000 – in press).

Since 1990, there has been in place in Spain a Programme for the Improvement of Olive Oil Quality, which is regulated by the European Union and financed by retaining 1.4% of the aid directed at olive production. This is used to monitor olive fly populations and co-ordinate and carry out control measures against it. During 1999, 1,313,858 ha of olives (in 24 provinces of 10 autonomous regions) were involved in the programme. This entailed having 1,142 monitoring points for adults and larvae and 82 meteorological stations. Data from these were sent via 24 provincial micro-processors to two central mini-computers where all the data was finally processed.

The Control, Alerting and Validating features of this system consists of the following characteristics: (1) A dividing of the olive growing areas into 10,000ha blocks; (2) a meteorological station in every block; (3) within each block, the olive groves are subdivided into homogeneous sub-blocks of about 100 ha each; (4) within each homogenous sub block a monitoring point is established consisting of 5 McPhail traps, and 5 yellow sticky traps with long life pheromone dispensers; (5) monitoring of adult populations of *B.oleae* is by weekly trap counts at each monitoring point; (6) monitoring of larval populations is weekly fruit samples.

Information Processing Levels are: (1) Monitoring points and meteorological stations integrate basic biological and climatic data; (2) transmission of level 1 data via telephone lines to the provincial micro-processors; (3) receipt and processing of data received in the provincial micro-processor (integration of provincial biological and climatic data); (4) receipt and processing of data at a national level in the mini computers. (integration of biological and climatic data with historical information)

Over the last decade, the collection and handling of data from such trapping devices has become clearly more sophisticated. The Spanish system described above has worked very well and has allowed the authorities there to foresee and prevent major damage by *B. oleae* in all the olive growing areas of Spain. Italy and Greece are also developing similar early warning systems as part of IPM packages for *B. oleae* and such systems will undoubtedly be extended to other countries in the Mediterranean basin.

Controlling *Bactrocera oleae*

Three main strategies involving Semiochemicals (mass trapping, lure and kill and mating disruption) have been pursued in the development of integrated pest management strategies for olive pests.

Mass trapping

The concept of mass trapping, in principle, is very simple; place a sufficiently high number of traps in an olive grove and achieve a satisfactory level of control through the capture of large numbers of adult flies. In practice, however, several factors influence the success of such efforts and cause some doubt as to the viability of such a technique for olive fly control on a large scale. Yellow traps coated with non-drying adhesive have been used successfully on both a small and a largescale for controlling olive fly populations. However, the high density of traps used required in some cases as many as five traps per tree, which made the technique uneconomic and very destructive to the natural enemy population in those olive groves (Economopoulos, 1979; 1980).

The number of traps required can be reduced substantially if the traps are baited with and olfactory attractant, a food attractant, a sex pheromone or both. Experiments carried out

in Italy have demonstrated that, with one yellow trap per tree baited with ammonium carbonate and the sex pheromone, very acceptable results were obtained if the olive harvest was good and the population of olive fly was low. However, the results obtained in those years where the population of olive fly was high and the olive harvest was low were not satisfactory (Delrio, 1985). Traps baited in a similar way, but used at a density of 1 per 9 trees, when used on a grand scale in Greece have lead to a reduction in the number of treatments required to control olive fly from 3 to 1 (Broumas *et al*, 1983).

Several factors therefore make the technique non-viable on a large scale. These include: (1) The lack of good attraction of females by the attractant source used; (2) the lack of highly efficient traps; (3) the problem of high insect populations and trap saturation; (4) the destruction of natural enemy populations if they are attracted to the same traps; (5) the need for a high density of traps per unit of surface area which in turn renders the technique too costly.

Many of these problems were initially found to apply in the case of *B. oleae* but have been overcome by abandoning sticky traps and turning instead to `target' devices with low visual stimulation for the flies but which were treated with insecticides to kill them once they made contact with them. In this way, problems of trap saturation were overcome because the insect once having picked up a lethal dose of insecticide from the target device, then flies or walks away from it until the toxic effects of the insecticide manifest themselves. Similarly, to use targets with grey, green or brown colours reduces the attraction of beneficial insects and thus conserves their populations in the olive grove.

Lure and Kill

Lure and Kill strategies for tephritid fruit flies fall into two groups; those that employ some form of target device and those that rely on attracting the insect onto a natural surface, e.g. host tree foliage, which has been treated with an attractant/insecticide mixture; this second technique will be referred to as sprayable formulations.

Target devices for Bactrocera oleae.

Work in Greece over the last ten years, and more latterly in Spain, has been aimed at overcoming the short-comings of mass trapping using sticky traps through the development of target devices which carry an insecticide for killing the attracted flies instead of adhesives. The target devices used in most of the large scale trials undertaken in Greece consisted of plywood rectangles (15 x 20 x 0.4 cm) dipped into appropriate concentrations of the pyrethroid insecticide Deltamethrin for a sufficiently long period of time to saturate the wood with the insecticide solution. These devices were baited with ammonium salt dispensers (food attractant) and one target in 3 or 5 was also baited with a sex pheromone dispenser.

Fewer sex pheromone dispensers than the food attractants were thought necessary since their distance of attraction was shown in earlier experiments to be 60 - 80 m while that for the food attractants was only 15 - 20 m. Great logistical problems had to be overcome during the installation period of the devices in June and July, especially in years where over 2,000,000 trees were treated. As the controlled release devices for the food and sex attractants became more advanced it was possible to install the target devices during early summer and reasonably expect them to last until late autumn when olive fly populations reduce in importance through decreasing temperatures and the olives were harvested. Young agriculturalists monitored the effectiveness of the target devices throughout the periods of operation and they monitored olive fly populations by the use of traps and through taking samples of olive fruit for periodic examination of damage levels. They also took samples of the target devices back to the laboratory to verify by bioassay that the insecticide content of

the plywood boards was still sufficient to kill the fly.

The results over five years from the area-wide application of these target devices in Greece can be summarised as follows:- Fly populations as measured by McPhail traps were consistently lower in target-device treated areas compared with conventionally treated controls. The average number of bait sprays that had to be used in target treated areas during the early years of the programme (1984/1985) was 1 as opposed to 2.5. No supplementary bait sprays were required in later years in the target-device treated areas. In most years, fruit infestation was lower than or equal to that in the controls where bait sprays were applied (Haniotakis *et al.*, 1991).

The target device method of controlling *B. oleae* therefore was very effective as a method of eliminating insecticide bait sprays and significant increases in beneficial insect numbers were observed in target-device treated areas (Paraskakis, 1989). However, for the method to work to its greatest effect, it has to be applied on a large area. In small plots, large-scale adult movements over short distances can significantly over-ride the effects of the devices. Similarly, when the system fails to contain pest populations, complementary measures are almost invariably required, significantly affecting the cost effectiveness of the technique.

In Spain similar target device technology has been tested on a relatively large scale since 1992. The device used in initial trials was similar to that used in Greece but it was made out of cloth, which was then soaked in deltamethrin rather than wooden boards. The types of baits used depended on the ease of access to the olive groves and the target devices. In groves where the trees and the targets are easily accessible the targets have been baited repeatedly with sprayable protein (1ml of protein hydrolysate) and/or sprayable pheromone (1ml of micro-encapsulated spiroacetal 'Polycore SKL'). In groves which are difficult to access such as in steep mountainous terrain, the targets were baited with long life baits such as those used on monitoring traps (5g dispensers of ammonium salt and 80mg long life dispensers of spiroacetal). These baits usually last the whole season from June to November. The systems involving sprayable attractants need to be replenished several times during the season but are very low cost in materials but labour-intensive. With the long life lures, the up-front costs are greater but require very little maintenance thereafter during the rest of the season. The results obtained in both large and small plots were satisfactory using both systems. Reductions in infestation of at least 50% were observed in treated plots compared with untreated controls.

Since 1998, two industrially produced target devices have been tested in Spain. One is produced in Greece (Vioryl) and the other in the UK (Agrisense). Given the industrial scale of the production of these two products, the costs involved have been substantially reduced. The Greek target device consists of a green coloured bag containing 60g of ammonium bicarbonate and is covered on the outside with deltamethrin. It is also baited with a sex pheromone dispenser containing 80mg of spiroacetal. The target is used at a rate of one per tree and half the traps are placed in the grove at the start of the summer and the other half are placed in the autumn when the fly begins to attack the fruit.

The traps of UK origin consist of a square brown-coloured carton (19 x 19 cm) which is impregnated on both surfaces with at least 20mg of a pyrethroid such as Deltamethrin. Every target carries an ammonium bicarbonate dispenser and one in every three targets has a spiroacetal long life dispenser. The target device is easily attached to small branches by a hook and eye system that leaves the device in the form of a cone, which protects the attractant dispensers. All the devices are installed at the beginning of summer. Results obtained over the last two seasons have been very satisfactory with the commercial target devices although fly populations have not been very high. The levels of control obtained were in most cases similar to those obtained with bait sprays.

The target device method of managing *B. oleae* populations, although more labour intensive generally, will nevertheless be pursued in most olive growing countries since legislative and environmental pressures will eventually restrict the broad scale use of bait sprays.

Sprayable formulations for Bactrocera oleae.

The wide scale use of protein/insecticide bait sprays for controlling *B. oleae*, has become well established in most Mediterranean olive growing countries, together with several disadvantages to its use. Probably the most important problem with this technique is its lack of selectivity. Many important insect predators and parasites are known to be attracted by the protein hydrolysate component of the bait spray mix and this often leads to substantial reductions in their populations with continued use of this technique.

With the isolation and identification of the sex pheromone of *B. oleae*, a selective attractant became available which could substitute the protein hydrolysate. Trapping experiments showed the 1,7 dioxaspiro [5.5] undecane major component to be strongly attractive to the males (Jones *et al.*, 1983).Observations made during attempts to disrupt the mating of *B. oleae* using techniques similar to those used for Lepidoptera, showed that instead of producing mating disruption, the wide-scale treatment of experimental olive groves with the pheromone produced in some instances only large immigration of *B. oleae* adults, both male and female. It appeared therefore that the pheromone, in addition to attracting the males in a clearly directed manner as seen with monitoring traps, was also attractive to females but not in such a strongly directed way. Attention was therefore moved from trying to achieve mating disruption with the pheromone to using the pheromone as a substitute attractant in bait sprays which were much more selective.

A sprayable formulation of the pheromone was therefore required which would slowly release it over a period of time consistent with the effective life of the insecticide once applied in the field. The pheromone was micro-encapsulated in Poly-urea type micro-capsules (5-10 micrometer) and in polymer entrapped micro beads (5-10 micrometer) (Polycore SKL□) containing 20 g spiroacetal per litre. More recently a concentrated polymeric formulation has been introduced with 150g spiroacetal per litre which has a similar field performance but which is much more cost effective. The formulated pheromone in every case is tank mixed with either malathion or dimethoate and applied aerially or from the ground.

In aerial sprays the plane delivers a 20 m wide swathe every 100 m of grove so that only 20% of the crop is treated. From the ground, the pheromone/insecticide mixture is applied either from a tractor mounted sprayer which applies the mixture to the south side of each row of trees or, if a knapsack sprayer is used, only 0.5 to1 square metre of foliage, again on the south side of each tree, needs to be treated. Trials carried out over many years in Southern Spain have shown that both application methods give consistently good results (Montiel, 1989). Indeed this technique is now being used on large areas of environmentally sensitive National Parks of Cazorla and Segura in Southern Spain totalling 13,800ha in 1996 (MAPA 1996).

For organic olive oil production the same techniques can be used but the killing agent has to be changed for one approved by the various organic farming accreditation bodies. In Spain, about 1,200ha of olives are treated with the sprayable pheromone formulation mixed with natural pyrethrum and rotenone (two botanical insecticides approved by the Spanish organic growers association). Such olive oil commands a very good price premium for the producers and can more than compensate for the higher costs of the control mechanisms involved.

The introduction of semiochemical based products as control methods against *B.oleae*

has been very successfully accomplished on a pilot scale in a number of olive growing countries and is now being applied on an area wide basis in Spain, Italy and Greece. Much further work is now required to transfer these newly developed techniques to the remainder of the olive growing regions of the Mediterranean Basin.

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Use of pheromones and other semiochemicals in integrated production
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The use of geostatistics and GIS as tools for analyzing pheromone trap data at a landscape level: an update

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Abstract: Spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), is the most important defoliator of forest trees in the boreal forest of North America. The species undergoes dramatic and periodic outbreaks, which result in extensive defoliation and tree mortality of fir-spruce forests. A network of pheromone traps for monitoring populations of the budworm has been placed, throughout the distribution of the moth, annually since the mid-1980's. Cooperators from government agencies and private forestry companies in Canada and the United States deploy traps using standardized sampling protocols. A computerized software system has been developed using geostatistics to convert the male moth counts, at point locations, to complete spatial coverage maps for use in geographic information systems (GIS). The system uses variograms to model autocorrelation between sample points and a technique known as kriging to interpolate between sample points. The resultant maps can be used to predict incipient outbreaks and predict defoliation. Benefits of the system for predicting changes in budworm dynamics and problems associated with coordinating a network with many diverse collaborators over an extensive geographic area are discussed.

Key words: spruce budworm, *Choristoneura fumiferaraa,* geographic information systems, geostatistics, spatial analysis, pheromone

Introduction

A brief description of the biology and recent history of spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), in North America is discussed in the context of its population periodicity. The dynamics of budworm populations and its economie impact on spruce-fir forests necessitated the development of a spruce budworm pheromone-trapping network for monitoring population densities. Herein, the evolution of the network is discussed. The advent of using spatial analysis techniques in ecology at a landscape level and the adoption of these techniques to spruce budworm data analysis are presented. A set of software tools was developed to use these techniques to analyse budworm pheromone-trap data. Some of the issues that have arisen in coordinating and managing this data set are described. How these techniques can be employed to manage budworm populations is presented.

Spruce Budworm Periodicity

Spruce budworm is an outbreak species and the most destructive defoliator in the firspruce forests of North America. Larvae of this native pest feed on the needles of balsam fir and white, black and red spruce. Populations undergo dramatic increases in population density during outbreaks and the outbreaks exhibit a cyclical periodicity. Unlike other species of outbreak insects, the period of spruce budworm outbreaks is very long and can last for 35-40

years (Royama 1992). Sanders (1996) graphed the eruptive change in larval densities that occurred during the course of the recent outbreak at one location in northern Ontario (i.e., Black Sturgeon Lake). He also demonstrated that male moth numbers captured in pheromone traps were correlated with these larval densities.

Annual data on defoliation by the spruce budworm for Canadian provinces and territories for 1975 to 1998 were extracted from the National Forestry Database Program (Canadian Council of Forest Ministers): http://nfdp.ccfm.org/frames2-e.htm

Defoliation in Nova Scotia and Prince Edward Island began in 1977 and declined to low levels by 1987. The area of defoliation in Newfoundland and New Brunswick was extensive in 1975 and steadily declined in the former province by the mid-80's but persisted into the mid-90's in New Brunswick. Recent defoliation in the Maritimes has been negligible. In Ontario and Quebec, defoliation was at a very high level in the late 70's and early 80's. In Quebec, the area of defoliation had declined to moderate levels by the late 80's and has been relatively insignificant in recent years. Defoliation levels remained high longer in Ontario as a result of regional differences in timing of the outbreak in that province (Candau et al. 1998). In western Canada, defoliation by the spruce budworm was very low in the late 70's and early 80's (except in Manitoba where it was variable). From the late 80's to the present, defoliation has increased in the west. However, the area defoliated in the west is relatively small compared to the huge areas of defoliation in the east during the 70's and 80's. The overall trend in defoliation history across Canada has been a steady decline from 1975 to the present, with a 50-fold reduction in area defoliated. Extremely low levels of defoliation by spruce budworm in eastern North America in recent years and the prospect of another spruce budworm outbreak, suggests that this would be an opportune time to use an early warning system for predicting population increases.

Spruce Budworm Pheromone Trapping Network

The elucidation of the sex pheromone of the spruce budworm in the 1970's (Sanders and Weatherston 1976) provided a method for sampling populations of male moths. It was envisioned that a network of pheromone traps placed throughout the distribution of spruce budworm would provide a cost-effectave method of sampling bud-worm populations. The technique would be less labour intensive than were conventional methods of sampling egg or larval populations (Sanders 1980). The network would not only provide an early warning system for impending outbreaks, but would also provide an index of population size at higher densities. The spruce budworm pheromone-trapping network had its origins in the Canada/US spruce budworm (CANUSA) project in the mid-1980s (Allen et al. 1986). Standard sampling protocols were advocated for use by all collaborators. This facilitated making comparisons between jurisdictions. The sampling protocol which dictated where and when traps should be deployed is described by Sanders (1996). The standard trap used in the network is the non-saturating Multipher trap that allows large numbers of moths to be captured. All trap locations were georeferenced using Universal Transverse Mercator (UTM) coordinates at a 10-km by 10-km resolution. Traps were deployed throughout the distribution of *C. fumiferana* in Canada and the United States (excluding Alaska, British Columbia, Yukon Territory and Northwest Territories). At the peak of trapping in 1995, 1110 pheromone-trap sampling locations were used across the continent (Figure 1). Collaborators have included provincial, state and federal government agencies in the United States and Canada, as well as industrial participants.

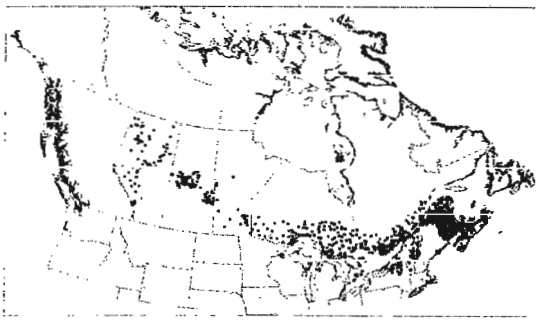

Figure 1. Distribution of pheromone-trap sampling locations for spruce budworm across North America in 1995.

Spatial Analysis

Liebhold et al. (1993) demonstrated that spatial analysis techniques, employing both geostatistics and geographic information systems (GIS), could be used to analyze population processes in insect populations at a landscape scale. Herein, the geostatistical technique known as kriging is used to interpolate point data (i.e., the number of male moths) from the trapping locations and construct surface maps that can be employed in a GIS. The interpolation technique relies on an autocorrelation function, known as a variogram, to provide weighting of nearby points used in the estimates.

Data Management Software

A set of software tools was developed to analyze data from the spruce budworm pheromone-trapping network (Lyons et al. 1997; Lyons and Sanders 1998). The tools were designed to be inexpensive and user friendly, and run on a desktop computer under the Microsoft Windows operating environment. It was anticipated that cooperators would be able to analyze and manipulate their own data, as well as data from adjacent jurisdictions. The software system includes both commercial and inhouse developed software. The graphic user-interface, written in the Microsoft Visual Basic programming language, links the program modules. Modules used in the system include Microsoft Access as the database software and Idrisi as the GIS software. Output maps can be enhanced using the structured drawing program CorelDRAW. The Geographic Calculator is used for projection conversions. The Kriging module was originally written in Visual Basic and C++ but has now been entirely converted to the former. We have invested considerable effort in correcting deficiencies in the software. A recurring problem with the software involved problems with conversion of database files to newer versions of Access. The solution to this problem in the latest version (2.1) of our software has been to simplify the database structure that was described by Lyons et al. (1997). Data are now stored in two separate tables within the database file. Georeferenced data are stored in one table, while trap catch data is stored in a second table. A unique identifier in each table facilitates linking the two. In addition, the system is now capable of producing maps for all provinces (except British Columbia) and

several regions (Maritime Provinces, Prairie Provinces, Northeast and North America). Typical output from the software system is a contour map showing categorized moth captures (e.g. Figure 2A). Since the process interpolates between sample points, extrapolates beyond sample points and uses points around the vicinity of the region being mapped, the GIS can be used to limit the contour map to the boundaries of the area in question (e.g. Figure 2B) and to a fixed radius around sample points (e.g. Figure 2C) (see Lyons et al. 1998).

Issues

Some of the issues or problems that have been identified over the course of the project include: 1) changes in cooperators, 2) changes in sampling protocol, 3) forest cover differences, 4) taxonomie problems, 5) variable georeferencing systems, 6) changes in lure potency, 7) changes in trap numbers and 8) a sample point density bias.

Over the course of the network, a significant number of changes have occurred in participating agencies and companies, and in the staff conducting the survey work. This has resulted in problems in continuity of reporting. Some jurisdictions, for various reasons, have implemented changes to the original sampling protocol. For ex Fleming et al. (1999) described problems in the interpolation method when some sample points are clustered in areas of high density and variability. Supplemental sampling of second-instar larvae in New Brunswick in such an area (Figure 3), resulted in a variogram that indicated greater semivariance in classes of nearby points. Removal of the supplemental points from the analysis produced a more conventional-shaped variogram. Similar clustering of pheromone trap sites has occurred during the history of the network, especially when trap densities in a jurisdiction have undergone dramatic changes.

Applications

At low densities of spruce budworm, intensive sampling of branches produces few larvae. At some point in the increasing phase of the population, sampling once again becomes practical. Sanders (1996) suggested that a moth density of 100 moths/trap was an appropriate threshold for instigating conventional larvae sampling methods. This value is correlated with approximately three larvae/branch. Output maps from the software system can be reclassified in the GIS to display areas where moth densities exceed this value and threshold maps can be produced (Figure 4).

The province of Alberta has specific densities of moths that they use to predict damage in the following year. These forecasts are unique to this province. Densities of less than 500 moths per trap predict a low risk of outbreak, 500 to 2000 moths predict a moderate risk, while densities greater than 2000 moths/trap predict a high risk of outbreak. Thus the output maps for Alberta can be reclassified in the GIS to reflect these moth densities and produce what they would refer to as a risk of outbreak map (Figure 5).

Two simple GIS functions can be used to manipulate output maps from our system and create a useful management map. Difference maps can be constructed by subtracting maps from two consecutive years (i.e., $map_D = map_t - map_{t-1}$). The resulting map can be reclassified to show areas of increasing populations (I.e., positive values), decreasing values (i.e., negative values) and stable populations (i.e., zero values). For example, the moth density map for Ontario in 1998 (Figure 6A) was subtracted from the moth density map for 1999 (Figure 6B) and a difference map was produced (Figure 6C).

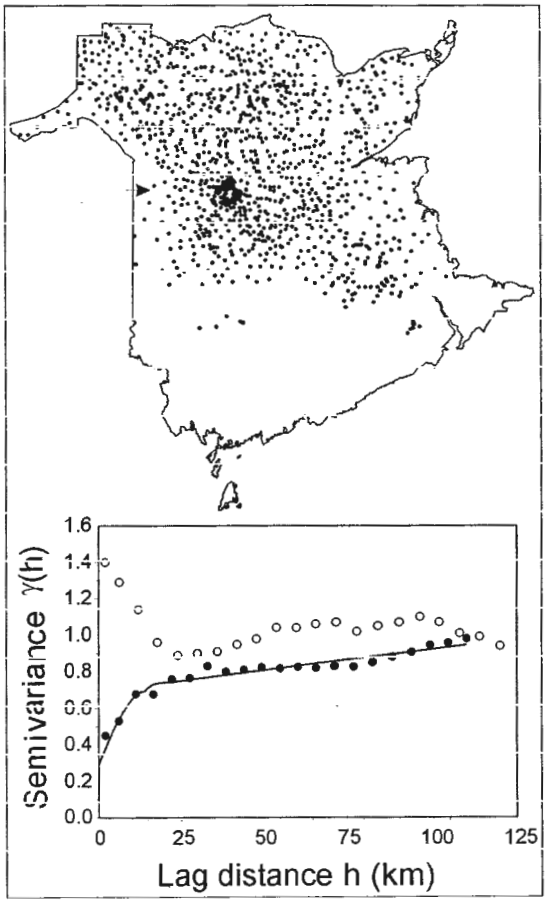

Figure 3. Distribution of sample locations for second-instar larvae of spruce budworm in New Brunswick and the corresponding variograms including (open circles) and excluding (closed circles) supplemental sample points (after Fleming et al. 1999). The arrow indicates the location of supplemental points.

One of the more powerful applications using these kriged maps surfaces is when these maps are used as variables in predictive models. In the following example a logistic regression model was constructed that uses the interpolated pheromone trap catch map (phero) as an input variable along with maps of previous year's defoliation (defol) and defoliation frequency (deffreq) to predict defoliation probability (p). Defoliation frequency was the sum of all defoliation maps for the province of Ontario, generated from aerial surveys, for the years 1941 to 1999, divided by the number of maps.

Figure 4. Areas of Ontario where male moth densities exceed the 100 moths/trap threshold in 1998.

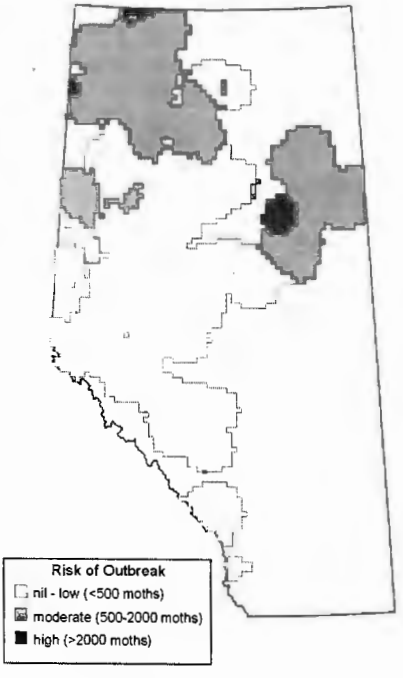

Figure 5. Risk/hazard map for spruce budworm in the province of Alberta in 2000 based on pheromone trap catches in 1999.

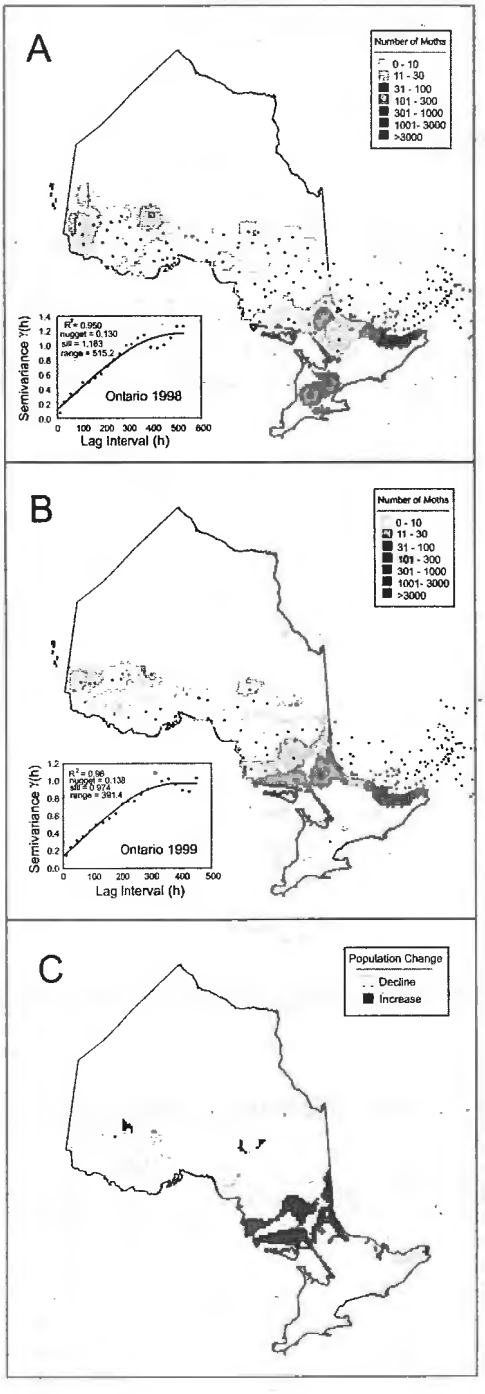

Figure 6. Estimated numbers of male moths of the spruce budworm in Ontario in 1999 (A) and 1998 (B). The positive and negative differences in the estimates for the two years indicate increasing and decreasing populations (C). Estimated numbers of male moths greater than 100 moths/trap indicate areas where larval sampling should be conducted (D).

The following equations are the model:
logit(p) = -13.51 + 3.44 log(phero +1) + 25.07 log(deffreq +1) + 2.73defol

$$p = \frac{e^{\text{logit}(p)}}{(1+e^{\text{logit}(p)})}$$

The concordance value (Table 2) for this model was extremely high at 97%. Solving the equations using the pheromone trap catch map for 1999 and the defoliation map for 1999 as input values results in an output map (e.g. Figure 7) which predicts the probability of defoliation in Ontario for the year 2000. Since moth captures are low in Ontario and there was only limited previous defoliation, the model only predicts two very small areas with probabilities of defoliation greater than 0.10 for 2000. A complete description of the model will be published elsewhere.

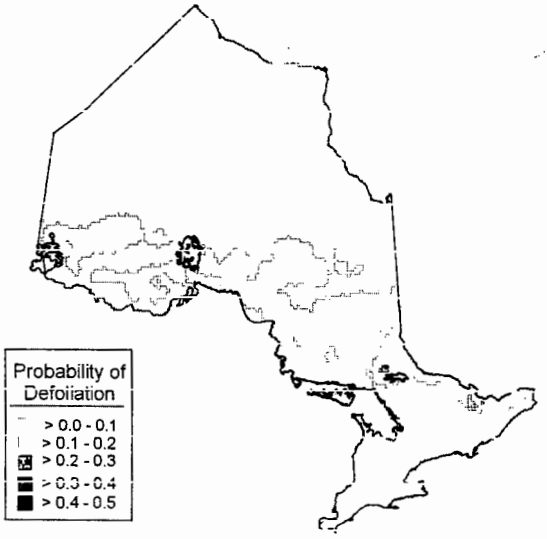

Figure 7. Probability of defoliation by the spruce budworm in Ontario as predicted from the logistic regression model.

Table 2 Statistics for logistic regression models incorporating individual and combined variables

Variable(s)	Concordant (%)	Discordant (%)	Tied (%)
deffreq	68 .3	26.7	5 .0
defol	79.1	0. 8	20.2
phero	92.3	7.4	0.3
deffreq, phero	94.7	4.7	0.6
deffreq, defol	90.5	8.2	1.4
defol, phero	96.6	3.2	0.2
deffreq, defol, phero	97.0	2.8	0.2

Future Directions

The data-analysis system for the spruce budworm pheromone-trapping network has now been developed to a stage whereby it is capable of quickly generating maps for a variety of jurisdictions and regions. It is now essential that the system be integrated into the spruce budworm management program of the individual jurisdictions. This requires feedback from the end users. To ensure that region-to-region and year-toyear comparisons can be made, a consistent source of pheromone must continue to be available to all cooperators every year and sampling must be undertaken using consistent protocols. In addition the lures must be calibrated by comparing them with the previous year's lures. This requires that current year's lures and previous year's lures be placed together in the field in representative locations covering a range of budworm densities. The moth captures at paired locations are then compared using regression analysis. Data generated from the spruce budworm pheromone-trapping network traps must be analysed by a single agency so that continent-wide maps can be produced.

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Use of pheromones and other semiochemicals in integrated production
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Juvenile hormone: action in regulation of sexual maturity in Caribbean fruit flies and potential use in improving efficacy of sterile insect control technique for tephritid fruit flies

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Abstract: Tephritid fruit flies, including the Caribbean fruit fly, pose a serious invasive threat to citrus production. These invasive species are quarantine pests and strict monitoring protocols are in place to detect introductions. The Sterile Insect Technique (SIT) is ideally suited to control tephritid fruit fly outbreaks and provides an environmentally safe and species specific method to eradicate tephritid fruit flies of agricultural importance world wide. Control is achieved in SIT by mass release of sterile males who mate with wild females. Females, mated with sterile males, do not produce offspring and rarely mate more than once. Optimization of SIT requires that sterile males compete with wild males for mates. We have discovered that loss of virginity enhances the sexual prowess of young males of the Caribbean fruit fly. After mating for the first time, males release twice as much sex pheromone, and they acquire another mate in less than half the time required by virgins. Additionally, we discovered that hemolymph of mated males contains significantly more juvenile hormone (JH) than that present in hemolymph of virgin males of the same age. Application of JH or the potent JH mimic, methoprene, to males on the day of adult eclosion induced precocious release of pheromone and mating by males. Thus, males, treated with JH, mated 4days earlier than control treated males. This discovery has the potential to improve efficacy of the SIT method because incorporating hormone supplement therapy into mass rearing of sterile males will allow for release of sterile males that are more competitive than those currently in use.

Key words: Caribbean fruit fly, Mediterranean fruit fly, juvenile hormone, sterile insect technique

Introduction

The Tephritid fruit flies, like the Caribbean fruit fly (*Anastrepha suspensa* (Loew)), have evolved complicated sexual communication systems. These systems rely on male produced auditory, visual and chemical signals for attraction of females and mating (see Sivinski and Burk, 1989). The signaling modalities are coordinated so that optimized signaling occurs during daily periods (Burk, 1983; Hendrichs, 1986; Hendrichs and Hendrichs, 1990; Landolt and Sivinski, 1992; Epsky and Heath, 1993). Pheromones appear to be responsible for long distance attraction (Perdomo *et al.*, 1975, 1976; Webb *et al.*, 1983; Heath *et al.*, 1993; Landolt *et al.*, 1992; Sivinski *et al.*, 1994) and, when coupled with auditory signals, maximize the probability of females finding and landing in the vicinity of male leks. The complexity of the signaling system is even more evident when considering the individual signaling modalities. For example, initial studies on pheromone communication indicated that, although only males produced and released pheromones (Feron, 1959; 1962; Nation, 1972), both sexes responded to male pheromone in the field (Perdomo *et al.*, 1976; Ohinata *et al.*, 1977). This suggests that the pheromone serves as both an intra- and intersexual function, particularly given the lek

forming behavior of males (Dodson, 1982; Burk, 1983; Sivinski, 1984; McDonald, 1987; Kaspi and Yuval, 1999). To date nine chemicals have been identified from volatiles released by Caribbean fruit fly males. These include: (Z)-3-nonen-1-ol and (Z,Z)-3,6-nonadien-1-ol (Nation 1983), anastrephin and epianastrephin (Battiste *et al.*, 1983), suspensolide (Chuman *et al.*, 1988), b-bisabolene (Tumlinson, 1988; Nation, 1991; Rocca *et al.*, 1992), E,E-a-farnesene and a-trans-bergamotene (Rocca *et al.*, 1992), and (Z)-b-ocimene (Tumlinson, 1988; Nation, 1991; Rocca *et al.*, 1992).

Age is also a major factor that regulates the sexual signaling system. Studies on mating of wild flies showed that insects do not engage in mating until they are 9-10 days old while laboratory reared insects begin mating several days earlier (Mazomenos *et al.*, 1977; Wong and Nakahara, 1978; Dodson, 1982). Additionally, female reproductive behavior, including response to pheromone, is directly correlated with ovarian maturity (Nation, 1972). Similarly, males undergo a period of maturation before they engage in sexual signaling (Nation, 1972; Landolt and Davis-Hernandez, 1993). Evidence from feeding studies has indicated that both sexes require protein sources for reproductive maturity (Galun *et al.*, 1985; Landolt and Davis-Hernandez, 1993) because correlations have been found among protein consumption, ovarian development and male calling behavior. However, although flies consume more protein during the maturation period, the amount consumed declines when they are sexually mature (Landolt and Davis-Hernandez, 1993). The fact that dietary protein is not required for sexual signaling by mature flies (Landolt and Sivinski, 1992; Epsky and Heath, 1993) suggests that the flies undergo a period of hormonally regulated adolescence during which time gametes mature and secondary sexual characters, including the ability to produce pheromone, develop. This apparent requirement for a period of hormonally regulated sexual development led us to explore the factor(s) responsible for coordination of reproductive maturity with sexual signaling in the Caribbean fruit fly.

Methods

Behavioral and mating observations.

Pupae, obtained at least seven days prior to adult eclosion from laboratory cultures maintained by the Florida Division of Plant Industry, Gainesville Fl, were housed in a greenhouse. On the day of eclosion, adults were segregated by sex, transferred to separate 30x30x30cm cages and provided with water and a 3:1 mixture of sugar and hydrolyzed brewers yeast. Experiments were conducted during the reproductive period that extended from between 12:00-18:00h (Heath *et al.*, 1993). We observed groups of five males for calling (exposure of the lateral abdominal, anal glands and other behavioral criteria associated male sexual signaling behavior) throughout the reproductive period on each day until flies were 10-days old. Five virgin males and virgin females were caged together each day after adult eclosion and observed for mating throughout the reproductive period. Mating pairs were removed from cages. New males and females were used each day. Thus, all flies had not been exposed to the opposite sex prior to pairing. Additionally, groups of five males, mated on the 5^{th} day after emergence were caged with five sexually mature females on the next day and the time each took to mate was recorded. These times were compared to times it took for virgin males 6-days-old to mate. In other experiments groups of five males were combined with females on the 5^{th} day. We removed mating pairs and held them separately. All males mated by the end of the 6^{th} reproductive period. We then caged the males who mated on either the 5^{th} or 6^{th} day with virgin females for a second time on the 7^{th} day. We compared the time it took for these mated males to acquire mates with the time it took for virgin males of the same age to mate.

The experiment was repeated with groups of 8-day-old virgins or males mated on either the 6^{th} or 7^{th} day caged with females for a second time on the 8^{th} day.

Collection and analysis of pheromone.
We collected pheromone from groups of five males, placed in volatile collection chambers prior to the daily commencement of sexual signaling (Teal *et al.*, 1999, 2000a). The system was purged with air for 1h prior to collection of pheromone during the first 4h of the reproductive period. Pheromone was collected from virgin males on each day after emergence. Pheromone was collected on the day after mating from groups of five males mated on days 5,6 and 8. At the same time we collected pheromone from groups of virgin males who were 6,7 and 9-days-old. The amounts of pheromone released by males were determined using capillary gas-liquid chromatography (Teal *et al*, 1999).

Hormone supplement therapy and identification of JH.
We used synthetic JH III and the JH agonist, methoprene, for hormone supplement therapy and applied a dose of 5µg of one or the other to the thorax of virgin 5-day-old males in a 1µl drop of acetone (Teal *et al.*, 2000a). This dose was selected because it has been used effectively by others (Yin *et al.*, 1995). Control males were treated with only acetone. Pheromone was collected from treated and control males on day 6. In other tests we applied either hormone or just acetone to males on the day of adult eclosion and collected pheromone and observed mating on each day.

We synthesized mixed diasteriomers of JH IIIB as described by Richard *et al.* (1989) and purchased JH III from Sigma Chemical Company for use as analytical standards in mass spectral analyses. To identify both JH III and JH III bisepoxide we collected hemolymph separately from 12-day-old males and mated and virgin 7-day-old males and extracted with hexane containing farnesyl acetate as a quantitative internal standard (Teal *et al.*, 2000b). The hexane extract was subjected to GC-chemical ionization (isobutane) mass spectral analysis using a Finnigan-Matt ITS 407 ion trap MS interfaced to a Varian Star 34007 GC. The GC was equipped with a cool-on-column injector. The 30m x 0.25 mm (id) analytical column used in the GC, a DB5-MS7 (J&W), was interfaced to a 10 m x 0.25 mm (id) uncoated, deactivated fused silica retention gap. Conditions of chromatography were initial injector temperature= 40° for 30 sec; injector temperature increased at 170°/ min to 270°; initial column temperature= 40° for 5 min; column temperature increased at 5°/ min to 210°; He carrier gas linear flow velocity= 24 cm/sec; GC-MS transfer line temperature= 230°. Diagnostic ions used for identification and quantification of JH III included m/e = 267 (M+1), 235 (M+1-CH_3OH), 217 (M+1-CH_3OH-HOH), 189 (M+1-CH_3OH-HOH-CO), 147 (M+1-$C_2H_4O_2$-C_3H_8O) (see Teal *et al.*, 2000b). Diagnostic ions used for identification and quantification of JH IIIB included m/e = 283 (M+1), 265 (M+1-HOH), 251 (M+1-CH_3OH), 233 (M+1-CH_3OH-HOH), 205 (M+1-CH_3OH-HOH-CO), 187 (M+1-CH_3OH-CO-2(HOH).

Results and Discussion

Male Caribbean fruit flies form mating leks (Nation, 1972; 1989; Sivinski and Burk, 1989; Webb *et al.*, 1983). Females, visiting leks rely on male size and elaborate interactions between auditory, visual and chemical signals emitted by males to select mates ((Nation, 1972; 1989; Sivinski and Burk, 1989; Webb *et al.*, 1983). The expression of male and female sexual behavior is closely coordinated with physiological development of reproductive maturity (Nation, 1972; 1974). In our laboratory strain of Caribbean fruit flies we found that all males engaged in calling behavior by the eighth day after emergence and that by day 9 all males released the maximum amount of pheromone and mated (Fig. 1). Interestingly, we also

found that if we introduced virgin males to females on the fifth day and left them together, all males mated by the end of the sixth day (Fig. 2). However, only 37% of the six-day old virgin males mated on that day (Fig. 2). Similarly, males caged with females continuously during days six and seven all mated where as only ca 51% of the seven-day old virgin males paired with females, for the first time on day seven, mated (Fig.2). These results indicated that the experience of being in proximity to virgin females over a 24h period induced males to mate.

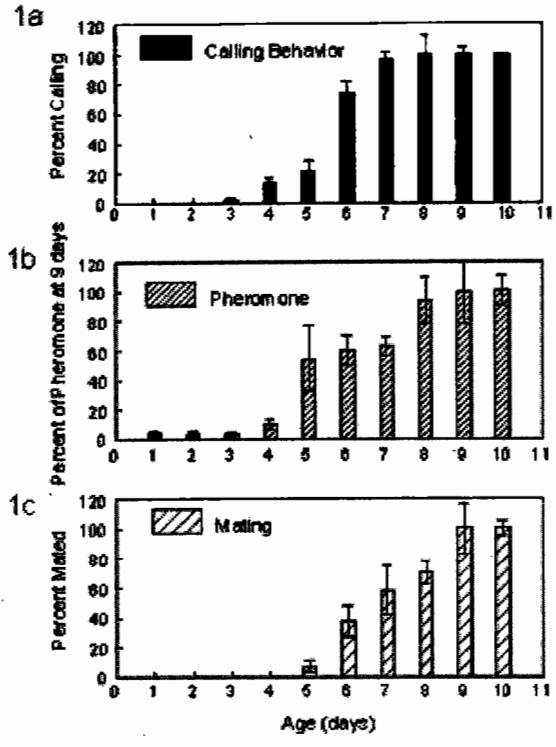

Figure 1. Effect of age on calling behavior, amount of pheromone released and mating by virgin males of the Caribbean Fruit fly. Data for calling behavior (12 groups of five males) and mating (17 replications of five males and females) represent the cumulative percentages of the total number of animals observed over 10 days. Data on pheromone release (10 groups of five males) represents the percentage of the average amount of pheromone released by males who were 10-days old.

Another feature of our study was the discovery that, once mated, males engage in mating much more readily than do their virgin counterparts of the same age. When we allowed males to mate, for the first time, on day 5 and 6 and then paired them with virgin females for a second time on day seven we found that all males mated with females for a second time within 2h after being combined with females (Fig. 3). However, only 30% of the naïve virgin seven-day old males mated on that day (Fig. 3). Similarly, when we caged males together over the sixth and seventh days and allowed all to mate and then re-caged the mated males for

a second time on day eight all of them mated within 90 min. However, only 60% of the virgin 8-day-old males mated with females on day eight. The remaining 8-day old virgin males required a second day to complete the initial mating. Results of these studies indicated that prior mating experience, and not size, was the key factor that influenced the male's ability to successfully attract, court and mate with females because males were selected at random, without regard for size, in all of these experiments.

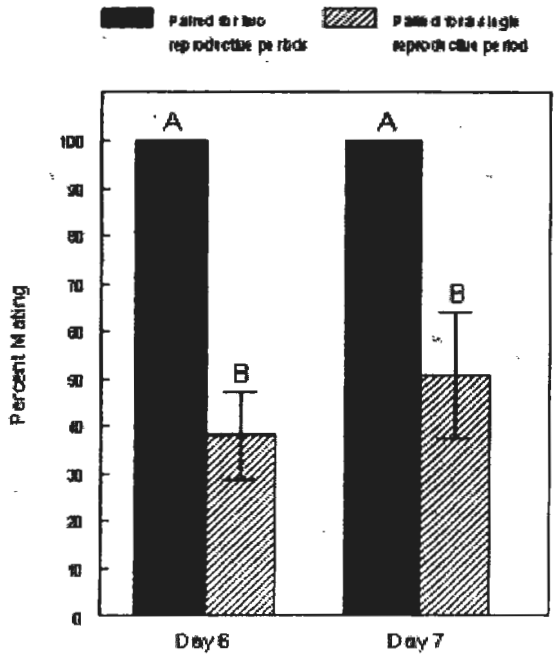

Figure 2. Comparison of percent of virgin males mating when paired continuously sexually mature virgin females for two reproductive periods, day five and day 6 or day six and day seven, with percent of virgin males mating when paired with sexually mature females for a single reproductive period on either day six or day seven. When mating occurred the mating pair was removed form the cage. All males paired with females for 2 successive days mated by the end of the second reproductive period whereas significantly fewer males mated when paired with females for a single day (t-test, $p=0.05$, 6 replicates).

Taken collectively, the results of our mating experience studies suggest that males have evolved an adaptive mating strategy that allows them to take advantage of mating opportunities even if they have not reached the peak of sexual maturity. Such a strategy could have evolved because the probability that tropical species of Tephritid fruit flies, like the Caribbean fruit fly, will find mates when oviposition sites are available is limited because host plants are widely distributed both in time and space. If few females were available early

in a male's life it would be more efficient to wait until greater numbers of females arrive before engaging in sexual behavior. This would allow for accumulation of sufficient energy reserves to maintain sexual signaling and territory defense. Alternatively, if large numbers of receptive females are available, then younger males have mating opportunities. Indeed, females avoid recently mated males and select naïve, less competitive, virgins for mating (Sivinski, 1984). Thus, if a male has an opportunity to mate early in life he may need to take it in order to achieve reproductive success and use the experience as an indication that other females are available. Thus, he may contribute proportionally more to the gene pool.

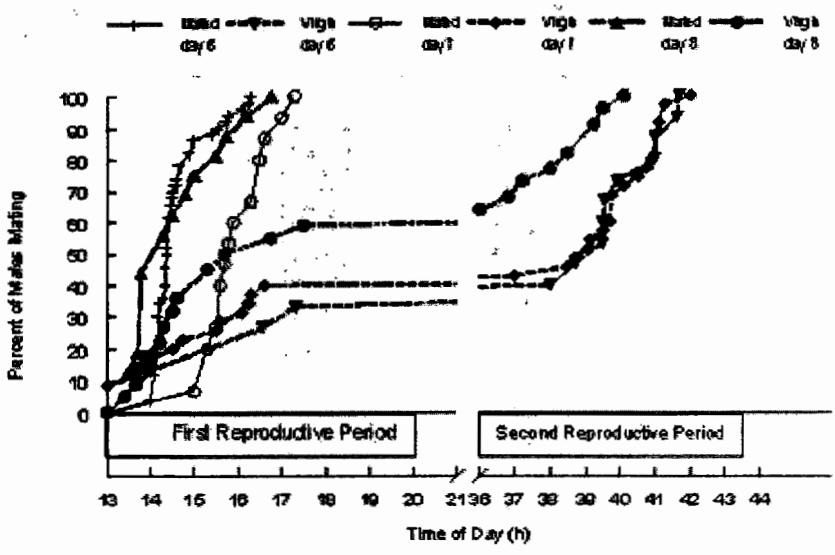

Figure 3. Comparison of the times required for mating by mated or virgin individuals after initiation of the 6th, 7th or 8th reproductive periods. Each data point represents the cumulative percent of the total population of a given treatment mated at that time. N=50 mated day six, 15 virgin day six, 15 mated day seven, 20 virgin day 7, 20 mated day eight, 20 virgin day 8.

Our research on mating by virgin and mated males suggested strongly that the act of mating induced physiological changes in males that caused them to engage in effective sexual signaling much more readily than their virgin counterparts. If this were so then we hypothesized that quantifiable differences in sexual behavior would be obvious between virgin and mated males of the same age. Male produced sex pheromones are key elements in the sexual communication system (Perdomo *et al.*, 1975; 1976; Nation, 1989; Sivinski and Burk, 1989; Heath *et al.*, 1993) and, in combination with visual and auditory cues, affect all aspects of sexual behavior. Therefore, we considered that release of sex pheromones would be a good diagnostic tool to monitor reproductive behavior and sexual competence. If this were so then we hypothesized that mated males should release more pheromone than virgins of the same age. When we measured the amount of pheromone released by six- and seven-day old virgin and mated males we discovered that mated males released at least twice as much pheromone as did their virgin counterparts (Table 1). In fact, the amounts of pheromone

released by these mated males were no different from that released by virgins on day nine, the age at which pheromone production peaks. Thus, the act of mating caused a physiological change in males enabling them to produce as much pheromone as males, either mated or virgin, who were at the peak of their sexual prowess. The benefit of this is clear if one considers that males must compete directly for the affections of females because if a mated 6-day-old male is to compete effectively in leks containing males who are nine or more days old, and at their sexual peak, then he must present the same qualities that render mature males attractive to females.

Table 1. Comparison of amount of pheromone released by mated and virgin 6-, 7- and 9-day old males. Five replications per treatment. Means are significantly different in a Fisher's least significant difference test ($p = 0.05$) if the letter is different in the significance column.

Male age and mating status	Mean amount pheromone (± SE)	Statistical significance
Day 6 Mated	412 (± 69.1)	A
Day 6 Virgin	180 (± 64)	B
Day 7 Mated	637 (± 63.2)	A
Day 7 Virgin	375 (± 66.5)	B
Day 9 Mated	643 (± 64.9)	A
Day 9 Virgin	683 (± 128)	A

Juvenile hormone (JH) coordinates development of sexual signaling with gamete maturity in many insects (Blomquist and Dillwith, 1983) and we had previously identified JH III from sexually mature (9-day old) males but not from immature 1-day old males (Teal *et al.*, 2000b). Analysis of extracts of hemolymph from 12-dy old virgin and mated males resulted in identification of JH III and for the first time the bisepoxide homologue of JH III (JH IIIB). However, no differences in the total amount of JH in extracts from either mated or virgin males were found. When we analyzed extracts of the hemolymph from 7-day old mated males we also identified JH III and JH IIIB (Fig.4) in a ratio of 1:3 (Fig. 5). The identification of JH IIIB was important because this JH homologue had only been identified from extracts obtained from tissue culture media in which the corpora allata had been incubated (Richard *et al.*, 1989; Yin *et al.*, 1995; Yin and Stoffolano, 1997). Thus, although JH IIIB was synthesized by the corpora allata of Diptera, it had not been found in the circulatory system and skepticism existed about the validity of the assumption that JH IIIB was, in fact, a functional hormone. We determined that each µl of hemolymph from a mated 7-day old male contained an average of 16pg (±2.0) of JH. This was significantly more (4.5fold) JH than was present in hemolymph from virgins of the same age (Fig. 5).

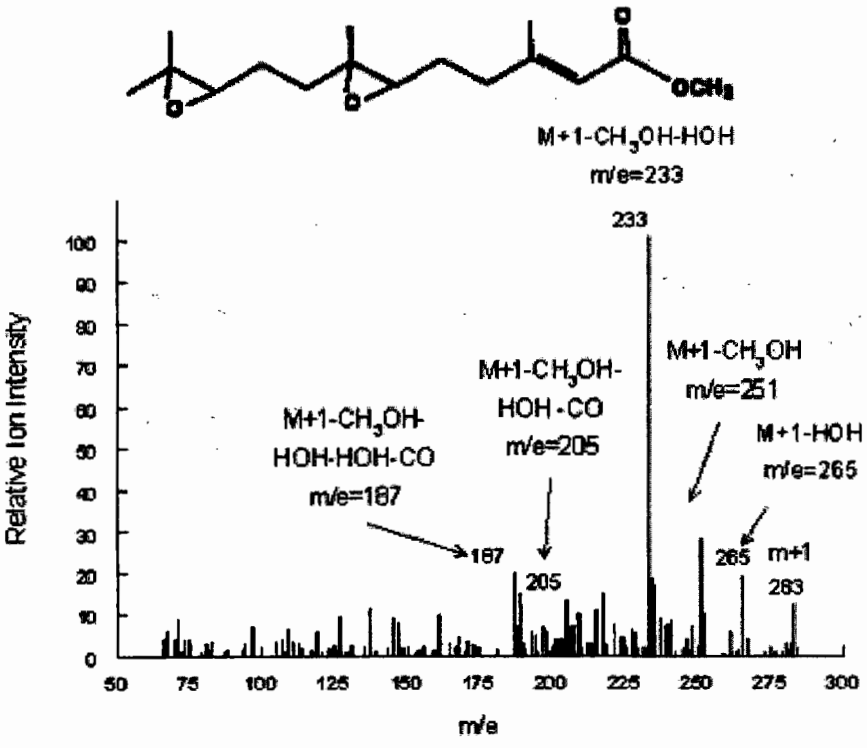

Figure 4. Chemical ionization (isobutane reagent gas) mass spectrum of naturally occurring JH IIIB isolated from 15:1 of hemolymph from 7-day old mated male Caribbean fruit flies. The structure of JH IIIB is shown above the spectrum and major diagnostic fragments are indicated along with the fragment losses. The structure was elucidated by comparison of retention time and fragmentation pattern of the natural compound with that of synthetic JH IIIB.

Knowing that circulating amounts of JH were higher in mated than in virgin males we hypothesized that JH was a pivotal hormone in regulating all aspects of sexual signaling and reproductive competence in these flies. If this were so then we believed that we could induce precocious expression of sexual behaviors and accelerate reproductive development times by application of JH to male flies. When we applied hormone to 5-day old virgin males we found that these males released three times more pheromone than did the control group of virgins on day six. Indeed, application of hormone to males on the day of emergence caused precocious reproductive development and expression of sexual signaling when compared to control males (Fig. 6). In fact, all treated males mated on the fourth day after emergence. Results of these experiments showed that JH levels increased after mating and that this hormone alone can stimulate young virgin males to increase pheromone production and mate.

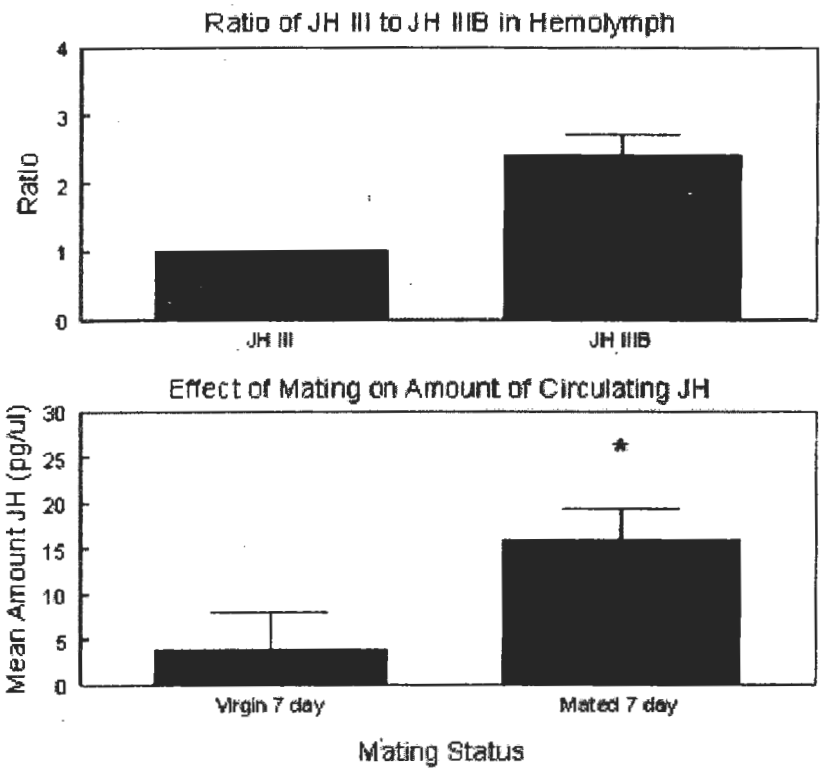

Figure 5. Ratio and amounts of JH III and JH IIIB found in extracts of hemolymph. Top: Ratio of JH III to JH IIIB obtained from samples from 12-day old virgins (n=5). The ratio was no different in samples obtained from 7-day old virgin or mated males. Bottom: Comparison of total amount of JH (JH III plus JH IIIB) in samples obtained from 7-day old virgin or mated males. The amount of JH in mated males was significantly greater in mated than in virgin 7-day old males (T test, p=0.05, 4 replicates).

Tephritid fruit flies including the Caribbean, Mediterranean and Mexican fruit flies pose the most serious invasive threat to citrus produced in the United States. Damage by these pests is not limited to citrus. More than 260 different hosts, including stone fruits like plums and peaches, and cash crops like tomatoes and peppers, have been recorded for the Mediterranean fruit fly alone.

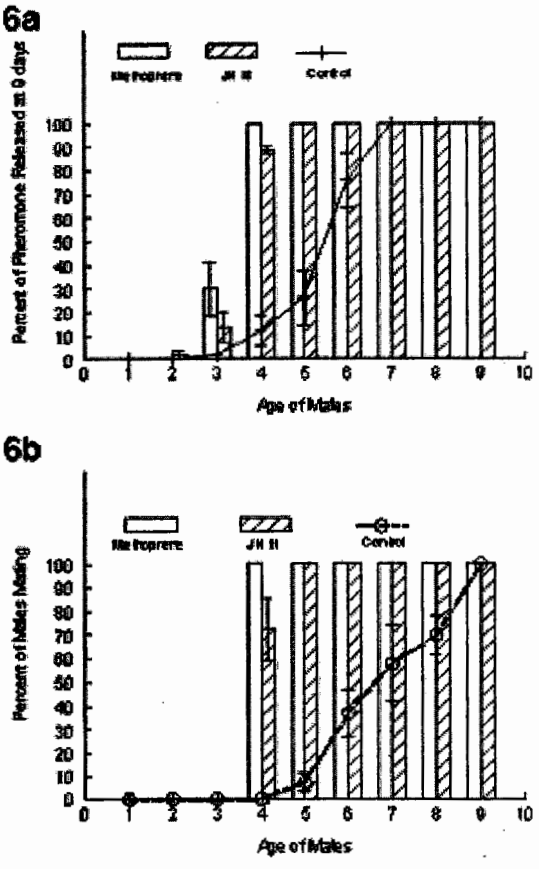

Figure 6. Effect of application of the JH agonist, methoprene, to newly eclosed males on amount of pheromone released and mating by males on each day after emergence compared with amount of pheromone released and mating by control males. 5a: Amount of pheromone released as a percentage of that released by virgin males at their sexual peak (9-days old) (n=6 replicates per treatment). 5b: Percent of males mating each day after emergence (n=4 replicates per treatment).

These invasive species are quarantine pests and strict monitoring protocols are in place to detect introductions. Once detected, the infested areas are subjected to immediate quarantine to eliminate movement of infested fruit to other areas, fruit from host plants are stripped from infested sites and ground and aerial pesticide application control protocols are initiated. In the past, fumigant treatment of imported fruit using ethylene dibromide (EDB) significantly limited invasions of these flies. However, registration for EDB use has been withdrawn and no substitute has been found. Thus, we are left with three practical alternatives for control: 1) stripping and destroying fruit from infested areas; 2) bait sprays using Malathion and more recently Spinosad; and 3) release of sterilized males in Sterile Insect Technique (SIT) protocols. The use of bait sprays has come under constant criticism due to perceived

environmental and health related problems and several law suites have been filed to stop application. The use of SIT provides an environmentally safe and species specific method to eradicate Tephritid fruit flies of agricultural importance throughout the world. Control is achieved in SIT by mass release of sterile males who mate with wild females. Females, mated with sterile males, do not produce offspring and rarely mate more than once. Thus, optimization of SIT requires that sterile males compete with wild males for mates. We believe that incorporating hormone supplement therapy into the rearing protocols used to mass produce sterile males of Tephritid fruit flies for use in SIT will improve efficacy of the technique significantly. Currently, protocols for release of sterile males of both Caribbean and Mexican fruit flies indicate that males need to be held for several days prior to release in order for the males to become sexually mature. Accelerating reproductive maturity by inclusion of hormone supplement therapy into mass rearing protocols would allow for release of sexually mature insects several days earlier than prescribed by the current protocols. This would reduce significantly the cost of holding adult flies prior to release and reduce the negative effects of holding large numbers of males in small cages, which results in physical damage to the flies. Additionally, sterile flies have a much shorter life span than do non-irradiated flies. Thus, when flies are held, as adults, prior to release many die. The ability to release sexually mature flies at a much earlier age would minimize this negative effect of mortality on mating and more sterile flies would mate prior to death. Finally, sterile males are considered to be effective for only a single mating. Thus, each male released is considered to remove only a single wild female from the reproductive population. It is very possible that the reason that sterile males fail to function effectively in more than one mating is that they fail to replenish the secretions from the accessory glands in the reproductive system after mating. Compounds in the accessory glands of the Mediterranean fruit fly have been shown to inhibit remating and to induce females to engage in searching for fruit and oviposition (Miyatake *et al.*,1999; Jang, 1995; Jang *et al.*, 1998). Wild males replenish the secretions within a few hours after mating. In other fruit flies, secretions from the accessory glands are responsible for inhibiting females from remating. JH has been implicated in inducing replenishment of accessory gland secretions. If this is so for Tephritid fruit flies then hormone supplement therapy could allow sterile males to replenish the accessory gland secretions after the initial mating. Therefore, sterile males could be capable of effectively mating more than once with wild females. Multiple mating by released sterile males will add even more to the cost effectiveness of the SIT technique.

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Use of pheromones and other semiochemicals in integrated production
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Effectiveness of a pheromone-baited multi-Lasiotrap in surveying and mass trapping of the tobacco beetle in Greek stores

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Abstract: In a recent study, a multi-surface adhesive trap for *Lasioderma serricorne* adults was designed and evaluated in the Laboratory of Entomology, Agricultural University of Athens, Greece. A new experiment was conducted in a horizontal-type store of Piraeus containing baled tobacco. Four types of traps (pheromone-baited multi-surface, unbaited multi-surface, pheromone-baited single and unbaited single) were used. Traps were checked at weekly intervals from January to December 1998. Catches were recorded from the end of April up to the first days of November with a population peak in the middle of August. As compared to unbaited multi-surface trap, pheromone-baited single and unbaited single trap, the pheromone-baited multi-surface trap caught approximately 4, 8 and 64 times more *L. serricorne* adults, respectively. All correlation coefficient values, corresponding to pairs of catches of different types of traps on the same date, were significantly different from zero ($P<0,01$). Although more adults were recorded in the central trap of the multi-surface traps, no significant differences were noted ($P>0,05$). Significantly higher numbers of adults were found on the illuminated sides of each trap type ($P<0,05$). Thus, apart from pheromone, the location of the trap influences significantly the trapping efficacy.

Key words: *Lasioderma serricorne*, stored tobacco, trapping, anhydroserricornin, multi-Lasiotrap

Introduction

The tobacco beetle, *Lasioderma serricorne* F. (Col. Anobiidae), is the main pest in fermented and processed tobacco leaves and their products (Ashworth 1993). The main uses of stored-product insect pheromone traps for this species is monitoring and the evaluation of pesticide application (Chambers 1990). However, the primary objective of a sampling program is detection of the infestation at its initial stages (Athanassiou and Buchelos 2000), and in the case of processed tobacco, zero tolerance is the standard regulation (Mueller 1990). As a result, "blind treatments" in stored tobacco increase significantly the cost of storage (Pierce 1994). Nevertheless, there is well-established information that these traps can be used successfully for mass trapping or mating disruption, which can lead to insectinstasis, because in storage facilities, we do not have the limiting influence of the immigration of mated females (Trematerra 1997; Jones 1998).

The sex pheromone of the tobacco beetle (anhydroserricornin) has been commercially available for almost two decades. In fact, this pheromone is one of the most widely used worldwide, usually in combination with sticky traps (Levinson and Levinson 1987; Mueller 1990; Buchelos and Trematerra 1998). A few years ago, a multi-surface adhesive trap for *Lasioderma serricorne* adults was designed in the Laboratory of Entomology, Agricultural University of Athens, Greece. Full description of the trap and its mode of function is given by Buchelos and Levinson (1993). This trap has proved economic, efficient, reliable and easy to use. This study is a continuance of this work; moreover, this study aims to evaluate some additional factors that influence *L. serricorne* capture.

Materials and methods

A large store room (27x12x4 m), belonging to the National Tobacco Board of Greece, in Piraeus, was used. The building was made of bricks, and had a solid roof. This room contained about 150 tons of balled tobacco (5000 bales). The tobacco had been collected in 1995, three years before the experiment.

The multi-surface trap that was used consists of 5 bilaterally sticky cardboard stripes (27x8 cm), (Lasiotraps, BAT Cigarette Factories, Hamburg, Gemany), being vertically suspended from a cruciform wooden device in such a way that 4 peripheral traps equidistantly surround the central stripe (for a detailed description see Buchelos and Levinson 1993). The central stripe was provided with a replaceable polyethylene capsule, containing 10 mg of anhydroserricornin (2,6-diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran), the sex pheromone of the tobacco beetle (Levinson and Levinson 1987; Levinson and Buchelos 1988).

Two multi surface traps of which one was baited and the other was unbaited were vertically suspended in the diagonal corners of the store room, while the remaining store corners were occupied by a baited and an unbaited single Lasiotrap. A series of glass windows existed across the store room's northern wall. Thus, this area was more illuminated (600-1800 Lux) as compared to the southern area of the store room (100-650 Lux). Renewal of the sticky traps was performed on a weekly basis from January to December 1998, while replacement of the pheromone capsules was made on a monthly basis. Baited and unbaited traps were rotated weekly clockwise from the one corner to the next, in order to minimize the influence of trapping location. Temperature and relative humidity were recorded indoors and outdoors, with thermohydrographs. No insecticidal treatments were taken place during the trapping period.

In order to evaluate the co-alteration between pairs of catches among different types of traps on the same date, the correlation coefficients' values were calculated. These values were tested for the departure from zero with the two-tailed *t*-test at p=0,01 (Snedecor and Cochran 1980). In addition, trap catches were compared a) between the central and the peripheral sticky surfaces, b) between the illuminated and non-exposed to light sticky and c) between the internal and the external sticky surface of the peripheral traps.

Results and Discussion

Temperature indoors was constantly higher than this outdoors, by an average of 8 ° C; on the other hand, relative humidity was higher indoors only during summer months (Figure 1). More than 96% of the total number of adults was captured during summer months and September (Figure 2). However, catches were recorded from the end of April up to the first days of November. In a previous study in the same area (Piraeus), Buchelos and Levinson (1993) stated that the highest numbers of adults were caught during July and, especially, August. The influence of temperature in the flight activity is in accordance with previous studies for *L. serricorne* in Greek storage facilities (Buchelos 1981; Levinson and Levinson 1987; Levinson and Buchelos 1988; Buchelos and Levinson 1993). Buchelos and Trematerra (1998) stated that temperature is the determinative factor for the adults' flight activity while in temperatures below 16 ° C, the insect's vital activities are restrained. Nevertheless, apart from flight, the release of the sex pheromone of the tobacco beetle is highly dependent on the climate indoors (Ashworth 1993; Buchelos and Trematerra 1998).

The total number of beetles counted on all trap types during the entire trapping period was 243346. The baited multi-Lasiotrap caught 71.7 % of the total number of adults, followed by the unbaited multi-Lasiotrap (18.4 %), the baited singe Lasiotrap (8.8 %) and the unbaited single Lasiotrap (1.1 %) (Figure 2). The superiority of the multi-Lasiotrap (as compared to the

other three trap types) was more obvious during July and August, at high population densities. On the other hand, early in the trapping period, as well as during September and October, this difference was relatively reduced. The peak for the baited multi-Lasiotrap was observed on the second week of August, while for the other three traps the peaks were recorded 1-3 weeks earlier. This fact must be seriously taken into account, because it is well known that, different trap types in the same storage facility can produce different results (Athanassiou and Buchelos 2000). In addition, higher numbers of captured adults do not always reflect actual changes in population densities, as those are obtained by absolute estimation methods. In the present case, all correlation coefficient values, corresponding to pairs of catches of different types of traps and on the same date, were significantly different from zero (Table 1). This is indicative of the co-alteration of captures between different trap types. The response of the insect is dependent of the addition of the pheromone in the sticky surface; this is partially the reason for the earlier peak occurrence for the unbaited traps.

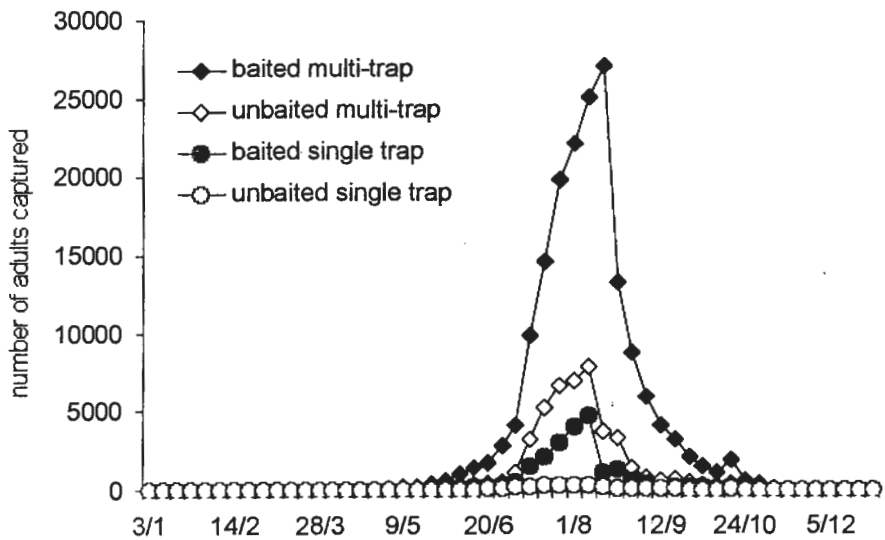

Figure 1. Temperature (° C) and relative humidity (%) during the trapping period.

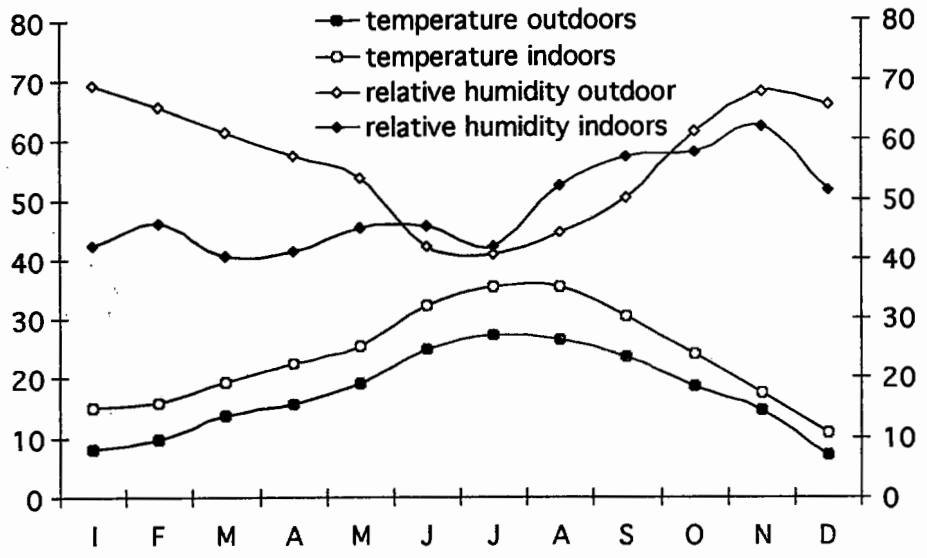

Figure 2. Captures of *Lasioderma serricorne* adults during the sampling period, on each trap type.

One of the most interesting results of this study is the performance of the unbaited multi-Lasiotrap; particularly, this trap type caught more than two times more adults than the pheromone baited single Lasiotrap. It is assumed that the visual stimulus provided from this trap is likely to increase captures, even in the unbaited traps. Also, the existence of pheromone in the air of the store room was not restricted only around pheromone traps, and can cause "air permeation" (Mueller et al. 1990; Trematerra 1997). On the other hand, the larger sticky surface can increase the captures significantly, even without the addition of pheromone. Similar results have been produced during the first assessment of the multi-surface trap (Buchelos and Levinson 1993).

Table 1. Correlation coefficients for pairs of catches of different trap types on the same date*

	Baited multi-trap	Unbaited multi-trap	Baited single trap
Unbaited multi-trap	0,942	-	-
Baited single trap	0,892	0,981	-
Unbaited single trap	0,922	0,941	0,892

*In all cases the coefficients' values are significant different from zero (two tailed *t*-test, p=0.01)

One other factor that influences captures is trap location; for all trap types, significantly higher number of adults were noted in the illuminated surfaces (for the combined trap data: df=1,60; F=4.76; P=0.0330). Nevertheless, the rotation of the traps rendered the interpretation

of these results rather complex, because only two traps were exposed to the illuminated areas (near the glass windows) each week. In general, although the orientation of the traps is likely to increase captures, practically the addition of pheromone is considered of higher significance than the perception of visual stimulus or trap location (Buchelos and Levinson 1993; Mullen et al. 1997).

Although more adults were found in the central sticky surface of the pheromone multi surface trap, no significant differences were recorded with the peripheral traps (df=1,102; F=0.09; P=0.7612, adult ratio 1.2: 1). Furthermore, more adults were found in the external surface of the peripheral traps, but also in this case, no significant differences were observed (df=1,122; F=0.83; P=0.3640, adult ratio 1.1: 1). The evaluation of these factors (as in the case of illuminated areas) is a very complex procedure, because in the case of *L. serricorne* captured adult numbers were very high, regardless of the characteristics of the adhesive surfaces. Lower captures, can signify these interactions on the basis of increased efficacy, as in the case of pyralid moths (Vick et al. 1990; Mullen et al. 1997).

Apart from monitoring and estimation of the population density, the capture of as many male adults as possible can be used for mass trapping, or at least for suppression, provided that the number of traps (or the trapping capacity) is high (Levinson and Buchelos 1988; Buchelos and Levinson 1993; Pierce 1994). Levinson and Buchelos (1988) used single baited Lasiotraps, in a storage facility with stored tobacco for three consecutive years. Those traps were found to reduce the population density of the tobacco beetle by approximately two thirds after two successive years of trapping. Although the current experiment lasted only for a year, it is assumed that partial insectistasis can be achieved with the multi-surface trap, due to its higher effectiveness. This kind of pheromones' utilization is more feasible when trapping is combined with other control measures (Levinson and Buchelos 1988; Pierce 1994).

To conclude, the use of the sex pheromone of the tobacco beetle for mass trapping is a very promising practical application, with the combination of traps that capture more insects. The satisfactory performance of adhesive surfaces for the capture of *L. serricorne* males, is an advantage that does not occur in the case of other stored product beetles (Athanassiou and Buchelos 2000). On the other hand, *L. serricorne* (along with the pyralid moth *Ephestia elutella*) is the major pest of stored tobacco and this renders the use of a single pheromone a very important tool in order to minimize routine preventive insecticidal treatments and residues in processed tobacco (in contrast with other stored products, which several beetle species co-infest, and as a result the used of various pheromones at the same time is required). For the aforementioned reasons, it becomes evident that mass trapping is much more feasible in stored tobacco, as compared to other products (such as stored cereals).

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Predicting the efficacy of modified modes of action of a pheromone-based attracticide: a bisexual attractant and autosterilisation

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Abstract: A simulation model was developed to study factors affecting the efficacy of a pheromone based attracticide product for control of the codling moth, *Cydia pomonella.* The model includes abiotic factors, such as spacing and durability of the attracticide formulation, as well as biotic factors, such as temporal dynamics and life history parameters of the pest species. Two different modes of action of the attracticide were investigated: attraction based on a non-pheromone bisexual attractant and auto-sterilisation as control agent. The addition of kairomones to a pheromone-based attracticide increases the performance of the lure and kill strategy. Kairomone that are novel in the environment, such as volatiles from alternative host plants, perform better than host plant volatiles, which have to compete with the natural odour sources. Replacing the knockdown insecticide with a chemosterilant increases the control impact of an attracticide.

Key words: Sex pheromone, plant volatile compounds, attracticide, population dynamics, simulation model, pome fruit orchard, *Cydia pomonella*

Introduction

The use of synthetic pheromones to suppress moth populations by disrupting sexual communication between the sexes is becoming more popular as a control method (for references see: Cardé & Minks, 1995; Witzgall & Arn, 1997). Since the introduction of commercial mating disruption products, efforts to develop more efficacious formulations have continued. This led to the concept of adding an insecticide to the device or formulation releasing the pheromone. The use of a formulation containing an insecticidal agent allows the necessary density of killing point sources needed to compete effectively with the attraction of the natural sex-pheromone source, the "calling" female insect. Point source formulations have been described for the control of *Anthonomus grandis* (McKibben *et al.*, 1990), *Pectinophora gossypiella* (Hofer & Brassel, 1992), *Cydia pomonella* (Hofer & Brassel (1992), Charmillot et al. (1996), Lösel *et al.* (2000)) and *Rhagoletis* spp. (Liburd *et al.*, 1999).

Several authors have stressed the fact that more fundamental research is necessary before we can predict the most fruitful strategies of using semiochemicals in direct control of pest insects. To investigate the mechanisms and principles of biocontrol techniques we have to rely on population models based on parameters from the agro-ecosystem, the target pest and the biocontrol method. Recent examples of models that simulate the dynamics of biocontrol methods are Cooke & Régnière (1996) for *Bacillus thuringiensis* efficacy against the spruce budworm, Roermond *et al.* (1997) for parasitoid efficacy (*Encarsia formosa)* against the whitefly and Barclay & Judd (1995) for pheromone-based control techniques.

In this paper we simulate the control efficacy of two modified modes of action of an pheromone-based attracticide for population control of the codling moth, *C. pomonella.* First we will investigate if the addition of a plant-based attractant enhances the control efficacy of the attracticide, following the recent studies of Knight & Light (2000) on a potent bisexual attractant for the codling moth (plant volatile 'DA2313'), which affects males, virgin females and gravid females. Secondly, we simulate the dynamics of an attracticide based on male auto-sterilisation, instead of male removal. This simulation is based on the recent studies of Charmillot *et al.* (2001) on field trials of auto-sterilisation of the codling moth using an attracticide where the knock-down insecticide is replaced by a sterilising agent (insect growth regulator).

Model outline

The model is based on the model presented by Roelofs *et al.* (1970) and modified for codling moth control by Potting et al. (2000). In figure 1 an overview is given of the structure of the model used to simulate the control efficacy of an attracticide against the codling moth. Parameters of the crop system serve as the basis for the determination of the economic threshold level. These include fruit density and natural mortality factors acting on egg and larval stages of the codling moth. Pest input-parameters include pupal density, daily survival rate and immigration rate. Key model state variables include male moth density, virgin female density and mated female density. The cumulative number of mated females determines, in combination with the crop system parameters, the level of expected damage (i.e. larval infested apples). The number of virgin calling (i.e. pheromone releasing) females determines the level of competition with the attracticide sources. Parameters of the attracticide application include the droplet density and droplet potency. Droplet potency is a combination of relative attractiveness of the pheromone component and relative knockdown potential of the insecticide component. Maximum attractiveness of the synthetic pheromone is set at a nominal value of 1, which equals attraction towards a calling female. In sex pheromone systems it is usually not possible to develop an attractant which is more attractive than the natural source (i.e. a calling female). For the codling moth we found a distinctive pheromone dose-response curve (Lösel *et al.*, 2000). Droplet potency decreases with exposure time to ambient weather conditions (Lösel *et al.*, in prep.). This was incorporated in a degradability rate parameter (default value: 0.025 day^{-1}). For more detailed information on the formula used, model parameters and default values we refer to Potting et al. (2000). For more details on the application of the attracticide we refer to Lösel et al. (2000).

Probability of mating

The key factor to the success of an attracticide approach is a significant reduction in the number of matings taking place. The probability that a male mates with a female is dependent on the number of attracticide spots and their relative attractivity compared to a calling female. In the model the probability of mating in the absence of competition from attracticide spots is set at 1. Thus the probability of mating decreases with a high density of attracticide spots and a high relative attractiveness per spot:

$$P_i = V_i / (A_i N) + V_i$$

where: P_i is the probability of a male to mate with a female on day i.
Vi is the total number of virgin females/ha on day i.
Ai is the relative attractiveness of the formulation on day i.
N is the number of attracticide spots/ha.

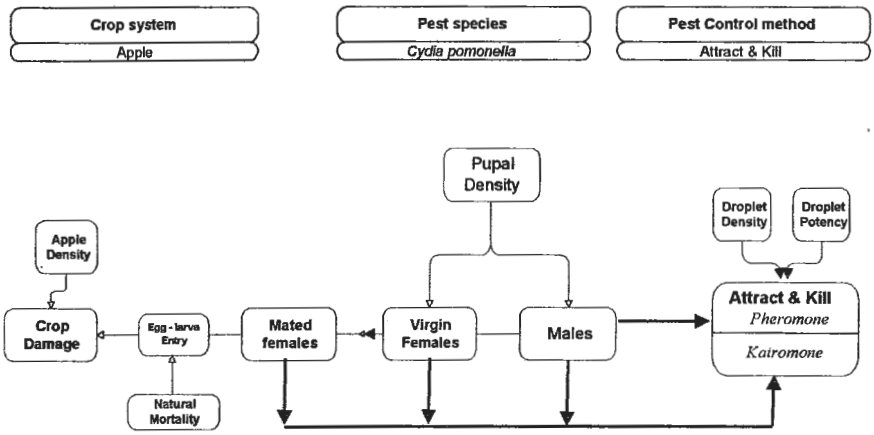

Figure 1. Overview of the attract and kill model structure. The simulation model is constructed with parameters confined in: (1) Orchard environment (2) *Cydia pomonella* dynamics (3) attracticide application characteristics. The attract and kill component is divided in a pheromone part affecting male behaviour and a kairomone (i.e. plant volatile) part affecting males, virgin females and mated females.

Factors affecting field performance of attracticide

Moth density, point source density and timing

Potting et al. (2000) made an assessment of the role of abiotic factors (attractant release characteristics, spacing and durability of the attracticide formulation) and biotic factors such as the population biology and behavioural characteristics of the pest species in the control efficacy of a pheromone-based attracticide. They found that a proper matching of the attracticide point source density with the moth density was an important factor determining reliable population control as well as a proper timing of the attracticide application due to the degradability rate of the formulation in ambient weather conditions.

Spatial aggregation of pest population

Pest species with a non-uniform spatial distribution structure in an agro-ecosystem can be difficult to control with a semiochemical-based control method (Barclay & Judd, 1995). In extreme cases of aggregation, some areas will have a low pest density and will be easy to control, whereas other areas will have high densities and will be more difficult to control. For the codling moth it is often reported that damage levels are higher near the border of the

orchard. This effect may be due to aggregation of moths near the border, for instance of dispersing moths that reach the margin and do not want to leave the orchard. An increase in moth density per tree will result in a decrease of control efficacy of the attracticide spots in the tree (Fig. 2a).

Mating frequency

In the model it is assumed that the sequence of events is emergence from pupae, mating and overnight mortality. Males are assumed to orient to a pheromone source only once per day. For several insect species this may not be realistic. The mating frequency of individual males can have a significant effect on the control effect of an attracticide with a decrease in efficacy with increasing mating frequency per day (Fig 2b). However, at higher attracticide point source densities this effect is negligible, because the competitiveness and attract and kill rate of the point sources is high from the first mating attempt onwards (Fig 2b).

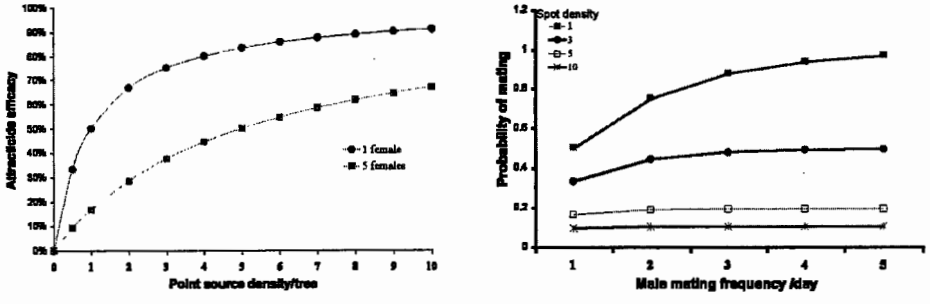

Figure 2. A - Attracticide efficacy in relation to point source density and moth density (i.e. number of calling females per tree). B - The influence of daily mating frequency of individual males on mating rate in attracticide treated plots with different point source densities.

Addition of other (non-pheromone) attractants

A disadvantage of a sex pheromone based control technique is that only males are affected. The addition of non pheromonal attractants in a pheromone-based attracticide could increase the impact of population reduction. Many insect species use olfactory cues from their host plant (i.e. kairomones) to find mating sites and suitable oviposition sites. For several moth species it has been demonstrated that male response to sex pheromone is enhanced in the presence of host-plant volatiles (Landolt & Philips, 1997). Female *C. pomonella* deposit their eggs near (young) apples and they probably use specific plant volatiles to locate the fruitlets (Yan et al., 1999). A bisexual attractant for the codling moth has recently been identified (Knight & Light, 2000). Trap catches with this plant based attractant are as high as pheromone catches with a ratio of 50% males, 25 % virgin females and 25% gravid females.

Addition of non-pheromone attractant – simulation results

A kairomone-based attracticide competes with natural sources in attracting insects. The use of a kairomone-based attracticide is generally density independent with respect to pest mortality, since the point sources compete with the natural odour sources and not with the pest itself as

in a pheromone-based attracticide. The daily depletion rate of males with a kairomone-based attracticide is fixed assuming that the number and quality of natural odour sources is constant.

The addition of plant-based attractant to a pheromone attracticide was simulated as well as the impact of a solely plant-based attracticide. Two types of kairomone were simulated: one that has to compete with the natural sources, i.e. a volatile component of a natural blend of host plant (i.e. apple fruitlet volatiles) and one kairomone that does not compete with the natural sources because it is novel in the environment, e.g. a component from an alternative preferred host plant (e.g. specific pear volatiles). In the simulation one apple-based attracticide source was assumed to be 10x more attractive than a fruitlet source and a pear-based attracticide source 50x more attractive. The simulation results are presented in figure 3.

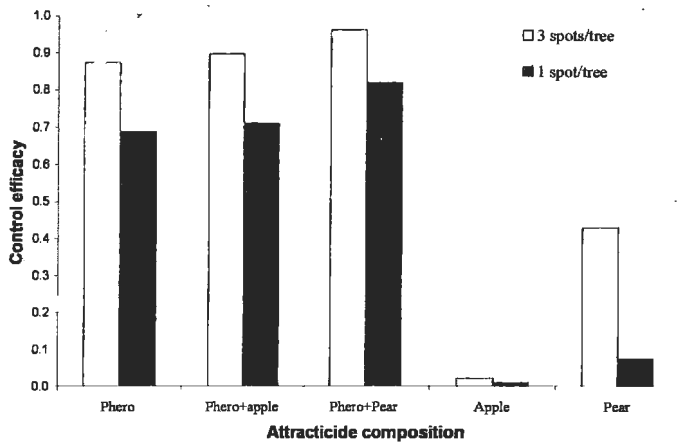

Figure 3. Simulation results of the control efficacy of a sex pheromone based attracticide with or without the addition of plant volatiles (crop volatiles: Apple or non-crop volatile: Pear) and attracticide based on plant volatile attraction only (Apple or Pear). Black bars indicate efficacy of attracticide at spot density of 1/tree, white bars indicate efficacy at 3 spots/tree. Main model parameters: Apple odour spot is 10x and pear-spot 100x more attractive as apple on tree; 200 apples/tree, pupal population 3000/ha, daily moth survival rate 0.75; Pheromone attracticide attracts 100% males; catch rate with plant volatile based attracticide: 50% male, 25% virgin female and 25% gravid female.

The addition of a kairomone increases the attraction potential of the attracticide sources and results in a slight higher impact on the population because it not only affects males but also virgin females and mated females (Fig. 3, Phero *vs* Phero +Pear). A non-host volatile, such as specific pear volatiles, competes better in an apple orchard with the natural odour sources (fruitlets) than a kairomone of apple origin. One apple tree can have 100-200 (odour releasing) fruitlets, thus the potential attraction of a kairomone-based attracticide has to be several orders of magnitude higher to have any significant impact on the pest population.

If the attracticide is solely based on kairomone-based attraction the 'novel' plant odour (in this case pear volatiles in apple environment) attracticide performs much better than a host-plant based attracticide (Fig. 3 Apple *vs* Pear). There is no significant difference in the

removal of male or virgin females, but there is a drastic reduction when only mated females are affected (not shown).

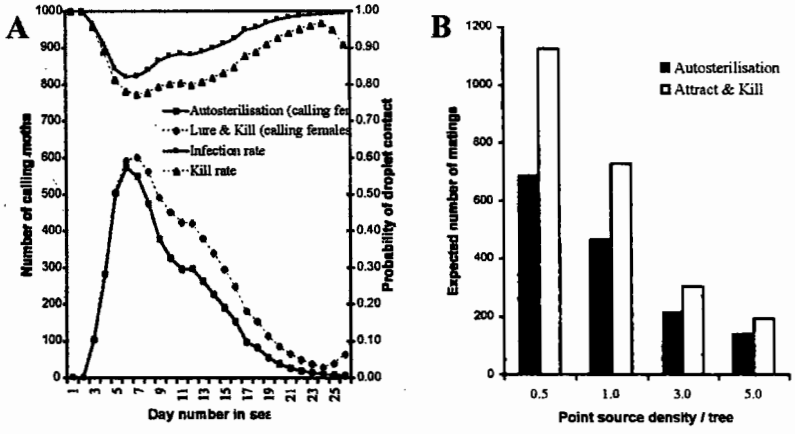

Figure 4. Comparison of efficacy of attracticide based on autosterilisation with insect growth regulator (solid lines, black bars) and knock down insecticide (dotted lines, white bars). **A)** Dynamics within a season. Lower graphs indicate simulated daily number of calling (virgin) females, top graphs indicate probability of droplet contact by male, which leads to sterilisation or direct mortality. **B)** Efficacy in relation to point source density. Main model parameters as in figure 3, Day number 1 = 8 May, flight curve based on Hofchen 1995, only first generation is plotted. Expected number of matings is 427 for attract & kill and 289 for attract and sterilise.

Autosterilisation

An interesting different mode of action of the lure & kill principle was recently successfully tested by Charmillot et al. (2001). They replaced the knock down insecticide in the attracticide paste with an insect growth regulator (IGR, Fenoxycarb) that sterilises males and females upon contact (Charmillot & Pasquier, 1992). Thus males that are attracted to attracticide sources are not killed, but sterilised by pick up of traces of IGR. Laboratory studies showed that males pick up enough IGR from the formulation upon contact to transfer a sterilising dose to every female they subsequently mate with. To simulate the autosterilisation strategy two extra adult states were added to the model: S_{δ} the number of sterilised males and, S_{φ} the number of females that mated with a sterile male.

Compared to A&K the number of calling females is lower in autosterilisation (Fig 4a). This is due to the fact that more matings take place in autosterilisation, since males are not killed. The majority of these matings does not lead to fertilisation of the females and furthermore decreases the number of natural sources of pheromone (females cease calling after mating), which will increase the competitiveness of the attracticide sources (i.e. probability of droplet encounter instead of calling female).

A chemosterilant in the attracticide formulation is about twice as effective as a knockdown insecticide, assuming a perfect take-up and transfer of the sterilant by the male to

the female. This better performance of autosterilisation compared to knockdown decreases at higher point source densities (Fig 4b). Barclay (1988) also found that an insecticide based attracticide required a greater trapping effort than the use of chemosterilant, especially when the daily survival and fecundity of the pest were high.

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Eco-Trap: efficient tool for the control of the olive fruit fly *Bactrocera oleae* in the Mediterranean area

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Abstract: The Eco-Trap is a green envelop 15 X 20 cm, containing ammonium bicarbonate and a dispenser of the 1,7-dioxaspiro[5.5]undecane, the pheromone of the olive fly. The ammonium bicarbonate decomposes slowly to ammonia, a powerful food attractant for all the fruit flies. The olive fly is attracted by both, the food and sex attractant to the surface of the trap, which is impregnated with deltamethrine, a powerful insecticide, and is exterminated. The ingredients of the Eco-Trap are active for all the period of the olive fly. Application of the Eco-Trap at the proper time, provide an efficient control of the olive fly Bactrocera oleae Gmelin, keeping the fly population in low level and the final infestation in lower or at least the same level with either bait sprays (hydrolyzed proteins-dimethoate) or cover sprays (dimethoate). Representative results of applications over the last four years in Greece and Italy are presented

Key words: Bactrocera oleae, olive fruit fly, pheromone, food attractant, attracticide, Eco-Trap

Introduction

The olive fruit fly Bactrocera oleae Gmelin is the most important pest of olives in the Mediterranean area causing great economic damages to the production, if efficient control measures are not applied. Current control methods rely on the use of broad spectrum insecticides in bait or cover sprays. In Greece and in Spain bait sprays with hydrolyzed proteins and dimethoate or fenthion are usually applied (Broumas, 1994) while in Italy, cover sprays with dimethoate, are the more common (Ricciolini, 1997).

After the identification and the synthesis of the sexual pheromone of the olive fly in 1980 (Baker et all 1980; Mazomenos and Haniotakis, 1981), great efforts have been made in all the Mediterranean countries, in order to develop efficient alternative control methods based on the pheromone, which could reduce or even eliminate the dispersion of toxic insecticides.

From the techniques using pheromones for the control of insect pests, the mating disruption was very soon abandoned, because of the volatility of the pheromone and the very long active period of the olive fly.

Special consideration has been given on the mass trapping, due to the availability of a variety of food, visual and pheromone attractants (Haniotakis 1981; Jones 1983). Several types of traps using one or more of these agents have been tested against this major pest. The most promising results have been obtained by a trap, which combines an entomotoxic surface, a food attractant and the pheromone. The traps that were used at the end of the decade of 80, designed by Haniotakis (1991), were plywood rectangles of natural brown color, impregnated with a solution of deltamethrine, sugar and glycerin. On this rectangle a dispenser of the pheromone and a small envelope containing powder of ammonium bicarbonate were attached. These traps achieved an acceptable olive protection, applied at small to medium size orchards using traps prepared in the laboratory. The massive production of the traps for large-scale

applications did not give at that time reliable results. The weak point of the massive production was the deficiency of the active components of the trap. It has been proved by systematic analysis that the insecticide was degradated by the daylight and was very soon exhausted. So even if the insects were attracted to the trap they were not killed.

At the beginning of the decade of 90 an improved trap was developed. This trap known under the trade name Eco-Trap is the trap that is in use today.

Material and methods

The Eco-Trap consists of (1) a green paper envelope 15 X 20 cm, lined inside by a polyethylene film for tightness. The external surface of the envelope is impregnated with 15 mg of deltamethrine and treated with a stabilizer in order to prevent the rapid degradation of the insecticide by the daylight. The density of deltamethrine on the trap is 30 μg/cm2; (2) an amount of 70 gr. of ammonium bicarbonate that is in the envelope. Decomposition of this salt at ambient temperature liberates ammonia that is a powerful food attractant for the olive fly; (3) a pheromone dispenser, containing 80 mg of synthetic racemic (±)1,7-Dioxaspiro[5.5] undecane.

The racemic compound has (1) a long range male attractant activity due to the (S)-(+)-enantiomer which is the natural pheromone of the insect (Haniotakis 1986) and (2) An aggregation pheromone activity of the (R)-(-)-enantiomer which it is also aphrodisiac and attracts males and females. The pheromone is incorporated into a special type wax that guarantees a slow release of the active component over six months.

The combination of food attractant and the pheromone resulted in an overall increase of female captures as compared to those with ammonia alone (Haniotakis1987; Broumas 1994).

The insects are attracted to the trap by the pheromone and the ammonia and even if they stay on the toxic surface for a few seconds, they are killed by the contact of the deltamethrine.

The manufacturing process guarantees a long life efficiency of the trap, concerning both the attraction and the killing capacity. More precisely: The insecticide lasts at least six months. Analyses of the residual deltamethrine on the trap were periodically made and at the same time in the laboratory was determined the mortality of insects that stay on the surface of the trap for 10 seconds. It has been found that after six months in the field, an amount of 4-6 mg of deltamethrine remains on the trap, quantity that is enough for a mortality of 100%. (Tomazou 1995), Figure 1.

The release rate of the pheromone, is determinate by analysing the remaining active compound in the dispenser, throughout the period of application. At the same time the efficiency of the dispenser was compared to the efficiency of a new dispenser. It has been found that after six months in the field the quantity of the pheromone that remains in a dispenser is 8-12 mg. At that time the efficiency of the dispenser is about 70% compared to a new one Figure 2.

In most applications the amount of 70 gr. of the salt lasts more than six months. The decomposition of the ammonium bicarbonate is slow and regular at temperatures below 35°C. At higher temperature the decomposition is rapid and the salt can be exhausted before the six months. In that case a second application of traps in the same area is necessary because only the pheromone is not enough to keep low the population of the insect. Figure 3.

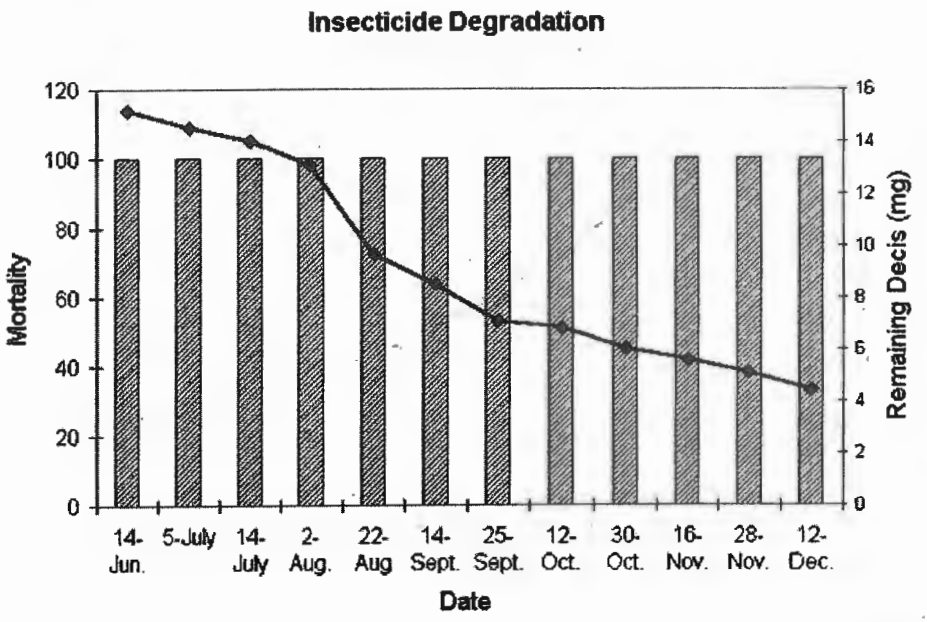

Figure 1

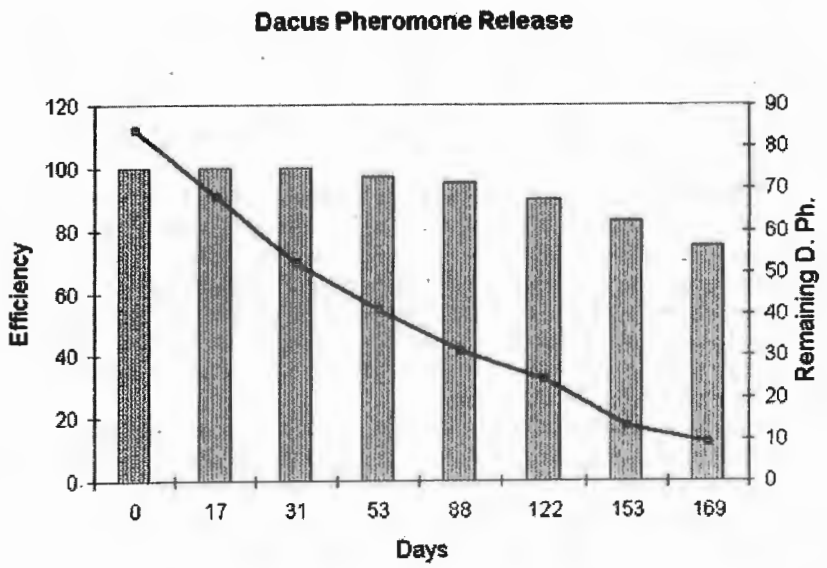

Figure 2

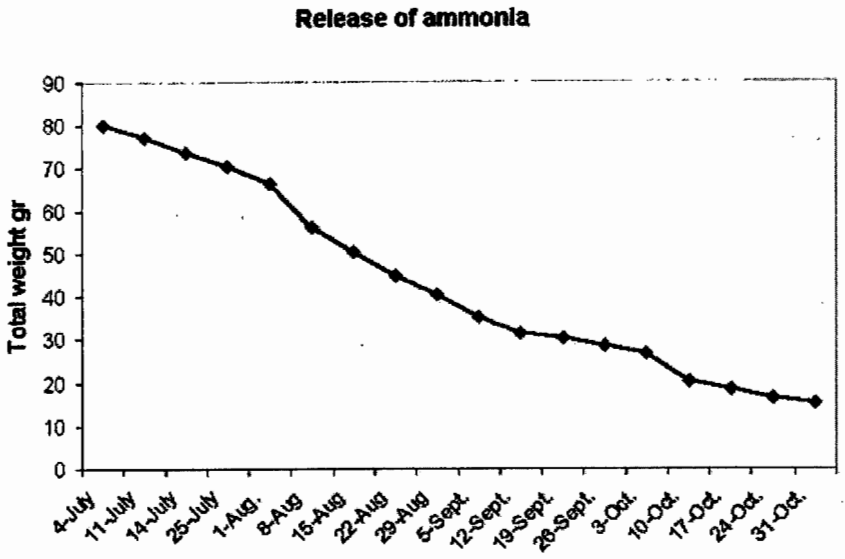

Figure 3

Application method

Before placing the Eco-Trap on the tree, two small holes 1mm in diameter are opened through the upper part of the green envelop in order to start the release of ammonia. With the same sharp object, one hole is opened through the pheromone capsule to start the release of the pheromone. The trap is then placed approximately in the middle of the foliage of the olive tree, in the shade, without coming in contact with leaves or branches. The rate of the application depends on the nature of the olive grove, the variety of the plants and the presence of the olive fly in the area.

In a normal plantation (100-150 trees per hectare) of trees of medium size (3-5 m high) and a maximum distance between the trees of 8 meters, a trap every other tree is placed at the beginning of the active period of the insect. In a plantation of large size trees (more than 5 m high) and a longer than 8 m distance between trees, one trap every tree is necessary.

The mass trapping is a preventive method and therefore the traps have to be placed in the grove shortly before the emergence of the first generation of the insect and before the olive fruit becomes susceptible to be infested by the olive fly. This stage can be practically identified by the hardening of the olive stone.

For example in Southern Greece and in Southern Italy, the traps are placed in the orchard at the end of May, beginning of June. In Northern Greece and Northern Italy the traps are placed at the beginning of July. In some cases if the emergence of the first generation of the olive fly is late, the traps can be placed at the end of July or even in August.

Systematic evaluation of the mass trapping technique using the Eco-Trap has started since 1993 in Greece and in Italy and now is extended in all the Mediterranean countries. The evaluation of the method is based on: (1) the olive fly population density expressed by the catches per five days or per week in either MacPhail traps or yellow sticky traps with a pheromone dispenser, and (2) the fruit infestation level These data are compared to the

population density of the olive fly and the infestation level in the neighbouring areas where bait sprays or cover sprays are applied.

In Greece the olive fly population density is monitored by a network of MacPhail traps baited with 2% ammonium sulphate water solution (one every 1000 trees) in the test area and the control area. In Italy usually a yellow sticky trap baited with a pheromone dispenser is used in the same rate. Catches are counted every five days or every week and are expressed as catches per day per trap or as catches per five days per trap. Fruit infestation is expressed as total infestation (live and dead eggs, L1, L2, L3, pupae, exit hols and stings infested by the fungus Camarosporium dalmatica (Macrophoma). Two typical examples of application of Eco-Trap are presented below.

Results

Tanagra, Voeotia, Greece: Results of four year applications (Broumas et al. 2001)

The mass trapping method was applied to a homogeneous, 300 hectares area (45.000 trees) for four successive years from 1996 to 1999. The results are compared to those of two neighbouring areas, one of 270 hectares at Shimatari and one of 235 hectares at Arma where bait sprays were applied. In all the experimental area the same variety "megaritiki" is cultivated and the trees are of medium size planted in a density of approximately 150 trees per hectare. Typical variation of the population density of the olive fly in the test areas is in Figure 4. Characteristic are the reduced densities of flies in the Eco-Trap area during all the period in contrast to the neighboring areas where low populations are observed only after the sprays applications.

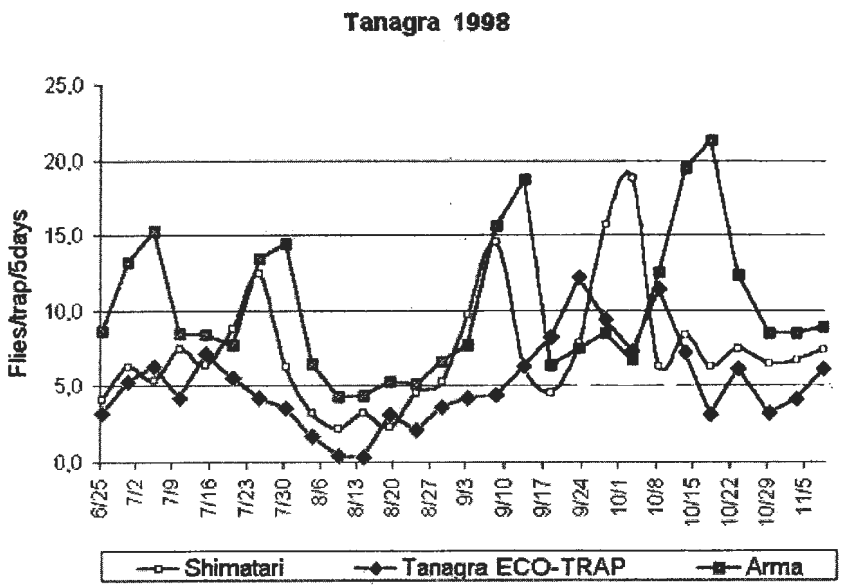

Figure 4

From 1996 to 1999 in the control areas at Shimatari and Arma three bait sprays were applied every year. Two bait sprays were applied, only once in Arma area in 1998. In November just before to start the harvest, the total fruit infestation was always lower in the Eco-Trap area than to the control areas. Last year the infestation was the double than the acceptable level of 10%, in the Eco-Trap area, but still three times lower than the infestation in the control area where three bait sprays were applied. Figure 5.

Toscana Italy (Ricciolini et all 2000)

The trials in the Toscana region in Italy are started in 1993. The last three years 1997-1999, the trials are realized in the context of the regulations CE 2132/96, CE 2430/97 and CE 528/99 of the European Community for the "amelioration of the quality of the olive oil". ARSIAT was the coordinator of the project, which was realized by the two olive farmers associations AIPROL and OTA. Every year 500 to 700 hectares were treated with the ECO-TRP shared into 10 rural communes.

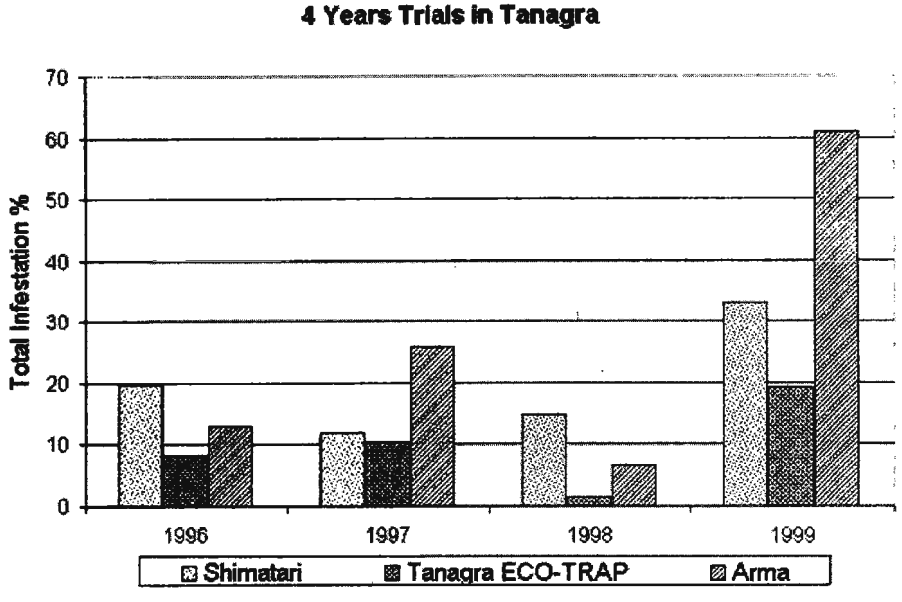

Figure 5

A typical result is summarized in Figure 6, where the variation of the infestation in the Eco-Trap area and the control area in the commune of Guardistallo for 1999 is presented. In the Eco-Trap area the infestation was kept low all the season exempt at the end of October when the infestation reached but not overpass the limit of 10%. In the control area the infestation was always higher despite the two cover sprays applied and at the end of October reached the 15%.

The results in all the communes in Toscana for 1999 were satisfactory. Always the infestation in the Eco-Trap area, where no sprays with conventional insecticides were applied, was inferior compared that of the neighboring areas, where one to three cover spray with

dimethoate were applied.

The mass trapping with the Eco-Trap has been proved to be a very efficient method for the control of the olive fly, with an efficacy comparable to the conventional sprays with chemical insecticides.

The use of the Eco-Trap is simple and safe for the farmer. The application of the Eco-Trap does not leave any residual insecticide on the fruit and the leaves.
The deltamethrine on the surface of the trap is attached on the paper in such a way that cannot be washed by the rain and as a consequence it is not dispersed into the environment.

According to the regulation of the European Union CE 1488/97 the Eco-Trap is a product allowed in the biological agriculture of the olive. Actually the Eco-Trap is the most efficient tool for the control of the olive fly, allowed in the biological agriculture.

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Insect semiochemicals

Use of pheromones and other semiochemicals in integrated production
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Female calling behaviour and male response to the synthetic sex pheromone components of *Palpita unionalis* (Lepidoptera: Pyralidae)

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Abstract: The pheromone biology of the jasmine moth *Palpita unionalis* was studied under Laboratory conditions. Female began calling during the second day following emergence. Calling activity and pheromone production is periodic and synchronous. Maximal calling and pheromone production was obtained the fourth day. The peak of the female calling occurred during the dark phase, six hours after lights off, of a 14:10 (Light: Dark) regime. Male response to each one of the two synthetic pheromone components [(E)11-16:Ac], [(E)11-16:Ald] and their blend was tested in a wind tunnel at different dosages. The (E)11-16:Ald stimulates more males to take flight, but the vast majority of them did not approach the pheromone source. With the (E)11-16:Ac fewer males took flight, but most of them flew close to the pheromone source and some landed on the source expanding their hair pencils. A two component blend at the ratio of 7:3 [(E)-11-16:Ac:(E)11-16:Ald] was the one that evoked the full behavioural repertoire by males. The proportion of males responding was lower at the dose of 2 μg and remained relatively unchanged for the other doses tested with a trend toward decreased responses at the higher dose of 32 μg. Field tests revealed that funnel type traps baited with 1 mg of the two compounds blend captured significant number of males.

Key words: *Palpita unionalis*, calling behaviour, pheromone titter male response, wind tunnel, pheromones (E)-11-hedecenal, (E)-11-hexadecenyl acetate.

Introduction

The jasmine moth *Palpita unionalis* Huebner (Lepidoptera: Pyralidae) is a serious pest of *Jasminunm* sp., *Ligustrum* sp., *Olea europea* and *Phiirea media,* causing severe damages on the foliage of these plants. *P. unionalis* occurs throughout the Mediterranean region (Balachowsky, 1972). In olive trees, larvae usually attack young leaves and shoots, while in years of high population densities; they attack also olive fruits, making them unsuitable for marketing.

The sex pheromone of *P. unionalis* was reported to consist (E)11-hexadecenyl acetate [(E)11-16:Ac] and (E)11-hexadecenal [(E)11-16:Ald] (Mazomenos *et al.*, 1994). The blend of the two components at the ratio of (7:3) [(E)11-16:Ac:(E)11-16:Ald] attracted males in field test. Further field tests indicated that the efficacy of the traps baited with the blend was limited and male catches were low. In order to improve the pheromone efficacy in monitoring and control studies it is essential to know the pheromone biology of *P. unionalis*

In many moth species, female calling and pheromone production is synchronous and usually depended on moth age as well as on other endogenous and exogenous factors. (Howlader and Gerber, 1986; Raina *et al.*, 1986; Snir *et al.*, 1986; Dunkelblum *et al.*,1987;

Noldus and Potting, 1990; Babilis and Mazomenos, 1992; Kakimura and Tatsuki, 1993).

Wind tunnel and field studies as well, have shown, that specific pheromone components or their blends can be responsible for several quantitative and qualitative aspects of male mating behaviour for many moth species (Baker and Carde, 1979; Linn and Gaston, 1981a, 1981b; Quartey and Coaker, 1993). Therefore knowledge of the role of each component is essential for understudying the behavioural mechanisms associated with male mating behaviour.

In this paper we report results on the effect of age on the female calling behaviour, the pheromone production and the role of the synthetic pheromone components on male attraction in wind tunnel and field tests.

Material and Methods

Insects.

The insects used were from a laboratory colony established from larvae collected from infested olive trees. The colony was maintained on Ligustrum *ovalifolium* (L) leaves at 25 ± 2°C, 60 ± 5% relative humidity (rh), and 14:10 h light-dark (L:D) regime (Vasilaina-Alexopoulou and Santorini, 1973). Pupae were segregated by sex and were kept at 25°C on a 14:10 Light:Dark (L:D) cycle. Emerged females/males were transferred daily to 20 cm^3 Plexiglas cages in separate chambers and kept under the conditions mentioned above. Moths were provided with a 10% sucrose solution.

Female calling behaviour.

Thirty newly emerged females were caged individually in 10x10x10cm screen cages provided with 10% sucrose solution. The calling behaviour of the females was observed during the scotophase at 15 min intervals, observations were facilitated with red light.

Data on the percentage of female calling, daily, the age at which females initiated calling, as well as the mean onset time (time after lights off), and the mean time spent calling were collected on successive nights from the first night of emergence until the seventh day.

Pheromone collection and analysis.

To quantify the pheromone produced, ovipositors of individual females 1 to 7-old were excised and extracted in 10 µl dichloromethane for 20 min in the sixth hour of the scotophase. Ten samples were prepared and analysed for each day. The extract was transferred to 0.3 ml conical screw caps vials. Five µl 25 ng of dodecyl acetate was added as internal standard. The samples were concentrated to about 2 _l and were analysed on Varian Model 3400 chromatograph, with flame ionization detector (FID) and a splitless injector system. The column was a DB-5 30 m x 0.32 mm (id0) (J&W, Scientific, Folsom, Ca, U.S.A.). The column temperature program was 80°C (hold 2 min) at 10°C/min to 250°C. Helium was used as carrier gas, at a flow rate of 2 ml/min

Chemicals.

Synthetic (E)11-16:Ald and (E)11-16:Ac, used for the wind tunnel tests, were provided from (Vioryl Co. Kato Kifissia, Athens, Greece). The components were found to be 97% pure, when analysed on DB-5 30 m x 0.32 mm-ID capillary GC column (J&W, Scientific, Folsom, Ca, U.S.A.).

Male behaviour.
The male response to the pheromone source was studied in a 150 x 35 x 35 cm glass wind tunnel similar to that described by Carde and Hagaman (1979) maintained at 25 ± 2°C, 60 ± 5% rh. Male response was tested during the fourth to seventh hour of a ten-hour scotophase. The males 2 to 5-d-old were placed individually in 15 x 10 cm cylindrical screen cages. The cages were transferred into the testing room, operated under the same conditions described, before the onset of the scotophase. Each cage was placed close to the downwind end of the tunnel five minutes prior to testing for male acclimation to the light intensity and airflow. The chemicals and the baits (5x5 cm filter paper Whatman No 1) were kept and prepared outside the testing room. The piece of the filter paper impregnated with the pheromone component was hung 15 cm downwind of the fan. Filter papers impregnated with the same volume of hexane were also introduced into the tunnel. Twenty-five males were tested to the following dosages: 2, 4, 8, 16 and 32 μg, of each of the component and their blend at the ratio of 7:3 [(E)11-16:Ald:(E)11-16:Ac].

Field trapping.
The two compounds blend in hexane solution was formulated in white rubber septa. Three funnel type traps baited with 1 mg of the pheromone blend were hung on olive trees in an olive grove located in Island Crete from 23 April to 15 November. Traps were suspended at least 50 meter apart the number of males caught was recorded weekly and the pheromone dispensers were replaced every 30 days.

Data recording and analysis.
The responses of the male to either the pheromone component alone or the blend was recorded for 5 min. The behavioural steps recorded included: wing funning and taking flight; flight close to the pheromone source; landing on the pheromone source and hail pencil display. Data recorded from the wind tunnel were analysed using a chi-square 2 x 2 test of independence (Zar, 1984). Responses of males of individual components and their blend were compared with respect to each dose and to the specific behavioural steps. The P=0.05 level was set for the rejection of the null hypothesis.

Results

Calling behaviour.
Moths emerged during the scotophase, 2 to 3 h before lights on. The following scotophase (scotophase 1) none of the 50 observed females initiated calling. Calling was initiated during the second scotophase. Maximum calling occurred during the fourth scotophase (68.5%). The percentage of the females calling for scotophase 2 to 5 ranged from 42.5 to 61.5% and then decreased so that by the seventh scotophase only 12.4% of the females were observed calling. Calling activity was initiated during the 5 to 6 h after lights off and terminated during the 7 to 8 h of the scotophase. The mean onset of calling advanced from 6.4 h on scotophase 2 to 5.1 h on scotophase 7. The mean time spend calling was similar from scotophases 2 to 5 and was decreased for the next two scotophases (6 and 7) (Table 1).

Pheromone gland content.
The pheromone content in female gland was quantified from scotophase 1 until the scotophase seven. Age affected the pheromone production of *P unionalis*, not detectable quantities of both components were found in scotophase 1. The pheromone production begins from scotophase 2, increased progressively and reached at maximal level on scotophase 4

(Table 2). The pheromone production coincides with the female calling activity. The quantity of (E)-11-16:Ac produced was approximately 7 times more than the quantity of (E)-11-16:Ald.

Male response to pheromone blend and the two components.

The role of each component on male behaviour and the blend was studied at the 8 μg dosage (Fig. 1). At a dosage of 8 μg, the males exhibited all the stages of the behavioural sequence. The proportion of males responding was maximal when blend of (E)11-16:Ac, and (E)11-16:Ald was tested. Wing fanning and taking flight was 100%, significantly higher than the level observed for the (E)11-16:Ac alone ($Z=13.3$, $P=0.05$), but was not different from that observed for males responding to the (E)-11-16:Ald.

Table 1. Effect of age on calling, the mean time of onset calling and the mean time spent calling of virgin females *P unionalis*. The females emerged during the last two hours of the scotophase and were kept at 14:10 (L:D) regime (N=30).

Female Age Scotophase	% Calling	Mean onset	Calling Time (min)
1	-	-	-
2	42.5 ± 1.3	384.0 ± 11.7	55.4 ± 3.6
3	65.2 ± 2.6	387.6 ± 7.8	62.8 ± 1.4
4	68.4 ± 1.4	360.1 ± 11.3	58.8 ± 2.7
5	61.5 ± 3.4	351.9 ± 20.2	61.2 ± 4.3
6	17.3 ± 3.0	331.9 ± 18.6	38.4 ± 2.0
7	12.4 ±3.1	304.2 ± 19.3	31.2 ± 3.1

Table 2. Titre of (E)-11-16:Ald and (E)-11-16:Ac female *P. unionalis* sex pheromone components (ng) obtained from individual pheromone gland extracts of 2 to 7-d-old virgin females (N=10).

Female Age Scotophases	(E)-11-16:Ac	(E)-11-16:Ald	Ratio Ac/Ald
1	-	-	-
2	173 ± 11.3	23 ± 1.2	7.5
3	262 ± 48.7	36 ± 6.6	7.2
4	296 ± 8.2	45 ± 8.2	6.6
5	292 ± 33.3	40 ± 8.9	7.3
6	241 ± 24.5	36 ± 3.1	6.7
7	171 ± 18.9	24 ± 3.2	7.2
Mean	239.4± 22.7	34 ± 3.5	7.1

Comparing males exhibiting wing fanning and taking flight, when the two components were tested alone, significantly more males were found to be responding to the (E)11-16:Ald than to the (E)11- 16:Ac (Z=7.62,P=0.05).

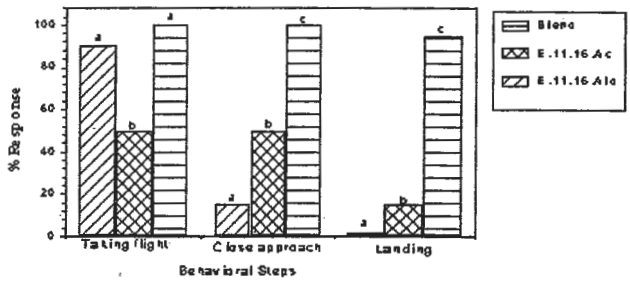

Figure 1. Proportional effect of (E)-11-16:Ald, (E)-11-16:Ac and their blend, on males *P. unionalis* response of various behavioral stages in a wind tunnel. Bars with different letters for each chemical are significant different (chi-square 2X2 test of independence P<0.05) (N=25).

The majority of males that made upwind progress in response to the blend flew close to the pheromone source and many of them landed on the filter paper, exposing their hair pencils. The number of males that flew close to the pheromone source with the (E)11-16:Ac was relatively high, however it was significantly lower than that of the blend (Z=13.33, P=0.05). Significantly more males landed on the filter paper with the blend, compared with the (E)11-16:Ac (Z=23.1, P=0.05). Few males landed on the filter paper with the (E)11-16:Ald alone.

Effect of pheromone dose on male behavioural steps.

The proportion of males that performed wing fanning and taking flight was not significantly different to all the doses tested with the (E)11-16:Ald and the blend (Fig, 2A). Male response and taking flight, at the dose of 2 μg was significantly lower than that of 4 μg (Z=3.95, P=0.05) for (E)-11-16:Ac. Above this dose, the proportion of males that performed wing fanning and taking flight were not significantly different and seemed to reach a plateau at the doses of 8 to 16 μg.

Few males flew close to the pheromone source, when (E)11-16:Ald was tested. The percentage of males that flew close to the pheromone source with the (E)11-16:Ac increased as the pheromone dosage increased from 2 to 4 μg and reached a plateau for higher dosages tested (Fig 2B). With the blend the proportion of males flying close to the source was high to all dosages and reached the 100% at the dose of 8 μg (Fig. 2B).

For all the doses of (E)11-16:Ald, very few males landed on the filter paper source and everted their hair pencils. The percentage of males landing and everting their hair pencils for (E)11-16:Ac increased as the dose increased and reached 35% at the concentration of 32 μg dose (Fig. 2C). The proportion of males that landed and everted their hair pencils when the blend was tested increased as the pheromone dose increased, 38% and 44% of males were landed on the filter paper at the 2 μg and 4 μg doses respectively, whilst at the higher doses (8, 16, 32 μg) the percentages of males landing reached 90 to 95% (Fig. 2C).

Field trapping.
Data collected from field experiments during 1998 and 1999 in Island Crete indicated that pheromone traps baited with the two-component blend attracted males. The number of males caught in pheromone traps compares well with the infestation levels recorded during the testing period on olive trees. During 1998 and 1999 the moth develops low population in Crete. Field results revealed that funnel traps baited with the pheromone blend are effective tools to monitor the moth population and time the application of control measures. Trap catches also indicated that in Island of Crete *P. unionalis* develops at least three generations per year (Fig. 3).

Discussion

Calling behaviour and pheromone production of *P unionalis* females is synchronous. Maximal pheromone content in the gland and maximal calling activity occurred during the scotophase the 6th h after lights off. It appears that P. *unionalis* follows a calling and pheromone biosynthesis pattern that is common for many moth species (e.g. Heliothis zea, Raina *et al.*, 1986; *Heliothis subflexa,* Heath *et al.* 1991; *Sesamia nonagrioides*, Babilis and Mazomenos, 1992; *Helicoverpa assulta*, Kakimura and Tatsuki, 1993; *Cydia pomonella* Bäckman *et al.*, 1997), In these species pheromone production occurs during the period where females are calling and releasing pheromone.

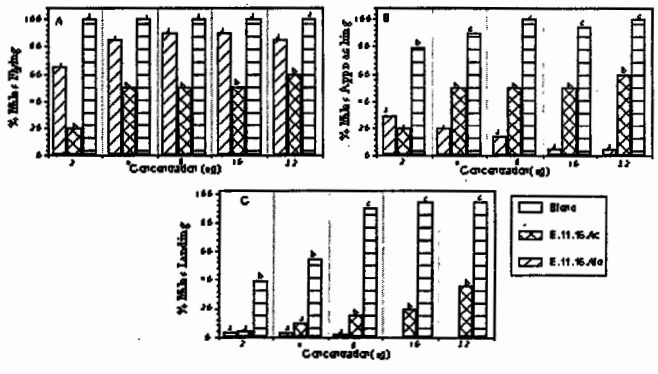

Figure 2. Percentage of males *P. unionalis* taking flight (A), flying close (B),and landing (C) on the pheromone source at various doses of the two-sex pheromone components (E)11-16:Ald, and (E)11-16:Ac and their blend. Bars with different letters for each dose are significant different (chi-square 2X2 test of independence P<0.05) (N=25).

Aside from the quantity of pheromone produced by females every day, the ratio of the two components was the same. The amount of (E)11-16:Ac produced was approximately seven times more compared to the amount of (E)11-16:Ald.

Evaluating the results obtained from the wind tunnel, a significant difference was observed between the two-pheromone components on the effects of the male *P. unionalis* mating behaviour. The (E)11-16:Ald stimulates more males to take flight, but the vast majority of them did not approach the pheromone source. On the contrary, for (E)11-16:Ac

fewer males took flight, but most of them flew upwind, approaching the source. Therefore, the males oriented themselves better towards the (E)-11-16:Ac source than when the (E)11-16:Ald was used. As a result, more males landed on the pheromone source, exposing their hair pencils in the presence of (E)11-16:Ac.

The two components blended at a ratio of 7:3 (E)11-16:Ald:(E)11-16:Ac evoked the whole sequence of behavioural steps. From these results it is clarified that the two-pheromone components act synergistically and the blend caused maximal response. Moreover, the blend elicited satisfactory male response, even at low concentrations. Synergism of the different components of sex pheromone blends is widely known amongst several species of Lepidoptera. Much work has been carried out with many moth species that use different multicomponent blends in their sexual behaviour such as: *Trichoplusia ni* (Linn and Gaston, 1981 a,b) *Diaphania nitidalis* (Klun *et al.*, 1986), *Cochylis hospes* (Underhill *et al.*, 1986). *Ephestia cautella* (Quartey and Coaker, 1993). In all of these species, it has been shown that the single presence of some of their pheromone components is not sufficient for successful male attraction and copulation. On the other hand, both in laboratory bioassays and field tests, most of these species use specific blends of appropriate components that act as a unit to evoke maximal stimulation of the males.

Age affected pheromone production the greatest amount of pheromone in the gland was measured, when the females were four days old. After the fourth day pheromone gland content and calling activity were decreased progressively. Similar results have been reported for other moths (Howlander and Gerber, 1986; Raina and Klun, 1986).

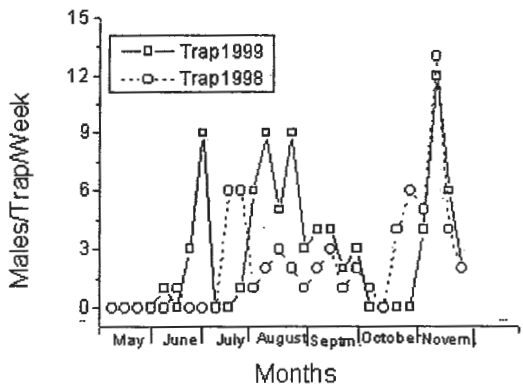

Figure 3. Males *P. unionalis* caught in funnel traps baited with rubber septa, loaded with 1 mg of the pheromone blend (E11-16: Ac: E11-16: Ald). Field tests were conducted in an olive grove in Island Crete during 1998 and 1999.

The different dosages of the two components and their blend tested quantitatively affected male *P. unionalis* behaviour. When the two components were tested alone, with the (E)11-16:Ald no dosage dependence was observed with respect to wing fanning, and taking flight. With the (E)11-16:Ac all the behavioural stages are dosage dependent. Dosage dependence to various behavioural steps has been reported for other moth species. The male response was influenced as the dosage of the pheromone increases for the species *T. ni* (Linn and Gaston, 1981) and *Lymanthia dispar* (Carde and Haganan, 1979).

The data presented shows that calling behaviour and pheromone production *of P. unionalis* is a synchronous process. The two pheromone components individually tested

affected male behaviour, however the blend of the two components at the ratio found in the pheromone grand elicited to males maximal response in wind tunnel experiments. Field data revealed that pheromone traps are effective tools to monitor *P. unionalis* population.

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Use of pheromones and other semiochemicals in integrated production
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Sex pheromone and analogs of the citrus mealybug, *Planococcus citri*: synthesis and biological activity

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Abstract: The citrus mealybug, is a cosmopolitan pest and affects many crops. The female sex pheromone has been identified by Bierl-Leonhardt et al., in 1981 as (+)-(1*R*)-*cis*-2,2-dimethyl-3-isopropenylcyclobutanemethanol acetate **1**. Several groups including our team have synthesized the pheromone. A number of analogs have also been prepared in order to study the structure-activity relationship. We present here a modified synthetic route for the pheromone, preparation of some analogs and the biological activity of these compounds. The starting materials for the pheromone and analogs are *cis*-pinonic acid or *cis*-pinonic aldehyde which can be easily obtained from cheap commercial (+)-α-pinene by cleavage with permanganate or ozonolysis. Conversion of the pinonic derivatives to the pinononic compounds was achieved either by a modified Hundsdiecker reaction (Wolk et al., 1986) or by ozonolysis of the enol acetate of *cis*-pinonic aldehyde (Barton and Fontana, 1996). In the present study, we report our results using the second method, which avoids the use of the unstable pinononyl halides. The key element of the synthesis of **1** is the use of pinononyl aldehyde **2** and its selective reduction to pinononyl alcohol **3**. The latter was submitted to a Wittig reaction and then acetylated; alternatively the sequence was reversed and the Wittig reaction was performed after the acetylation to form the pheromone **1**. The stereochemistry was preserved in all steps and no racemization was observed. Field tests indicated that a number of analogs display considerable biological activity. One of them, a homolog containing an elongated acetate side chain by one carbon **10** has a relatively high activity. This observation has practical importance because the synthesis of the homolog is shorter and more convenient than that of the pheromone. The field tests indicated that the acetate group and the double bond in the pheromone molecule are essential for biological activity.

Key words: Citrus mealybug, *Planococcus citri*, synthetic sex pheromone, analogs, structure-activity relationship, field bioassay.

Introduction

The citrus mealybug, (Risso) is a cosmopolitan pest, affecting subtropical fruits and ornamentals. The female sex pheromone has been identified by Bierl-Leonhardt et al., in 1981 as (+)-(1*R*)-*cis*-2,2-dimethyl- 3-isopropenylcyclobutanemethanol acetate **1**. Recently, we have initiated a field project to assess the potential of the synthetic pheromone for monitoring and mass trapping of the pest. To this purpose we have developed a modified synthesis of the pheromone and of a number of analogs.

This report describes the general scheme of synthesis of the pheromone termed planococcyl acetate (Wolk et al., 1986) and analogs, and evaluates the structure-activity relationship based on outdoor male trapping.

Materials and Methods

Preparation of the P. citri pheromone, planococcyl acetate 1.

The primary starting material was (+)-α-pinene, purchased from Aldrich containing 95% of

the (+) enantiomer according to GC analysis on a chiral 30 m x 0.25 mm column coated with a 0.25 micron film of Cyclodex - B. The (+)-α-pinene was cleaved with ozone to produce pinonic aldehyde **2** which was stored with 2.5% BHT as antioxidant, at low temperature. Aldehyde **2** was converted into the enol acetate **3** as an E/Z mixture. Ozonolysis of acetate **3** gave pinononic aldehyde **4**. This aldehyde is very prone to oxidation by air. It can be stored for a few days with 2.5% BHT under argon at low temperature. The ozonolysis and preparation of the enolacetate were carried out according to the procedure of Barton and Fontana (1996) with the following modifications: The solvent mixture of methanol + dichloromethane was replaced with dichloromethane and $NaHCO_3$ was added to trap any acid formed. BHT was added after reduction of the intermediate oxonides with dimethyl sulfide to prevent air oxidation of the formed aldehydes. This step was particularly important for pinononic aldehyde **4**, which is very prone to oxidation and conversion to pinononic acid.

The key step of the synthesis involved selective reduction of pinononic aldehyde **4** to pinononic alcohol **5** with $Zn(BH_4)_2$. The reagent was prepared in situ according to Ranu and Chakraborty (1990), from an etheral solution of $ZnCl_2$ and $Na(BH_4)$ in DME without filtering the formed $Zn(BH_4)_2$ from the by-product NaCl. The reagent solution was added to **4** in THF and the reaction was terminated after 15 min. by careful addition of water. Both steps were performed in an ice-bath, The conversion of **4** to **5** was ca. 95%. The reaction time was critical, less the 15 min reduced the conversion and a longer reduction time resulted in the formation of additional products.

The pinononic alcohol **5** was either submitted to a Wittig reaction with the ylide prepared from triphenylmethyl phosphonium bromide with butyllithium and then acetylated with acetic anhydride and pyridine; alternatively the sequence was reversed and the Wittig reaction was performed after the acetylation of **5**. The first route is shorter and therefore preferable for the preparation of technical grade pheromone (~ 85% purity by GC). Crude intermediate products were used in this sequence, starting from **2**; the pheromone **1** was cleaned at the end of the synthesis by column chromatography on silica with hexane and ethyl acetate as eluent. The overall yield of **1** from **2** was 20-25%. In the second route, pinononyl acetate **7** was used as intermediate. It was purified by chromatography on silica with hexane plus increasing amounts of ether. Wittig reaction of purified **7** with the ylide, prepared from triphenylmethyl phosphonium bromide with butyllithium, gave a mixture of the pheromone **1** and planococcyl alcohol **6** due to partial hydrolysis of acetate during workup. Reacetylation of the mixture and column chromatography gave ~95% pure pheromone **1** in 15-20% yield based on **2**. The general scheme of the synthesis, starting from **2**, is outlined in Figure 1. The pheromone **1** was identical with an authentic sample prepared by a different route (Wolk et al., 1986), as compared by capillary GC and GC-MS in the EI and CI mode.

***Preparation of the P. citri pheromone analog homoplanococcyl acetate** 10* **(Figure 1).** Homoplanococcyl acetate 10 was prepared from pinonic alcohol 8 which, in turn, was prepared by selective reduction of 2 with $Zn(BH_4)_2$. A Wittig reaction of 8 with and subsequent acetylation yielded 10 in 43% after chromatography on a silica column. This analog was identical to another sample of 10, prepared previously by a slightly different route (Dunkelblum et.al., 1987), as compared by capillary GC and NMR.

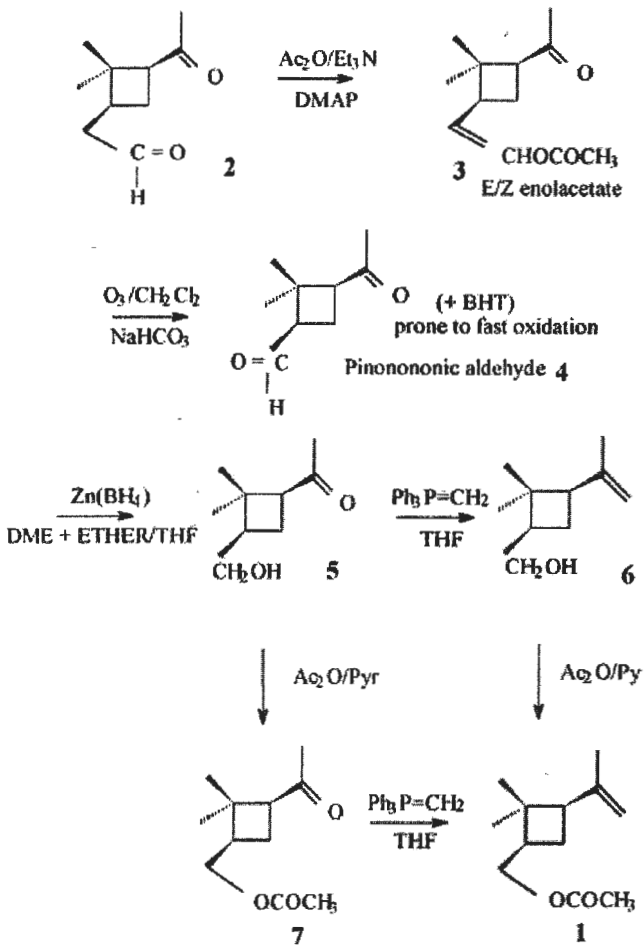

Figure 1

***Preparation of the P. citri pheromone acid methyl ester analog 13* (Figure 2).**
This analog was prepared from pinonic acid **11** (Wolk wt al., 1986). Methylation of **11** with diazomethane gave the methyl ester **12** and subsequent Wittig reaction with the ylide prepared from triphenylmethyl phosphonium bromide and butyllithium provided **13**. The last step proceeded in low yield and analog **13** was obtained in 20% yield from **11** after chromatography on a silica column.

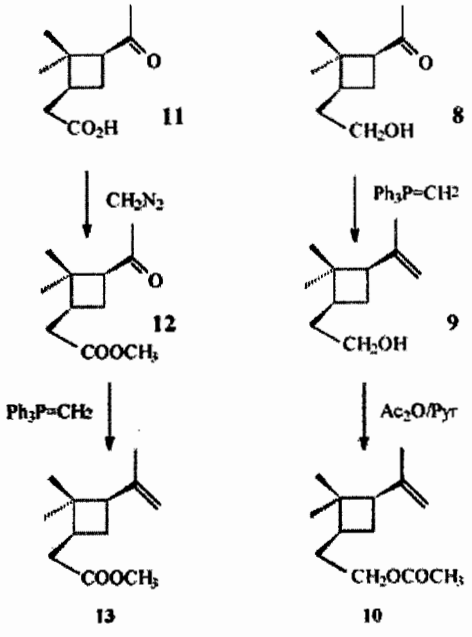

Figure 2

***Preparation of the ethylidene analog 14* (Figure 3).**
The ethylidene analog **14** was obtained from **5** by a Wittig reaction with the ylide prepared from triphenylethyl phosphonium bromide with butyllithium. A Z/E mixture of **14** was obtained in 35% yield after column chromatography on a silica column and characterized by GC-MS and NMR. Analog **16** was prepared and tested before (Dunkelblum et al., 1987).

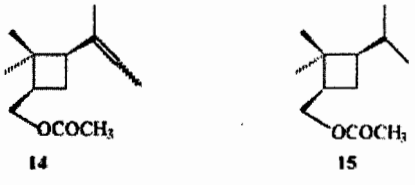

Figure 3

Preparation and analysis of racemic P. citri pheromone 1R.
The racemic pheromone was prepared from commercial racemic *cis*-pinonic acid (Aldrich) by our previous procedure (Wolk et al., 1986). The racemic and chiral pheromones were analyzed on a chiral 30 m x 0.25 mm capillary column coated with Cyclodex-B phase of 0.25 μm film thickness. The column was kept for 2 min. at 60^0 C and then programmed at 10^0 C/min to 130^0 C. The analysis was performed in the split mode with a total flow of 30 ml/min

with a split ratio of 20:1. An almost base-line separation was achieved; the (+) chiral pheromone enantiomer eluted at 50,75 min and the (-) enantiomer eluted at 51,10 min.

Field bioassay:

Tests were conducted in Sweetie grapefruit, avocado and parsimon plantations. Triangular sticky traps were used, baited with rubber septa impregnated with synthetic pheromone or analogs in 200 µl hexane. Control traps were baited with dispensers impregnated only with hexane. Traps were suspended in the canopy of the trees at least 25 m apart. The sticky plates were transferred to the laboratory and the trapped *P. citri* males were counted using a stereomicroscope.

The pheromone was tested in a dose response experiment of 25µg - 800µg. All analogs were tested a number of times and their activity was compared to that of the pheromone **1**. In most tests, a dose of 50µg - 200µg was used. The analog **10** (homolog) and the ethylidene analog **14** were tested also at a dose of 400 µg. In all field tests five replicates were used for each treatment.

Statistical Analysis.

Trap catch data were transformed to $\sqrt{x} + 0.5$ and then subjected to analysis of variance, followed by the Student-Neuman-Keuls multiple range test (at $P < 0.05$), to determine significance between means.

Results and discussion

The citrus mealybug, *P. citri* in Israel is a serious pest of citrus and parsimon plantations. We were interested in the practical use of the pheromone for the management of the pest and in the analogs for the study of structure-activity relationship. The pheromone is not commercial, which is why we devised a new synthesis based on simple reactions (Figure 1). Our previous synthesis (Wolk et al., 1986) was short but involved two complex steps. The key steps in the present method are the isolation of the air-sensitive pinononic aldehyde **4** and its selective reduction with $Zn(BH_4)_2$ to pinononyl alcohol **5**. The aldehyde **4** can be stored with BHT under argon at -18^0 C. For the synthesis of technical grade pheromone (80-85%), crude intermediates **4**, **5** and **6** were used. The alcohols **5** and **6** were difficult to purify by column chromatography due to strong absorption and loss of material. The pheromone **1**, obtained after acetylation of **6**, was purified by column chromatography. The technical grade pheromone was tested in the field and its activity was statistically comparable to that of 95% pure pheromone. For the preparation of 95% pure pheromone, pinononyl acetate **7** was prepared and purified by column chromatography before the Wittig reaction. This route requires an additional acetylation step due to hydrolysis during the workup of the Wittig reaction.

The synthesis of the homolog **10** is shorter and the yield is higher (~40%) than that of the pheromone (~20%). The yield of the analogs **13** and **14** was relatively low and they were prepared solely for the structure-activity relationship study.

Chiral analysis of the pheromone **1** and its racemate on a Cyclodex-B capillary column indicated that no racemization occurred during all steps of the synthesis. The commercial starting material, (+)-α-pinene contained 95% of the (+) enantiomer, and the final product **1** was approximately of the same chiral purity. The present chiral separation of the (±) *P. citri* pheromone represents the first successful separation of the enantiomers of this pheromone. Previously, only the enantiomers of the corresponding planococcyl alcohol **6R** could be

separated (Novotny et al., 1989).

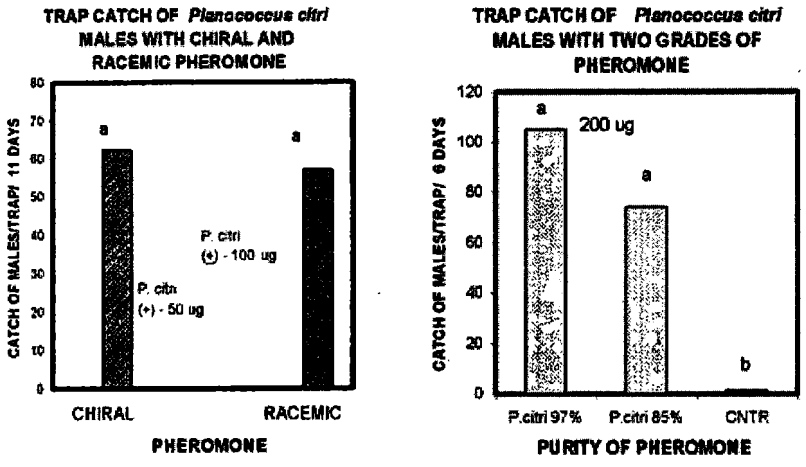

Figure 4

The first two field tests compared the attractiveness of the racemic and technical grade pheromone with high grade chiral pheromone (Figure 4). The racemic pheromone **1R** was prepared specifically for this purpose by our former method (Wolk et al., 1986), starting from commercial racemic pinonic acid **11R**.

The results indicate clearly that the antipode of the natural pheromone is benign, and therefore the chiral purity of the pheromone is less important. We did not observe any racemization during our synthesis but, even if some occurred, it would not inhibit the activity of the pheromone. The chiral purity of the pheromone was ca. 95%, comparable to that of the chiral commercial (+)-α-pinene. The technical grade pheromone was somewhat less active then 97% pure pheromone but the trap catch of the two pheromone grades did not differ statistically.

A dose response test of the pheromone, using Israeli rubber dispensers, indicated that doses of 100μg - 800μg attracted statistically the same number of males; lower dosages caught fewer males (Table 1). Therefore, in most tests a dose of 200μg of pheromone or analogs was used.

The attractiveness of the pheromone analogs **10**, **13** and **14** was assessed in several field tests and their activity was compared to that of the pheromone **1**. Of particular interest was the homolog **10**, which had shown significant activity in previous tests conducted several years ago. The results of a few representative tests are shown in Table 2.

The homolog **10** showed significant activity; about 50% in most tests as compared with that of the pheromone **1**. In Test 2 (Table 2), the activity was the same as that of the pheromone. This result was not be repeated in any other test conducted in sweetie, avocado or parsimon plantations (some tests are not presented). The ethylidene analog **14** has also considerable activity, about 50% as compared with pheromone **1**. However, its synthesis is similar to that of the pheromone, having therefore no advantage for practical application. The analogs **13** and **15** (tested previously) displayed no activity.

Table 1. Dose response of *Planococcus citri* pheromone.

Pheromone dose 1 (μg)	Male captures/trap/week
0	2.9c
25	245 b
50	252b
100	337a
200	381a
400	352a
800	397a

Table 2. Trap catch of *Planococcus citri* males (mean/trap/week) with pheromone and analogs. Each treatment was tested in five replicates.

Compound	Amount (μg)	Test 1 August 99 Sweetie	Test 2 October 99 Avocado	Test 3 August 00 Parsimon	Test 4 September 00 Sweetie
Pheromone 1	200	98.7a	182bc	110a	91.6a
Homolog 10	200	56.3b	210ab	59b	
Homolog 10	400		343a		
Analog 13	200	1c			
Analog 14	200		126c		47.4b
Analog 14	400		140c		
Control	Hexane	1c	0.7d	1c	1.8c

The rationale for preparing a series of analogs was: 1) to assess the structure-activity relationship of the pheromone in order to determine which structural functions are essential for biological activity. 2) to obtain an active analog which would be easier to prepare than the pheromone. Both goals were achieved.

The present field bioassays of the analogs, combined with previous results (Dunkelblum et al., 1987) with additional analogs, clearly indicate that the double bond and the acetate group are essential for biological activity. Saturation of the double in analog **15** or replacing the acetate by a carboxy-methyl group analog **13** eliminated the attractiveness. Conversely, elongation of the double bond with a methyl group, ethylene replacing methylene, analog **14**, or addition of one carbon into the acetate side chain in analog **10** has maintained considerable activity, attracting about 50% of males as compared with the attractiveness of the pheromone. The ethylene analog **14** is a 1:1 mixture of *E* and *Z* isomers and we do not know if both or only one geometric isomer is active. The active analogs **10** and **14** maintain the same molecular shape as the pheromone, keeping all essential functional groups of the pheromone.

Conclusions

A practical synthesis of the *P. citri* pheromone has been developed, using two ozonolysis steps and a selective reduction of an aldehydic group in a ketoaldehyde intermediate. Screening all available analogs, showed that analog (homolog) **10** is highly and constantly active. The synthesis of this compound is much more convenient then that of the pheromone or any other analog, therefore, it is of practical importance. It could replace the more expensive pheromone in field work.

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Sexual behavior of the *Maladera matrida* male beetle as affected by female cuticular components

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Abstract: The Maladera matrida beetle is a noxious polyphagous pest. Both larvae and adults destroy crops, the former feeding on underground crops such as peanut, sweet potato, potato, carrot, and the latter on flowers and foliage of many plants. In studies conducted in peanut fields it was found that the beetles emerge at sunset from the ground, where they stay all day and most of the night, for a brief (45-90 min) feeding and mating episodes, both taking place at the same time. The males come out first for early feeding, while the females emerge a little later and form a 1:1 (M:F) ratio in aggregations after 15-25 min and most of the active time in the field. In laboratory studies it was demonstrated that females attract males and that the chemicals involved are probably located on the cuticle of mature females. In this paper we describe studies of the behavior of males towards live or frozen females and show that the attracting chemicals may be washed from the cuticle by organic solvents. Both apolar or low polar (hexane or dichloromethane) and polar (methanol) solvents are required for removal of the active components from the cuticle. Washings of females frozen in the morning are not as attractive as those obtained from females at dusk. Preliminary studies show that applying cuticular extracts to washed females restores the sexual activity of males toward females.

Key words: *Maladera matrida*, beetle, female sex pheromone, cuticular components, removing and restoring sexual activity of males.

Introduction

The *Maladera matrida* beetle was first discovered in Israel in 1983 and declared a new species to science (Argaman 1986). The larvae develop in the soil and the adults emerge at sunset from the soil to feed on plants and mate. The males emerge first being followed after a short time by the females, which are probably attracted to volatiles released from the damaged leaves. After 15-25 min a ratio of 1:1 between the sexes is being established. (Harari *et al.*, 1994, Yarden and Shani, 1994, Harari *et al.*, 2000). The females and males form aggregates on the plants and stay in these groups for 45-90 min to feed and mate. It was also found that traps baited with volatiles collected from females together with peanut leaves (beetles' food) attract flying beetles to the same extent as do live females together with food (Yarden and Shani, 1994). By the use of gas chromatograph-electroantennodetector (GC-EAD), a bioactive volatile released by the female, identified as (*Z,E*)-_-farnesene, triggered the antennae of both males and females (Yarden *et al.*, 1996). Its activity in both sexes indicates that this compound is probably not the sex pheromone. The high ratio of males to females trapped (80M:20F) in black traps baited with females and food (Falach and Shani, 2000) hints at a long-distance sex pheromone released by the females. Thus we notice two types of behavior, namely, attraction of males to females in traps from long distance at a high proportion of males to females and aggregation of females and males on plants at a ratio of 1:1 between the sexes. The latter aggregations may have a role in the sexual attraction, or they are used for the excitation of the males in a short-range distance. In order to learn more about the sex pheromone complex of the *M. matrida* beetle the cuticular components of the female

beetles were washed with organic solvents and their effect on the sexual behavior of the males was studied. The preliminary results are reported here.

Materials and Methods

Rearing of beetles.
Third-stage grubs of *M. matrida* were collected in the field and reared individually in 5-ml plastic vials containing humid sand. The grubs were fed with wheat roots. The adult beetles, after being sexed, were reared in sand in groups (up to 100 beetles of the same sex in a 1-liter plastic container) and fed with rose flowers. The containers and vials were kept in an incubator at 16 h light:8 h dark regime at 30:26°C and 60% relative humidity.

Wild beetles for laboratory studies.
The study of Harari *et al.* (1997) and our own show that females can mate more than once in their life. The first time takes place at the age of 10-11 days and the second time (under laboratory conditions) is about 14 days later. We therefore used females collected in the field – by traps or manually in the evening – and separated them from males for laboratory studies two weeks later.

Sexing of the beetles.
Fast sexing was based on viewing of the last segments of the lower abdomen of adults (see later). For verification sexing was performed by the method described by Gerling and Hefez (1990). Adult beetles were inspected after the pigydium had been opened gently. Females are distinguished by the presence of two elastic chitinic plates located on each side of the pigydium aperture across the abdomen tip. Males are distinguished by a sex organ, which widens into a funnel-like asymmetric chitinic appendage, reminiscent of the stinger of a bee, for grasping the female (Argaman 1986, 1990).

Male behavior in laboratory studies.
Males were confronted with frozen-and-thawed females in order to eliminate the effects of non-receptivity and selectivity of females, which drastically reduce the number of incidents of certain modes of sexual behavior. Ten living adult males (marked with white correction fluid) were placed on sand bedding in a glass container (30x30x30 cm) in a dark hood (red light, 3-5 lux) at least five hours before the onset of the experiment. Five females that had been frozen (at -13□C for 10-15 min or for 1-2 days) and thawed were inserted into the glass container 5 min prior the onset of the experiment. Thawing was done a few minutes before the experiment for females that were frozen in the same evening of the experiment and three hours before the experiment for those frozen one or two days before the experiment. Each experiment was performed in five replications and lasted for 60 min.

Cuticular washings.
Adult females were washed with three different solvents of increasing polarities consecutively: n-hexane (non-polar), dichloromethane (low polarity) and methanol (polar). Twelve beetles were immersed twice in 7-8 ml of each solvent for a period of 10 min each time. The washing solvents were combined and evaporated by gentle blowing of N_2 to reduce their volume to 0.07 ml. The beetles were dried in air for 40 min before they were frozen.

Statistical analysis.
All results were analyzed by χ^2 test.

Chemicals.
n-Hexane, dichloromethane (CH_2Cl_2) and methanol AR grade (Frutarom, Haifa, Israel) were used after checking that no residuals were left after evaporation.

Results and Discussion

External features of the beetle.
Emerging beetles are white and within a day change to light brown in color, which turns brown-dark brown within a few days.

Adult female and male beetles can be distinguished by the appearance of the last segments of the lower abdomen (Fig. 1). The last abdominal sternite in the male is round, whereas in the female it is straight. In an earlier study by Ben-Yakir *et al.* (1996) it was found that there are differences between the sexes in the pupae stage, which is sensitive to handling.

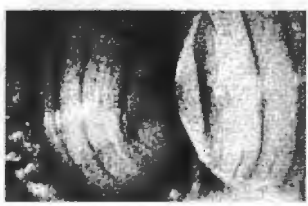

Figure 1

Sexual behavior patterns.
Five behavioral patterns of the males were identified: mating, mating attempt, mating disturbance, touching a female, wing fanning. The first three patterns seem to have a direct relation to the sexual behavior of the males toward the females, while the last two patterns are not necessarily related to the sexual ritual and hence were not included in the following report. Some of the behavior patterns are illustrated in a series of photographs (Fig. 2).

The mating posture (mounting of the male on the back of the female) and the copulation with live females lasts for 30-40 min. This behavior was designated "mating". For mating both excited males and receptive females are required. The case of female refusal to the mounting male was designated "mating attempt". There is no indication whether this behavior is due to the discrimination of the female against the attempting male or whether it is because the female is not yet receptive. In few cases with live mating females, an attempt of another male to push the mating male from the female was observed. This behavior was designated "mating disturbance".

Experiments with live pairs of beetles (5 females and 5 males in a glass container) gave low numbers of mating and other sexual activities. Increasing the number of beetles in order to increase the number of mating would make it too complicated to watch and observe the behavior patterns of each male and to record accurately the behavior of all beetles involved. We therefore used another procedure, in which live males were confronted with frozen females. Cooling the female beetles for 10 min at $-13^{\circ}C$ is enough to kill them without modifying their shape or harming the chemicals on their cuticle. To verify that this procedure does not interfere with the normal behavior patterns, the behavior of males toward live and frozen females was compared (Table 1). The data in Table 1 indicate that the sexual activity

of the males is preserved and that the number of incidents in each category of behavior is increased.

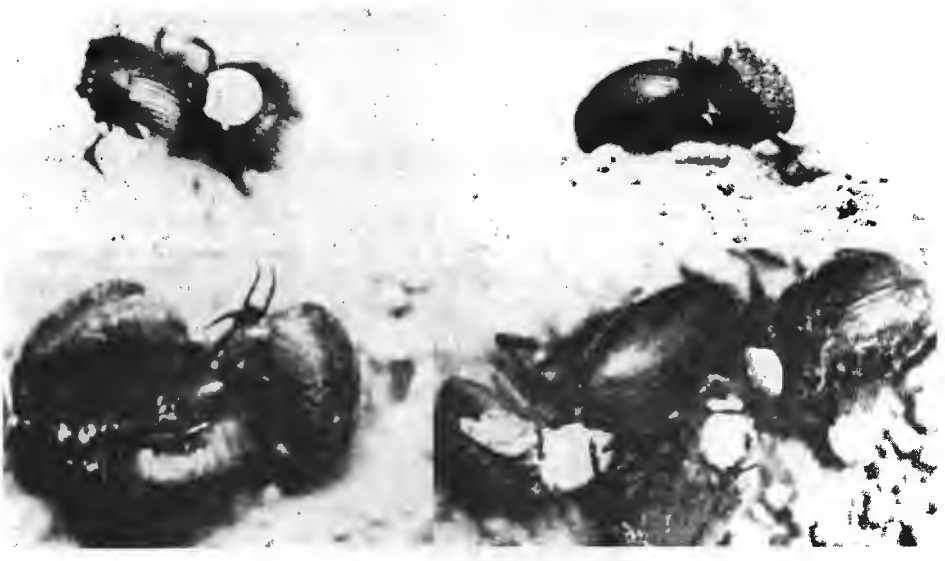

Figure 2

Table 1. Sexual behavior of males toward frozen and live females. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Sexual behavior	Frozen females	Live females	χ^2
Mating	7.6±1.1	2.2±1.1	14.9 [a]
Mating attempt	7.2±1.5	1.2±1.1	21.4 [a]
Mating disturbance	5.0±3.0	0.0±0.0	25.0 [a]

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Mating.
The number of mating events per female is much larger with dead females than with live ones, because the former do not refuse, do not have preferences and are inactive. But the chemical cues on the females are still active and attract the males. Moreover, since the length of the copulation is shorter with the dead females (10-15 min) than with live ones, more males mate with the same female, thus the average number of mating per female is increased.

Mating attempt.
As for mating, the number of attempts to mate per female is increased due to the inability of the dead females to refuse the males, thus more males have the chance to try to copulate.

Mating disturbance.
It is clear that this type of behavior is relatively rare with live females, but is very frequent with dead females. The reason for this difference might be the small chance of a male to push away a mating male; such a behavior may be considered a waste of energy and time for the disturbing male. But with dead females, which do not react, the trials of many males to replace the mating male are more frequent and a large number of events are recorded.
Time of sexual attractiveness. Since the beetles emerge at sunset to feed and mate, it was assumed that this time is the only period of sexual activity of the adults of the day. It was therefore important to verify that the males would not react to females at other times (Table 2). The small number of sexual events toward the females frozen in the morning as compared with those frozen in the evening indicates that no chemical cues were present on their cuticle to excite the males. To test this possibility, we compared the organic-solvents washes of the cuticle of females either frozen in the evening or in the morning (to be published). Preliminary results revealed several differences between the chemicals present on the cuticle at the different times of the day, but no biological studies have yet been conducted with the unique chemicals found in the evening.

Table 2. Sexual behavior of males toward females that were frozen either in the morning or in the evening hours and live females as the control. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Sexual behavior	Frozen females		Live females (control)	χ^2 Morning/ control	χ^2 Evening/ control	χ^2 Morning/ evening
	Morning	Evening				
Mating	2.6 ± 1.7	9.2 ± 2.6	2.6 ±1.7	0.0	18.5 [a]	18.5 [a]
Mating attempt	0.0 ± 0.0	1.8 ± 1.9	1.4 ±1.1	7.0 [a]	0.3	9.0 [a]
Mating disturbance	0.2 ± 0.4	4.8 ± 3.3	1.6 ±1.5	5.4 [a]	8.0 [a]	21.2 [a]

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Effectiveness of the frozen beetles.
The findings presented in Table 2 and the pattern of behavior of the beetles in the field suggested that both the behavioral studies and the washing should be limited to a short period of 30-60 min at sunset. The consequence was that we could not conduct both washing and behavioral observation at the same evening. This meant that we had to keep the frozen beetles for at least 24 hours before conducting the experiment. The results in Table 3 demonstrate that freezing for as long as 48 h did not impair the attractiveness of the females to males.

Repellence by frozen males.
To assure that males do not attract males by an aggregation pheromone, we checked the behavior of males toward frozen males. The deterrence of males from frozen males, shown in Table 4, verifies previous observations (Yarden and Shani, 1994) and living males emerging from the soil were repelled by frozen males and immediately returned to their holes in the soil. This behavior may have resulted from the occurrence of repellents on the cuticle of the

males or from the absence of female chemical attractants.

Table 3. Effect of freezing duration before the experiment on male sexual behavior. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Sexual behavior	Duration of freezing					
	24 h	0 h (control)	χ^2	48 h.	0 h (control)	χ^2
Mating	7.2±0.8	7.6±0.9	0.1 [a]	8.4±1.1	7.4±1.5	0.3 [a]
Mating attempt	2.8±1.9	3.6±1.9	0.5 [a]	3.8±2.6	2.6±3.7	1.1 [a]
Mating disturbance	8.4±6.3	8.4±1.3	0.0 [a]	5.8±1.8	6.4±2.4	0.1 [a]

[a] Values are not significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Table 4. Sexual behavior of males toward frozen males as compared with that toward frozen females. Values are means ± SE of incidents of each type of behavior per a group of 5 beetles of each sex

Sexual behavior	Frozen males	Frozen females	χ^2
Mating	0.0±0.0	7.4±1.3	37.0 [a]
Mating attempt	0.0±0.0	9.8±4.7	49.0 [a]
Mating disturbance	0.0±0.0	6.0±4.4	36.0 [a]

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Effect of solvent trickled in frozen females.

As mentioned above, the difference in male behavior in the evening and morning hours could stem from the presence of attracting or exciting chemicals on the female cuticle in the evening but not in the morning. Washing of the cuticle by three solvents of increasing polarities (hexane, dichloromethane and methanol) is expected to release most of chemicals adhered to the cuticle. The solvents themselves were found to have no ill effect on the male behavior, as no reduction in the normal sexual behavior of the males was found after the evaporation of 10 _L of each of the three solvents trickled on frozen females (Table 5).

Cuticle washing with organic solvents.

The sexual activity of males toward females was totally eliminated by washing the females with three solvents of increased polarities: hexane, dichloromethane and methanol (Table 6). Two solvents only were also effective if one of them was methanol. The nonpolar hexane plus the low polar dichloromethane solvents were incapable of complete removal of the sexual activity. These results indicate that a mixture of both polar and less polar components constitute the short-range sex pheromone of the female *M. matrida* beetle.

Table 5. Male sexual behavior as affected by solvents trickled on frozen females. Frozen females were trickled with 10 µl of solvent and dried for 3 h before the experiment. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Solvent	Mating			Mating attempt			Mating disturbance		
	Trickled	Not trickled	χ^2	Trickled	Not trickled	χ^2	Trickled	Not trickled	χ^2
n-Hexane	4.2±2.3	5.2±2.6	0.5	0.8±0.8	2.0±1.0	2.5	3.6±4.1	2.0±2.0	2.3
CH_2Cl_2	2.8±1.1	3.2±2.1	0.1	1.0±1.2	0.2±0.4	2.7	1.0±1.2	3.6±3.5	7.3 [a]
Methanol	4.4±2.1	4.2±1.8	0.0	2.4±3.4	2.4±1.8	0.0	3.0±2.0	4.0±1.2	0.7

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Table 6. Sexual activity of males toward frozen females washed with different solvents. Values are means ± SE of incidents of each behavior per a group of 5 females

Washing solvent				Mating		Mating attempt		Mating disturbance	
Hexane	CH_2Cl_2	Methanol		Washed females	Unwashed females	Washed females	Unwashed females	Washed females	Unwashed females
+	-	-		6.6±2.9	5.0±0.7	4.4±1.8	2.8±1.6	3.4±3.2	6.2±3.8
-	+	-		5.4±1.7	7.8±2.0	1.0±1.2	1.8±0.4	3.8±2.6	6.4±1.5
-	-	+	*	3.2±1.3	6.8±3.8	1.2±0.8	2.8±0.4	2.0±2.0	5.6±5.5
+	+	-	*	2.4±1.1	6.0±1.0	1.4±1.3	1.8±1.1	0.8±1.3	3.0±1.9
+	-	+	*	0.4±0.5	6.8±1.1	0.0±0.0	1.0±1.0	0.4±0.9	7.2±0.4
-	+	+	*	0.4±0.5	6.8±0.4	0.0±0.0	0.6±0.5	0.0±0.0	6.8±1.6
+	+	+	*	0.0±0.0	7.8±1.3	0.6±1.3	9.8±5.5	0.0±0.0	8.4±3.5

[a] Values are significantly different according to χ^2 tests for all types of behavior ($P = 0.05$).

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Identification of pheromone of the greater wax moth *Galleria mellonella* from the different regions of Russia

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Abstract: The composition of pheromone volatiles from calling males of the greater wax moth (GWM) *Galleria mellonella* from six regions of Russia was studied by GC-MS. The volatiles from calling males from all the regions contain nonanal and undecanal as the main components, but in different ratio for the males of GWM from different regions. Hexanal, heptanal, octanal, decanal, undecanol and 6,10,14 -trimethylpentadecanon-2 were found as minor components also in different combinations. The structure of the ketone was proved by the comparison its mass-spectrum with spectrum of synthetic ketone.

Key words: sex pheromone, male volatiles, *Galleria mellonella* , hexanal, heptanal, octanal, decanal, undecanol, 6,10,14-trimethylpentadecanon-2, Lepidoptera, Pyralidae.

Introduction

The greater wax moth (GWM) *Galleria mellonella* (Lepidoptera: Pyralidae) is an important pest of the honeybee *Apis mellifera*. The larval stage of the GWM feeds on the honey, pollen, and wax produced by honeybees. However, an effective method of control of this pest has not been developed. Physical, chemical, and biological methods are imperfect (Ali *et al.*, 1973, Burges 1977, 1978, Cantwell and Smith 1970), and further studies are needed to find more effective control methods. Some studies have been conducted on the use of pheromone traps for capturing GWM. It was found that GWM male adults produce a sex pheromone in glands located on their forewings (Barth 1937, Roller *et al.*, 1968). The pheromone was identified as a mixture of two aldehydes, nonanal and undecanal (7: 3) (Leyrer and Monroe 1973). However, the response of females to the synthetic bait in laboratory tests was not as high as their response to live males (Finn and Pyne 1977,), and in field tests this mixture was practically inactive (Flint and Merkle 1983). Two additional components, nonanol and undecanol, were found among volatiles collected from GWM males from Canada during their calling period. The ratio of the main components, nonanal and undecanal, of this population was found 1: 3 (Romel et al. 1992). The ratio of these components in volatiles of GWM from USA was 7 : 3 (Leyrer and Monroe 1973).

In the work reports we analysed the volatiles of GWM calling males from four regions of Russia: the central part of Russia (Penza, Nizhnij Novgorod and Ivanovo), middle Ural (Ufa), Altai (Barnaul), and Far East to ascertain the differences in their pheromone compositions.

Material and methods

Insect source and collection of effluvia.

The GWM larvae, collected from the domestical hives, were kept in 2 L glass containers (50-75 specimens) with frilles filter paper for pupation. Larvae, pupae, and adults were kept in

darkness at 28-31° C and 60% humidity. Adults were obtained from the first, second, or third generation of laboratory colonies started from wild stock. Insects were reared either on comb wax from their native hives. Before using the comb wax was kept frozen at -15°C. After 5-7 days from the start of pupation the cocoons were dessected, and males were separated from females. Five to ten of 2- to 7-day-old GWM males were kept in a glass cylinder (60 x 230 mm) in the dark at 28-31°C for 1-3 days after exclosion. Effluvium of males was collected once a day (at 2-5 PM) for half an hour using a glass tube with a charcoal disk (d = 4 mm; l = 3 mm), which was connected to the narrow end (d = 5 mm) of cylinder and a vacuum source (20-30 cm/sec), using a Personal Air Sampler (PAS_1000, SUPELCO, USA). The mean amounts of components were obtained as the ratio of its absolute quantity to (number of males x number of collection periods).

Isolation and analysis of volatiles.

The volatiles were rinsed from the charcoal disk with 300 µl methylene chloride and were first analyzed by GC-MC without evaporation of solvent to estimate the quantity and ratio of the main components; then they were concentrated to the desired volume for analysis of minor components. Capllary GC-MS analysis was performed on an LKB 2091 EI system at 70 eV, coupled with LKB-CLINICON (LKB Sweden) 2130-310 data system (PDP-11/05). A 30m x 0.25 mm SE-30 fused silica column was programmed 30 min at 40°C and then to 230°C at 4°/min. The column was operated in the splitless mode. Before analysis, 40 ng of nonadecan was added as standard to the samples for comparison of retention times and quantification of unknown substances. For identification of small amounts of minor components we used the reconstruction of total ion mass chromatogram by characteristic ions. Collection and identification of male volatiles were repeated two to four times for insect from each region.

Results and discussion

The main and some minor male pheromone components of *Galleria mellonella* from different regions of Russia were identified before. The structure of C_6-C_{11} aldehydes and C_{10} alcohol was determined by their mass-spectra (Ponomarev et al. 1997). Another minor component, 6,10,14-trimethylpentadecanon-2, was identified by mass spectrometry. The compound of similar structure, 6,10,14-trimethylpentadecanol-2, was discovered in female pheromone of *Corcyra cephalonica* (Pyralidae) (Hall *et al.*, 1987). The volatiles of *Galleria mellonella* males from Ivanovo (Table 1) do not include this ketone, hexanal and undecanol. The ratio of main components, nonanal and undecanal, is various for the populations from different regions: 100: 50 (Ufa), 100: 60 (Ivanovo), 100: 70 (Nizhniy Novgorod), 100: 80 (Far East), 100: 100 (Barnaul) and 80: 100 (Penza).

The ratio of minor components in volatiles of GWM males from different area is various substantially. Only the ratio of all components (exept undecanol) in male volatiles from N.Novgorod is similar to that in GWM from Far East.The application of mixture nonanal: undecanal (3: 1) for the protection of 173 hives in Ivanovo resulted twofold reduction of damage caused by *Galleria mellonella.*

Conclusion

The volatiles of male pheromone of *Galleria mellonella* from six different area consist of similar components in different ratio of main and minor compounds.

Table. Ratio of the components in the volatiles of calling males *Galleria mellonella* from different regions.

Compound	Ivanovo	N.Novgorod	Penza	Ufa	Barnaul	Far East
Nonanal	100	100	80	100	100	100
Undecanal	60	70	100	50	100	80
Hexanal	-	1	0.2	1	1	1
Heptanal	2	5	1	3	20	7
Octanal	12	18	4	6	40	20
Decanal	6	6	5	3	30	20
Undecanol	-	0.1	2	0.3	2	0.1
6,10,14,Trimethyl-pentadecanol –2	-	3	2	1	7	4

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On the origin of pine sawflies caught in pheromone traps

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Abstract: This study investigated behaviour of male European pine sawflies, *Neodiprion sertifer* Geoffr. (Hym., Diprionidae), that were released downwind from pheromone traps. Releases were done at three distances; either at 5 m from one trap, or at 50 m or 200 m from five traps, placed in a line perpendicular to the current wind direction. As a control, males were released identically but without any pheromone source present. The behaviour of the males prior to take-off was studied on a release platform. The following different types of behaviour were recorded: grooming, wing fanning, orientating and take-off. The frequency of grooming was significantly higher in the pheromone treatments compared to the control, whereas the frequency of wing fanning and orientating increased, although not significantly. The direction in which the males displayed the various types of behaviour was more concentrated towards the wind when pheromone was present than during the control experiment. By colour marking of Ecology, Lund University, d travel speed could be calculated. The minimum recorded time from take-off to landing was 1 min, 6 min and 45 min for the 5 m, 50 m and 200 m experiments, respectively. The stimulation- and attraction range of the trap was at least 200 m, and the sampling range after 24 hr was calculated to approximately 400 m (c.i. 140–1600 m).

Keywords; Diprionidae, *Neodiprion sertifer*, sampling range, attraction range

Introduction

Traps baited with species-specific pheromones are becoming common tools for monitoring insect pests. However the correspondence between catches and damage is often poor (Trumble, 1996), partly because of an unknown sampling range of the trap. The sampling range is the maximum range from which insects can be shown to reach an attractive (odour) source within a given time period (Wall & Perry, 1987). Another definition is attraction range, the maximum distance over which insects can be shown to direct their movement to a source (Wall & Perry, 1987). Hence, the sampling range will increase with time up to a maximum level where it levels off, whereas the attraction range is constant.

By performing well designed mark-release-recapture experiments one can gain increased knowledge about the function of the monitoring trap: How many insects are recaptured from different distances within a given time? How fast do the insects reach the trap? When combined with information from a weather station the influence of weather on trap catch can also be determined. Studies of this kind are rare, but are useful when designing a monitoring program for an insect.

The European pine sawfly, *Neodiprion sertifer* Geoffr. (Hymenoptera: Diprionidae), is one of the most harmful insects to forestry in Europe (Day & Leather, 1997). The larvae consume pine needles, and following an outbreak trees loose growth capacity at least for the following ten years (Austarå *et al.*, 1987). The adult *N. sertifer* female contains approximately 10 ng of the sex pheromone precursor (2*S*,3*S*,7*S*)-3,7-dimethyl-2-pentadecanol (diprionol) (Wassgren *et al.*, 1992). Males respond to the female-released pheromone: either acetate or propionate of the alcohol, both in electrophysiological recordings (Hansson *et al.*, 1991) and

in field trapping (Anderbrant *et al.*, 1992a).

In the present study we investigated if there exists any pheromone-modulated behaviour in males of *N. sertifer*. By releasing individually marked males we calculated the recapture rates from different distances and the travel speed of males flying upwind towards the pheromone, together with the sampling range.

Material and Methods

Study site and insects.

The study was performed in a young, 2 to 3 m tall, birch (*Betula pendula* Roth.) plantation, surrounded by old pine plantations (*Pinus sylvestris* L.). in Aug.-Sept., from 1996 to 1998, 35 km east of Lund, southernmost Sweden. Larvae of *Neodiprion sertifer* were collected in June from various places in Sweden. The larvae were reared outdoors in a protected place. All the males were marked before release; in 1996 and 1997 a colour dot was painted on thorax dorsally with instant markers. In 1998, we released individually marked males. By dividing the thorax into four fields and painting one to three dots of seven different water based and water-resistant colours, there were enough combinations for releasing differently marked males. After marking the males were kept individually in test tubes, and stored at +4 to +8 °C until use.

Experimental setup.

Males were either released 50 m or 200 m downwind of five pheromone traps (inter-trap distance 25 m), or downwind of one trap 5 m away (Fig. 1 in Östrand *et al.*, 2000). The traps were of Lund-II type (Anderbrant *et al.*, 1989). The pheromone, 100 µg acetate of the attractive isomer (2*S*,3*S*,7*S*)-3,7-dimethyl-2-pentadecanol, > 99% stereochemically pure, prepared at and obtained from the Mid Sweden University (Högberg *et al.*, 1990; Anderbrant *et al.*, 1992a) was added to a cotton roll, Celluron® No. 2 (Paul Hartmann, S.A., France),. The traps were placed 1.7 m above ground. New baits were used in every pheromone experiment, and each bait released approximately 45 µg of the pheromone during the first day (calculated from Anderbrant *et al.*, 1992b).

The test tubes containing the males were opened and stacked horizontally in a plastic jar. The jar was covered on the outside with black opaque plastic. Five holes were drilled in the lid. After leaving the test tubes, the males moved towards the light coming through the holes in the lid, and on to a white cardboard platform that was attached to the lid. When > 40 males were released two such platforms were used.

Experimental procedure.

The prevailing wind direction was determined by aid of a wind vane and the platforms and the traps were re-located every experimental day, so that the males always were released downwind of the trap(s). One or two persons stood behind the platforms and recorded which males that took off using a tape recorder, while a second person patrolled the five traps and recorded incoming males.

The ground speed of male *N. sertifer* flying upwind to the pheromone source(s) could not be determined. Instead, we calculated the time elapsed from take off to landing = travel time. The exact time for landing could not be recorded when patrolling the five traps in the 50 m and 200 m experiments, and instead we present a range. The median of such ranges was used when the 'exact' travel speed was not available.

Results and Discussion

No unique pheromone-stimulated behaviour was recorded in *N. sertifer* males on the release platform. However, significantly more groomings were recorded at the presence of pheromones compared with the control (Fig. 1). The frequency of males displaying wing fanning and orientation on the platform increased compared to when pheromone was absent, but these differences were not significant, presumably due to the frequency of wing fanning shifting with wind speed (Östrand *et al.*, 2000), and too little data on orientation. An increased frequency of wing fanning is often seen in pheromone-stimulated male moths (e.g. Kishaba *et al.*, 1970; Elkinton *et al.*, 1984).

Significantly more males displayed wing fanning against the wind in all three pheromone experiments compared to the control, and more males took off into the wind at all three distances, although at 200 m the difference was not statistically different from the control (Fig. 2). Thus, the attraction range of the traps were at least 200 m. To compare, males of pea moth *Cydia nigricana* (F.) and oriental fruit moth *Grapholita molesta* Busck. were stimulated at 500 m (Wall & Perry, 1987) and 80 m (Baker & Roelofs, 1981) from 100 µg pheromone sources, respectively. Differences between species are partly due to different sensitivity in the insects, but also the release rate of the pheromone is important.

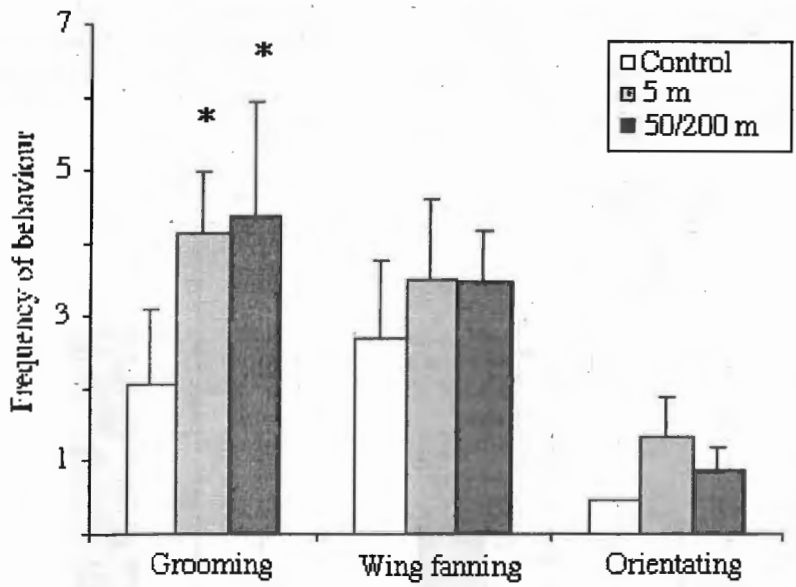

Figure 1. Recorded frequencies of grooming, wing fanning and orientation from pheromone and control experiments. Frequencies are expressed as number of occurrences per hr and number of males present on the platform during the time period, lasting from 30 to 50 min. Data from 50 and 200 m pooled. Values marked with * are significantly different from the control following Students *t*-test. Data from Östrand *et al.*, 2000.

The fastest recorded travel times from the release platform to the trap were approximately 1 min, 5 min and 45 min for males flying from 5 m, 50 m and 200 m, respectively (Fig. 3). If these travel times are re-calculated to travel speeds the fastest ones, 5–10 m/min, were comparable for the different distances. This travel speed is lower than recorded in moths flying upwind to odours, e.g. 50-75 m/min in pea moths (Wall & Perry, 1987) and 20 m/min in cabbage loopers, *Trichoplusia ni* (Hübn.) (Kishaba *et al.*, 1970). The difference is most likely explained by the fact that our experiments were carried out in a young, relatively dense, birch plantation, as compared with the open fields used in the studies on moths, allowing for the pine sawflies to make stops along their way to the pheromone traps.

The recapture rates after 24 hr were on average 3.7 %, 14.0 % and 20.0 % for the 5 m (n=8), 50 m (n=8) and 200 m (n=9), respectively. The recaptures varied with the recorded wind speed (Östrand *et al.*, subm.). At 50 m the recapture was highest at intermediate wind speeds of 1.5-2.0 m/s, whereas it decreased at wind speed > 1.5 m/s at 200 m. Optimal wind speed for capturing insects in odour-baited traps have also been reported in various field experiments on flies (e.g. Nottingham, 1987; Aluja & Prokopy, 1992; Brady *et al.*, 1995).

The sampling range after 1 hr, 2.5 hr and 24 hr were calculated to 190 m (confidence interval following Sokal & Rohlf, 1995: 70–630 m), 290 m (90–1250 m) and 400 m (140–1600 m) (Data from Östrand *et al.*, subm.) (Fig. 3). In order to achieve homogenous variances and a linear relation recaptures were arcsin√recapture transformed while distances were log transformed (see Schlyter, 1992). The sampling range was then calculated as the X-intercept of this linear regression. Although these estimate of sampling ranges have wide confidence intervals it is the first estimates of sampling range of a monitoring trap for pine sawflies and it will be helpful when designing monitoring programmes for these insects. Zhang & Schlyter (1996) calculated the sampling range of a trap used for the arctid moth *Hyphantria cunea* (Drury) to 340 m (190–710 m) after 60 hr, using a similar experimental set-up.

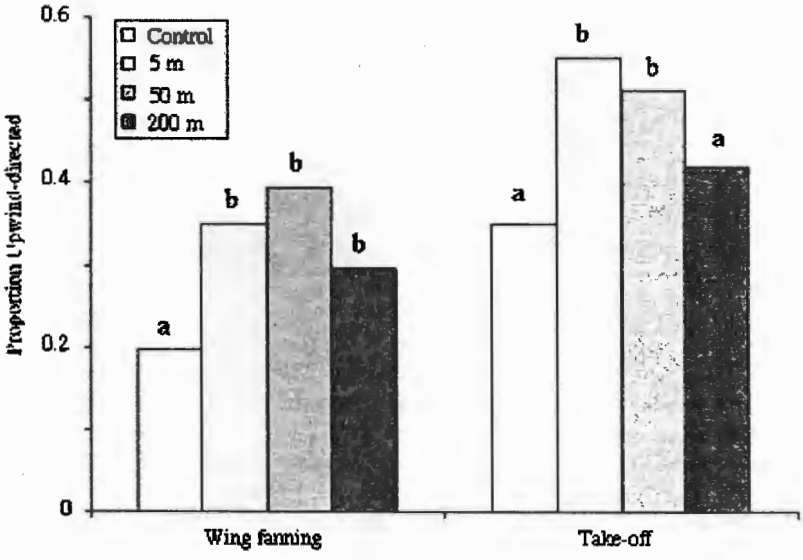

Figure 2. Proportion of upwind-directed wing fanning and take-off in the different pheromone experiments and control. Values having different letters are significantly different from each other following *G*-test.

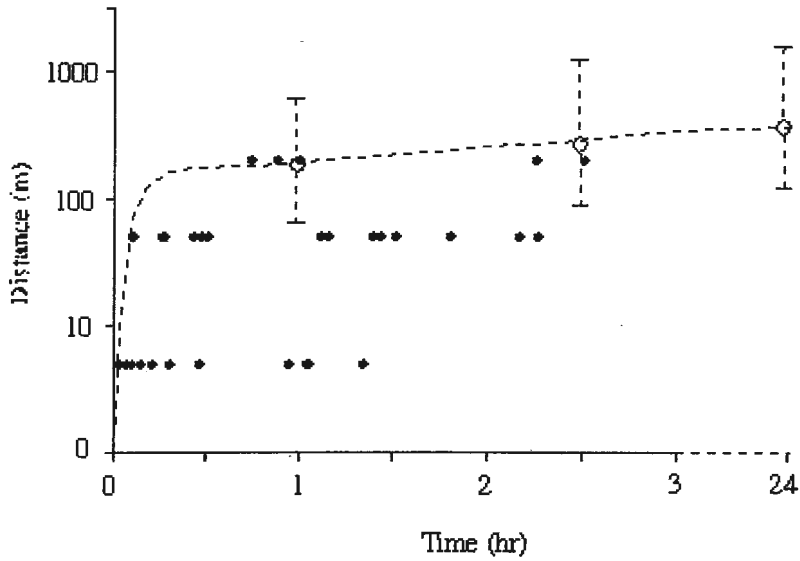

Figure 3. The relationship between distance from pheromone traps and arrival times of caught males during the first 3 hr in the 5 m (n=12), 50 m (n=16) and 200 m (n=5) experiments performed in 1998 (black dots). In many cases the median time of catch has been calculated, as the exact time for take-off and / or landing was not determined. Calculated sampling ranges, with 95% confidence intervals, after 1 hr, 2.5 hr and 24 hr of sampling are shown as unfilled circles. The dashed line illustrates the relationship between time and sampling range.

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Use of pheromones and other semiochemicals in integrated production
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Pentatomid bug pheromones in IPM: possible applications and limitations

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Abstract: Male-produced pheromone components have been identified from several species of agriculturally important stink bugs, including the red-shouldered stink bug *Thyanta pallidovirens*, the green stink bug *Acrosternum hilare*, the conchuela stink bug *Chlorochroa ligata*, Uhler's stink bug *C. uhleri*, and Say's stink bug *C. sayi*. The pheromone of *T. pallidovirens* consists of a thermally unstable ester, methyl (2*E*,4*Z*,6*Z*)-decatrienoate, in combination with one, two, or all of the sesquiterpenes zingiberene, sesquiphellandrene, and α-curcumene. The pheromone attracted only females, as well as a specialist predator, the sphecid wasp *Astata occidentalis*. The pheromone of *A. hilare* consisted of two isomers, (4*S*)-*cis*- and (4*S*)-*trans*-(*Z*)-bisabolene epoxides, in a 19:1 ratio. Both compounds were required for attraction of females. Male *C. sayi* produced methyl geranate as a major component, with traces of two other components, methyl citronellate and methyl dihydrofarnesoate. Only methyl geranate appeared to be required for attraction. Males of *C. uhleri* and *C. ligata* produced very similar blends, with methyl dihydrofarnesoate as the major component, and methyl farnesoate and a novel compound, methyl (E)-5-2,6,10-trimethyl-5,9-undecadienoate, as minor components. Only methyl dihydrofarnesoate appeared to be required for attraction of females. Overall, relatively low numbers of bugs were caught in field trials of pheromone-baited traps, probably due in part to inefficient trap designs. In addition, phytophagous stink bugs communicate over shorter distances by means of substrate-borne vibrational signals, and these signals may be critically important in attracting bugs right up to pheromone sources. Examples of the vibrational signals for two species, *Nezara viridula* and *A. hilare*, are given.

Key words: Pentatomidae, stink bug, pheromone, attractant, vibrational signals

Introduction

Phytophagous stink bugs (Heteroptera: Pentatomidae) are occasional to chronic pests in all types of crop systems, including annual crops such as grains, cotton, alfalfa, beans, and tomatoes, and perennial crops such as tree fruits and nuts (Schaefer and Panizzi 2000). Damage is caused by both immatures and adults, but only adults are winged and capable of long-distance movement. Injury to young seeds, fruits, or nuts produces necrotic lesions and often results in premature abortion, while attacked leaves may wilt and die. Stink bugs are also known or implicated as vectors of plant pathogens such as yeast, fungi, and bacteria, particularly in crops such as pistachio.

Many stink bug species are polyphagous and the adults are highly mobile, which complicates their monitoring and control. Bugs migrate into crops in response to natural events such as the senescence of native vegetation in the habitat, or in response to mowing or harvesting of nearby crops harboring large bug populations. Effective bug control hinges on the rapid detection of these invasions so that appropriate control measures can be

implemented before serious crop damage occurs. However, sampling methods for most bug species are still relatively primitive, consisting mainly of sweep-net or beating tray sampling, or visual inspection of fruits for feeding damage or excrement. Monitoring methods based on pheromones or other attractants have not yet been developed for most of the major pest bug species.

Until recently, stink bugs often were kept under control by insecticide sprays applied to control primary pest species such as lepidoptera. However, recent changes in crop protection including the introduction of highly selective methods such as genetically modified plants that produce Bt toxins, or pheromone-based mating disruption, coupled with a corresponding decrease in the use of broad-spectrum insecticides, has resulted in a resurgence of problems with true bugs in crops such as apples, pears, and cotton. Continued high levels of damage by stink bugs and other pests may hinder the continued implementation of these new control methods. Thus, new methods of monitoring and control of stink bugs are urgently needed.

We summarize here our efforts to identify the pheromones of some of the most important stink bug pests of agriculture in California, including the red-shouldered stink bug *Thyanta pallidovirens*, the green stink bug *Acrosternum hilare*, the conchuela stink bug *Chlorochroa ligata*, Uhler's stink bug *C. uhleri*, and Say's stink bug *C. sayi*. We also describe problems encountered during the development of pheromone-based traps for these stink bug species. Finally, we describe some preliminary results relating to substrate-borne vibrational signals that stink bugs use for communication at short ranges, once males and females are on the same plant or substrate.

Materials and methods

Insects.

Stink bug colonies were started from bugs collected by sweep-netting of agricultural crops or native vegetation at sites in southern California. Bugs were reared on a diet of organically grown green-beans (*Phaseolus vulgaris* L.), raw shelled peanuts (*Arachis hypogaes* L.), and raw sunflower seeds (*Helianthus annuus* L.), supplemented with bouquets of alfalfa (*Medicago sativa* L.), or seasonal weeds including mustard (*Brassica campestris* (L.)), London rocket (*Sisymbrium irio* L.), Russian thistle (*Salsola iberica* Sennen), shepherd's purse (*Capsella bursa-pastoris* L.), and cheeseweed (*Malva parviflora* L.) depending on availability. Bugs were reared in a controlled environment chamber, on a 16:8 L:D cycle , with lighting provided by banks of 8 fluorescent light tubes (Sylvania Octron, 32W, F032/T35), at 23±2°C, and >50% relative humidity. Eggs were collected from the colony every other day and were held in covered Petri dishes through to the second nymphal stage. After the 2nd molt, nymphs were transferred to 1.9 liter cardboard ice-cream containers with muslin lids. Nymphs were fed as described above, with food changed every other day. After the final molt, adults were collected and sexed and cohorts of virgin adults were maintained with food in clean ice-cream cartons (5-7 bugs per 0.95 liter container, 10-20 bugs per 1.9 liter container) until used for collection of volatiles or bioassays.

Collection and analysis of insect-produced compounds.

Sexed, virgin adult bugs and a few green beans were put into glass aeration chambers lined with hardware cloth screen for the bugs to perch on. Humidified, charcoal-filtered air was drawn through the chamber, and entrained bug volatiles were collected on activated charcoal traps made from 4 mm id. glass tubes loaded with a 0.4 cm bed of 80-100 mesh activated charcoal, precleaned by heating at 200°C under a flow of clean N_2 (~100 ml/min) overnight.

Aerations were conducted continuously for 2-3 wk at ~25°C with cohorts of bugs of known age, changing the food and the collectors every other day. Collectors were eluted with pentane (500 µl), and extracts were stored in glass vials with Teflon-lined screw caps at ~-20°C until needed. Aeration chambers were set up near the window so that bugs had natural light, and supplementary fluorescent light was provided with a light bank directly overhead to provide long day conditions (lights on from 6:00 to 22:00). Bugs that died were replaced with virgin individuals from a cohort of the same age. Dead bugs were removed as soon as discovered to minimize contamination; as muscles relaxed in dead bugs, the contents of the defensive metathoracic glands were released (Ho, pers. obs.). As a control, green beans were aerated, collecting the volatiles as described above. Extracts were analyzed by GC-MS, looking particularly for sex-specific compounds produced only by sexually mature virgin bugs.

Laboratory bioassays.

Laboratory bioassays were carried out with a vertical glass Y-tube olfactometer (i.d., 4.5 cm, arms 14 cm long, center tube 18 cm long). Each arm of the Y terminated in a female ground glass fitting, with matching male fittings terminating in hose nipples. Teflon tubing connected the Y-tube to stimulus flasks, and vinyl tubing connected the bottom outlet to a vacuum source.

Test bugs were reared under long day conditions as described above. Lighting was provided by a light bank fitted with a daylight fluorescent lamp and a wide-spectrum "grow-light" fluorescent lamp (Sylvania Octron 32W) suspended 30 cm above the olfactometer. The light level as the upper end of the Y-tube was ~ 600 lux, and at the lower end, ~ 300 lux. Bioassays were conducted at ambient temperature and humidity conditions in the laboratory (26°C ± 3°C and 50 ± 15% humidity). Depending on bug species, bioassays were conducted between 10:00 and 22:00.

Field bioassays of pheromone lures.

Field bioassays generally were carried out with rubber septum lures loaded with hexane solutions of test compounds. Traps used included commercially available (Trécé Inc., Slainas CA) jug traps, consisting of a clear plastic jar with two inward-pointing screen cones, or a custom built screen trap designed especially for trapping stink bugs. We also experimented with a "trap plant" concept, in which individual plants were baited with a pheromone lure, and the number of bugs on treated and control plants were counted.

Recording vibrational signals.

Recordings were made with virgin, sexually mature bugs in a quiet room with fluorescent lighting provided with Sylvania Octron 32W lights. Spectral and temporal characteristics of songs were determined from recordings made from bugs singing on the membrane of a 10 cm diam low-midrange loudspeaker (40-6,000 Hz frequency response, impedance 8 Ω, #WS 13 BF, Visaton, Germany). A pair of insects was placed on the speaker cone, and prevented from escaping by placing a 10 cm diam translucent Fluon®-coated plastic cylinder over the speaker. Signals were amplified with a custom-built amplifier, then digitized and recorded on the hard drive of a Pentium 4 computer equipped with a Lexicon Core2 PCI recording system for PC (Sweetwater Sound, Fort Wayne, IN), using Cool EditPro™ Special Edition version 1.1 software (Syntrillium Software, Phoenix, AZ). Digitized data files were rerecorded onto CDs. Signals were followed in real time with headphones. Most insects emitted signals within a couple of minutes of being placed onto the speaker membrane, or after they had contacted each other.

Mating behaviors related to song production also were analysed from pairs of bugs

placed on a potted bean plant with most of the side branches removed. Signals transmitted through the plant were recorded by placing the membrane of a dynamic microphone cartridge (impedance 600 Ω, 22 mm diameter, 40-22000 Hz frequency response ; #D 3800, AKG, Austria) in contact with the stem 3 cm above the ground. Contact between the plant and the microphone was made by a 2 x 2 mm piece of double-sided sticky tape placed between the membrane and the plant stem. The signal from the microphone was amplified, digitized, and recorded. Along with the recordings, behavioral observations were recorded in a notebook so that behaviors could be correlated with song characteristics.

Recordings from both types of substrate were analyzed using Sound Forge version 4.5 software (Sonic Foundry Inc., Madison WI). Data extracted from recordings included the dominant frequencies, durations and repetition times (the latter defined as the time interval between the start of two sequential signals) of particular pulses or pulse trains.

Results and discussion

Identification and bioassay of pheromones.

In all of the stink bug species studied, males began producing sex-specific compounds with the onset of sexual maturity. These compounds were entirely distinct from the defensive compounds, and with one exception, males of each species produced a unique blend. Thus, male *T. pallidovirens* produced a novel compound, methyl (2*E*,4*Z*,6*Z*)-decatrienoate **1**, in combination with three sesquiterpenes, zingiberene **2**, sesquiphellandrene **3**, and α-curcumene **4** (Fig. 1) (Millar 1997, McBrien and Millar 1999). The ester **1** is thermally unstable, which complicated its identification and synthesis. In laboratory and field trials, the ester alone or the sesquiterpenes alone were not attractive. However, the ester in combination with any one, two, or all of the sesquiterpene components was attractive specifically to females, indicating that the pheromone is a sex pheromone. This degree of redundancy in an insect pheromone signal is unusual. During field trials, significant numbers of a specialist parasitoid of stink bug adults, the sphecid wasp *Astata occidentalis*, were also caught (Millar et al. 2001). Only females were caught, clearly indicating that this predator eavesdrops on the pheromone to locate its prey for provisioning its nest. The attraction of significant numbers of this specialist predator also provided indirect evidence for the correct identification of the pheromone. Further tests determined that the ester alone was attractive to the wasp.

Male *Acrosternum hilare* produced a 19:1 mixture of (4*S*)-*cis*-(*Z*)-bisabolene epoxide 5 ((4*S*)-*cis*-*Z*-BAE) and (4*S*)-*trans*-*Z*-BAE 6 (Fig. 1) (Aldrich et al. 1993; McBrien et al. 2001). These two components are also produced by the southern green stink bug *Nezara viridula*, but in a different ratio of about 1:3 (Aldrich et al. 1987; Baker et al. 1987; Brézot et al. 1993). Neither compound alone was attractive to female *A. hilare*, but a 95:5 *cis*:*trans* blend, mimicing the ratio naturally produced by males, was attractive to females in Y-tube bioassays. Bioassays in a field cage showed that significantly more *A. hilare* females were attracted to lures treated with a 95:5 blend of synthetic (4*S*)-*cis*-*Z*-BAE and (4*S*)-*trans*-*Z*-BAE placed inside a bouquet of alfalfa than to an alfalfa bouquet containing a pentane-treated control. In field cage studies, attraction of females was greatest during the late afternoon and evening hours, and female *A. hilare* approached the synthetic pheromone source almost exclusively by walking. Full scale field trials are continuing, but have been hampered by difficulties in locating sites with large bug populations, and by inappropriate trap design (see below).

The major component of the male-specific compounds from *Chlorochroa sayi* was identified as methyl geranate **7** (Fig. 1) (Ho et al. 2001). Two other components, methyl citronellate **8** and methyl dihydrofarnesoate **9**, were also detected in trace amounts. In

laboratory and field bioassays, methyl geranate as a single component appeared to be as attractive as the three-component blend. Furthermore, significant numbers of male *C. sayi* were caught in traps baited with *C. sayi* pheromone, suggesting that the pheromone may be an aggregation pheromone rather than a sex pheromone. However, other explanations cannot be excluded, such as the attraction of males by females once the latter are in the trap.

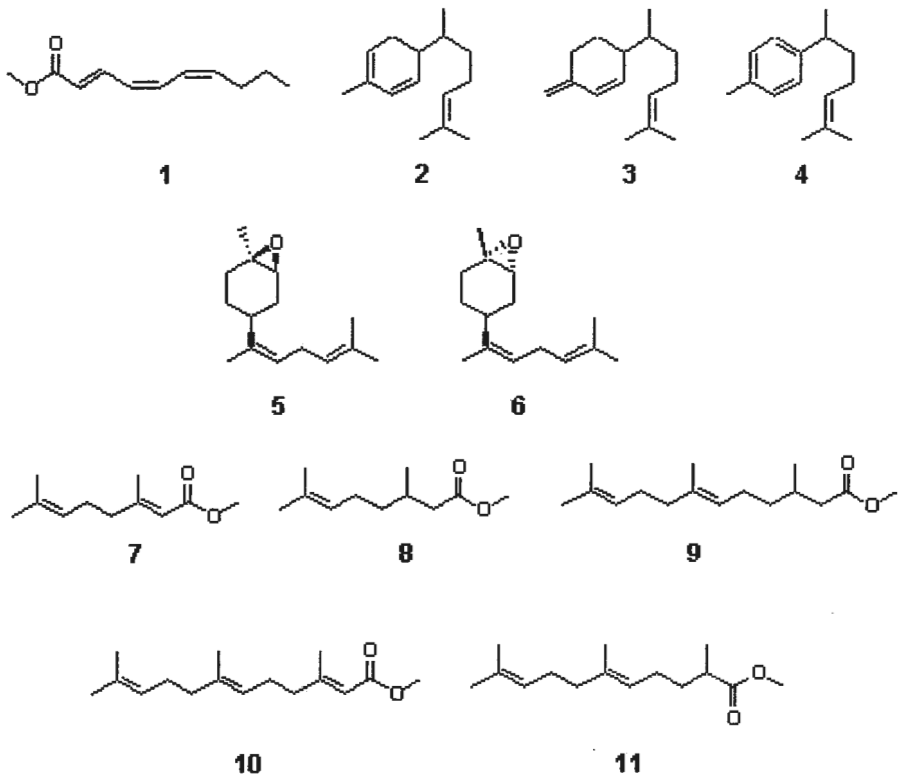

Figure 1. Structures of sex-specific compounds produced by sexually mature male stinkbugs. Compounds: **1**, methyl (2*E*,4*Z*,6*Z*)-decatrienoate; **2**, zingiberene; **3**, sesquiphellandrene; **4**, α-curcumene; **5**, (4*S*)-*cis*-(*Z*)-bisabolene epoxide; **6**, (4*S*)-*trans*-(*Z*)-bisabolene epoxide; **7**, methyl geranate; **8**, methyl citronellate; **9**, methyl dihydrofarnesoate; **10**, methyl farnesoate; **11**, methyl (E)-5-2,6,10-trimethyl-5,9-undecadienoate.

Males of *C. uhleri* and *C. ligata* produced blends of male-specific compounds that were indistinguishable (Ho 2000). The major component consisted of methyl (*R*)-3-(*E*)-6-2,3-dihydrofarnesoate **9** in combination with traces of methyl farnesoate **10** and a novel compound, methyl (E)-5-2,6,10-trimethyl-5,9-undecadienoate **11** (Fig. 1). The latter compound is an analog of methyl dihydrofarnesoate that has been chain-shortened by one carbon. Laboratory and field trials indicated that the major component alone was attractive to females. In addition, as had been found with *C. sayi*, significant numbers of *C. uhleri* males

were caught in pheromone-baited traps, although it cannot be certain that they were actually attracted by the pheromone, and not by cues associated with trapped females. It is also not clear why the male-produced volatiles of these two species, which are sympatric over at least part of their ranges, are virtually identical.

Stink bug trap development.

To date, we have demonstrated that the pheromone blends for the various stink bug species are species specific, and are attractive to one and in some cases possibly both sexes, in laboratory, field cage, and full field bioassays. However, trap catches, using pheromone lures placed in several standard types of insect traps, have been lower than expected for several possible reasons. First, the relatively long-lived adult bugs, which feed and mate multiply throughout their lives, may respond less strongly to pheromones than short-lived, non-feeding species such as some of the lepidoptera, which are under intense pressure to mate and reproduce before they exhaust their limited energy reserves and die. Second, typical insect traps that are designed to catch flying nocturnal insects may be inappropriate for catching stink bugs. Observations of stink bugs responding to pheromone baits have provided several pieces of information that proved important for designing more effective stink bug traps.

Specifically: (1) Stink bugs show a strong tendency to walk upwards on a plant, or, if on the ground, they walk towards the nearest vertical object and climb upwards. (2) Stink bugs do not like to enter dark spaces. (3) At medium range (10 cm to several meters) from a pheromone source, bugs walk towards the pheromone source rather than flying. Thus, traps must be made accessible to walking insects, so they must be in contact with the ground or better, with plant material. (4) Bugs are not easily captured in sticky traps. Bugs walk into traps, and when their feet touch the stickum, they stop, and avoid getting caught. (5) Bugs move around a lot inside traps, and frequently find their way out. Thus, traps must be easy for the bugs to enter, but difficult for them to find their way out again.

In the past year, we concentrated our efforts on designing a trap based on these design criteria, and preliminary results suggest that the new trap is much more effective, at least in row crops, with individual trap catches as high as ~40 bugs per trap over 3-7 d trapping periods (McBrien and Millar, manuscript in prep.). In the coming year, our efforts will be focused on applying the same design concepts to a trap suitable for use in tree crops.

Substrate-borne vibrational signals.

It has been known for some time that stink bugs produce substrate-borne vibrational signals for communication at short range (Gogala 1984; Çokl 1985; Ota and Çokl 1991; Ryan et al. 1996; Çokl et al. 1999, 2000). These signals are generated with a tymbal organ that stretches across the dorsal surface under the elytra. The vibrations are transmitted into the plant stem through the insect's legs, and are propagated along the plant as bending waves (Michelsen et al. 1982). The signals are detected by vibration sensors in the legs of the receiving insect. Both males and females appear to produce several different vibrational songs. To locate each other once on the same plant, males and females produce a duet of calling songs (Figs. 2 and 3), with one or both insects following the signals to their sources. Once at close range or having contacted each other, the insects may begin producing courtship songs. There is virtually no airborne sound associated with these signals, and their primary transmission medium is clearly the plant substrate (Michelsen et al. 1982). Because these signals are transmitted through the plant, they may be less prone to eavesdropping by parasitoids and predators than a more widely dispersed airborne acoustic signal.

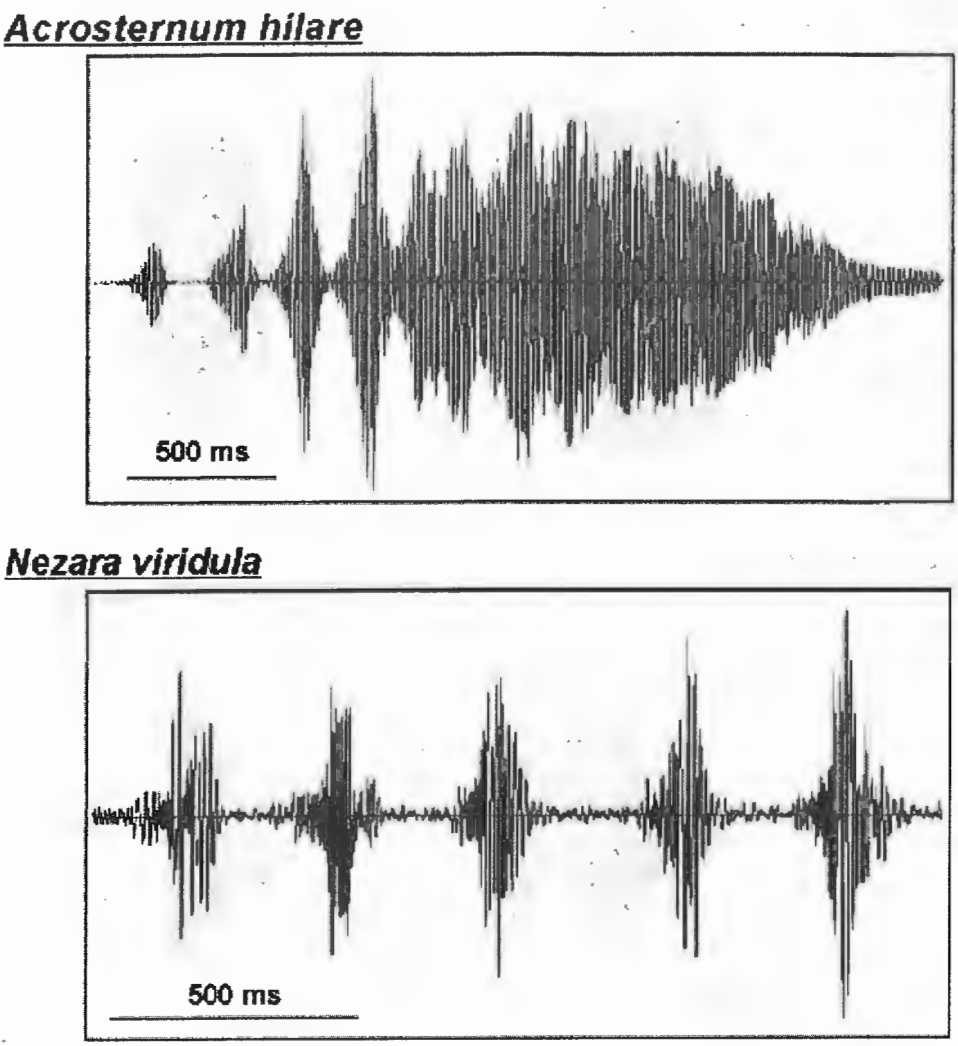

Figure 2. Oscillograms of calling songs produced by male *Acrosternum hilare* and *Nezara viridula*.

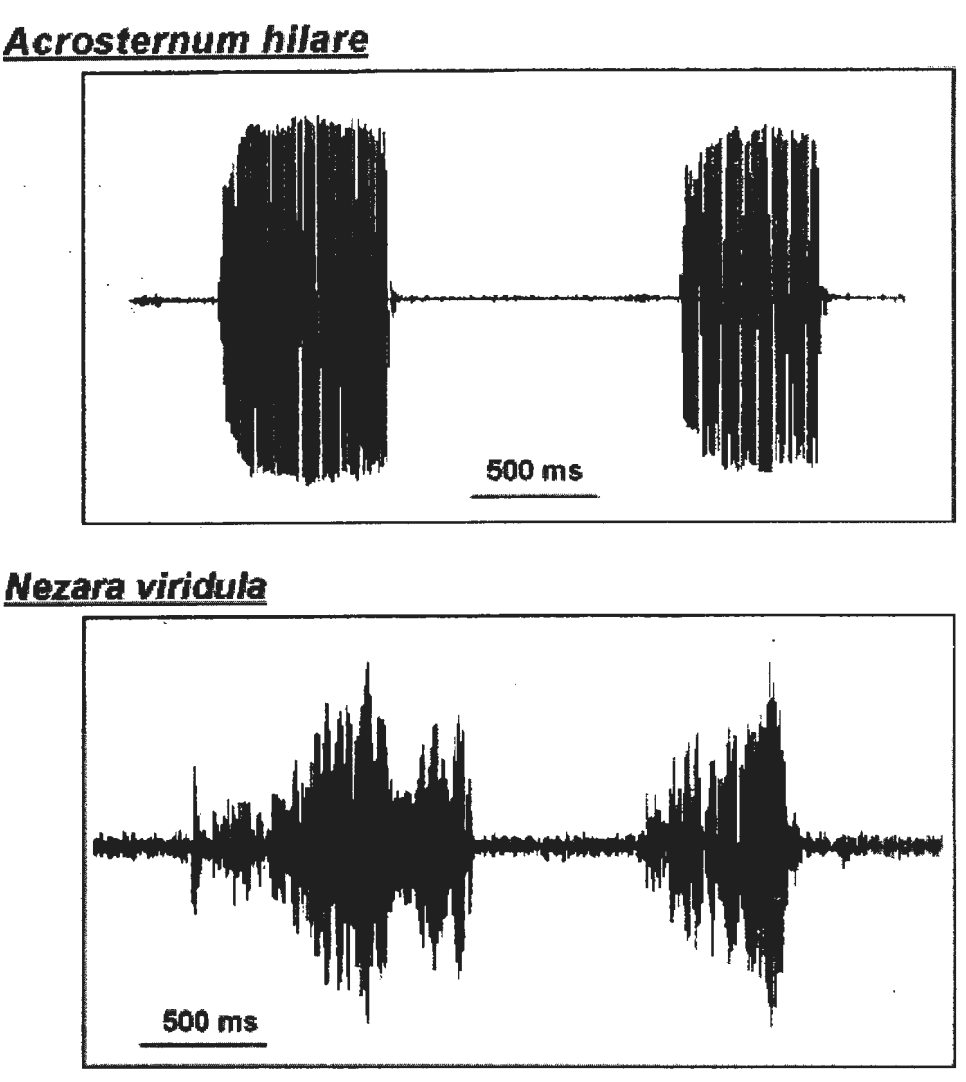

Figure 3. Oscillograms of calling songs produced by female *Acrosternum hilare* and *Nezara viridula*.

Preliminary investigations of the song repertoire of California populations of *Nezara viridula* and *Acrosternum hilare*, chosen for study because they utilize the same pheromone components, have shown distinct differences in the song repertoires of the two species (Üokl et al. 2001). Female *A. hilare* produce a calling song, and the production of this song is stimulated by male presence or calling. The temporal characteristics of this song are similar to the narrow-band *N. viridula* female calling song. Male *A. hilare* produced two different songs, each associated with a different phase of mating behavior. In the calling phase, males produced a song composed of regularly repeated, complex pulse trains whose temporal

structure resembled that of the *N. viridula* male courtship song. In the courtship phase of behavior, males of *A. hilare* emitted a courtship song which terminated female singing. This song had similar temporal characteristics to those of the narrow-band *N. viridula* male calling song. Immediately after initiating copulation, *A. hilare* pairs emitted another song which had no counterpart in the repertoire of *N. viridula*. In contrast, males of *A. hilare* did not appear to produce a song equivalent to the *N. viridula* male rival song, and neither sex of *A. hilare* produced songs equivalent to the broad-band pulses and pulse trains found within male and female *N. viridula* calling songs. Orientation of males towards females was mediated by the female calling song, which had similar spectral and temporal characteristics in both species. Overall, male songs of the two species, although they shared some spectral characteristics, differed in temporal structure and in the contexts in which they were emitted.

Because these vibrational songs bring males and females together once they are on the same plant, they may be important in sampling stink bugs with pheromone traps. Insects may be attracted to the vicinity of a trap by the pheromone, but if the shorter-range vibrational signals are not present, the insects may not move into the trap towards the pheromone source. This may explain observations by several researchers of bugs clustered in the vicinity of pheromone traps, but with few bugs actually in the traps (Aldrich et al. 1991; James et al. 1996). The interplay between the pheromonal and vibrational signals is the subject of ongoing research.

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Use of pheromones and other semiochemicals in integrated production
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Volatile chemicals released by pentatomid bugs having a kairomonal effect on *Trissolcus basalis*: their role on host specificity and prospects for IPM

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Abstract: Volatile semiochemicals emitted by the host, or by different products excreted or secreted by the host itself, play a major role in enabling host microhabitat location by parasitoids. The response of the egg parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) to volatile compounds, from four species of pentatomid bug females (Heteroptera: Pentatomidae) in ovipositional state, was analysed in a Y-tube olfactometer. *Trissolcus basalis* was attracted by volatiles from its coevolved host, *Nezara viridula* (L.), thus confirming previous reports, whereas it did not respond to *Murgantia histrionica* (Hahn), suggesting that a previous host record from the field may be unreliable. In addition, this parasitoid did not respond to volatiles of the non coevolved host *Graphosoma semipunctatum* (F.) and of the non-host *Eurydema ventrale* (Kol.) (Heteroptera: Pentatomidae). Although the host range of Scelionidae can vary greatly, several species are reported as monophagous or oligophagous. The analysis of these results may help in explaining host specificity in *T. basalis* in order to use this parasitoid with improved efficacy and safety in biological control programmes.

Key words: Pentatomidae, *Trissolcus basalis*, kairomone, host selection, host specificity.

Introduction

Models concerning host foraging behaviour of parasitoids are usually divided into a series of hierarchical steps: host habitat location, host location, host recognition and host suitability (Vinson, 1985), which, among numerous ecological and physiological factors, are mediated by physical and chemical cues, the latter also termed semiochemicals or infochemicals (Vinson, 1985, 1998; Vet and Dicke, 1992). The ensemble of physical and chemical characters of the host egg, the substrate and the combined material and/or organisms, that elicit host location and acceptance by egg parasitoids, has been proposed to be defined as "host unit" (Conti et al., 2000a). In the case of pentatomid bugs, a general host unit is represented by the characters of the host egg, the plant substrate, the secretion used by host female to glue the eggs onto the substrate, the contact traces left by gravid females on the substrate and volatile chemicals from adult bugs (Bin et al., 1993; Mattiacci et al., 1993; Colazza et al., 1999b; Conti et al., 2000a, 2000b; Salerno, 2000).

These information sources have, as a yet, not been used as clearly defined and quantifiable elements for the definition of host specificity, although it has been suggested (Conti et al., 2000a; Salerno, 2000). Host specificity is an important issue for researchers and practitioners in developing biological control programs, and particularly when natural enemies are introduced (i.e., classical biological control). In fact, most parasitoid species are restricted to development in relatively few hosts that either share similar life history traits or

live in the same habitat. However, both in natural and laboratory conditions such parasitoids may switch (Conti et al., 2000b) or shift host (Follett et al., 2000), thus revealing an unsuspected wider host range.

This paper deals with laboratory studies carried out in order to evaluate the influences of the volatile cues (Mattiacci et al., 1993; Colazza et al., 1999b) released by four species of pentatomid bugs (Heteroptera: Pentatomidae) on host location by *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae). This cosmopolitan egg parasitoid appears to be the most polyphagous species among the pentatomid egg parasitoids, as it attacks 51 species (Salerno, 2000). In spite of that it has been used, on a worldwide scale, mainly for biological control of the southern green stinkbug, *Nezara viridula* (L.) (Jones, 1988; Jones et al., 1996) a serious cosmopolitan pest that can attack more than 30 different crops (Todd, 1989).

The pentatomid bugs tested were: 1) the coevolved host *N. viridula*; 2) a factitious (non co-evolved) host, *Graphosoma semipunctatum* (F.), which attacks Umbelliferae and, occasionally, Gramineae (Voegele, 1966); and two non-hosts, 3) the European *Eurydema ventrale* (Kol.) (Bonnemaison, 1952) and 4) the American *Murgantia histrionica* (Hahn) (McPherson, 1982), both of which attack Cruciferae and Capparidaceae. In order to better understand the role of volatiles from bug females in the determination of host specificity by the parasitoid, laboratory data are discussed in relation to those from the literature on host associations.

Material and methods

Insects.

All pentatomid bugs were reared in a controlled condition chamber (14-h photophase, at a temperature of 25 ± 1°C; and RH 70 ± 10%), inside clear plastic food containers (300mm x 195mm x 125mm-high) with 5-cm diameter mesh-covered holes. Separate containers were used for nymphs and adults. All stages were fed with seeds, fruits and vegetative parts of their preferred food plants. Sunflower seeds (*Helianthus annus* L.) and French beans (*Phaseolus vulgaris* L.) were used to feed *N. viridula*, fresh celery (*Apium graveolens* L.) and parsley seeds (*Apium petroselium* L.), fennel (*Phoeniculum vulgare* Mill.) and *Ferula communis* L. for *G. semipunctatum*, cabbage and broccoli (*Brassica oleracea* L.) for *E. ventrale* and *M. histrionica*. Food was changed every 2-3 d and water was provided weekly through cotton wool soaked in small containers. Only mated females in ovipositional state, i.e., with enlarged and slightly bloated abdomens, were used for the experiments.

Parasitoids were reared in 16-ml glass tubes and kept in an incubator (15-h photophase, 24 ± 2°C; and RH 80 ± 5%). Adult wasps were fed with a solution of sugar (10%), honey (10%), benzoic acid (10%), yeast and water (Safavi, 1968). Host eggs were exposed to parasitoids for 48h, and were removed and stored in another tube for incubation. After emergence, male and female parasitoids were kept together to allow mating. Mated females used in the experiments were isolated almost 24 h before the assays, provided with a drop of the Safavi (1968) diet, and were used when they were 2-5 d old.

Volatile chemicals bioassays.

The Y-tube olfactometer (stem 9-cm; arms 8-cm at 130° angle; ID 1.5-cm) used and the device adopted for the observations were similar to the ones described by Colazza et al. (1997). Medical-grade compressed air flowed through both arms creating an air stream of 144 ml/min per arm. The flow was regulated by flowmeters, and bubbling through a water jar humidified the air before it passed into the olfactometer. The Y-olfactometer was surrounded by a black fabric curtain to minimise possible cues from the room, and was illuminated by

two 22-W cool white fluorescent tubes located above the device and by infra-red illumination (homogenous emission of wavelengths at 950 nm provided by 108 LEDs) from below. The temperature in the bioassay room was continuously maintained at □ 25°C. For each replicate, one adult bug, caged in a small brass mesh box (2.5 x 1.5-cm), was randomly assigned to one arm and placed near the orifice. Each bug was bioassayed separately, employing a new host adult after each set of two replicates per parasitoid, with a total of 6. Female wasps were tested singly and tests were repeated from □ 9:00 to 19:00. After each experimental trial, the whole system was cleaned with hexane, acetone and distilled water. A single wasp was introduced into the Y-tube at the entrance of the stem, and its walking pattern was recorded for 10 min. using a monochrome CCD videocamera (Sony SSC M370 CE) fitted with a 12.5-75 mm /F 1.8 zoom lens. The camera lens was covered with an infrared pass filter (Kodak Wratten filter 87Å) to remove visible wavelengths. Analog video signals from the camera were digitalized by a video frame grabber. Digitalized data were processed by XBug, a video tracking and motion analysis system developed for Linux (Colazza et al., 1999a).

The following parameters, that better describe differences between arms, were calculated: - residence time, computed as the percentage of time spent in each of the olfactometer arm (%), and - average linear speed in each arm (mm/sec).

Statistical analysis.

Data on parasitoid response to volatiles in the test *vs.* control arms of the olfactometer were analysed with *t* tests for paired comparisons. The possible presence of bias was evaluated in the blank tests by also comparing the two arms of the olfactometer with *t* tests for paired comparisons. Data from the test arms with the different bugs and from the blank were analysed with 1-way analysis of variance (ANOVA) and the Tukey's Honestly Significant Difference test for multiple comparisons between the means. Because both arms in the blank were obviously bug-free, after having verified the absence of bias as described, data for analysis were randomly chosen from one of the two arms. Eteroschedastic data were subjected to angular or square-root transformation (Statsoft, 1997; Sokal and Rohlf, 1998).

Results

Trissolcus basalis walking behaviour in the blank trials showed absence of bias between the olfactometer's arms, both in terms of residence time (t=0.96; df=15; p=0.35) and linear speed (t=0.45; df=15; p=0.66) (Fig. 1). Instead, as expected, female *T. basalis* exhibited a clear preference for its coevolved host, *N. viridula*, in ovipositional state (Fig. 1).

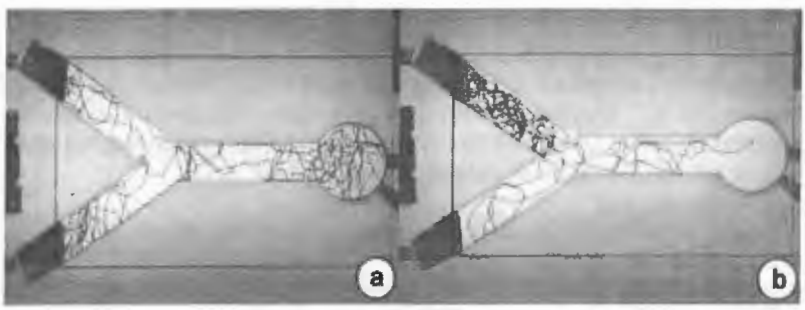

Figure 1. Tracks of *Trissolcus basalis* in Y-tube olfactometer without bugs (blank) (a) or with a gravid female of *Nezara viridula* in the right arm (b).

In fact, residence time was significantly higher (t=4.38; df=16; p<0.001) and linear speed significantly lower (t=5.09; df=14; p<0.001) in the arm containing the host compared with the control (Fig. 2). When the non coevolved host *G. semipunctatum* was tested, *T. basalis* females did not show any preference for the test arm compared with the control. Seemingly, this parasitoid spent less time in the arm containing the bug, although only at the 0.08 probability level (t=1.91; df=15; p=0.075), whereas, the linear speed was similar in both arms (t=0.41; df=13; p=0.69). Finally, *T. basalis* did not respond to volatiles from the other bugs tested (Residence time. *E.v.*: t=0.08; df=13; p=0.93. *M.h.*: t=0.98; df=14; p=0.34. Linear speed. *E.v.*: t=0.63; df=13; p=0.54. *M.h.*: t=0.70.; df=14; p=0.50) (Fig. 2).

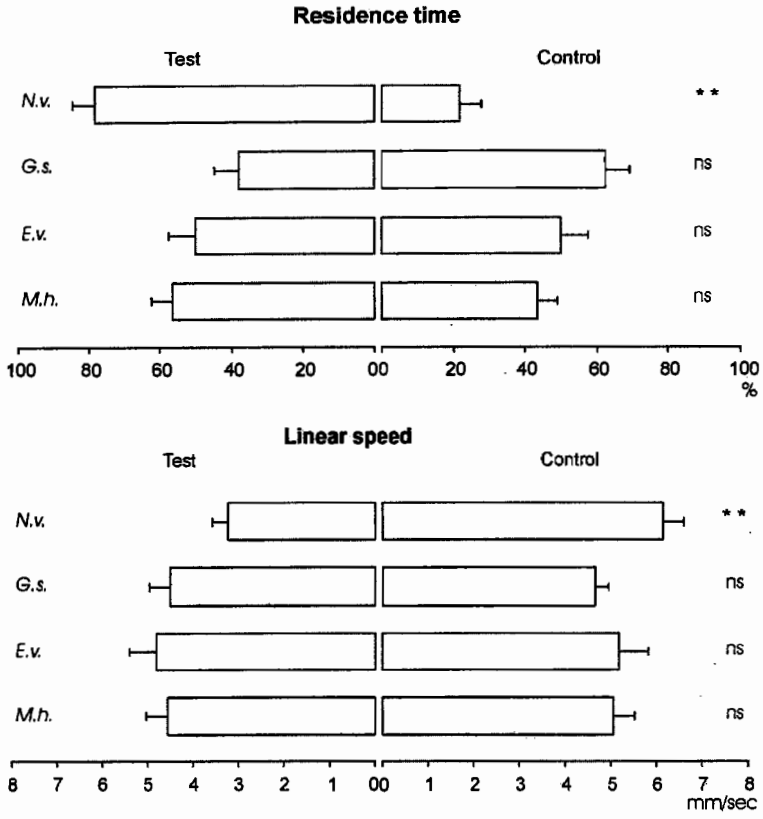

Figure 2. Response of *Trissolcus basalis* to volatiles of *Nezara viridula* (*N.v.*), *Graphosoma semipunctatum* (*G.s.*), *Eurydema ventrale* (*E.v.*) and *Murgantia histrionica* (*M.h.*). Comparisons of residence time and linear speed (Mean ± SE) between test and control arms (** p<0.01; ns not significant; t-tests for paired comparisons).

Differences in parasitoid residence time and linear speed were also found when blanks and test arms containing the different bugs were compared (Fig. 3). Residence time (F=6.51; df=4, 73; p<0.001) was significantly higher in the presence of cues from *N. viridula* compared with *G. semipunctatum*, *E. ventrale* and the blank, whereas it was intermediate on *M. histrionica*.

Linear speed (F=3.11; df=4, 71.; p=0.02) partially confirms these results, being significantly lower on *N. viridula* compared with *E. ventrale* and the blank (Fig. 3).

Discussion

Adults of the coevolved host *N. viridula* release both volatile and contact cues that have a kairomonal effect on *T. basalis* females. Volatile cues are used by *T. basalis* to locate the potential host community (Mattiacci et al., 1993, Colazza et al., 1999b), whereas adult traces left on the substrate provide additional information to the parasitoid about the presence of host egg masses (Colazza et al., 1999b). Once the host eggs are encountered, the wasp's acceptance is affected by a contact kairomone present on the egg surface (Bin et al., 1993). Parasitoid response to volatile cues is characterised by an increase of residence time and a reduction in linear speed. Both behaviours suggest an increased searching in the area contaminated by host volatiles (Bell, 1990).

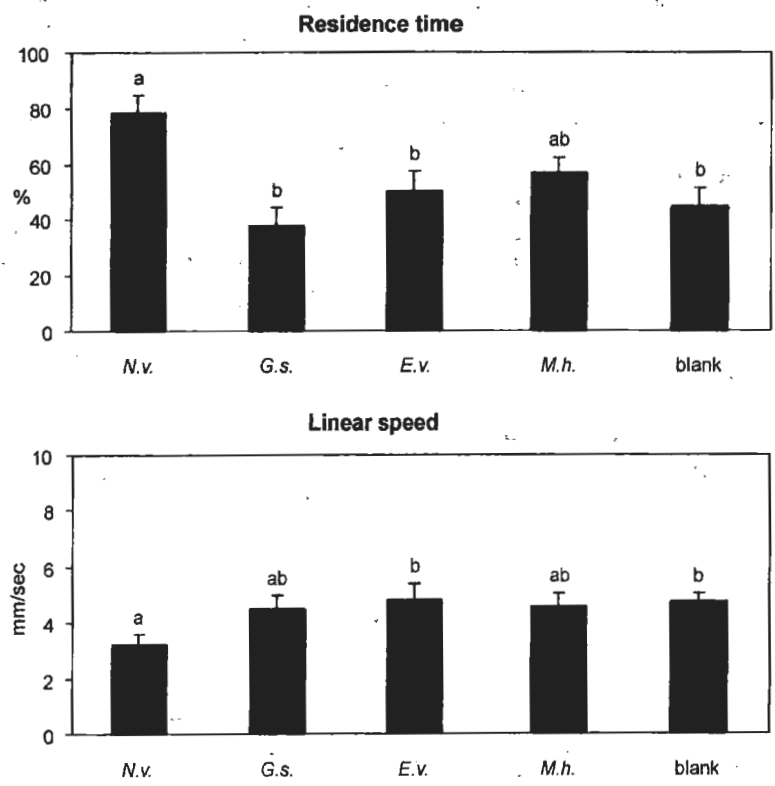

Figure 3. Comparisons of residence time and linear speed (Mean ± SE) of *Trissolcus basalis* as response to volatiles of *Nezara viridula* (*N.v.*), *Graphosoma semipunctatum* (*G.s.*), *Eurydema ventrale* (*E.v.*) and *Murgantia histrionica* (*M.h.*). Columns with the same letter are not significantly different (p<0.05; ANOVA, Tukey HSD).

Trissolcus basalis also recognises and accepts the eggs of *G. semipunctatum* (Voegele, 1966; Kartavtsev et al., 1977), and such response may be chemically mediated by the adhesive secretion present on eggs, which most likely have properties similar to those of *N. viridula* (Bin et al., 1993). However, because this host selection process lacks cues necessary for host location, it seems improbable that *T. basalis* could also attack the eggs of *G. semipunctatum* in field conditions. In fact, this parasitoid does not respond to volatiles from adult females of *G. semipunctatum* and shows a behaviour that may even suggest some kind of repellence. However, this hypothesis needs to be confirmed with more tests.

In addition, *T. basalis* does not respond to volatiles from adult females of *E. ventrale*. It also rejects the eggs of this non-host pentatomid bug, both in field and laboratory conditions (unpublished data), indicating that different cues are involved in such steps of the host selection sequence.

Finally, one field association reported in the literature, was not confirmed by laboratory tests. This regards a record of *T. basalis* on *M. histrionica* in Brazil (Correa-Ferreira and Moscardi, 1995). In the laboratory, this parasitoid did not respond to volatile cues, although partially responded to contact chemicals left on the substrate (Salerno et al., 2000) and partially recognised *M. histrionica* eggs, but did not develop in the eggs (unpublished data). Therefore, parasitoid adaptation to such a potential new host appears unlikely because at least part of the cues used are not shared with its coevolved host.

Host-parasitoid associations are characterised by complex interactions that are often difficult to understand. Comparing such interactions with those implying possible novel hosts and non-hosts may help to explain the mechanisms involved in the host selection steps. This may help defining host preference in *T. basalis* in order to use this parasitoid with improved efficacy and safety (Nechols et al., 1992) in IPM programmes. Finally, this system may be used as a model for host range studies that can also be applied in other host-parasitoid systems.

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Use of pheromones and other semiochemicals in integrated production
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Aggregation pheromone of the almond bark beetle *Scolytus amygdali* (Coleoptera: Scolytidae)

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Abstract: The almond bark beetle (ABB), *Scolytus amygdali* (Coleoptera: Scolytidae), is a pest of stone fruits in the Mediterranean region and southern Europe. Adults feeding on buds cause most of the damage. Applications of non-selective insecticides, burning of dead trees and pruning slash are environmentally unsafe and are often ineffective for ABB control. Preliminary experiments with ABB colonizing branches indicated the existence of an aggregation pheromone, and prompted us to identify it. Volatiles emitted by female ABB boring into plum branches were collected on Porapak Q and eluted with hexane. GC-EAD analyses of volatile extracts, using female antennae as an electroantennographic detector, revealed four EAD-active candidate pheromone components, as follows: (3*S*,4*S*)-4-methyl-3-heptanol (**SS-I**), most abundant and EAD-active component; (3*S*,4*S*)-4-methyl-3-hexanol (**SS-II**); (5*S*,7*S*)-7-methyl-1,6-dioxaspiro[4,5]decane (**III**); and 7-methyl-1,6-dioxaspiro [4,5]dec-8-ene [**IV**], the first unsaturated spiroaketal found in insects. In field experiments (1994-1998) using funnel traps baited with polyethylene pheromone dispensers, **SS-I** unlike **SS-II** was attractive by itself, while **SS-I** plus **SS-II** at a ratio of 2:1 was optimally attractive. Addition of stereoisomeric mixtures of **III** and/or **IV** did not affect trap captures. Candidate kairomones ethanol and propanol did not affect total trap catches. Methanol, in contrast, strongly inhibited attraction of beetles to pheromone-baited traps and prevented colonization of cut branches. It failed, however, to reduce damage to tree buds caused by ABB maturation feeding. Although **SS-I** plus **SS-II** was twice as attractive as the stereoisomeric mixtures of 4-methyl-3-heptanol plus 4-methyl-3-hexanol, these readily obtainable stereoisomeric mixtures can be used for both pheromone-based monitoring and control of ABB populations.

Key words: pheromone, bark beetle, Scolytidae, stone fruits, ethanol, trap

Introduction

The almond bark beetle (ABB), *Scolytus amygdali* Geurin-Meneville, is a pest of cultivated species of stone (*Prunus*) and poem (*Malus*) fruits in the Mediterranean region and southern Europe. During the last two decades populations in Israel have again reached high densities. Most severely affected are plantations of plum, apricot and peach in Israel (Mendel et al. 1997), cherry in Spain (Teresa Garcia Becedas, pers. commu.), and almond in Morocco (Mahhou and Dennis 1992). Outbreaks often follow tree injury caused by flatheaded root borers, *Capnodis* spp. (Coleoptera: Buprestidae) (Ben-Yehuda et al. 1997). Current management of ABB by preventive applications of non-selective insecticide, burning of both dead trees and pruning slash are environmentally unsafe and often ineffective.

Only a few pheromones of *Scolytus* bark beetles have been described. Female *S.*

multistriatus in Britain produce (3*S*,4*S*)-4-methyl-3-heptanol, α- and δ- multistriatin (Pearce et al. 1975), the latter also being a pheromone component of *S. scolytus* in Germany (Gerken et al. 1978). Male *S. scolytus* in Britain produce both (3*S*,4*S*)- and (3*R*,4*S*)-4-methyl-3-heptanol (Blight et al. 1979a), but only the former is attractive in the field. (Blight et al. 1979c). Female *S. scolytus* produce (3*R*,4*S*)-4-methyl-3-heptanol and α-multistriatin, the latter serving as an anti-aggregation pheromone (Blight et al. 1983a). 4-Methyl-3-heptanone is produced by female *S. multistriatus* and male *S. scolytus* (Blight et al. 1983b).

Attractants of four other *Scolytus* species were found in field screening tests. *Scolytus pygmaeus* and *S. laevis* responded to lures containing (3*S*,4*S*)-4-methyl-3-heptanol, α-multistriatin plus α-cubebene or (-)- limonene (Vité et al. 1976; Minks and van Deventer 1978; Bejer 1979). *S. mali* was attracted to volatiles produced by conspecific females (Rudinsky et al. 1978). On the other hand, fir engravers *S. ventralis,* select and attack trees being lured by their host primary attractants (Macías-Sámao et al. 1998a,b).

Most native American *Scolytus* spp. colonize gymnosperm trees and are bigamous, suggesting that pheromonal communication is controlled by males (Wood 1982). Most West Palearctic *Scolytus* spp. colonize angiosperm trees and are monogamous, with females (except male *S. scolytus*) attacking the tree (Balachowsky 1949).

In preliminary experiments we observed aggregation behavior of female and male ABB on cages containing female-infested branches, whereas few beetles alighted on cages containing uninfested branches. These observations suggested that female ABB emit an aggregation pheromone.

Our objectives were to: 1) identify the aggregation pheromone of ABB; 2) test attraction of ABBs to synthetic pheromone components; 3) examine whether candidate kairomones enhance attractiveness of the pheromone; and 4) explore the potential of synthetic semiochemicals to manipulate ABB in fruit orchards.

Materials and Methods

Insects and host material.

Branches of plum (*Prunus communis)* and nectarine (*Prunus persica*) infested with ABB were collected in a stone-fruit farm at Kefar Tabor in eastern Galilee. All beetles were carefully identified to ensure that only ABB, but not congeneric *S. regulosus,* were included in a mass rearing program.

Collection and extraction of volatiles.

Seven to 10 branches (4-5 cm in diam.) infested with several thousand mostly unmated females were placed in a metal container (30 x 40 x 50 cm). Charcoal-filtered air was drawn through the chamber at 10 l/min, and volatiles were collected in a glass column filled with 1 gram of Porapak Q. Columns were replaced at 48 h intervals and trapped volatiles were eluted with hexane. In addition, beetle-produced frass (boring dust plus fecal matter) was extracted in hexane.

Analyses of extracts.

Extracts were analyzed by coupled gas chromatographic electroantennographic detection (GC-EAD). The analyses were performed on a Hewlett Packard 5890 gas chromatograph equipped with a fused silica column (30 m x 0.25 mm ID), coated with HP-5 or FFAP. The HP-5 column was programmed at 50°C for 1 min rising at 3°C/min to 110°C and then at 20°C/min to 240°C; the FFAP column was programmed at 50°C for 5 min rising at 5°C/min to

140^{0}C and then at 10^{0}C/min to 220^{0}C. EAD-active compounds were identified by coupled gas chromatography mass spectrometry (GC-MS) of insect-produced and authentic standards.
Chemicals. (3*S*,4*S*)-4-Methyl-3-heptanol (**SS-I**) and (3*S*,4*S*)-4-methyl-3-hexanol (**SS-II**), 7-methyl-1,6-dioxaspiro [4,5] decane (**III,** containing four stereoisomers) and 7-methyl-1,6-dioxaspiro [4,5] dec-8-ene (**IV,** containing four stereoisomers) were prepared at the University of Hamburg. 4-Methyl-3-heptanol (**I**) containing all four stereoisomers at a ratio of 3:3:2:2 (30% of **SS-I**) was purchased from Aldrich Chemicals. 4-Methyl-3-hexanol (**II**) containing all four stereoisomers at a ratio of 1:1:1:1 (25 % of **SS-II**) was prepared by a Grignard reaction of 2-bromopentane and propionaldehyde in dry THF at the Volcani Center.

Preparation of lures.
All compounds tested in field experiments are listed in Table 1. Chemicals were dissolved in hexane and aliquots of 200 µl were applied to 1 cc polyethylene-capped vials (Just Plastic Ltd, UK). The lures were dried at room temperature. This polyethylene dispensers performed better than rubber septa (Yogev Ltd, Rishon Le'zion, Israel) as shown in preliminary tests.

Field experiments.
Field experiments were conducted at Kefar Tavor in eastern Galilee in a plantation with small blocks of almond, plum, apricot and nectarine, and at Mishmar ha'Emeq (Yizre'el Valley), in a plantation of almonds of several varieties. Lures were deployed in black flat funnel traps (Röchling, Haren KG) which were suspended 1m above ground between pairs of trees at intervals of 30-50 m. Experimental treatments were arranged in randomized blocks, replicated 8-20 times.

Table 1. List of field-tested attractants.

Pheromone components
1. 4-methyl-3-heptanol (containing four stereoisomers) = **I**
2. (3*S*,4*S*)-4-methyl-3-heptanol = **SS-I**
3. 4-methyl-3- hexanol (containing four stereoisomers) = **II**
4. (3*S*,4*S*)-4-methyl-3-hexanol = **SS-II**
5. 7-methyl-1,6-dioxaspiro [4,5] decane (containing four stereoisomers = **III**
6. 7-methyl-1,6-dioxaspiro [4,5] dec-8-ene (containing four stereoisomers) = **IV**
Potential kairomones
1. methanol
2. ethanol
3. propanol

Experiments 1-8 (Tables 2, 3) were designed to determine the most attractive pheromone blend. Experiments 9-12 (Table 4) tested whether candidate kairomones (methanol, ethanol or propanol) affected attractiveness of pheromone components. The alcohols were applied to 12 cc polyethylene-capped vials (Just Plastic Ltd, UK). Since methanol was found to inhibit the response of beetles to pheromone-baited traps, experiments

13-15 (Table 5) tested whether methanol also inhibited colonization of branches by beetles. In these experiments, treatments were spaced > 20 m and consisted of 10-12 freshly cut branches (4-5 x 50-60 cm long) placed in the shade of trees. Numbers of beetle-produced entrance holes and attempted egg galleries were compared between untreated branches and branches treated with pheromone, candidate repellent or both. Experiment 16 (Table 6) tested whether release of methanol in orchard trees reduced the incidence of ABB bud feeding compared to control trees.

Analyses of results.

Trapped male and female beetles were counted. Differences between means were tested by a parametric one-way ANOVA. The procedures used were PROC GLM, and PROCMEAN type III, and the sum of squares was used for computing all F values. Means were transformed into square roots + 0.5. Percentages of males were transformed into arcsin. Differences between means were tested for significance, using the Student-Neuman-Keuls test (P=0.05).

Results

GC-EAD analyses.

In GC-EAD analyses of volatile extracts, four components consistently elicited antennal responses (Figure 1). The most EAD-active component was identified as (3*S*,4*S*)-4-methyl-3-heptanol (**SS-I**). The other components were identified as (3*S*,4*S*)-4-methyl-3-hexanol (**SS-II**), (5*S*,7*S*)-7-methyl-1,6-dioxaspiro [4,5] decane (**III**) and 7-methyl-1,6-dioxaspiro [4,5] dec-8-ene (**IV**).

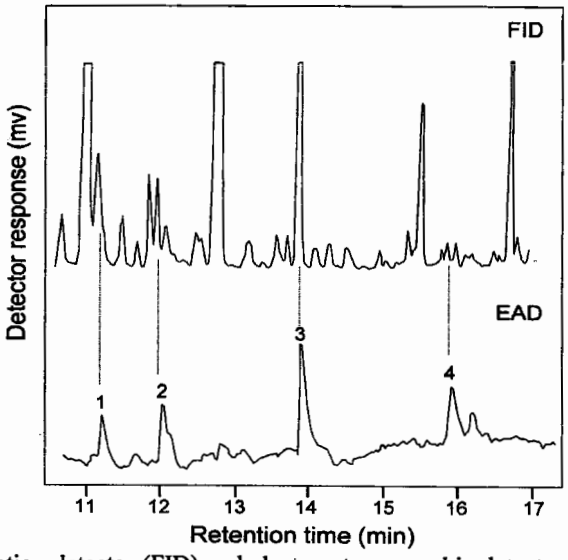

Figure 1. Flame ionization detector (FID) and electroantennographic detector (EAD: antenna of female *Scolytus amygdali*) responses to volatiles from female *S. amygdali* boring into plum branches. Chromatography: FFAP (30m x 0.25 mm ID) column; temperature program: 50^{0}C (5 min), 5^{0}C/min to 140^{0}C, then 10^{0}C/min to 220^{0}C. EAD-active components were identified as follows: **SS-I** = (3*S*,4*S*)-4-methyl-3-heptanol; **SS-II**= (3*S*,4*S*)-4-methyl-3-hexanol; **III** = (5*S*,7*S*)-7-methyl-1,6-dioxaspiro[4,5]decane; and **IV** = 7-methyl-1,6-dioxaspiro [4,5] dec-8-ene.

***Field experiments. Experiments 1-6* (Table 2).**
Preliminary field tests (1995) had indicated that **SS-I**, unlike **SS-II**, was attractive by itself. Increasing the amount of **SS-I** resulted in enhanced attraction of ABBs (Exp. 1). Addition of **SS-II** to **SS-I** at a ratio of 1:1 or 2:1 doubled trap captures (Exps. 2, 3). Spiroaketals **III** and/or **IV** failed to enhance attractiveness of **SS-I** plus **SS-II** (Epps. 4, 5), and did not affect the sex ratio of trapped beetles.

Table 2. Experiments 1-6 (at Kefar Tavor): Attraction of *Scolytus amygdali* to baits containing single stereoisomers or stereoisomeric pheromone mixtures.

Bait	Treatments Components (μg)	Captures Beetles per trap per week	%
Exp 1 (November 1996)			
A	**SS-I** (50)	2.6 b	-
B	**SS-I** (200)	8.0 a	-
C	**SS-I** (500)	10.8 a	-
D	**SS-I** (2000)	7.0 a	-
Exp. 2 (November 1996)			
A	**SS-I** (50)	5.3 b	-
B	A + **SS-II** (10)	7.3 ab	-
C	A + **SS-II** (25)	8.8 ab	-
D	A + **SS-II** (50)	14.4 a	-
Exp. 3 (November 1996)			
A	**SS-I** (200)	27.4 b	-
B	A + **SS-II** (20)	35.9 b	-
C	A + **SS-II** (50)	39.7 ab	-
D	A + **SS-II** (100)	74.3 a	-
E	A + **SS-II** (200)	64.3 a	-
Exp. 4 (November- December 1996)			
A	**SS-I** (50) + **SS-II** (50)	15.9 a	-
B	A + **IV** (10)	17.5 a	-
C	A + **IV** (25)	16.7 a	-
D	A + **IV** (50)	30.1 a	-
Exp. 5 (April 1997)			
A	**SS-I** (50) + **SS-II** (50)	61.8 a	26.4a
B	A + **IV** (25)	75.3 a	25.7a
C	A + **IV** (25)	67.7 a	29.5a
Exp. 6 (November 1997)			
A	**SS-I** (500)	21.6 b	42.4 a
B	A + **SS-II** (250)	31.8 a	45.9 a
C	A + **IV** (250)	32.1 a	44.2 a

***Experiments 7, 8* (Table 3).**
The **SS-I** stereoisomer attracted significantly more beetles, and proportionately more males, than the stereoisomeric mixture of **I** (Ex.). Similarly, the blend of **SS-I** plus **SS-II** was significantly more attractive than blends in which **SS-I**, **SS-II** or both were replaced by **I** or **II** (Exp. 8)

Table 3. Experiments 7, 8 (at Kefar Tavor): Attraction of *Scolytus amygdali* to baits containing single stereoisomers or mixtures of stereoisomeric pheromone components.

Bait	Treatment Components (μg)	Capture Beetles per trap per week	%
Exp. 7 (July 1996)			
A	**SS-I** (200)	45.4 a	17.7 a
B	**I** (400)	21.9 b	2.3 b
C	**I** (800)	22.1 b	3.1 b
Exp. 8 (April 1998)			
A	**SS-I** (500) + **SS-II** (250)	192.5 a	44.90 a
B	**SS-I** (500) + **II** (1000)	161.7 a	40.49 a
C	**I** (2000) + **SS-II** (250)	112.7 b	24.11 b
D	**I** (2000) + **II** (1000)	106.0 b	20.77 b

***Experiment 9-12* (Table 4).**
Ethanol, unlike methanol or propanol, attracted more beetles than did the control, but fewer than did the stereoisomeric pheromone mixture (Exp. 9). Addition of methanol to the pheromone strongly inhibited the response of beetles, whereas addition of ethanol or propanol had no effect (Exps. 10-12). Ethanol, however, increased the percentage of captured males.

***Experiment 13-16* (Tables 5, 6).**
Methanol, unlike ethanol or propanol, significantly reduced numbers of ABB attacks on cut branches, both in the presence or absence of synthetic pheromone (Exps. 13-15). However, release of methanol in the crown of orchard trees failed to reduce the number of buds destroyed by beetles (Exp. 16).

Discussion

The alcohol **SS-I** is also the major pheromone component of *S. multistriatus* (Pearce et al. 1975) and *S. scolytus* (e.g. Blight et al. 1978). Other stereoisomers of 4-methyl-3-heptanol have been identified in ant species (Nacscimento et al. 1997 and literature cited therein). The second pheromone component of ABB, **SS-II**, is not known from other insects. However, (3*R*,4*S*)-4-methyl-3-hexanol was found in the head of the ant *Tetramorium impurum* (Pasteels et al. 1981). Unsaturated spiroacetal **IV** is reported for the first time. The saturated spiroacetal **III**, in contrast, had been identified in several coniferophagous bark beetles, including

Pityogenes chalcographus (Francke et al. 1977), *Pityophthorus* spp. and *Cryphalus piceae* (Francke et al. 1995), *Conophthorus* spp. (de Groot et al. 1991, Birgersson et al. 1995, Pearce et al. 1995), and *Leperisinus varius* (Kohnle 1985). It repells male *C. coniperda* and *C. resinosae* (Birgersson et al. 1995, Pearce et al. 1995).

Table 4. Experiments 9-12 (at Kefar Tavor and Mishmar ha'Emeq): Effects of aliphatic alcohols on attraction of *Scolytus amygdali* to baits containing single stereoisomers or mixtures of stereoisomeric pheromone components.

Bait	Treatment Components (μg)	Capture Beetles per trap per week	%
Exp. 9 (September 1997)			
A	**I** (200) + **II** (100)	77.6	9.1
B	Methanol 10 cc	1.0	-
C	Ethanol 10 cc	9.7	40.1
D	Propanol 10 cc	1.1	-
E	Non-baited traps	0	-
Exp. 10 (October –November 1997)			
A	**SS-I** (500) + **SS-II** (250)	6.2 a	21.9 b
B	A + ethanol 10 cc	9.0 a	39.7 a
Exp. 11 (June-July 1998)			
A	**I** (200)	40.7 a	4.5 a
B	A + methanol 10 cc	5.2 b	>0.1 b
C	A + ethanol 10 cc	27.3 a	5.4 a
D	A + propanol 10cc	50.8 a	8.6 a
Exp. 12 (July 1998)			
A	**SS-I** (200) + **SS-II** (100)	30.4 a	25.1 a
B	A + methanol 10 cc	0.8 b	>0.1 b
C	A + ethanol 10 cc	32.3 a	27.3 a
D	A + propanol 10 cc	19.0 a	23.5 a

The identification of the aggregation pheromone of ABB enabled the preparation of pheromone-baited traps to monitor and possibly mass trap beetle populations. Because **SS-I** and **SS-II** were not commercially available and difficult to synthesize, readily obtainable stereoisomeric mixtures of **I** and **II** were tested. Attractiveness of **I** and **II** was lower than that of **SS-I** plus **SS-II**, probably due to an inhibitory stereoisomer present in the mixture of **I**. In contrast, unnatural steroisomers present in **I** are not inhibitory to *S. scolytus and S. multistriatus* (Blight et al., 1979b). Similarly, attraction of *Rhynchophorus* palm weevils to their aggregation pheromones (5*S*,4*S*)-5-methyl-4-octanol, (3*S*,4*S*)-3-methyl-4-octanol and (4*S*,5*S*)-4-methyl-5-nonanol is not affected by unnatural stereoisomers (Perez et al. 1996;

Giblin-Davis et al. 1996, and literature cited therein).

Ethanol released from decaying wood and stressed plants is a known attractant for ambrosia beetles and bark beetles, including *S. intricatus* (Moeck 1970; Klimetzek et al. 1986; Byers 1992; Markals and Kalapanida, 1997). It is a synergist with other host kairomones and pheromones (Schroeder and Lindelöw 1989; Chénier and Philogéne 1989; Vite et al. 1976; Ross and Daterman 1995), and is used in Spain and Egypt for monitoring ABB. While ethanol was also weakly attractive to ABB in our study, neither ethanol nor propanol affected attractiveness of the pheromone. The lack of synergistic activity might have been due to high release rates (~380 mg/day) of the alcohol. Byers (1992) suggested that ethanol at low rather than high release rates attracted most *Tomicus piniperda* beetles. Unexpectedly, methanol strongly inhibited response of ABBs to pheromone-baited traps and reduced ABB colonization of branches. Similarly, when methanol was added to commercial pheromone lures of *Ips typographus,* it significantly reduced captures of the pine bark beetles *Orthotomicus erosus* and *Pityogenes calcaratus* (Mendel et al., unpublished). Despite the fact that methanol strongly reduced colonization of branches by ABB, it failed to disrupt bud feeding. Other repellents and/or release rates of repellents appear to be necessary to prevent colonization of plants and bud feeding, respectively.

Table 5. Experiments 13-15 (at Mishmar ha'Emeq): Effect of methanol on colonization of branches by *Scolytus amygdali.*

Treatment		Penetration holes per 5 cut branches	
Bait	Components (μg)		
		Plum	Almond
Exp. 13 (August 1998)			
A	Branches	4.1 a	-
B	Branches + methanol 10cc	0.9 b	-
Exp. 14 (October 1998)			
A	Branches + I (500) + II (250)	19.7 a	-
B	A + methanol 10cc	2.3 b	-
Exp. 15 (July 1998)			
A	Branches	1.6 b	0.8 b
B	A + I (500) + II (250)g	25.4 a	8.9 a
C	B + methanol 10 cc	1.3 b	0.6 b
D	B + ethanol 10 cc	19.2 a	14.1 a
E	B + propanol 10 cc	16.3 a	6.7 a

Our results indicate the possibility to establish a pheromone-based management of ABB. Traps baited with I and II have already been used to study the seasonal ABB flight, annual population trends, and occurrence of high populations in stone fruit plantations (Mendel et al.,

unpublished). We plan to investigate the relationship between numbers of captured beetles and damage caused by beetles, and to develop mass trapping of ABB, thereby providing growers with an alternative to insecticidal control of ABB.

Table 6. Experiment 16: Effect of methanol on bud feeding by *Scolytus amygdali.*

Bait	Treatment Components (μg)	Number of buds destroyed per 5 branches		
		April 2000		May 2000
	Location [a]	a	b	c
A	Tree			1.4 [b]
B	A + methanol 10cc x 2			1.5
C	A + I (500) + II (250)	2.7	7.5	
D	C + methanol 10cc x 2	3.9	8.3	

[a] Locations: 4-year-old plantations; two plum orchards at Yoqne'am (a) and Kerem Maharal (b); both near Mt Carmel; and a cherry orchard at Matat (c), in Upper Galilee. In each location, treatments were replicated 20 times. Timing of the experiment coincided with the major seasonal flight of ABB, according trap catch results obtained in 1999 (unpublished data).

[b] Means within locations are not significantly different

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Use of pheromones and other semiochemicals in integrated production
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Responses of *Aphytis chilensis* to the synthetic sex pheromone of the oleander scale

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Abstract: The role of the synthetic sex pheromone of the oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), in the long-range host-searching behaviour of the specialist parasitoid *Aphytis chilensis* Howard (Hymenoptera: Aphelinidae) was studied. Different concentrations in hexane of the (-) and (+) enantiomers of the synthetic host sex-pheromone were compared for their attractiveness in dual choice tests in an Y-olfactometer. Wasp females were significantly attracted from the synthetic sex pheromone at the concentrations from $3x10^{-4}$ up to $6x10^{-3}$ (μg/μl) of the (+) enantiomer, and at a concentration of $3x10^{-3}$ (μg/μl) of the (-) enantiomer. At the concentrations less than $6x10^{-5}$ (μg/μl), for the (-) enantiomer, or less than $3x10^{-4}$ (μg/μl) for (+) enantiomer female wasps did not show preferences. These results indicate that in searching behaviour of *A. nerii A. chilensis* is orientated towards areas likely to contain suitable host stages by host-derived information.

Key words: parasitoid, Y-olfactometer, kairomone, sex-pheromone, *A. nerii*, *A. chilensis*

Introduction

The Oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), is a cosmopolitan pest mainly spread in the Mediterranean basin. This scale insect is polyphagous and attacks citrus and olive trees and ornamental plants. In Sicily, the population level of this pest is generally below the economic threshold, nevertheless its density could increase causing serious damages on fruits in some areas and in lemon orchards heavily sprayed (Liotta *et al.*, 1973).

One of the most important biological control agents of Oleander scale in Sicily is *Aphytis chilensis* Howard (Hymenoptera: Aphelinidae) (Liotta, 1972) that develops as ectophagous on second and third instar larvae of host with a cycle of development of 21 days at 25±1 °C (personal observations).

In the field of our studies on foraging behaviour regarding the host-parasitoid system *Aspidiotus nerii – Aphytis chilensis* we are investigating on the possible role played by volatile cues from host. Parasitoids often use chemical stimuli, called infochemicals (Nordlund & Lewis, 1976), as information from their environment while are searching for their hosts, and these infochemicals can be associated to their host, such in the case with the host sex pheromone (reviewed in Godfray, 1994). Parasitoids can orientate towards these cues over a moderate distance that they associate in some way with the host. For many parasitoids species this means flying to the general source of the odors perceived (Vinson, 1991). For this reason Y-tube olfactometers have been used to behaviour analysis of parasitoids (Colazza *et al.*, 1997; Greany *et al.*, 1977; Lecomte & Thibout, 1986; Moneith, 1955; Morgan & Hare, 1998). Host finding process in the *A chilensis-A. nerii* system might involve some degree in infochemicals-mediated behaviour: it was observed that *A. chilensis* respond to host-

associated kairomones as water extracts of Oleander scale covers (Luck & Uygun, 1986). We thought be interesting evaluate the possible effects of volatile cues as the Oleander scale sex pheromone. We addressed a question: does *A. chilensis* is attracted from Oleander scale sex pheromone?

In order to give a response to the question we analysed the behaviour of the parasitoid females in Y-olfactometer using in tests the synthetic sex pheromone of *A. nerii*, recently isolated (Einhorn *et al.*, 1998) and synthesised (Boyer & Ducrot, 1998).

The aim of this work was to examine the response of *A. chilensis* to (+) and (-) enantiomers of the synthetic sex pheromone of *A. nerii* at different concentrations.

Materials and methods

Insects.

The colony of *A. nerii* was reared on fruits of lemon (*Citrus limon* L.) in cages kept in laboratory at 25±1 °C, 60-70 % RH, and a photoperiod 16 L: 8 D. The colony of *A. chilensis* was established from pupae collected in the field and reared on *A. nerii* and kept in the same laboratory condition.

Tested parasitoids were obtained form pupae removed from their rearing host, individually isolated in glass vials (Ø =1 cm) and supplied with a drop of honey solution. The females used in the experiment were 2-3 days old, had no oviposited previously, or been in contact with the host, and were used only once.

Host sex pheromone.

We used the synthetic sex pheromone of the Oleander scale diluted in hexane (1.5 g/l), provided by Dr. Einhorn and Dr. Ducrot of the Unité de Phytopharmacie et médiateurs chimiques, INRA, Versailles (France). We tested both (-) and (+) enantiomers in different concentrations in hexane as following: (-) enantiomer (μg/μl): $6x10^{-3}$, $3x10^{-3}$, $3x10^{-4}$, $6x10^{-5}$, $6x10^{-6}$, $3x10^{-6}$

(+) enantiomer (μg/μl): $3x10^{-3}$, $3x10^{-4}$.

Test protocol.

Wasps were bioassayed using a Y- olfactometer chamber made of transparent Plexiglas (two arms 8 cm long and common stem 8.5 cm long) sandwiched between two glass sheets (15 x 20 cm). Medical-grade compressed air flowed through both arms creating an airstream of 30 ml/min per arm: the flow was regulated by flometers. Both arms were connected to two glass vials (350 ml) holding the odour source sealed with Teflon tape. In each experiment as test we used a diskette of filter paper (Ø = 8,5 cm) impregnate with 500 μl of sex pheromone in the concentrations as above mentioned and as control a diskette of filter paper (Ø = 8,5 cm) impregnate with 500 μl of hexane. The diskettes were changed any 3 tests.

For each bioassay, a single female was introduced into the Y-olfactometer at the entrance of the common stem and observed for 10 min. Adults were observed under infrared light illumination (homogenous emission of wave-lengths at 950 nm provided by 108 LEDs) with a CCD camera connected with a video monitor. Analog video signals from the camera where digitalized by a video frame grabber and data was processed by a video tracking a motion analysis system for "Xbug" (Colazza *et al.*, 1999). Measurements were made of time spent by wasps in each arm of the olfactometer. Female wasps were tested singly. The temperature in the bioassay room was ≈ 26°C at all times and all tests were carried out from ≈ 9:00 to 15:00.

Choices between test and control arm within a test were analysed using Wilcoxon matched-pairs test (Statistica 5.1. Statsoft, inc. 1997).

Results

With only clean air passing through Y- olfactometer females spent an equal proportion of their time in each arm (42.13 ± 11.43 left vs. 52.98 ± 13.76 right, n= 15 t=-0.51 p= 0.61).

The responses of *A. chilensis* to the synthetic host sex pheromone are presented in figures 1 and 2. The (-) enantiomer (fig. 1) of synthetic sex pheromone at the concentrations of $6x10^{-3}$ µg/µl, $3x10^{-3}$ µg/µl and $3x10^{-4}$ µg/µl induced the wasp parasitoids to spend significantly more time in the test arm than in the control arm (n=21, t=5.71, p=0.000013, n=34, t=2.41, p= 0.0212 and n=40, t=3.15, p=0.0030 respectively). With concentrations of $6x10^{-5}$ µg/µl, $6x10^{-6}$ µg/µl, and $3x10^{-6}$ µg/µl wasps showed no significant preference for one of the two arms (n=33, t=1.48, n.s., n=38, t=1.79, n.s. and n=23, t=0.19, n.s. respectively).

In the experiments with the (+) enantiomer (fig. 2) of synthetic sex pheromone *A. chilensis* spent significantly more time in arm test with a concentration of $3x10^{-3}$ µg/µl (n=17, t=2.47, p=0.02) and it does not show any preference for the two arms with a concentration of $3x10^{-4}$ µg/µl (n=17, t=-1.01, n.s.).

Discussion

Successful parasitism by parasitoids of herbivorous insect hosts is preceded by several phase of searching that lead females into the close vicinity of their potential hosts (Vinson, 1991; Godfray, 1994). Since successful foraging is directly linked with their reproductive success, natural selection will favour animals that make optimal use of foraging cues. Vinson (1991) organised these various chemical cues in different groups in relation to the chemical characteristics of the compounds and the host selection level they effect. After parasitoid locates the host-habitat, it may initiate a host search for cues eliciting a behavioural response (named Group I). These cues are volatile, thus acting over a distance and they arise from the host's food (plant volatiles), volatiles as a consequence of associated organism, or volatile from a pre-host stage (female sex pheromones). These chemical cues result in the female parasitoid arriving at a potential host community or the microhabitat of the host rather than the host (Vinson, 1991).

Some parasitoid species use stimuli produced by host adults to help in the location of the immature stages that they attack and these cues can be sex pheromones of the hosts. Cues directly from the host tend to be non-volatile contact kairomones (Hare *et al.*, 1993), although some parasitoids exploit volatile pheromones of their host as kairomones (Hardie *et al.*, 1994; Feener *et al.*, 1996; Vet *et al.*, 1991). Sex pheromones, often, are not directly associated with the attacked host stages but serve to reduce host searching area (reviewed in Vinson, 1985; Godfray, 1994).

Our results suggest that synthetic *A. nerii* sex pheromone is used by *A. chilensis* as cue eliciting a positive response in the females. Both (-) and (+) enantiomers of sex pheromone attracted female wasps but the (-) enantiomer was attractive at three different values of concentration tested while the (+) enantiomer at only one concentration value. *A. chilensis* attacks immature male and II and III instar female scales; sex pheromone is produced by III instar female scale. Therefore these volatile cues could be used by *A. chilensis* as indirect cue to locate suitable search area or as direct cue to attach host stage producing it.

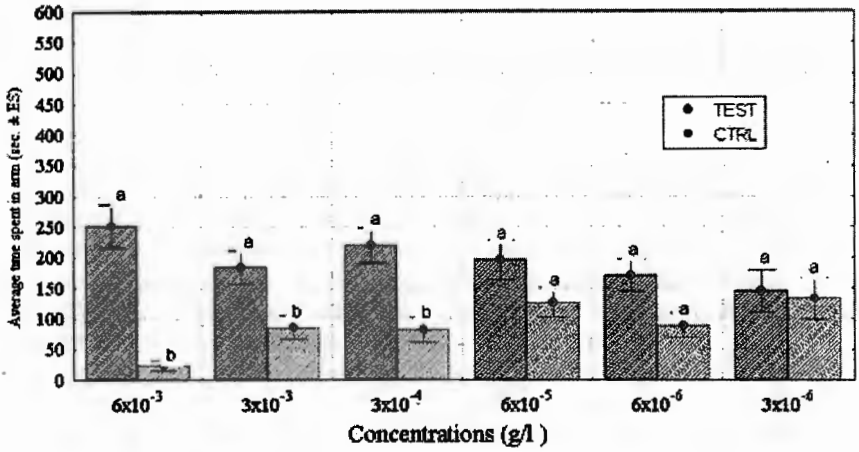

Figure 1. Response of *A. chilensis* in Y-olfactometer to (-) enantiomer of syntehtic *A. nerii* sex pheromone

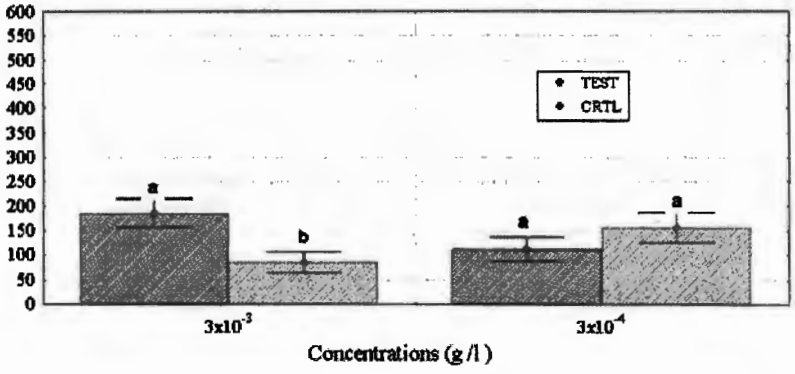

Figure 2. Response of *A. chilensis* in Y-olfactometer to (+) enantiomer of syntehtic *A. nerii* sex pheromone

In monitoring of *A. nerii* population by mean of pheromone traps to capture males it could be interesting evaluate any possible effect that the attraction of the synthetic sex pheromone might have on parasitism efficacy of *A. chilensis*, even if we have to consider that more complex cues depending on scale- host plant system could be important for foraging parasitoids (Morgan & Hare ,1998).

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Use of pheromones and other semiochemicals in integrated production
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Females sex pheromone of oleander scale: quantitative aspects of its production and release

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Abstract: Sex pheromone of the Oleander Scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) was investigated to define its chemistry. Quantification of sex pheromone emitted by female of Oleander Scale was performed using headspace solid phase microextraction (SPME) subsequently analyzed by gas chromatography/mass spectrometry (GC/MS), adopting a cyclobutane derivative standard of Oleander Scale sex pheromone chemically synthesized as a standard. The headspace SPME of the volatile emission from about 30 Oleander Scales virgin females of a Sicilian population monitored for several days allowed individuating the initial point of sex pheromone production in females twenty-seven d-old. The amount of pheromone production has been determined in 10 pg for single scale per day.

Key words: Sex pheromone, oleander scale, SPME, lemon

Introduction

In Sicily, commercial citrus orchards are infested by Diaspid pests, commonly known as scales (Liotta, 1970; Liotta *et al.*, 1977). These pests cause a general weakening of the tree, and infested fruits are graded down in the packinghouse making them unmarketable. Control of Scales consists mainly of insecticide applications which use has been rationalized due to the discovery and the synthesis of sex pheromones. The pheromones, in the scales Integrated Pest Management programs, are used to monitor scale male so that combining male capture data and degree day model upcoming events in the scale lifecycle the best time to make pesticide applications could be forecasted. So far, the sex pheromones of four species of Diaspididae have been chemically identified and adopted in IPM: the California red scale, *Aonidiella aurantii* (Maskell) (Roelofs *et al.*, 1977), the yellow scale *A. citrina* (Coquillett) (Gieselmann *et al.*1979a), the San Jose scale *Quadraspidiotus perniciosus* (Comstock) (Gieselmann *et al.*1979b; Anderson *et al.*, 1981) and the white peach scale *Pseudolacaspis pentagona*.(Heath *et al.*,1979). The sex pheromone of the Oleander scale *Aspidiotus nerii* Bouché, was recently chemically identified (Einhorn *et al.*, 1998; Boyer & Ducrot, 1998). In all identification processes the methodology used consisted in collecting in volatile substances emitted by a large number of females over a long time period. We used a different approach by means solid phase microextraction (SPME) followed by gas chromatography/mass spectrometry (GC/MS) that is an excellent concentration and preparation technique for the analysis of volatile and semi-volatiles compounds that can be found in the headspace above the sample. This solvent free technique is reliable because it has great sensitivity, it is fast, does not damage insects and can be applied over several consecutive days on the same sample. In this study SPME analysis was adopted for detection of *A. nerii* pheromone on lemons and for tentatively quantifies its production over several days.

Materials and methods

Insect cultures.
The Oleander Scale colony was established from materials collected in Sicily from lemon trees. Insects were reared on lemon fruits previously coated with paraffin over about the 2/3 of their surface to avoid desiccation. Daily lemon fruit infestations were manually performed transferring on fresh fruit crawlers from gravid females and infested fruits were kept isolated in a plastic bottle cap at 24 ±1 °C, 70 ± 5% RH with a 16L:8D photoperiod. Twenty-four days after the infestation second instar males were manually removed, then two days before the second molt, lemons, infested with about 30 virgin females, were used for SPME collection.

Sample collection.
Volatile collection was performed by SPME from the headspace over two infested lemons with Oleander Scale virgin females, held in a 300 ml glass vial sealed with a silicon septum, and left under laboratory conditions at 25 ±1 °C. Volatile was collected by a 70μm SPME (Supelco) CarboWax divinylbenzene (CW/DVB) coated fiber, for 18 hours starting at 2.00 p.m. on each day of the sampling period. Virgin females of Oleander Scale were sampled from twenty-five d-old to thirty-seven d-old. Between two volatile samplings, the infested lemon fruits were held sealed in the same glass vial.

Chemical analysis.
All the samples were analyzed by GC on a fused capillary column HP5-MS (30m length, 0.25 mm I.D., 0.25 μm film thickness, Agilent) in a Agilent mod. 6890 chromatograph equipped with the mass selective detector Agilent 5973 in the following condition: the injector temperature was held at 250 °C; He as carrier gas at 10^{-3} l/min.; oven temperature program: 5 min. isotherm at 45°C followed by linear temperature increase of 4° C/ min. up to 300° C held for 10 min.

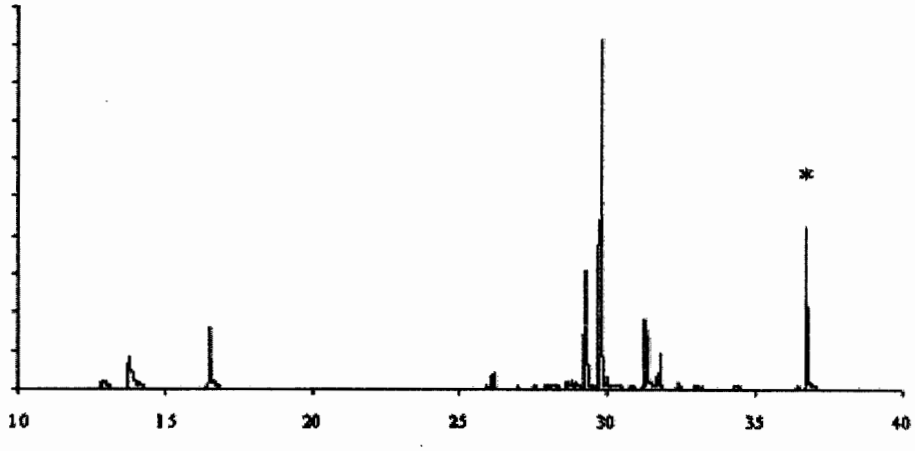

Figure 1. SPME (CW-DVB) chromatogram of synthetic pheromone of oleander scale on lemon substrate for 18 h (asterisk = pheromone peak)

A cyclobutane derivative, standard of *A. nerii* pheromone, was tested in order to identify the response factor, the retention time on the chromatogram and the most characteristic fragments in the mass spectrum. Capacity of SPME to detect a pheromone concentration of 1.5 μg, in our experimental system, was tested to investigate the presence of some lemon peaks from the examined area (Fig.1). To improve the detection limit, the SIM mode was set on mass spectrometer.

Results and Discussion

The retention time of standard pheromone was found to be 36,8 ± 0.1 min. and the mass spectrum showed the following, more intense, ions 68, 79, 93, 107, 121, 133, 161, 189, 204 amu. In Figure 2 were reported the results of these experimentation. The y axis represents the total amount of pheromone collected by fiber in the glass vial closed with a silicon. The amount has been calculated from integrate area under the peak at ~36.8 min. of R.T. In the first two day no peak was detected over the instrument detection limit, for this reason we consider the first part of the curve as the "zero level" of pheromone presence. On the twenty-seventh day, one peak begun to grow up at ~36.8 min. of R.T. and on the twenty-eightieth day we had the maximal integrated area. After the twenty-eightieth day, the peak slowly decreased down to a constant value.

Assuming constant daily pheromone production for each individual, the shape of the growing part of experimental curve yields from different pheromone production start time among all the individuals. According to this supposition on the twenty-eightieth day all the scales were probably on production. The supposed amount of pheromone produced by all virgin scales in one day was determined by subtracting the amount of pheromone collected on the twenty-seventh day to the amount collected on the twenty-eightieth day. The result was 10 pg per single scale per day.

This experiment, under established conditions, gave us three important results agreeing with morphological aspects of scales: first, we were able to identify the beginning of pheromone production; second, it the last day of pheromone production was determined; third, the pheromone production of a single female scale has been measured.

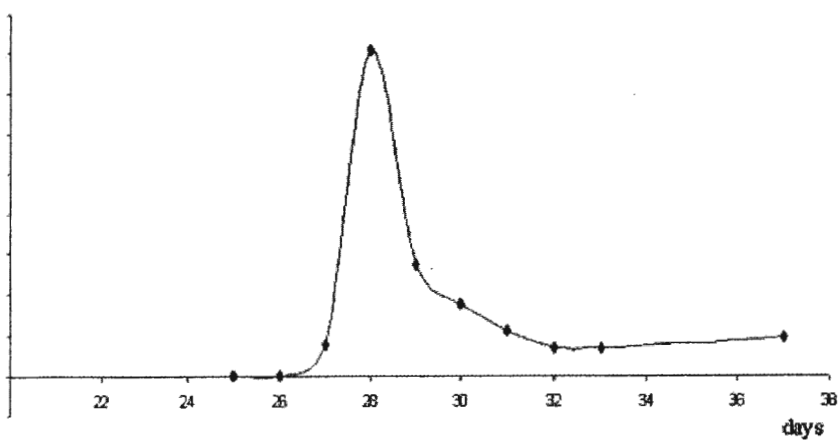

Figure 2. Sex pheromone of oleander scale - timing of production

In order to assess the importance of semiochemicals as sympatric speciation factors (Tremblay e Rotundo, 1978), as showed in some species by the presence of so-called "pheromone strains" (Aldrich *et al.*,1987), further research developments could be aimed at verifying whether the sex pheromone could be produced also by the *A. nerii* partenogenetic population and whether colonies of *A. nerii* collected from sicilian citrus orchards release only a cyclobutane derivate as attractive substance or other substances are involved instead, at least in determining pheromone specificity.

Acknowledgement

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Use of pheromones and other semiochemicals in integrated production
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Applied research of ecochemicals in Estonia

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Abstract: This paper reviews applied research of ecochemicals in Estonia.

Key words: Sex pheromone, monitoring, Lepidoptera

Introduction

Pheromone and kairomone traps can offer a reliable technique for monitoring and controlling different pest moths. To achieve good correlation between population density and trap catches, the attractant dispenser should have stable attractivity over the flight period of pest. Amount of trapped males, species specificity, and aging of dispensers depend on the load amount of substances and their ratio, amount of degradation products, type of substrate, and other characteristics of dispenser. Due to different evaporation rates, the ratio of attractant components in effluvia will change over the aging period. Consequently, the attractiveness of a dispenser is not constant in time and the component ratio of the emitted blend differs from the optimal. It may be concluded that in many cases the dispensers should be designed for specific climate conditions. Beside the chemical structure of blend components, evaporation rate depends on the shape of the dispenser. In our earlier experiments we used rather large Feroflor type dispensers produced by AS Tartu Flora. To achieve better evaporation characteristics and to minimize the decomposition of pheromone substances in dispenser, the small-size Minifer and Miniket dispensers were designed and tested for many pest insect pheromone blends, to avoid competition between traps, the loading of the attractant should be minimal. These requirements apply for a monitoring system as well as for mass-trapping. The substrate of Miniket dispensers is a special rubber composition (Mõttus et al., 2000a). In this paper we summarise our results on creating and introducing pheromone and kairomone preparations for practical plant protection in Estonia.

The field screening has been a successful tool to find pheromone type attractants and for some moths, attractive blends are optimised using this method. In 1980 – 1990, a co-ordinated field-screening project was accomplished in the European part of the former Soviet Union. Most of the results were published in different local journals in Russian and were therefore not reviewed by abstract journals and by Pherolist. Table 1 lists some attractive blends ascertained by research scientists of our laboratory, participating in this project.

Pest insects

In areas where the codling moth, *Cydia pomonella* L., has one generation, the control of pest insects can sometimes be achieved by mass trapping methods. To control the *C. pomonella* population, pheromone traps were used in an isolated apple orchard (Madsen et al., 1976). In Estonia, Leivategija (1982) introduced this method to many small private orchards after a successful demonstration of the technique. The pheromone kits, consisting of Feroflor dispensers, available from AS Tartu-Flora, Estonia were used. The mass trapping method was introduced in Velikie Luki, in the region with climate closer to that of Estonia (Yemelyanov et al., 2000).

Eleven compounds were identified in the pheromone blend of *C. pomonella* (Causse, 1988). Despite repeated identification by several investigators, the mode of activity of pheromone blend components is not clear and McDonough et al. (1993) considered the only active compound to be (E,E)-8,10-dodecadienol (I). Arn et al. (1985) have published experimental data on activity of more blend components, dodecan-1-ol (12:OH) included. In Minifer dispenser, a mixture of (I) and 12:OH was used in two different ratios. We discovered that the Miniket UMD dispenser, loaded with the two-component attractant blend, had elevated attractivity to *C. pomonella,* compared with Minifer and Feroflor dispensers containing the two components or only (I).

The pea moth, *Cydia nigricana* F., uses (E,E)-8,10-dodecadien-1-yl (II) acetate as the main component of female sex pheromone (Greenway, 1984; Witzgall et al., 1993). Male attraction to synthetic E8-E10-12Ac declines after a few days in the field due to isomerisation, 5 % of isomeric impurities caused significant inhibition (Witzgall et al., 1993). The isomerisation of conjugated dienes in dispensers is influenced by the substrate used (Vrkoc et al., 1988). There is no evidence of any substrate suppressing isomerisation sufficiently in field conditions, but a propheromone compound, [(8,9,10,11-n)- E8-E10-12Ac] iron, releases isomerically pure (II) (Streinz et al.,1993, Witzgall et al., 1996).

A pheromone mimic, (E)10-dodecenyl acetate, was recommended to monitor *C. nigricana* (Macaulay et al., 1977). This compound is less attractive than E8-E10-12Ac, but ensures constant attraction over the whole flight period (Greenway and Wall, 1981). According to Tuovinen (1982), it can be used for the timing of a spray in Finland, but the correlation between total catch and damaged peas was not strong enough for prognosis.

High-dosed Feroflor HM dispensers, loaded with 3 mg of (II) have been found to be suitable for indication of economic threshold and timing of sprays (Tschmyr, 1981; 1985) in Voronezh region, Russia.

The blend of (II) and the false isomers of (II) (30 g/ha) was an effective mating disruptant in Sweden. Males were observed to fly out of the treated field and their attraction to calling females in the cages was almost entirely suppressed. Larval infestation decreased from 36 % to 2 % , if the immigration of mated females from other fields was prevented (Bengtsson et al., 1994; Witzgall et al., 1996).

Different sex attractant preparations containing Z-11-hexadecenyl acetate (Z11-16:Ac) and Z-13-octadecenyl acetate (Z13-18:Ac) in variable ratios have been used to determine the population density of the Apple fruit moth, *Argyrestia conjugella* Zell. Female pheromone has not been identified, attractant was ascertained by field screening (Booij et al, 1984). On the basis of many-year experiments in Finland, Tuovinen (1991) concluded that the attractant preparation, which contains Z11-16:Ac and Z13-18:Ac, is not specific enough for growers' use, but it may be used by trained personnel to determine the need of sprays. AS Flora produces Feroflor type dispensers of different dose and composition for *A. conjugella.*

Table 1. Some sex attractants evaluated by field screening method in Estonia, Byelorussia and in Voronezh and Krasndar regions of Russia . Species marked with an asterisk: the tested blend had attractivity similar to pheromone preparations and was used for monitoring population density. In most cases, the blend compositions are optimized (overleaf)

Species	Blend components	Ratio	References	Host plants
Eupoecilia sanguisorbana H-S*	Z11-14:Ac	0.7	Ryabtcinskaya et al,	*Sanguisorba*
	E11-14:Ac	0.3	1986b	*officinalis* L.
	12:Ac	1.5		
Aleima loeflingiana L.*	Z11-14:Ac	0.2-0.3	Ivanova et al., 1986	*Quercus* L.
	E11-14:Ac	0.8-0.7		
Arhips lafauriana Bag*	Z11-14:OH	1.0	Ivanova et al., 1986	polypha-gus
Choristoneura diversana Hbn *	Z11-14:Ac	1	Ryabtcinskaya et al,	Rosacea,
	12:Ac	1-2	1986	*Corylus* L
			Ivanova et al., 1986	
			Bykhovets et al, 1986	
Thiodia caradjana Kenn	Z11-14:Ac	0.9	Ryabtcinskaya et al,	Compo-sitae ?
	E11-14:Ac	0.1	1986	
	Z9-12:Ac	0.2		
Eucosma cumulana Gn.	Z8-12:Ac	0.6	Ryabtcinskaya et al,	*Inula salicina* L.
	(E,E) 8,10-12:Ac	0.4	1986	
Gypsonoma nitidulana L.*	Z11-14:OH	0.1	Ryabtcinskaya et al,	*Populus* L.
	Z11-14:Ac	0.9	1986	
	E11-14:Ac	0.1		
G. oppressana Tr.	Z11-14:Ac	0.2	Ryabtcinskaya et al,	*Populus* L.
	E11-14:Ac	0.8	1986	
G. dealbana Fröl.	Z8-12:Ac	0.4	Ryabtcinskaya et al,	*Betula* L.
	(E,E) 8,10-12:Ac	0.6	1986	*Corylus* L
	E11-14:Ac	0.2		
Cydia medicaginis Kuzn*	(E,E) 8,10-12:Ac	1.0	Tchmyr & Mõttus,	*Medicago* L.
			1980, 1981	
Cydia (Grapholita) compositella	(E,E-)6,8-10:Ald	0.9	Tchmyr et al., 1981	*Medicago* L.
F*	Z8-10:Ac	0.1	Mõttus et al., 1982	*Trifolium* L.
Grapholita delineana Walk.*	(E,E-)6,8-10:Ald	1.0	Mõttus et al., 1983	*Cannabis* L.
Pammene germana Hbn	Z8-12:Ac	1.0	Ryabtcinskaya et al,	*Acer* L.
			1986	*Crataegus* L.
Dicrorampa petiverella L.*	Z11-14:Ac	0.2	Bykhovets et al, 1986	*Achillea* L.
	E11-14:Ac	0.8		*Tanacetum* L.

Feroflor CA-71, having load ratio of Z13-18:Ac and Z11-16:Ac of 3:7, was used widely in Estonia (Mõttus et al., 1996). In field tests with the small-size Miniket type dispensers, 1:1 blend had the highest attractivity (Mõttus et al., 1999a). At the same time, the need of identification of *A. conjugella* pheromone blend is evident, none of the tested mixtures had attractivity similar to a female pheromone. *A. conjugella* is cued to different mountain ash tree (Sorbus spp.) species and its biology in Armenian mountains is reviewed by Mirzoyan and Grigoryan (1990). In the habitat of *A. conjugella*, about 80 species of ash trees grow, and habitats of *A. conjugella* are strongly limited by climatic conditions.

In regions with two generations the Large fruit-tree tortrix, *Archips podana* Sc., is considered to be an important pest (Persoons et al., 1974), in regions of one generation, it may cause damage sporadically (Paternotte, 1999). Lately it was demonstrated that pheromone traps offer a worthwhile method of investigating *A. podana* population densities and other biologically interesting correlations resulting from the effect of human activity or changes in climate (Kozlov and Haukioja, 1993).

Sex pheromone of *A. podana* was identified by Persoons et al. (1974) as 1:1 mixture of Z11-14Ac and E11-14:Ac. The optimum ratio of Z11-14Ac and E11-14:Ac in pheromone blend in Byelorussia (Bykhovets et al., 1986), Central Russia (Ryabtchinskaya et al., 1986), and in Carpathian region (Safonkin, 1990) has been described as 6:4 of Z/E.

The Gaussian normal distribution curve was used for calculation of the optimal ratio of compounds in the latest experiments in Estonia. Calculated values of optimal content of Z11-14:Ac for *A. podana* in two different habitats were close, being respectively 58.6 % and 60.0 % (Ojarand et al., 2000). Our field experiments are aimed of optimizing the dispenser and of using pheromone methods for plant protection and monitoring climate changes.

Polyphagous moths *Hedya nubifereana* Hawk., *Choristoneura diversana* Hbn., *Epiblema foenella* L., and *Ptycholoma lecheana* L. may have economical significance in Estonia only sporadically, in case of outbreaks. In regions close to Estonia, for instance Lithuania, Byelorussia, and Pskov district of Russia, significantly higher population densities of these moths are noted (Bykhovets et al., 1986). In Estonia they may be surveyed as an indicator factor of biodiversirty as it may be expected that population density of these moths depends on climate and human activity, which was the reason to test the pheromone dispensers.

Control of the Currant-clearwing, *Synanthedon tipuliformis* Cl., in red, white, and black currants is difficult due to the larvae hidden in the wood and the short flight period (2–3 weeks) of adults. A mixture of 2E,13Z -18:Ac and 3E,13Z-18:Ac in a ratio of 100:3 at doses of 0.010-1.0 mg is recommended for species-specific field monitoring of *S. tipuliformis* in Hungary and New Zealand (Szocs et al., 1990, 1998, Ujvary, 1993). According to Buleza (1992), the effectiveness of the sex attractant is influenced by the colour of the trap, light yellow traps captured reliably more males than white, red, green, or black traps.

Mating disruption with pheromone blend (100:3) provided sufficient control of *S. tipuliformis* in Netherlands (Bal, 1998). Mass trapping using of 25 traps/ha decreased larval damage by 50 % and increased black currant yield by 1.56 t/ha in Byelorussia (Samersov et al., 1998). In Estonia, S. tipuliformis is considered an economically significant pest and Feroflor type dispensers have been successfully used for population survey (Laanmaa and Luik, 1997). Unfortunately, results are not published in a scientific journal. In Lithuania, investigation of 16 blackcurrant plantations in 1989– 91 showed that S. tipuliformis damaged up to 1% of branches in young bushes not yet producing berries and 1– 63% (average 13%) of those producing berries (separate branches were regarded as those commencing no higher than 30 cm above the ground). Normally, there was 1 larva per branch, while 2 larvae occurred less often and 3 only when over 20% of branches were damaged; 3 being considered

a suitable criterion of heavy infestation. Study of seasonal dynamics using pheromone traps showed that the time of commencement of flight varied by 2 weeks according to weather conditions and most adults flew in the last 10 days of June (Buda, 1993).

The Currant shoot borer, *Lampronia* (*Incurvaria*) *capitella* Cl., is a significant pest of red and white currant, causing damage to its pods. On the basis of our experiments, we estimated the amount of trapped male insects to be about about 50 000 per hectare. Severe damage may be caused and in Estonia some red currant plantations were cut off due to too high population of *L. capitella*. It is considered a harmful pest in neighboring areas as well (Samersov, 1998, Tuovinen, 1997).

The Antler moth, *Cerapteryx graminis* L., (Noctuidae, Hadeninae), is a Holoarctic species with the southern range limit of about 45° N (Balachowsky, 1972). The species has one generation per year and its larvae feed on almost all species of grasses. Infestation of extensive meadows and pastureland, leads to a decline in grassland productivity (Zolk, 1931). In Estonia, outbreaks of *C. graminis* are described in 1936 and in 1980 – 1990.

There are two known imaginal forms and some variable color morphs of *C. graminis* (Forster and Wohlfahrt, 1971, Pyöronilä et al., 1979). Maercks (1943) and Schenker (1950, 1956) described the life history of *C. graminis*, but there is little scientific understanding what factors cause antler moth outbreaks.

One reason for higher population, as we consume, may be higher rainfalls during the period of feeding and pupating of *C. graminis*. The monitoring of *C. graminis* may have some importance because the influence of environmental changes on its population, as it occurred in 1980s in Sudeten Mountains, Poland (Klukowski, 1993), and in Denmark (Berris and Graveland, 1988). Danell and Ericson (1990) have concluded that in northern latitudes, the larval density was negatively correlated with the mean temperature in June.

The sex pheromone of *C. graminis* has not been identified, field tests have shown the attractivity of Z11-16:Ac (Mõttus et al, 1981) and 1:1 mixture of Z11-16:Ald and Z11-16:Ac (Priesner,1986). The attractant blend was optimised in experiments in Estonia and Poland as 7: 3 – 4:6 of Z11-16:Ac and Z11-16:Ald (Mõttus et al.,1998).

The Timothy tortrix, *Aphelia paleana* Hbn., is cued to many Pocaceae (Gramineae) and its economical significance is not known. Mixture of Z11-14:Ac and E11-14:Ac (9:1) is attractive to A. paleana (Booij and Voerman, 1984). This mixture had the highest attractivity for A. paleana in Krasnodar Territory (Ivanova et al., 1986). In a detailed study, it was demonstrated that the pheromone communication channel for *A. paleana* was narrower than that for A. podana and the attractant dispensers should have component ratio accuracy better than ±3% (Mõttus et al., 2001).

Hoplocampa flava L. and *Hoplocampa minuta* Crist. are important pests of plums in Estonia. No evidence of investigations of the chemical communication of Hoplocampa spp. has been found. White sticky traps were effective in monitoring *H. testudinea* Klug., a pest of apples (Lukas and Kocourek, 1998). There are no publications on usage of non-toxic methods to control this significant pest in Estonia. In our laboratory, the research on colour traps is in progress.

In Estonia there are not many cruciferous pest insects having long distance attractant pheromones. Consisting Z11-16:Ac dispenser Pheroflor MB2 for the Cabbage moth, *Mamestra brassicae* L. has been successfully tested together with other blends for Noctuidae (Zolotov and Mõttus, 1985). Economically more impotant *Plutella xylostella* L., the Diamondback moth, has, in recent years, caused economic damage to cabbage and some other cruciferous plants, such as oilseed rape, in Estonia and measures to control the pest are needed (Mõttus et al, 1997). It has one generation per year. How it overwinters successfully in temperatures below 0 0C for 5 - 6 months (November - April) is not clear. In Estonia,

pheromone traps usually start catching male *P. xylostella* in early June, when cabbage plants are transplanted to fields

The pheromone blend of *P. xylostella* has been identified by Tamaki et al. (1977) as mixture of (Z)-11-hexadecen-yl acetate (Z11-16:Ac) and (Z)-11-hexadecenal (Z11-16:Ald). Koshihara and Yamada (1980) found that the attractiveness of mixtures of Z11- 16:Ac and Z11-16:Ald to males was synergized by (Z)-11-hexadecen-1-ol (Z11-16:OH). Besides of *M. brassicae*, the cruciferous plants are host plants of *Phyllothreta cruciferae*, *P. striolata* and more *Phyllotreta* species. *Phyllotreta* spp. can severely damage several cruciferous cultures, such as cabbage, kale, radish, oilseed rape, and other at the beginning of growth period (Alford et al., 1991).

Isothiocyanates have been implicated as chemical cues mediating host-finding behaviour of crucifer-feeding specialist insects and their natural enemies (Pivnik et al., 1994). Cruciferous plants are preferred host plants of *M. brassicae* and it was demonstrated that only mated females flew upwind to allyl NCS, but not virgin females or males (Rojas, J. C. 1999).

Allyl NCS was attractive to *Phyllothreta cruciferae* and *P. striolata* in field trapping experiments. (Pivnick et al., 1992,). Evaporation rates used in field tests were between 0.2 and 3.0 mg/day, this exceeds the evaporation rate of pheromones about a 1000 times. It has been noted that release rate of 4 mg/day is equivalent to the release from 2000 freshly damaged plants or 270 000 intact plants of *Brassica juncea* at the flower bud stage (Pivnick et Jarvis, 1991). In our experiments some other isothiocyanates and thiocyanates were used in similar doses but remarkably higher efficacy was achieved.

The pollen beetles, *Meligethes* spp., are attracted to different isothiocyanates (Fenwick et al, 1983, Finch 1977). Later it was demonstrated that mixtures of the alkyl isothiocyanates with or without 2-phenylethyl NCS are generally more attractive than individual isothiocyanates, but this may be ascribed to the high total release rates, 35 – 53 mg/day (Blight and Smart, 1999).

Plant semiochemicals

Use of pheromones and other semiochemicals in integrated production
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Chemical characterisation of corn plant compounds by different extraction techniques and the role of potent chemicals in the reproductive behaviour of the corn stalk borer *Sesamia nonagrioides*

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Abstract: Plant chemistry is probably the most important source of information contributing to the final decision by an insect to select a host and it is actually the balance of opposing positive and negative cues evoked by phytochemicals that determines whether a plant is accepted or rejected by a herbivore. Two different techniques, a modified steam distillation extraction and a solid phase microextraction (SPME) technique for the extraction of plant volatile chemicals from different corn hybrids were utilised and the extracts were analysed and characterised by GC-MS. For the steam distillation technique, plant material was steam distilled for 4 h in a modified apparatus with a water-cooled oil receiver, to reduce hydrodistillation overheating artifacts and the extract after appropriate treatment was analysed by GC-MS. The SPME fibre was introduced in a 40ml vial that contained plant material and left to collect analytes for 60 min. Following collection, analytes were chromatographed and analysed by GC-MS. The two techniques are compared in terms of extraction efficiency and for both qualitative and quantitative differences in the chemical profile of the extract. The oviposition preference of the corn stalk borer, *Sesamia nonagrioides*, was evaluated in bioassays using artificial substrates impregnated with the steam distillation extract. The chemical composition of the steam distillation extract of different corn hybrids obtained is correlated to the egg laying behaviour of the insect and the role of potent chemicals on this behaviour is discussed.

Key words: *Sesamia nonagrioides*, plant chemicals, oviposition preference, corn hybrids,

Introduction

Host plant recognition and selection in Lepidoptera is primarily a function of the ovipositing female and since newly emerged larvae are often limited in their dispersal abilities, oviposition is particularly crucial as it determines survival of their progeny (Renwick, 1989).

Visual factors such as shape, colour and size, in many cases studied, were significant (Renwick and Chew, 1994), but chemical cues unambiguously play the major, if not decisive, role in host selection (Udayagiri and Mason, 1995). The latter must be especially true for insects that are active during the night. Plant chemistry is probably the most important source of information contributing to the final decision by an insect to oviposit or not and it is actually the balance of opposing positive and negative cues evoked by phytochemicals that determines whether a plant is accepted or rejected by a herbivore (Huang and Renwick, 1993, Renwick and Chew, 1994).

Chemical cues of corn stimulate oviposition in the European corn borer (Lupoli et al., 1990). Pentane leaf extracts of corn containing *n*-alkanes stimulate oviposition of *Ostrinia nubilalis* (Udayagiri and Mason, 1995, 1997). On the other hand corn methanol extracts received fewer *O. nubilalis* eggs than the methanol controls (Udayagiri and Mason, 1995). A methanol soluble oviposition deterrent chemical has been discovered in the frass of fifth instar larvae of *O. nubilalis* (Dittrick et al., 1983). Derridj et al. (1986, 1992) have reported that

females of *O. nubilalis* discriminate corn cultivars based on carbohydrate content.

The corn stalk borer *Sesamia nonagrioides* (Lef) major pest of maize in the Mediterranean countries is considered as an oligophagus pest and it attacks plants of the *Graminae* family, with its major host being the corn plant. Corn hybrids that are resistant to corn stalk borer have been identified but the defensive mechanisms involved are as yet undermined (Butron et al., 1998, Velasco et al., 1999). Selection of corn hybrids that are not preferred as host plants for oviposition by the corn stalk borer is valuable for the development of insect-resistant plants.

This paper reports comparative studies on the chemical composition of corn hybrids, employ two extraction methods steam distillation and solid phase microextraction and on the effects of steam distillation extracts to the egg laying behaviour of the corn stalk borer.

Material and methods

Insects.

The insects used were obtained from a laboratory colony maintained under a 16:8 h L:D regime at 25 ± 1 °C and 65 ± 5 % RH, on artificial diet (Tsitsipis et al., 1983). Aqueous sugar solution 10% was offered as food to the adults.

Plants.

Eight corn (*Zea mays*) hybrids 33A14, 3211, 31B13, 3283W, 33R87, Konstanza kindly provided by Pioneer Hi-Bred International, Inc and two Greek corn hybrids (Dias and Aris) were used. Plants from seeds (F1 generation) derived from commercially available Konstanza hybrid in Greece were used as a susceptible control. Corn seeds were planted in small pots, in a greenhouse under 25 ± 1 °C and 65 ± 5 % RH. When the plants had developed 6-8 leaves they were used for the collection of chemicals.

Collection of plant chemicals - steam distillation.

Corn leaves (25 g, 6-8 leaf stage) were steam distilled for 4 h in a steam distillation apparatus with a water-cooled oil receiver, to reduce hydrodistillation overheating artifacts (Roussis et. al., 1995). The chemicals carried by water vapours were condensed and trapped in a layer of diethyl ether. The ether layer was dried over magnesium sulfate to remove residual water, concentrated to 200 µl under a gentle stream of nitrogen and stored at –20 °C until further analysis. For each hybrid, three replicates were performed. For the oviposition preference test, the concentrated extract of each hybrid was properly diluted to give an equivalent concentration of 1 mg of leaves per µl of extract.

Collection of plant chemicals - SPME.

Corn leaves (1g, 6-8 leaf stage) were placed in a 22 ml headspace vial and covered with 11 ml of a saturated NaCl solution. The vial was placed on top of a hot plate, continuous stirring was applied by means of a magnetic stirrer and the content of the vial was heated to 40 °C. A 100 µm polydimethylsiloxane (PDMS) fibre (Supelco, USA) was exposed on the headspace for 90 min and was subsequently desorbed into the injection port of the GC-MS system for 5 min.

Oviposition preference tests.

Oviposition preference of females was examined in a two-choice bioassay. A sheet of 20 cm x 20 cm common filter paper (65 g/m^2, Filtrak, Spezialpapier Filtrak Niederschlag, D-09484,

Germany) was placed on top of a 21 cm x 21 cm A4 paper (80 g/m^2) and the two sheets were rolled together diagonally to form a tube. The paper rolls were placed in small vials containing water to keep the moisture high. In each cage (30 cm x 30 cm x 30 cm) there were two paper rolls. One was loaded with 500 µl of the steam distillation extract of a hybrid (equivalent to 500 mg of leaves) and one with 500 µl of solvent. One pair of 2-d-old insects was released and the number of eggs deposited was recorded 48 h later. Data for volatiles of each hybrid and the controls were collected from 10 replications for each hybrid.

Gas chromatography-mass spectrometry.

Gas chromatography-mass spectrometry analysis was carried out on a Hewlett Packard 5890 Series II gas chromatograph interfaced to a Fisons VG Trio 1000 (Manchester M23 9BE, UK) quadrupole mass spectrometer. Electron impact ionization was used, with an electron energy of 70 eV and a trap current of 200 µA. All extracts were chromatographed on a 60 m x 0.25 mm(id) x 0.1 µm film thickness DB-5 column (J&W Scientific, Fisons). The oven temperature program was 50 °C for 2 min, then 5 °C/min to 250 °C hold for 1 min, then 2 °C/min to 280 °C and hold for 50 min. Helium was used as the carrier gas at a flow rate of 1 ml/min. Splitless injections were made (1 µl) at an injector temperature of 250 °C and a splitless period of 90 s. Prior to GC-MS analysis, 50 µg of *n*-tetradecane was added into the vial to serve as an internal standard.

Statistics.

For statistical analysis, an index of oviposition, the oviposition index (OI) was calculated according to the formula (Udayagiri and Mason, 1997).

$$OI = \frac{(\text{N eggs on hybrid} - \text{N eggs on control})}{(\text{N eggs on hybrid} + \text{N eggs on control})}$$

The OI takes positive or negative values indicating hybrids that are preferred by females for oviposition (positive values) and those that are not preferred (negative values). For comparison of the mean OI in the oviposition preference experiments the non-parametric Kruskal-Wallis test was used.

For statistical analysis of the chemical compounds present in the steam distillation extracts, only the peaks with a relative area (area of peak/total area) greater than 0.1% that had been detected in at least two of the three replicates for each hybrid were used as variables. To investigate any association of chemical composition and oviposition preference, data of all constituents, which met the above-mentioned criteria, were subjected to principal component analysis. Principal component analysis was performed on the correlation matrix and principal components were rotated using the varimax orthogonal rotation method. (Jolliffe 1986)

Results

Extraction techniques.

Comparative GC-MS analysis of the steam distillation and the SPME extracts of corn leaves revealed both qualitative and quantitative differences for all hybrids evaluated. Approximately forty compounds were detected, characterised and quantified on the basis of retention time data and mass spectra, as constituents of each of the steam distillation and the SPME extract. The results, in terms of chemical compound classes are summarised in Figure 1.

The two techniques compare well for compounds such as aldehydes, esters, hydrocarbons and ketones. The major differences are mainly on the alcohol and the terpenoid content of the two extracts. Although the alcohol content of the two extracts was qualitatively

the same (four compounds were detected and quantified for both techniques), steam distillation extracted a higher amount of alcohols with phytol, a hydlolysis product of chlorophyl, accounting for 53% of the total 63% of the alcohol content of the extract. For SPME, phytol accounted for only 5.7% of the total area. The efficiency of SPME to extract compounds depends, among other factors, on the affinity of the fibre coating to certain chemical characteristics, such as polarity, of the compound to be extracted. During this investigation a non-polar polydimethylsiloxane fibre was used and this can explain the lower content of polar compounds on the SPME extract. The two techniques differed both qualitatively and quantitatively on the terpenoid content. A total of fourteen compounds that accounted for the 43.5% of the total area were quantified on the SPME extract, whilst the steam distillation extract contained a total of six compounds accounting for only the 2.9% of the total area. In order to investigate possible role of corn leaves chemicals on the reproductive behaviour of *S. nonagrioides*, a physical means of performing bioassays was needed and thus the use of the steam distillation extracts in two choice bioassays was further explored.

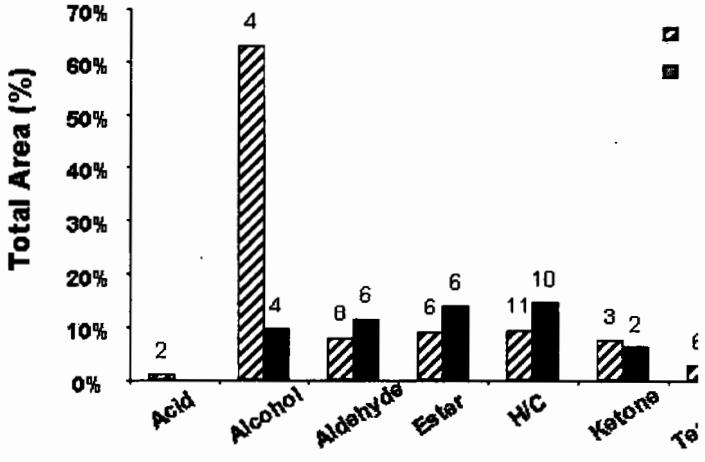

Figure 1. Distribution of chemical compound classes in corn leave extracts as determined by SPME and SD.

Oviposition preference.

Statistical analysis (Wilcoxon signed-ranks test, P=0.05) between solvent (diethyl ether) and steam distillation extracts of each hybrid revealed that there is a significant difference when chemicals of hybrids 3211, 31B13, 33A14 and Dias were compared to their respective controls (Table 1).

OI values (Kruskal Wallis test, X^2=15.734, df=7, P=0.028) make evident that there is a significant difference in the OI values between the eight hybrids tested: the most distinct difference occurs between the hybrids 33R87 and Dias (Fig. 2).

Table 1. Oviposition response of *Sesamia nonagrioides* females to hybrid volatiles in two-choice bioassays. Statistical analysis of the Oviposition Index (OI) for all hybrids and hybrid volatiles evaluated.

Hybrid	Mean OI Plants volatiles
3211	0.45 [a] (P=0.047)
Aris	-0.20 (P=0.594)
33A14	0.60 [a] (P=0.041)
33R87	-0.46 (P=0.059)
Pioneer	0.19 (P=0.646)
31B13	0.58 [a] (P=0.037)
Dias	0.59 [a] (P=0.021)

[a] Significant difference in egg laying between the control and the hybrid as determined by Wilcoxon signed-ranks test at P=0.05.

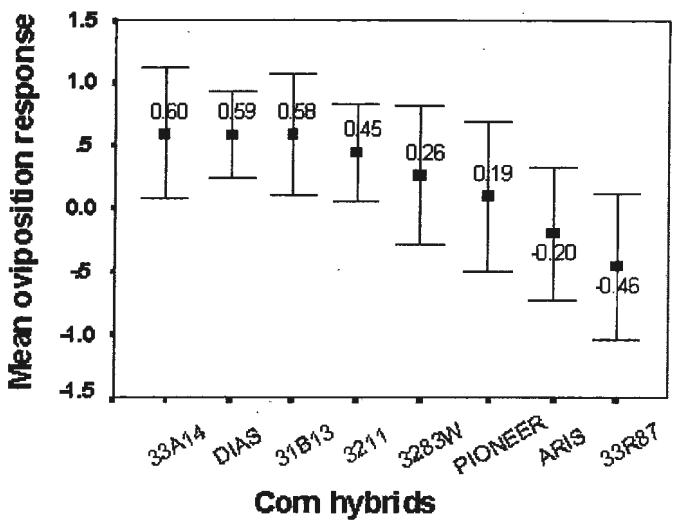

Figure 2. Oviposition response of *S. nonagrioides* females to corn hybrids volatiles [Kruskal-Wallis test, P=0.05 (Mean OI +/- SE)]

Steam distillation extracts.

All peaks detected in the steam distillation extracts of the eight corn hybrids were subjected to principal component analysis resulting to the extraction of 7 principal components (PCs) that accounted for 100% of the total variation of the original data set. In order to evaluate any association of the oviposition preference of the corn stalk borer females as indicated by the bioassays and the chemical composition of the different hybrids, Pearson's correlation

coefficients were computed for the OI and all 7 principal components. It was found that OI for plant chemicals was significantly correlated to Principal Component 6 (PC6) [Pearson's correlation coefficient (two-tailed) = -0.752, P = 0.031] (Figure 3) .

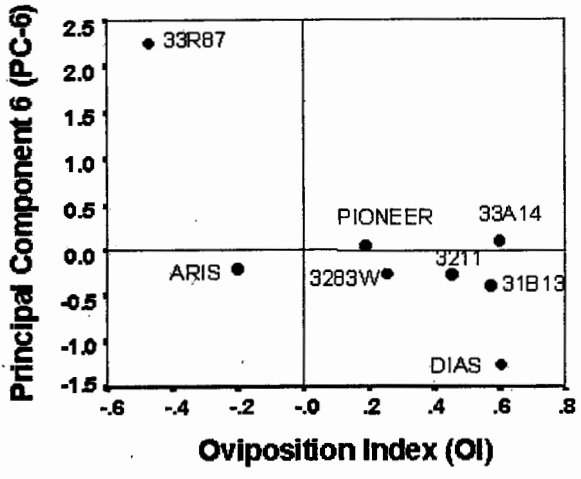

Figure 3. Scaterplot of PC-6 and Oviposition Index (OI)

Table 2. Simple correlation coefficient of chemical compounds used for statistics and Principal Component factor 6 (PC6) as extracted from principal components analysis.

Compound	PC6	Compound	PC6
(Z)-3-Hexenol	-0.118	α-Farnesene	-0.070
Terpenoid	0.031	Tetradecanal	0.295
(Z)-3-Hexenyl acetate	-0.193	Nonadecanol	0.111
Linalool	0.272	Cadinol	-0.036
Nonanal	-0.177	Unknown III (Terpenoid)	-0.156
Decanal	0.533	Pentadecanal	0.855
Propenoic Acid, Hydroxy Phenyl	-0.071	Neophytadiene	-0.042
Trans-caryophyllene	-0.083	Hexahydrofarnesyl Acetone	0.789
Ionone	-0.306	Neophytadiene (isomer)	-0.172
Uknown II(Terpenoid)	-0.157	Eicosatrienoic acid, methyl ester	0.789
Nerolidol	-0.147	Heptadecanal	-0.246
Heptadecanal	-0.246	Hexadecenol tetramethyl	-0.162
Hexadecenol tetramethyl	-0.162	Octadecenoic acid	0.531
Octadecenoic acid	0.531	Heneicosane	0.010
Heneicosane	0.010		
Phytol	-0.123		

The coefficients presented in Table 2 indicate how these two principal components are correlated to the original variables, that is the chemical compounds detected in the steam distillation fraction of the eight hybrids. The coefficient for each compound determines how 'important' that compound is for the particular principal component. A high positive or negative value (a high loading) for the coefficient of a certain compound indicates that this compound has a strong influence on the particular principal component.

Principal component 6 separates hybrid 33R87 from the rest of the other hybrids examined. Statistical analysis of the OI values for plant chemicals (Figure 2) revealed that the two hybrids that differ most are 33R87 and Dias. These two hybrids lie on the two extreme parts of the PC6 axis [33R87 positive part of the axis and Dias negative part of the axis (Figure 3)].

In terms of chemical compounds, PC6 is dominated by pentadecanal, hydroxyfarnesyl acetone and eicosatrienoic acid methyl ester, all with a high positive loading. Hybrids with high positive values on that component (that is hybrids that are not preferred for oviposition; negative OI values, e.g 33R87) have large amounts of pentadecanal, hexahydrofarnesyl acetone and eicosatrienoic acid methyl ester and these could be the compounds responsible for oviposition deterrent activity. Further studies with pure synthetic compounds are in progress to verify these findings.

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Use of pheromones and other semiochemicals in integrated production
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Utilisation des composés allélochimiques des *Allium* en tant qu'insecticides

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Résumé: Le bromure de méthyle, le fumigant le plus largement utilisé, est un des facteurs importants de la réduction de la couche d'ozone. Son interdiction d'utilisation est prévue pour 2005. Une alternative serait l'emploi de fumigants naturels issus de plantes. Les espèces du genre *Allium* (poireau, oignon, ail) présentent des propriétés insecticides et fongicides bien connues. Elles sont utilisées en cultures associées et leurs composés allélochimiques soufrés présentent des propriétés répulsives et des effets anti-appétents vis à vis des insectes. Les propriétés des composés volatils des *Allium* (les thiosulfinates, R-S-S-O-R' ; R,R' = Me, Pr, Al) ont été évaluées sur des insectes des denrées stockées et comparées à celles de leurs produits de dégradation (les disulfures) déjà testés et avec celles d'un fumigant commercial de synthèse. Un disélénure présent en faible quantité chez les *Allium* a aussi été évalué sur *Tineola bisselliella*. Le disélénure de diméthyle a une LC50 environ 10 fois plus faible que le disulfure correspondant. Les thiosulfinates ont été testés sur *Callosobruchus maculatus*, *Sitophilus oryzae*, *S. granarius*, *Ephestia kuehniella* and *Plodia interpunctella*. Les thiosulfinates de diméthyle et de diallyle apparaissent plus toxiques que les disulfures sur tous les insectes testés. Ils ont une LC50 (24h) comprise entre 0.02 et 0.25 mg/l. Ils montrent même une activité insecticide plus élevée que le bromure de méthyle. Ceci suggère que les thiosulfinates pourraient être utilisés pour le contrôle des ravageurs des denrées stockées, spécialement en protection intégrée puisque ce sont des composés labiles rapidement dégradables.

Mots clefs: disulfures, thiosulfinates, insecticides, fumigation, bromure de méthyle

Potential of Allium allelochemicals for safe insect control

Abstract: Methyl bromide, the most widely used fumigant, is responsible for 10% of all the factors causing the depletion of the ozone layer. Its deregistration is schedulated for 2005. An alternative strategy is the use of natural fumigants of plant origin. For centuries, *Allium* species (garlic, onion, leek) are used in inter-cropping as they show well-known effects on many polyphagous insects and fungi . They produce characteristic sulfur allelochemicals with repellent and antifeedant effects against insects. *Allium* volatiles (thiosulfinates, R-S-SO-R' ; R,R' = Me, Pr, Al) were evaluated as insecticides against stored products pests comparatively with their degradation compounds (disulfides) already tested and with synthetic commercial fumigants. An *Allium* minor diselenide was also evaluated. The LC50 of this dimethyldiselenide seemed 10 times lower than the LC50 of the corresponding disulfures on *Tineolla bisselliella*. Thiosulfinates were tested on *Callosobruchus maculatus, Sitophilus oryzae, Sitophilus granarius, Ephestia kuehniella* and *Plodia interpunctella*. Me and Al thiosulfinates appeared to be more toxic than disulfides to all the insects tested. Their LC50 (24h) varied between 0.02 to 0.25 mg per liter. Thiosulfinates showed an insecticide activity stronger than methyl bromide itself. It is thus suggested that thiosulfinates could be used for controlling stored products pests, specially in Integrated Control as they are very labile compounds rapidly degradated.

Keywords: disulfides, thiosulfinates, insecticides, fumigation, methyl bromide.

Introduction

Les composés secondaires sont souvent considérés comme étant un moyen de défense de la plante productrice contre divers organismes comme les pathogènes et les ravageurs (Fraenkel, 1959). Ces composés sont très nombreux et variés, et certains sont largement distribués, comme les alcaloïdes, les terpènes et les tanins, tandis que d'autres ont une répartition plus restreinte comme les composés soufrés présents notamment chez les *Allium*.

Dans le genre *Allium* (Liliaceae), on trouve principalement des acides aminés soufrés non protéiques, les alk(en)ylcystéine sulfoxides. Leurs dérivés dipeptidiques de l'acide glutamique sont également présents en grande quantité, leur proportion pouvant atteindre 5 % du poids sec (Lancaster et al., 1988). Ces alk(en)ylcystéine sulfoxides sont au nombre de quatre (Granroth, 1970) : la S-méthyl-L-cystéine sulfoxide (MeCSO), présente en faible proportion dans tous les *Allium* cultivés mais prépondérante chez certaines espèces sauvages et ornementales, la S-propyl-L-cystéine sulfoxide (PrCSO), présente surtout chez le poireau *Allium porrum*, la S-1-propényl-L-cystéine sulfoxide (PeCSO), prépondérante chez l'oignon *Allium cepa*, et la S-allyl-L-cystéine sulfoxide (AlCSO) ou alliine, prépondérante chez l'ail *Allium sativum*. Les proportions de ces 4 composés varient non seulement d'une espèce à l'autre (Freeman, 1975), mais également à l'intérieur d'une espèce selon l'organe, la variété, le stade de développement et les conditions environnementales considérés (Boscher et al., 1995).

Ces dérivés de la cystéine sont très spécifiques des *Allium* puisqu'il n'en a été trouvé qu'en petite quantité dans d'autres monocotylédones, notamment des Liliacées (Akashi et al., 1975).

Chez les *Allium*, les dipeptides sont stockés dans le cytoplasme des cellules (Lancaster et Collin, 1981) et libérés sous l'influence de la γ-glutamylpeptidase (Austin et Schwimmer, 1970).

La plupart des activités pesticides liées aux *Allium* sont cependant dues à des substances volatiles dérivées de ces acides aminés. Celles-ci sont émises lors de la destruction des cellules. Les acides aminés précurseurs sont alors mis en présence d'une enzyme, l'alliinase ou alliinealkylsufinate lyase (EC 4.4.1.4) (Stoll et Seebeck, 1949), présente dans les vacuoles (Lancaster et Collin, 1981), qui provoque, après la coupure de la liaison C-S (Kupiecki et Virtanen, 1960), la synthèse de toute une série de composés soufrés volatils (Schwimmer et Friedman, 1972 ; Ferary et Auger, 1996 ; Ferary et al., 1996 ; Jaillais et al., 1999). Cette enzyme est absente chez les autres Liliaceae (Tsuno, 1958) mais existe par exemple chez les Crucifères (Mazelis, 1963) et chez des Légumineuses (Schwimmer et Kjaer, 1960 ; Mazelis et Fowden, 1973).

Cette réaction conduit à la formation d'acide sulfénique dont les molécules se réarrangent deux à deux pour former des thiosulfinates (Ti) (Cavallito et Bailey, 1944 ; Stoll et Seebeck, 1949) symétriques ou mixtes selon les précurseurs présents. Dans le cas du PeCSO, il y a formation essentiellement d'oxyde de propanethial responsable de l'effet lacrymogène de l'oignon (Virtanen et Spare, 1961).

Les Ti, eux-mêmes assez instables excepté en solution aqueuse et à l'état gazeux, n'ont été mis en évidence que récemment (Auger et Thibout, 1981 ; Auger et al., 1990 ; Ferary et al., 1998). Le thiosulfinate de diallyle ou allicine ($TiAl_2$) donnera ainsi 66 % de disulfure (DS), 14 % de sulfure (S) et 9 % de trisulfure (TS), des thiosulfonates (To), des vinyldithiines et de l'ajoène en proportions variables selon les conditions de dégradation (Block et al., 1984). A cette chimie secondaire soufrée est associée une chimie secondaire du sélénium (Se) beaucoup moins explorée (1000 à 10000 fois moins abondant) où le sélénium se substitue au soufre dans la plupart de ses composés aussi bien précurseurs que volatiles

comme les disélénures (DSe) (McSheehy et al., 2000).

Ce sont, par ordre de fréquence, les DS, les TS et les Ti dont les activités biologiques ont été les plus étudiées car en fait les DS et TS sont mis en évidence en premier lieu lorsqu'on utilise les méthodes d'analyse classiques (Arnault et al., 2000).

Plusieurs études réalisées sur divers ordres d'insectes ont montré la toxicité de ces composés. Mais à côté des effets négatifs des composés soufrés des *Allium* sur plusieurs insectes, souvent des généralistes ou des insectes non inféodés aux *Allium*, ces composés peuvent également avoir des effets positifs sur les insectes spécialistes se développant aux dépens des *Allium*. Plusieurs travaux sur deux des principaux insectes ravageurs des cultures d'oignon et de poireau, la mouche de l'oignon, *Delia antiqua* et la teigne du poireau, *Acrolepiopsis assectella*, ont étudié les comportements locomoteurs et particulièrement l'attraction de ces deux insectes par les composés soufrés. Ces études ouvrent la voie à l'utilisation efficace de pièges pour protéger les cultures.

Chez *A. assectella*, 7 composés soufrés des *Allium* ont été testés. A l'exception du disulfure de diallyle ($DSAl_2$) peu attractif, le disulfure de dipropyle ($DSPr_2$), disulfure de diméthyle ($DSMe_2$), le thiosulfonate de dipropyle ($TOPr_2$) et thiosulfonate de diméthyle ($TOMe_2$) sont attractifs pour les femelles comme pour les mâles, mais ce sont le thiosulfinate de diméthyle ($TiMe_2$) et surtout le thiosulfinate de dipropyle ($TiPr_2$) qui sont les plus attractifs (Lecomte et Thibout, 1981 ; Thibout et al., 1982). La teigne du poireau est donc essentiellement attirée par les composés volatils réellement émis par leur plante-hôte.

Chez *D. antiqua*, les études à l'aide de pièges se sont restreintes à l'effet du $DSPr_2$. A la différence des mouches japonaises, celles d'Europe et d'Amérique du nord sont attirées par ce composé (Matsumoto, 1970 ; Ishikawa et al., 1981 ; Weston et Miller, 1985 ; Judd et Borden, 1991). D'autres mouches, telles *Phormia regina* et plusieurs espèces de *Lucilia* sont aussi attirées par ces pièges (Matsumoto, 1970).

Signalons enfin les effets des Ti et des DS sur le comportement locomoteur de l'hyménoptère parasitoïde de la teigne du poireau *Diadromus pulchellus*. Cet insecte est surtout sensible au $DSPr_2$ qui est émis en quantité par les fèces de son hôte (Lecomte et Thibout, 1984 ; Auger et al., 1989b).

Dans cette étude les composés soufrés des *Allium* ont été testés sur des insectes ravageurs des denrées stockées, coléoptères et lépidoptères, et sur un ravageur des vêtements et des textiles, la mite, *Tineola bisselliella*.

Afin d'analyser si l'emploi de telles substances en tant que substitut du bromure de méthyle est compatible avec la lutte biologique contre les insectes des denrées stockées, nous avons également testé l'influence de ces composés sur des hyménoptères parasitoïdes, ennemis naturels de ces ravageurs, notamment *Dinarmus basalis* qui se développe aux dépens des ravageurs *Callosobruchus maculatus* et *Bruchidius atrolineatus*.

Matériel et méthode

Elevage des insectes.

Tous les insectes, âgés d'une semaine, sont élevés avec une photopériode L D : 16-8 et une humidité relative de 75% +/- 10%. *Sitophilus orizae*, *Sitophilus granarius*, *Ephestia kuehniella* et *Plodia interpunctella* sont placés à une thermopériode de 25°C-17°C synchrone à la photopériode. *S. orizae* est élevé sur du blé, *S. granarius* sur du maïs, *E. kuehniella* sur la farine de blé, et *P. interpunctella* sur du maïs broyé additionné de glycérol. Les coléoptères bruchidae *C. maculatus* et *B. atrolineatus* ainsi que leur hyménoptère parasitoïde *D. basalis* sont élevés sur le niébé (*Vigna unguiculata*) à une température constante de 30°C . La mite *T. bisselliella* est élevée sur des peaux de lapin à la température constante de 22.5°C.

Composés utilisés.
Le bromure de méthyle (BrMe), le $DSMe_2$, le $DSAl_2$ et le disélénure de diméthyle ($DSeMe_2$) proviennent de chez Aldrich. Le thiosulfinate de diallyle ($TiAl_2$) et le $TiMe_2$ sont synthétisés au laboratoire avant utilisation. L'extrait d'ail (Gar Vitan) à teneur mesurée de 0.3mg/ml d'allicine est fourni par Sucaf Pharma (Israël).

Tests de fumigation.
Les chambres de fumigation sont des cristallisoirs en verre de 12 litres scellés, étanches. Les composés sont introduits dans l'enceinte sous forme liquide et sont déposés sur un papier filtre Whatman. Ils diffusent alors sous forme gazeuse à l'intérieur du cristallisoir. L'exposition des insectes aux composés dure 24 heures. Les CL 50, déterminées par la méthode des Probits, un jour après, sont exprimées en milligramme de fumigant par litre d'air.

Résultats

Le tableau 1 montre que les insectes utilisés dans nos expériences sont tous sensibles aux composés testés. Les CL 50 varient de 0.02 mg/l (*E. kuehniella* en présence de $TiAl_2$ et de $DSAl_2$) à 1.23 mg/l (*S. orizae* en présence de $DSMe_2$).

Tableau 1. CL 50 (exprimées en mg/l) pour 5 espèces d'insectes adultes des denrées stockées après une exposition de 24h aux thiosulfinates (Ti) et disulfures (DS) de diméthyle (Me_2), diallyle (Al_2) et au bromure de méthyle (BrMe).

Insectes	Fumigants				
	$TiMe_2$	$TiAl_2$	$DSMe_2$	$DSAl_2$	BrMe
Ephestia kuehniella	0.04	0.02	0.17	0.02	-
Plodia interpunctella	0.02	-	-	-	-
Sitophilus granarius	0.14	-	-	-	-
Sitophilus orizae	0.19	-	1.23	-	1.05
Callosobruchus maculatus	0.25	0.16	0.65	0.40	-

Toutefois les insectes présentent des différences de sensibilité en fonction de l'espèce et du composé considéré. Il semble que les deux espèces de lépidoptères *E. kuehniella* et *P. interpunctella* soient les plus sensibles notamment au $TiMe_2$. Quel que soit le composé testé, les trois espèces de coléoptères (*S. granarius*, *S. orizae*, *C. maculatus*) ont des CL 50 toujours supérieures à celle des lépidoptères.

Les CL 50 des Ti sont comparables pour une même espèce, par exemple chez *E. kuehniella*, elle est de 0.02 mg/l en présence $TiAl_2$ et de 0.04 mg/l en présence de $TiMe_2$. Chez *C. maculatus* la CL 50 est de 0.25 mg/l avec le $TiMe_2$ et de 0.16 mg/l avec le $TiAl_2$. Les Ti apparaissent comme étant plus toxiques que les DS et même que le BrMe pour une même espèce.

Le tableau 2 montre la sensibilité de deux espèces de coléoptères ravageur de denrées stockées et notamment des graines de niébé et de leur parasitoïde *D. basalis* vis à vis du $DSMe_2$ et du $DSAl_2$ ainsi que d'un extrait d'ail.

Tous les composés testés sont toxiques vis à vis des différents stades de développement des insectes étudiés et les CL 50 varient de 0.17 mg/l (pour les œufs de *C. maculatus*) à 2.04 mg/l (pour les larves L_4 de *C. maculatus*).

Tableau 2. CL 50 (exprimées en mg/l) chez deux espèces de ravageurs des denrées stockées *C. maculatus*, *B. atrolineatus* et leur parasitoïde *D. basalis* pour une exposition de 24h aux disulfures (DS) de diméthyle (Me_2), diallyle (Al_2) et au $TiAl_2$ présent dans un extrait d'ail.

Insectes		Fumigants		
		$DSMe_2$	$DSAl_2$	Extrait d'ail
Callosobruchus	Adultes	0.65	0.40	0.37
maculatus	Larves L4	2.04	-	-
	Oeufs	0.17	-	-
Bruchidus	Adultes	0.23	-	-
atrolineatus	Larves L4	2.0	-	-
Dinarmus	Adultes	0.31	0.35	0.14
basalis	Larves L4	1.58	-	-

Il apparaît clairement que les stades adultes sont plus sensibles que les stades larvaires pour les trois espèces considérées.

Les deux composés purs semblent par ailleurs moins efficaces que l'extrait d'ail, correspondant à une dose contrôlée en allicine, pour lequel les CL 50 sont toujours moins élevées, bien quc plus importantes pour le $TiAl_2$ (tableau 1).

Enfin nous pouvons constater également que les phytophages ravageurs sont moins sensibles que leur hyménoptère parasitoïde. Ce résultat implique des conséquences négatives sur une utilisation éventuelle des composés soufrés en lutte intégrée contre ces ravageurs en présence de *D. basalis*.

Tableau 3. CL 50 (exprimées en mg/l) chez la mite *Tineola bisseliella* après une exposition de 24h aux disulfures (DS) de dipropyle (Pr_2), diallyle (Al_2), diméthyle (Me_2) et au disélénure (DSe) de diméthyle.

Insectes	Fumigants		
	$DSPr_2$	$DSAl_2$	$DSeMe_2$
Tineolla bisselliella	1.26	0.013	0.002

Il apparaît enfin que la mite *T. bisselliella* est également très sensible vis à vis des composés des *Allium* (tableau 3) avec une forte différence de sensibilité en fonction des composés. Le $DSAl_2$ est beaucoup plus toxique que le $DSPr_2$. Le $DSeMe_2$ est le composé pour lequel la CL 50 est la plus faible, cette substance semblant très efficace contre cette espèce.

Discussion

Les composés soufrés des *Allium* sont donc toxiques pour de nombreuses espèces d'insectes en milieu clos. Outre les effets toxiques, des cas d'effets antiappétants ont été observés. Des extraits d'ail perturbent la prise alimentaire du coléoptère *Epilachna varivestis* (Nasseh, 1981). Le comportement de ponte chez deux lépidoptères *Pieris brassicae* et *P. napi* est inhibé par des extraits d'oignon (Lundgren, 1975). Pareillement, des extraits d'ail réduisent significativement le taux de ponte des femelles de psylle du poirier, *Cocopsylla pyricola* (Weissling et al., 1997). Des extraits d'ail et d'oignon perturbent également l'établissement du puceron *Myzus persicae* sur sa plante hôte et empêchent l'alimentation de l'insecte, entraînant le cas échéant la mort de celui-ci (Hori, 1996).

Les effets répulsifs les plus souvent décrits ont des conséquences sur le comportement locomoteur de nombreux insectes. Ainsi, l'odeur d'oignon est répulsive pour la mouche du chou, *Delia (brassicae) radicum* (Prokopy et al., 1983), ainsi qu'un extrait d'ail pour le moucheron *Simulium indicum* et le moustique *Culex fatigans* (Bhuyan et al., 1974).

Des extraits d'*Allium* où la teneur en Ti a été vérifiée, ont montré une activité répulsive pour trois espèces de coléoptères des denrées stockées (Trematerra et Lanzotti, 1999).

L'étude des effets négatifs des *Allium* a surtout porté sur la physiologie des insectes. Des perturbations du développement ont été observées chez la coccinelle *Epilachna varivestis* en présence d'extraits d'ail (Nasseh, 1981). Des applications topiques d'extraits d'ail chez les larves de 5ème stade de *Spodoptera litura* présentent une activité juvénomimétique qui perturbe le développement et la mue, entraînant la mort de l'insecte (Suryakala et al., 1984). Une mortalité larvaire plus importante est obtenue chez le parasitoïde *Diadromus pulchellus* lorsqu'il se développe sur son hôte la teigne du poireau, *A. assectella*, alimentée à l'aide d'une nourriture riche en *Allium* (Bekkaoui et Thibout, 1992).

Divers ordres d'insectes sont sensibles aux effets insecticides des *Allium*, en particulier aux extraits d'ail. Ils se révèlent toxiques pour les pucerons *Sitobion avenae* et *Rhopalosiphum padi* (Nasseh, 1983), pour le criquet *Schistocerca gregaria* (Thapar et Chandra, 1981), pour les larves de doryphore, *Leptinotarsa decemlineata*, et de piéride du chou, *P. brassicae* (Greenstock, 1970, in Amonkar et Banerji, 1971), pour la teigne de la pomme de terre, *Phthorimaea operculella* (Nasseh, 1992), pour cinq espèces de moustiques des genres *Culex* et *Aedes* (Amonkar et Reeves, 1970), pour les puces (Renapurkar et Deshmuskh, 1984), pour la mouche *Musca domestica* et pour le coléoptère *Trogoderma granarium* (Bhatnagar-Thomas et al., 1974). Chez ces deux dernières espèces, les auteurs ont montré que l'extrait avait une action anticholinestérasique.

Chez la mouche blanche des serres, *Bemisia argentifolii*, les œufs, les nymphes et les adultes sont sensibles à la présence de divers extraits d'ail (Flint et al., 1995). De même les œufs, les larves et les adultes des coléoptères des stocks *Tribolium castaneum* et *Sitophilus zeamais* présentent une mortalité qui dépend de la concentration de l'extrait d'ail utilisé (Ho et al., 1996). Une action ovicide de l'ail frais a été mise en évidence sur la punaise *Dysdercus koenigii* et les noctuelles *Earias vitella, Spodoptera litura* et *Helicoverpa armigera*. Les individus qui parviennent à éclore ne termineront leur développement qu'en très faible proportion (Gurusubramanian et Krischna, 1996).

Le poireau s'est révélé toxique pour *Drosophila melanogaster* (Lecuyer, 1975). Dans ce cas, la toxicité de la plante varie selon les saisons, l'apport en sulfate ou encore les diverses parties utilisées (Thibout et Auger, 1997). Il faut noter que, parmi les insectes testés, chez *Acanthoscelides obtectus*, la bruche du haricot, la ponte et le développement sont assez peu sensibles aux composés volatils émis par les gousses d'ail frais (Regnault-Roger et Hamraoui,

1993) et que, chez *Myzus persicae*, Nasseh (1983) observe une diminution de la mortalité après traitement à l'aide d'un extrait d'ail, ceci en contradiction avec les résultats de Hori (1996) cités plus haut.

Une autre manière d'utiliser les propriétés des *Allium* dans la lutte contre les insectes est celle des cultures associées dans laquelle les effets des diverses plantes sont censés perturber le comportement des spécialistes. A notre connaissance, quatre expériences de ce type ont été réalisées. La première associe l'oignon et la carotte, *Daucus carota* (Uva et Coaker, 1984). Les auteurs observent alors sur les carottes une diminution des attaques de mouche de la carotte, *Psila rosae*, et de puceron de la carotte, *Cavariella aegopodii*, de même qu'une diminution des attaques de *Thrips tabaci* sur les oignons. La deuxième associe l'oignon, l'ail et la pomme de terre, *Solanum tuberosum* (Potts et Gunadi, 1991). Les résultats sont dans ce cas moins intéressants que les précédents puisque les populations de *Myzus persicae, Aphis gossypii* et *Empoasca* sp. décroissent tandis que les populations de *Thrips palmi* et *T. parvispinus* augmentent.

La troisième associe la betterave, *Beta vulgaris*, et d'autres plantes dont l'oignon (Rottger, 1979). Aucune modification de l'attraction de la mouche de la betterave, *Pegomya betae*, par sa plante-hôte n'est observée.

Enfin, la quatrième associe le chou et diverses plantes odoriférantes dont l'oignon, l'ail, *Allium ascalonicum* et *Allium schoenoprasum* (Latheef et Ortiz, 1983). Dans ce cas, le résultat est inverse de celui espéré puisque les pontes de *P. rapae* sur le chou sont en augmentation.

Chez les phytophages, le comportement locomoteur de trois lépidoptères, *P. interpunctella, E. kuehniella* et *Plutella (maculipennis) xylostella*, a été observé en présence des $DSPr_2$, $TiPr_2$ et le $TSPr_2$. Seul le $TiPr_2$ a une activité répulsive sur *E. kuehniella* ; dans les autres cas, il n'y a pas de modifications du comportement locomoteur (Auger et al., 1989a). Par ailleurs, le $TiPr_2$ est répulsif pour le bruchidae *C. maculatus* (Lecomte, communication personnelle).

Les composés volatils des *Allium* peuvent avoir des effets négatifs sur certains insectes entomophages, ce qui risque d'avoir des répercutions sur les populations d'insectes phytophages. Ainsi, les disulfures séquestrés par le criquet *Romalea guttata* s'alimentant sur l'oignon sauvage, *Allium canadense,* sont répulsifs pour deux espèces de fourmis prédatrices, *Tapinoma melanocephalum et Solenopsis invicta* (Jones et al., 1989). Parallèlement, le $DSPr_2$ et le PrCSO (précurseur non volatil) repoussent les fourmis *Formica fusca* et *Formica selysi* qui attaquent plus efficacement les larves de la teigne du poireau, *A. assectella,* nourries sur milieu artificiel sans poireau que celles nourries sur milieu artificiel avec poireau (Nowbahari et Thibout, 1992). Le nombre de larves transportées au nid est plus faible en présence de $DSPr_2$ et le temps de transport inversement est plus long. Dans ces deux cas, répulsion et antiappétence sont observées.

Les autres effets négatifs des composés soufrés volatils étudiés chez les insectes concernent les effets physiologiques et plus précisément les effets toxiques. Chez le moustique *Culex pipiens*, les $DSAl_2$ et $TSAl_2$ sont larvicides (Amonkar et Banerji, 1971).

En plus des travaux sur les insectes, quelques résultats ont été publiés sur les nématodes et les acariens. Toutes les études sur les activités nématicides des *Allium* ont eu pour cible le nématode *Meloidogyne incognita*. Isolés des extraits nématicides d'*Allium grayi* et d'*Allium fistulosum*, le TSMePr, le $DSPr_2$, le $DSAl_2$, le $TiPr_2$, le TiMePe et le $TOPr_2$ se révèlent les plus actifs (Tada et al., 1988). Parallèlement, Sharma et ses collègues ont montré que les extraits d'ail sont actifs, l'alliicine pouvant être utilisée pour protéger les plants de tomate en plongeant leurs racines dans une solution à 25 ppm pendant 5 minutes (Gupta et Sharma, 1993). Enfin, signalons que l'acide asparagusique a été cité comme possédant une

puissante activité nématicide (Parry et al., 1982).

Très peu d'études ont été publiées sur le pouvoir acaricide des *Allium* et de leurs composés soufrés. Un travail démontre cependant que des extraits d'ail et des préparations à partir de ces extraits présentent des effets répulsifs contre l'acarien *Tetranychus urticae* (Boyd et Alverson , 2000). Signalons un brevet de 1994 de Ferrari, concernant l'effet d'extraits d'ail sur l'acarien *Varroa jacobsoni* nuisible aux abeilles.

Conclusion

Les propriétés pesticides des composés volatils des *Allium* semblent considérables.

Ces composés apparaissent donc comme potentiellement utilisables pour le contrôle des ravageurs tels que les insectes phytophages, les acariens, les nématodes. Ces composés pourraient être plus particulièrement utilisés en fumigation pour lutter contre les ravageurs des denrées stockées. De part nos résultats, les thiosulfinates et le disélénure de diméthyle semblent être les plus intéressants insecticides. Cependant, étant donné l'instabilité des thiosulfinates, les disulfures et le disélénure de diméthyle, plus stables, paraissent plus facilement utilisables et pourraient donc s'avérer être une bonne alternative au bromure de méthyle dont l'utilisation sera très prochainement interdite.

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Structure-activity relationships of phenolic and nonphenolic aromatic acids as oviposition stimuli for the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae)

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Abstract - In a study of carboxylic acids affecting oviposition preference of the spruce budworm, a major defoliator of conifers in North America, nonphenolic aromatic acids and phenolic acids were compared in a dual-choice bioassay to assess structure-activity relationships. Among a series of nonphenolic aromatic acids with a C_1 - C_6 alkanoic, C_3 - C_4 alkenoic or C_3 alkynoic side chain, females displayed the greatest preference for acids with a saturated C_3 - C_4 side chain (MW = 150 - 164) at dosages of 78.6 and 786 nmol/cm^2. This behavioral activity was consistent with the previously reported strong oviposition preference of female budworm for aliphatic acids of similar molecular weight; that is, for C_8 - C_{10} straight chain carboxylic acids (MW = 144 - 172) and cyclohexyl carboxylic acids (MW = 142 -170). In contrast, comparable phenolic acids at the same dosages acted as oviposition deterrents. In general, the deterrent effect of phenolic acids was greatest when the length of the acid side chain consisted of 3 carbons and the number of hydroxyl and/or methoxy groups on the aromatic ring was greater than one. Ferulic and sinapic acids exemplified this trend and were the strongest deterrents among the phenolic acids tested. Apparently, the presence or absence of an hydroxyl (or methoxy) group on the aromatic ring accounts for the difference in the observed behavioral effects between the two groups of aromatic compounds.

Key Words - phenolic acid, aromatic acid, carboxylic acid, ferulic acid, sinapic acid, *Choristoneura fumiferana*, oviposition stimulant, oviposition deterrent, structure-activity relationship

Introduction

The spruce budworm, *Choristoneura fumiferana* (Clemens), is a major defoliator of spruce and balsam fir forests in North America. It is a difficult insect to control and new pest control agents and strategies would be welcome. Phytochemicals that modify insect oviposition behavior could be useful as part of an integrated control strategy or have other practical applications. The spruce budworm also provides a useful model system to study oviposition stimuli. Recently, we found that aliphatic carboxylic acids applied to artificial substrate substantially increased oviposition preference of the spruce budworm for treated substrate (Grant et al. 2000). Peak activity was associated with a series of straight chain $C_8 - C_{10}$ carboxylic acids and cyclic analogs of similar molecular size, specifically C_8 - C_{10} cyclohexane acids (e.g. cylcohexanepropanoic acid). The structural similarity of the cyclohexane acids to simple aromatic acids such as benzoic and cinnamic acid derivatives, and to more complex phenolic acids (aromatic acids characterized by one or more hydroxyl groups on the aromatic ring), suggested that some aromatic acids might also act as oviposition stimuli for the spruce budworm. Benzoic acid, for example, is a suspected oviposition host cue for the silkworm moth, *Bombyx mori* (L.), which has antennal olfactory receptors specifically tuned to this compound (Popoff 1997). In contrast, p-coumaric acid, a phenolic acid from a nonhost plant, deters oviposition by *Etiella zinckenella* (Treitschke) (Hattori et al.

1992). Many aromatic and phenolic compounds are common constituents of plants, including conifers (Strack et al. 1989, Kraus and Spiteller 1997) that are hosts for the spruce budworm. Preliminary studies with various classes of phenolic compounds, including acids, have shown that phenolics tend to deter oviposition of the spruce budworm (Abou-Zaid et al. 2000).

The objective of this report was to expand our study of carboxylic acids as oviposition stimuli for the spruce budworm to determine how aromatic and phenolic acids affect oviposition preference, and how changes in the constituents of these acids modify this preference. The exploration of structure-activity relationships can provide useful insights into the active moiety of oviposition stimuli (Cole et al. 1989, Douglass et al. 1993, Breeden et al. 1996). This information could rationalize development of new compounds with greater stimulating or deterring effects, or with better environmental stability (Cole et al. 1989, Honda 1995).

Methods and Materials

Insects were obtained from a long-established laboratory colony at the Canadian Forest Service, Sault Ste. Marie, ON, Canada. Larvae were reared on artificial diet but females were provided host foliage for oviposition. Candidate compounds were evaluated in a dual-choice oviposition bioassay (Grant and Langevin 1994, 1995). Mating pairs of spruce budworm were placed singly in small screen cages (9 cm diam.) with a top and bottom made from plastic Petri dishes lined with filter paper (Whatman No. 1), which served as the oviposition substrate. The top and bottom dishes were separated by a 4.5 cm high aluminum screen spacer. Bioassays were conducted in a well ventilated room maintained at 23-25EC, 50-60% RH, and on a 16:8 (L:D) h cycle.

Test chemicals were obtained from Sigma-Aldrich Canada (Oakville, ON). Candidate nonphenolic aromatic acids included six compounds with an alkanoic side chain of one carbon (benzoic acid) to six carbons (6-phenylhexanoic acid), two compounds with an alkenoic side chain (3-phenyl-*trans*-2-propenoic acid [= cinnamic acid] and 4-phenyl-*trans*-3-butenoic acid), and one compound with an alkynoic side chain (3-phenyl-2-propiolic acid). Thirteen candidate phenolic acids with alkanoic side chains of 1 - 3 carbons or a 3-carbon alkenoic side chain (i.e. cinnamic acid derivatives) and with 1- 3 hydroxy and 0 - 2 methoxy groups on the aromatic ring were tested. The choice of test compounds was limited by commercial availability.

Test compounds were dissolved in 95% or absolute alcohol to produce concentrations of 10 mM and 100 mM (Grant et al. 2000). In addition, five of the phenolic acids with significant oviposition activity at the 10 mM level were tested further at a 1 mM concentration. A 0.25 ml aliquot of a test solution was pipetted evenly over one-half (31.8 cm^2) of the filter paper substrate (visibly divided by a light pencil line), resulting in dosages of 7.86, 78.6 and 786 $nmol/cm^2$, respectively, for the 1,10 and 100 mM concentrations. The 78.6 $nmol/cm^2$ dosage was deemed to be a physiologically realistic stimulus level (Zhao et al. 1998, Grant et al. 2000).

A 0.25 ml aliquot of the solvent used to dissolve the test compound was applied to the other half of the filter paper to serve as the control. Both the top and bottom filter papers in 25 arenas were treated for an experiment, which was replicated at least once.

Oviposition preference, as indicated by the number of egg masses on treated and control substrates after 2 days, was quantified by means of an oviposition preference index (OPI) = (treated - control) x 100 / (treated + control). Mean OPI values ranged from -100% to +100%; statistically significant positive values indicated preference while significant negative values indicated nonpreference (deterrence). Results of replicated experiments were pooled if

statistically homogeneous (i.e. $P > 0.05$), as indicated by the Mann-Whitney test for 2 replicates or the Kruskal-Wallis test for more than 2 replicates (Zar 1984). Statistical significance ($P \# 0.05$) of OPIs was assessed with the Wilcoxon matched-pairs, signed-rank test (Zar 1984).

Results

Female spruce budworm oviposited on substrate treated with several of the nonphenolic aromatic acids. At 78.6 nmol/cm^2, preference was limited to three compounds: 3-phenylpropionic acid and 4-phenylbutanoic acid with saturated side chains of 3 and 4 carbons respectively (Fig. 1), and 3-phenyl-2-propiolic acid (not shown in Fig.1) with a triple bond in the C_3 side chain (OPI = 35.1%, $p < 0.001$). In contrast to their saturated analogues, the two aromatic acids with a double bond in the C_3 side chain (cinnamic acid and 4-phenyl-*trans*-3-butenoic acid) were not active at this dosage. However, at the higher dosage (786 nmol/cm^2), these unsaturated aromatic acids had a significant effect on oviposition along with their saturated analogs (Fig. 1). The activity of 3-phenyl-2-propiolic acid increased at this higher dosage (OPI = 68.1%, $p < 0.001$).

In contrast to the above aromatic acids, the phenolic acids tended to deter oviposition by the spruce budworm. Of the 13 phenolic acids tested, five were significantly deterrent at 78.6 nmol/cm^2 and eight were deterrent at the 10-fold higher dosage (Fig. 2), including gallic acid, 3,4,5-trihydroxybenzoic acid (not shown in Fig. 2), which generated OPI values of -31.4% ($p < 0.01$) and -20.2% ($p < 0.05$) at the low and high dosages, respectively. In general, the deterrent effect of phenolic acids was greatest when the acid side chain consisted of three carbons (horizontal comparison of compounds in Fig. 2) and the number of functional groups on the aromatic ring was greater than one (vertical comparisons, Fig. 2). Sinapic and ferulic acids, as the most deterrent compounds tested, exemplified this trend. Unsaturation in the C_3 side chain of the phenolic acids did not appear to affect behavioral activity as occurred with two of the nonphenolic aromatic acids mentioned above.

There was no consistent difference in the effect of the phenolic acids at the 78.6 and 786 nmol/cm^2 dosages (Fig. 2). However, of the five phenolic acids bioassayed at a lower dosage (7.8 nmol/cm^2), only ferulic acid remained deterrent (Table 1). In contrast, the effect of gallic acid was reversed and females showed a significant preference for substrate treated at this dosage (Table 1). This result may illustrate the reversal in behavioral response sometimes observed in bioassays covering a range of dosages (Torto et al. 1991).

Table 1. Effect of selected phenolic acids at 7.8 nmol/cm^2 on oviposition of spruce budworm

Compound	OPI(%)[a]	P[b]
gallicacid	31.4	0.001
syringicacid	9.4	0.52
caffeicacid	-5.2	0.69
ferulicacid	-28.6	0.03
sinapicacid	-16.9	0.20

[a] Oviposition preference index

[b] Wilcoxon matched-pairs, sign rank test

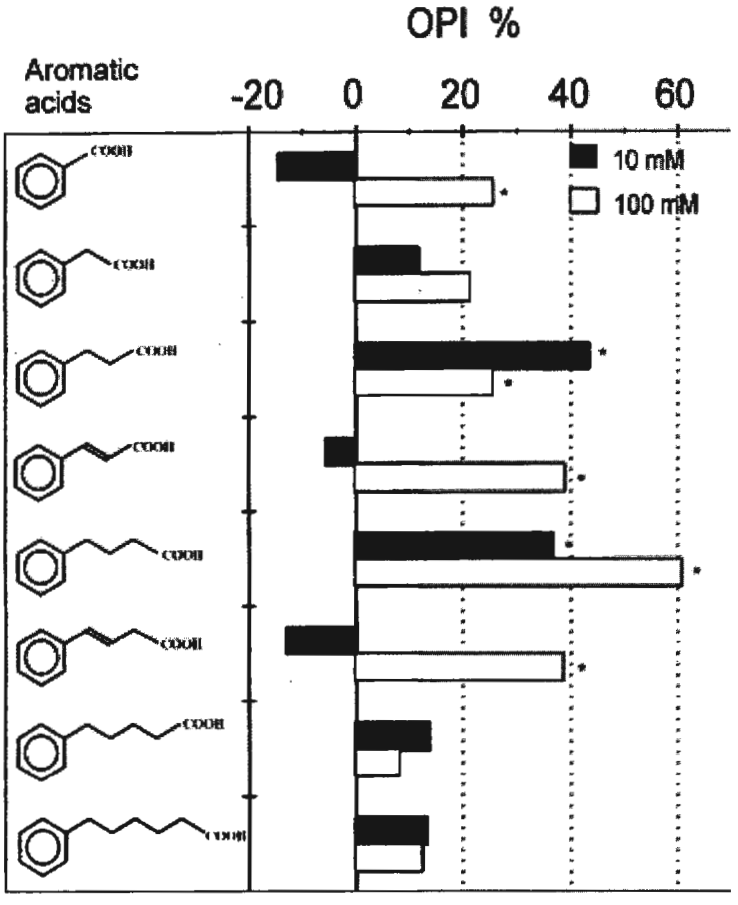

Figure 1. Effect of nonphenolic aromatic acids at two concentrations on oviposition preference of the spruce budworm as indicated by the oviposition preference index (OPI %). Aromatic acids illustrated from top of figure to bottom are respectively, benzoic acid, 2-phenylacetic acid, 3-phenylpropanoic acid, 3-phenyl-*trans*-2-propenoic acid, 4-phenylbutanoic acid, 4-phenyl-*trans*-3-butenoic acid, 5-phenylpentanoic acid, and 6-phenylhexanoic acid. An asterisk indicates a significant OPI value (P # 0.05, Wilcoxon matched-pairs, sign rank test).

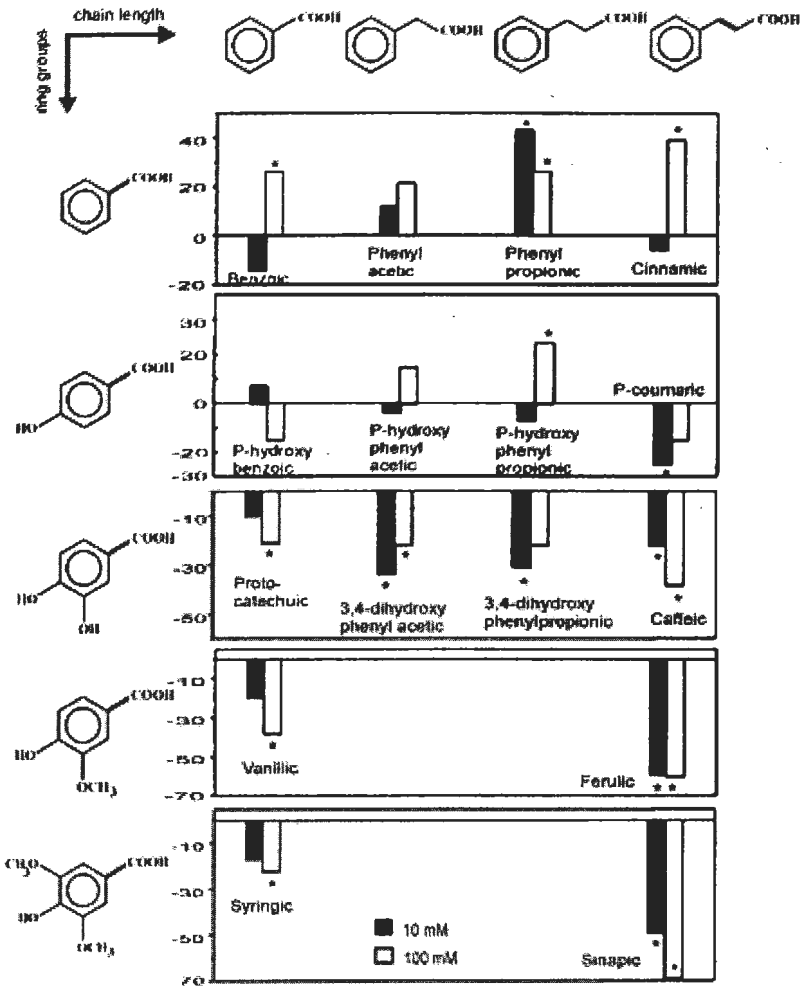

Figure 2. Structure-activity relationship between phenolic and nonphenolic aromatic acids and oviposition by spruce budworm. Each panel represents a group of aromatic acids with the same hydroxyl and/or methoxy substituents on the aromatic ring. Within each panel, the length of the acid side chain on the aromatic ring increases from C_1 to C_3, with unsaturation present in the last C_3 compound. The Y-axis for each panel is the oviposition preference index (OPI %). An asterisk over a column indicates a significant OPI value (P # 0.05, Wilcoxon matched-pairs, sign rank test). The first panel is modified from Fig. 1.

Discussion

The results suggest that spruce budworm preference for nonphenolic aromatic acids is dependent on the length of the acid side chain and dosage. The most stimulating aromatic acids had $C_3 - C_4$ acid side chains. They also closely matched the size (9 - 10 carbons, MW =

146 - 164) of the most stimulating straight chain and cyclohexane aliphatic acids (C_8 - C_{10}, MW = 142 - 172) reported in Grant et al. (2000). At the highest dosage (786 nmol/cm^2), these aromatic acids remained stimulating in contrast to the C_8 - C_{10} aliphatic acids, which became repellent or behaviorally inactive at this higher dosage (Grant et al. 2000). The lower volatility of the aromatic acids in contrast to the corresponding aliphatic acids probably accounts for this difference. Lower volatility would keep airborne concentrations from reaching too a high a level and thus becoming repellent.

The introduction of a double bond (*trans*) into the acid side chain of two stimulating saturated aromatic acids (i.e. 3-phenylpropanoic acid and 4-phenylbutanoic acid) apparently reduced their oviposition activity, as the unsaturated analogues were inactive at 78.6 nmol/cm^2 (although they were active at the higher dosage). The reason for this inactivity relative to the saturated analogues at this dosage is not clear. The result could be an anomaly, as 3-phenyl-2-propiolic acid with a triple bond in the side chain was an effective stimulus at the same dosage. However, Douglass et al. (1993) found that the introduction of a double bond into the acid side chain of analogs of $-bergamotenoic acid, a host-produced oviposition stimulus for *H. zea*, significantly reduced oviposition preference of this moth relative to the corresponding saturated analogues.

With respect to the phenolic acids, the results show that they tend to deter oviposition by the spruce budworm. Although the series of compounds tested was limited, it appears that the most deterrent phenolic acids have a $C_6 + C_3$ structural skeleton similar to the most stimulating aromatic acids ($C_6 + C_3$ and $C_6 + C_4$). Compounds with this structure, particularly phenylpropanoid ($C_6 + C_3$) derivatives, often have broad stimulating or deterring effects on the behavior of other adult insects (Metcalf 1987, Cowels et al. 1990, Hattori et al. 1992, Dudareva and Pichersky 2000). The difference in the respective behavioral effects of the simple aromatic acids versus phenolic acids for the spruce budworm is apparently due to the presence or absence of a hydroxyl (and/or methoxy) group on the aromatic ring, which would affect both chemoreception and volatility. As the phenolic acids have little or no volatility, they presumably act through contact chemoreceptors and thus function as deterrents rather than repellents. However, mechanical or visual effects of the phenolics cannot be ruled out (Tabashnik 1985).

Phenolic compounds are important oviposition stimuli for other Lepidoptera. Chlorogenic acid (a phenylpropanoid derivative) and several flavonoids are oviposition host stimulants for various butterfly species (Honda 1995, Haribal and Renwick 1996, Carter et al. 1998) and for *Helicoverpa armigera* (Hhbner), a moth species (Simmonds 1998). On the other hand, phenolics derived from nonhost sources have proved to be oviposition deterrents for a number of moth species, including *Plutella xylostella* (Tabashnik 1985), *Heliothis virescens* (Ramaswamy et al. 1992), *E. zinckenella* (Hattori et al. 1992) and *Sitotroga cerealella* (Ge & Weston 1995).

Structure-activity relationships of carboxylic acids as oviposition stimuli have been studied in a few other insects. Oviposition preference of female *H. zea* for $-bergamotenoic acid, a host stimulant, appears to be due primarily to the carboxylic function but, as in the spruce budworm, oviposition activity is modified substantially by the length of the side chain to which the carboxyl function is attached and by the presence or absence of a double bond in the side chain (Douglass et al. 1993, Breeden et al. 1996). In the case of the cabbage rootworm fly, *Delia radicum* (L.), the situation is much simpler. Sinapic acid is a naturally occurring oviposition deterrent for this fly, produced in the frass of a moth caterpillar feeding on the same plants (Jones et al. 1988). The deterrent activity of sinapic acid was dependent solely on the carboxylic acid function. Neither the phenolic ring nor other structural features

conferred or affected deterrent activity (Cole et al. 1989). It remains to be seen how a change at the carboxylic function would affect the spruce budworm response to the behaviorally active compounds reported here.

Few studies have examined how adult Lepidoptera detect nonvolatile phenolic compounds. Electrophysiological studies with a *Papilio* butterfly species have shown that tarsal contact chemoreceptors of females respond to host-derived phenolics (*trans*-chlorogenic acid and a flavonoid) (Roessingh et al. 1991). Hence phenolic acids can be expected to stimulate contact chemoreceptors on the tarsi or ovipositor of the spruce budworm, or possibly chemoreceptors on the proboscis (Rivet and Albert 1990). The deterrent effect of phenolic acids on the spruce budworm may reflect an ability of its sensory receptors to detect potentially noxious substances through a deterrent neuron (Schoonhoven 1991). We have shown previously that quinolizidine alkaloids have strong deterrent effects on ovipositing spruce budworm (Zhao et al. 1998) and the action of these deterrents may have a similar chemosensory pathway. The relationships between the chemical structure of carboxylic acids and their behavioral activity will provide useful comparisons for electrophysiological studies of the contact chemoreceptors of the spruce budworm stimulated with these compounds, and would help confirm the sensory mode of action of these compounds. Those results could also lead to a better understanding of the chemical constituents that stimulate or deter oviposition behavior.

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Use of pheromones and other semiochemicals in integrated production
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Laboratory and field evaluation of floral odours from African marigold, *Tagetes erecta*, and sweet pea, *Lathyrus odoratus*, as kairomones for the cotton bollworm *Helicoverpa armigera*

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Abstract: Significant increases in upwind flight of *Helicoverpa armigera* in a wind-tunnel were obtained with air entrained headspace samples of African marigold, *Tagetes erecta*, flowers (P=0.014), and sweet pea, *Lathyrus odoratus*, flowers (P=0.047). Identification of the compounds contained in the Porapak Q extracts was done by GC-MS. Direct EAG was used to screen for the most electrophysiologically active natural extracts and GC-EAG was used in screening for compounds to test in the wind-tunnel. Two 4-component synthetic kairomonal blends were identified which caused significant increases in upwind flight in the wind-tunnel (P<0.001 and P=0.014 for marigold and sweet pea blends respectively). Funnel traps baited with floral odours were tested for their ability to catch *Helicoverpa armigera* and other insects in the field in Israel. Significantly more *H. armigera*, another noctuid pest, *Autographa gamma*, the honeybee *Apis* spp., a wasp *Halictus* spp. and lacewings (Chrysopidae) were caught in traps baited with synthetic marigold, *Tagetes erecta*, and sweet pea, *Lathyrus odoratus*, floral volatiles than in unbaited control traps. The marigold blend contained benzaldehyde, (±)-linalool, phenylacetaldehyde and (S)-(-)-limonene, and the sweet pea blend (-)-linalool, phenylacetaldehyde, benzyl alcohol and diacetone (4-hydroxy-4-methyl-2-pentanone) in the natural ratio. Although the target specificity and level of attraction obtained with the floral traps was too low for mass trapping, the floral traps could possibly be used for monitoring female *H. armigera* populations. These findings are discussed in relation to the potential use of floral kairomones in integrated control of *H. armigera*.

Key words: *Helicoverpa armigera*, kairomone, floral odour, insect-host plant interaction

Introduction

Helicoverpa armigera is a serious pest of cotton, chickpea, pigeonpea, maize and other crops in many parts of the old world tropics and subtropics. Use of larvicides has been the predominant management strategy for several decades (King, 1994; Wilson, 1982). However development of insecticide resistance has caused major control problems. There are also safety and environmental contamination considerations associated with the large quantities of insecticide often used in cotton (Menn 1991) giving impetus to finding new control methods or improved pest monitoring to allow need based insecticide application. It was within this context that the possibility of using floral compounds for trapping *H. armigera* was tested. Since female insects can be caught with a floral lure in theory a floral baited trap could have more impact on reducing oviposition levels than a pheromone trap which only captures male insects, or when used for monitoring purposes it could give a better reflection of oviposition levels within a crop. However this depends on a reasonably high level of attraction to the floral baited trap occurring under field conditions.

Materials and methods

Headspace sampling.
Samples of floral headspace volatiles were collected by air entrainment using freshly cut *Tagetes erecta* and *Lathyrus odoratus* flowers. Four to 55 flowers were placed in clean glass quickfit flasks (typically 500 ml capacity, although flask size was varied with the number of flowers available). Charcoal filtered air was drawn through the flask at 2 l/min for typically eight hours. On exiting the container the air was drawn through a Porapak Q filter (200mg, 60-80 mesh size, Phase Separations, UK). The entrained volatiles were eluted from the Porapak Q filters with 2 ml of dichloromethane and stored at -20°C.

Experimental insects.
A laboratory strain of *H. armigera* was reared on a semi-synthetic chickpea-based diet. Adult moths were fed a 10% sucrose solution. The culture was maintained at 25°C, with a relative humidity of 50% and 14 : 10 h light-dark regime.

Electroantennography and GC-EAG analyses.
Electroantennography and GC-EAG were carried out using standard methods as described in Cork *et al.* (1990). Linked GC-EAG analyses of entrained flower volatiles were replicated five times using different insects and carried out on three different Porapak Q extracts. GC retention times of compounds identified were converted into Retention Indices by comparison with the retention times of saturated, straight-chain hydrocarbons, thus *n*-tetradecane = 1400. These analyses are reported in Bruce and Cork (in press). Direct EAG using a 1µg dose on filter paper was used with synthetic compounds to confirm their electrophysiological activity.

GC-MS analyses.
EAG-active compounds observed in the GC-EAG analyses were subjected to analysis by gas chromatography (Carlo Erba 5160 Mega Series) linked to a mass spectrometer (ITD 700, Finnigan MAT, Hemel Hempstead, U.K.). The EAG-active compounds were identified by comparing the electron impact MS with library spectra (Adams, 1995) and confirmed by comparing the GC retention times and EI-MS with synthetic standards. A cyclodextrin B column (50m x 0.22mm ID) on a Varian 3700 GC was used to identify which enantiomer(s) of chiral compounds were present in the Porapak Q extracts.

Wind-tunnel bioassay.
A wind-tunnel (225 x 60 x 60 cm, 50 cm/sec airspeed) was used to investigate behavioural responses of female *H. armigera* to natural and synthetic blends of putative kairomone components as described by Bruce & Cork (in press). Bioassays were carried out under reduced lighting (0.8 lux), at 25 °C and 50% R.H., during the first 2 - 3 hrs of scotophase which corresponded with the natural time of nectar foraging and oviposition of female *H. armigera* (Roome, 1975). Samples of floral volatiles were applied to Whatman No. 4 filter paper strips in 50 µl aliquots from stock solutions. The filter paper was then immediately clipped to a vertical support and positioned at the centre of the cross-sectional view, height 30cm. Test moths were released individually 200 cm downwind of the odour source. Maximum distance flown upwind and number of approaches to within 20 cm of the odour source were scored during a 12 min period.

Field trapping experiments.
Compounds used in lures, benzaldehyde, (±)-linalool, phenylacetaldehyde, (S)-(-)-limonene, were purchased from Sigma Israel Chemicals Ltd. 2,6-di-*tert*-butyl-4-methyl-phenol (BHT) was used (10% of the a.i.) as an antioxidant in lures that contained benzaldehyde and phenylacetaldehyde. Dioctyl phthalate (85%) was used to slow the release of (-)-limonene (15%) which had to be formulated in separate double-thickness sachets because of its high volatility. Lures were made by pipetting these compounds into polyethylene sachets cut from a roll of polyethylene tubing (500 gauge, 125_m thickness, Transatlantic Plastics, Southampton, UK) and sealed using a 'Futura' electrical heat sealing device (Audion Elektro, Holland). Unitrap' (IPS, S. Wirral, UK) cone traps were baited with floral lures and set up 50 cm above crop height. Distance between traps was 12m. Traps were situated in a chickpea field for the first month (10/5/99-31/5/99) and in a cotton field for the second month (1/6/99-29/6/99). Both fields had a uniform crop. To obtain an independent measure of the *H. armigera* population density during the field experiments, four pheromone traps (Unitraps) and a light trap were also set up. They were located in the same field as the traps baited with floral lures but approx. 60 m away from the nearest floral trap to avoid interference with the main experiment. Female moths caught in the floral traps were dissected and examined for the presence of spermatophores to determine their mated status.

Results

Electroantennography and GC-EAG analyses (Tables 1, 2)

Wind-tunnel bioassay
Wind-tunnel results are summarised in Table 3. Initially tests were conducted with aliquots of air entrained samples that had elicited EAG responses in GC-EAG analyses. Subsequently, once EAG active compounds had been identified, synthetic kairomonal blends, using the same ratio and concentration of compounds as in the natural sample were used.

Table 1. Female *H. armigera* EAG responses to Synthetic Compounds (n = 9) and the GC Retention Time Data Used for their Identification from *Tagetes erecta* Samples

Compound (1 µg)	Mean EAG response (-mV) ± SE	*P*-value[a]	Retention Index (polar column)		Retention Index (non-polar column)	
			Natural	Synthetic	Natural	Synthetic
(-)-limonene[b]	0.47 ± 0.02	0.012	1200	1200	1017	1019
Benzaldehyde	0.61 ± 0.07	0.001	1522	1522	925	926
(±)-linalool	0.59 ± 0.11	0.032	1539	1541	1085	1088
Phenylacetaldehyd	0.75 ± 0.08	<0.001	1642	1642	1007	1011

[a] Paired *t*-test comparing treated and dichloromethane control means.

Field trapping experiments

In the first week of trapping floral baits containing the synthetic *T. erecta* and *L. odouratus* blends were compared with each other and a combined blend containing volatiles from both *T. erecta* and *L. odouratus*. As shown in Fig. 1 there was very little difference between the floral baited treatments and combining volatiles from both plant sources did not increase catches of *H. armigera*.

Table 2. Female *H. armigera* EAG responses to Synthetic Compounds (n = 9) and the GC Retention Time Data Used for their Identification from *Lathyrus odoratus* Samples

Compound (1 µg)	Mean EAG response (-mV) ± SE	*P*-value[a]	Retention Index (polar column)		Retention Index (non-polar column)	
			Natural	Synthetic	Natural	Synthetic
Diacetone	0.48 ± 0.03	<0.001	1366	1369	_821	_818
(-) -Linalool	0.59 ± 0.11	0.032	1545	1547	1086	1088
Phenylacetaldehyde	0.75 ± 0.08	<0.001	1652	1654	1014	1015
Benzyl Alcohol	0.62 ± 0.03	0.001	1887	1888	1022	1022

[a] Paired *t*-test comparing treated and dichloromethane control means.

Due to the low *H. armigera* catches per trap per night in floral odour baited traps, different (weekly) experiments including the standard 4-component marigold trap bait (M1) and the unbaited control treatment were treated as different replicates instead of separate experiments. *H. armigera* catches were summed for these two treatments for each weekly experiment and statistical analysis comparing catches was carried out. There was a significant increase in *H. armigera* catches in traps with the standard 4-component marigold lure compared with the unbaited control trap catches over the whole season (Table 4), P=0.0023 (Mann Whitney 'U' test).

As shown in Table 5 floral odour baited traps were less effective than pheromone baited or light traps. However they had the advantage of catching female *H. armigera* and did not require a power source. Of 48 female *H. armigera* from the floral traps that were examined, 36 contained spermatophores i.e. 25% were unmated. Floral baited traps had little selectivity and other flower visiting insects were also captured. As well *H. armigera*, significantly more *Autographa gamma*, *Apis mellifera*, *Halictus* spp. and *Chrysopa carnea* were caught in traps baited with synthetic marigold, *T. erecta*, and sweet pea, *L. odoratus*, floral volatiles than in unbaited control traps. A large proportion of the insects caught were beneficial pollinators (*Apis*, *Halictus*).

Table 3. Wind-tunnel Responses of female *H. armigera* to air-entrained samples and synthetic blends of *T. erecta* and *L.odoratus* flowers in the wind-tunnel

Treatment	Furthest Flown Upwind (cm) ± S.E.	Mean No. of Upwind Approaches	No. of Replicates	*P*-value[a]
Control	98.9 ± 12.0	0.80 ± 0.23	69	
T. erecta extract	135.5 ± 12.7	1.86 ± 0.38	63	0.014
Control	89.4 ± 21.0	0.35 ± 0.21	17	
L. odoratus extract	109.7 ± 26.7	2.59 ± 1.15	17	0.047
Control	77.1 ± 14.0	0.54 ± 0.28	41	
T. erecta synthetic blend [b]	147.6 ± 15.4	4.00 ± 0.96	35	0.0008
Control	67.9 ± 20.8	0.53 ± 0.53	17	
L. odoratus synthetic blend [c]	132.2 ± 18.0	2.94 ± 1.24	18	0.014

[a] Result of Mann Whitney 'U' test comparing treated and control medians for number of upwind approaches

[b] 0.23µg phenylacetaldehyde, 0.5 µg benzaldehyde, 0.73 µg (±)-linalool, 8.6 µg (+)-limonene

[c] 5.9 µg diacetone, 3.3µg (±)-linalool, 3.1µg phenylactaldehyde, 0.44µg benzyl alcohol

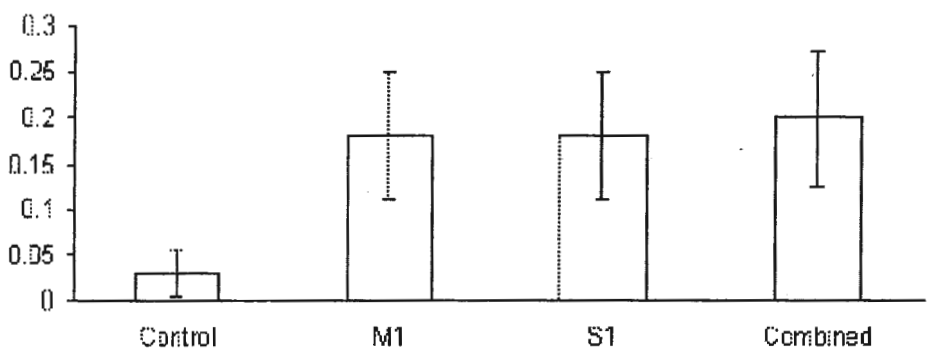

Figure 1. *H. armigera* catch during the first week of trapping. M1 = *T. erecta* blend: 1.5 x 5cm sachet containing 0.5ml of a 40:1:1 mixture of phenylacetaldehyde, benzaldehyde and (±)-linalool +10%BHT; 2.5 x 2.5cm sachet containing 0.5ml of (-)-limonene in dioctyl phthalate (15% (-)-limonene) inside a 4 x 5cm outer sachet. S1 = *L. odouratus* blend: 1.5 x 5cm sachet containing 0.5ml of a 1:1:1:1 mixture of diacetone, benzaldehyde, (-)-linalool and benzyl alcohol +10%BHT. Combined = 1.5 x 5cm sachet containing 0.5ml of a 1:1:1:1:1 mixture of benzaldehyde, benzyl alcohol, diacetone, (±)-linalool and phenylacetaldehyde +10%BHT and the (-)-limonene sachet as in M1.

Table 4. Mean *H. armigera* Catch in Standard Marigold Baited Traps and Unbaited Control Traps in Israel Trial (both sexes, per trap, per night)

Week	Unbaited Control	M1 [a]
1	0.025	0.175
2	0.000	0.208
3	0.000	0.028
4	0.000	0.056
5	0.000	0.111
6	0.000	0.111
Mean	0.004	0.115

[a] lure as in legend of Fig. 1

Floral baited traps had little selectivity and other flower visiting insects were also captured. As well *H. armigera*, significantly more *Autographa gamma*, *Apis mellifera*, *Halictus* spp. and *Chrysopa carnea* were caught in traps baited with synthetic marigold, *T. erecta*, and sweet pea, *L. odoratus*, floral volatiles than in unbaited control traps. A large proportion of the insects caught were beneficial pollinators (*Apis*, *Halictus*).

Table 5. Mean Catches in Different Types of Traps over the Whole Season

Trap Type	Mean No. H. armigera per trap per night
Unbaited Control	0.0004
Floral Odour Baited	0.115
Light Trap	1.35
Sex Pheromone	8.80

Conclusions

Experimental evidence for a role of olfaction in host-plant selection behaviour by *H. armigera* has been obtained in the current study. Natural selection would be expected to favour searching mechanisms in which a preliminary assessment of the host-plant could be made prior to alighting because this would mean that time and risk could be concentrated on plants more likely to provide an oviposition site (Damman & Feeny, 1988). It seems probable that there is a sequence of cues leading to acceptance of a host-plant and that olfaction is more important in the earlier stages prior to contact with the plant (Miller & Strickler, 1984, Hsiao; 1985). Host-plant odours stimulate searching behaviour and make foraging moths such as *H. armigera* more responsive to other cues associated with host-plants (Brantjes, 1978; Hurtel & Thiéry, 1988; Bell, 1990; Bernays & Chapman, 1994).

For mass trapping purposes the floral lure used in the field trials reported here has too low a trap performance in terms of moth capture per night (on average 0.11 per night with the standard marigold blend in Israel) and was insufficiently selective. With a low level of

attraction into the trap there is too much of a risk that a mated female moth could lay eggs in the crop before being attracted into the trap. One female can lay in excess of 1000 eggs (Fitt, 1989). Also the number of traps required per hectare would need to be higher and this might be uneconomic. A large number of traps could interfere with other cultivation practices where a tractor requires access to the crop.

Monitoring requires the trap catch to give a good indication of potential threat to a crop. Numbers caught per trap per night need not be high so long as they correlate well with the likelihood of crop damage by the larvae developing from the eggs of the female moths. Since a floral trap can catch female insects on which oviposition depends it is possible that they could be useful for monitoring purposes. Maini & Burgio (1999) found a good correlation between female European corn borer, *Ostrinia nubilalis*, captures in phenylacetaldehyde baited traps and ensuing crop damage. Another important consideration for monitoring is the sensitivity of the trap to early infestation. If the trap does not catch moths until there has already been considerable oviposition in the crop the warning given could be too late for applying a pesticide spray. The economic threshold can be exceeded by fewer than 5 females per acre (Lingren *et al.*, 1982). Large larvae are a more difficult spray target than small larvae because they require a higher dose of active ingredient to be killed (Lingren *et al.*, 1982). This means that monitoring should give as much warning as possible so that arrangements for an early insecticide application can be made. Because *H. armigera* is so mobile any improvements in monitoring its populations by keeping track of populations of adult female moths in an area could lead to better informed pest management decisions about pesticide applications. Catches of female moths might give a better reflection of egg-laying in the local crop.

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Use of pheromones and other semiochemicals in integrated production
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Release rate of ammonia - a key component in the attraction of female mediterranean fruit fly to protein-based food lures.

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Abstract: The Mediterranean fruit fly, *Ceratitis capitata*, is one of the most injurious pests around the world. The common way to control the fly is via poison-bait sprays directed mainly against the females. Hydrolyzed proteins are the customary, but not satisfactory, bait component. Ammonia is the most significant volatile breakdown product of protein decomposition. The relationship between ammonia emanation and the attraction of the medfly was studied earlier and indicated the dependence of attraction on the rate of release of ammonia. The aim of this study was to quantify the rate of ammonia release for optimal attraction of medfly females as a first step in devising a new and more efficient female-attractive bait based on chemically defined components. The rate of release of ammonia was measured in an ammonia trapping system, which was built for this purpose. The ammonia was released from 1 ml sample in a 3-cm-long x 1.5-cm i.d. glass tube. The most attractive concentration of ammonia solution was 0.01 N. The most attractive rate of release of ammonia calculated from the correlation of increasing concentrations of ammonia solutions and their release rates was 14.3 µg/tube/hour. The use of higher rates of release of ammonia up to ~100 µg/tube/hour may be also adequate for use because although they are somewhat repulsive, they are attractive enough to catch relatively higher numbers of flies.

Key words: Mediterranean fruit fly, *Ceratitis capitata*, Tephritidae, Diptera, food baits, attraction

Introduction

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Diptera: Tephritidae), is one of the most injurious fruit pests. It is widely distributed and the list of its host range is long and diverse (White and Elson-Harris, 1994). The medfly is a high-priority quarantine pest. The demanding regulations at its export destinations are the cause for the intensive control applications against the medfly in the fruit-growing areas as well as for seeking more efficient and environment friendly ways to control it. Females are the main target for control because they damage fruits and are the dominant factor for multiplication. Female-attractive baits are therefore needed in any applicative system against this pest for both monitoring and direct control.

The need for external supply of protein for ovary maturation is the reason for the attraction of females to protein-based baits. Hydrolyzed proteins are the customary, but not satisfactory, means to attract the females. The common commercial hydrolyzed proteins baits are black liquids, difficult to handle and variable in content affecting their attraction. The new fruit fly dry bait developed by Heath *et al.* (1995), although easy in use and more efficient then the liquid hydrolyzed proteins (Gazit *et al.*, 1998), is limited to traps only. The need for more powerful baits for use in both traps and in bait sprays is a must in the fight against this pest.

Ammonia is the most conspicuous end product of protein decomposition. The relationship between ammonia emanation and the attraction of the medfly was studied earlier (Mazor *et al.*, 1987). The quantification of the rate of ammonia release for optimal attraction

of medfly females reported here was considered as the first step in devising a new and more efficient female attractive bait based on recognized components.

Materials and Methods

The quantification of the rate of release of ammonia and the attraction of the medfly females were studied in two experimental setups: olfactometer and ammonia trapping system.

Laboratory-reared flies were obtained as pupae from the Citrus Marketing Board of Israel. Pupae and emerging flies were kept in a room with windows, under natural photoperiod conditions and a controlled atmosphere of 26±2^{0}C and 68±2% relative humidity. The behavioral tests were conducted at the same conditions. 200 protein-deprived and mostly unmated 3 - 10-day-old females were placed in an olfactometer described by Gothilf and Galun (1982) for the attraction tests. The flies were offered granulated sugar, and water absorbed on cotton wool. The ammonia stock solution was a concentrated volumetric solution (BDH) adjusted to several concentrations between 0.0001N and 0.1N. 1 ml of the desired ammonia solution was pipetted into a 3-cm-long x 1.5-cm i.d. glass tube. A 10-cm metal wire was joined to the side of the glass tube allowing to insert the bait into the trap. 6 traps, 3 with bait and 3 empty ones as control were suspended alternately from the horizontally rotating wheel of the olfactometer at a rate of 1 complete turn/10 min for 1 hour. At the end of the experiment, the entrance holes of each trap were plugged with a piece of cotton wool and the traps were transferred to the refrigerator for a few minutes to allow the counting of the captured flies. Flies trapped in all 3 baited traps in one olfactometer were considered as one replicate. The attraction of 0.01 N ammonia solution was tested also with 3-10-day old protein-deprived males.

The ammonia trapping system includes a 100 ml round bottom flask containing 1-3, 3-cm-long x 1.5-cm i.d. glass tube(s), with the tested solution (depending on the rate of ammonia release) connected to two consecutive water traps, 16-ml-long x 2-cm i.d. glass tubes containing 10 ml double-distilled water. Each glass tube contained 1 ml of several concentrations between 0.0001N and 0.1N of pure ammonia solution. The whole system was sunk in a water bath at a temperature of 30^{0}C. Fresh air was pulled into the trapping system by a vacuum pump at a rate of 100 ml/min. The air was drawn into and through the round glass flask containing the tested material and then through cintered glass filters to the first and the second 10-ml water tubes. Most of the emitted ammonia was caught in the first water tube. The trapping of ammonia lasted 1 to 8 hours (depending on the rate of release of ammonia).

The amount of ammonium ion in the water was determined by a colorimetric phenol chlorite method (Solorzano, 1969) and was calculated as ug ammonia released from 1 ml experimental material per hour.

Results and discussions

The rate of ammonia release affected the attraction of the flies. The most attractive ammonia solution, 0.01 N, caught per olfactometer during 1 hour the highest proportion of females, namely an average of 58% of the 200 females (calculated from Fig. 1). The observed rate of release of ammonia from this solution was 17.1 ± 3.5 µg/tube/hour. The calculated rate of release of ammonia from this ammonia solution was 14.3 µg/tube/hour, based on the equation $y=22040x^2+802.31x+4.0506$ describing the correlation between increasing concentrations of ammonia solution and the measured release rates of ammonia from them (Fig. 2). The slope

of the curve representing attraction against increasing rates of release of ammonia is very steep up to the most attractive point, while the subsequent descent is gradual (Fig. 3). The correlation between these two parameters up to this highest point fits best a logarithmic function (Fig. 4). The most attractive solution, 0.01 N, was attractive also to males, but the ratio between the catches of females to males in response to this solution was twice as much. A comparison between the catches of female and male medflies by 0.01N ammonia solution showed that 117 ± 23 females and 54 ± 13 males were captured ((n=64, n=16 respectively). Although the aim of the bait development is toward a more female-selective bait, evaluating the degree of male catches should be considered.

A source that releases ammonia at the optimal rate of release may serve as the principal component of female attractive food-lure baits. Only rates of release higher than the most attractive rate that was measured in our system, namely ~14 µg/ tube/hour should be taken into account, because of the large differences in attraction due to small changes in concentrations at lower release rates. The use of higher rates of release of ammonia up to ~100 µg/tube/hour may be adequate for use because although they are repulsive to some extent they are attractive enough to catch relatively higher numbers of flies.

In spite of the optimal captures (58%) as a response to a specific rate of release of ammonia, it is doubtful whether this can be still be improved, a point yet unknown.

The females that took part in these experiments were protein-deprived and mostly unmated because they were introduced into the olfactometers when they were 2-day-old. Preliminary results showed that equal numbers of females responded equally to ammonia at different ages when they were continuously protein-deprived. The ammonia probably symbolized a signal for the presence of protein. This point should and will be tested in the near future. Other components that may induce attraction in females in different physiological states such as mature fruit lures are the target for further study.

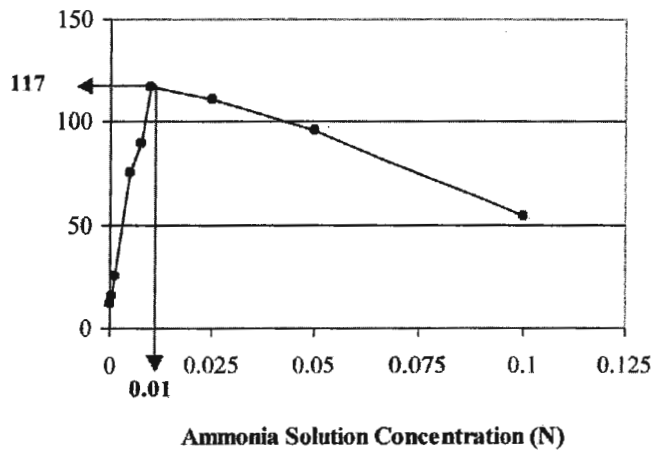

Figure 1. Female catches in response to increasing concentrations of ammonia solutions. (n=16)

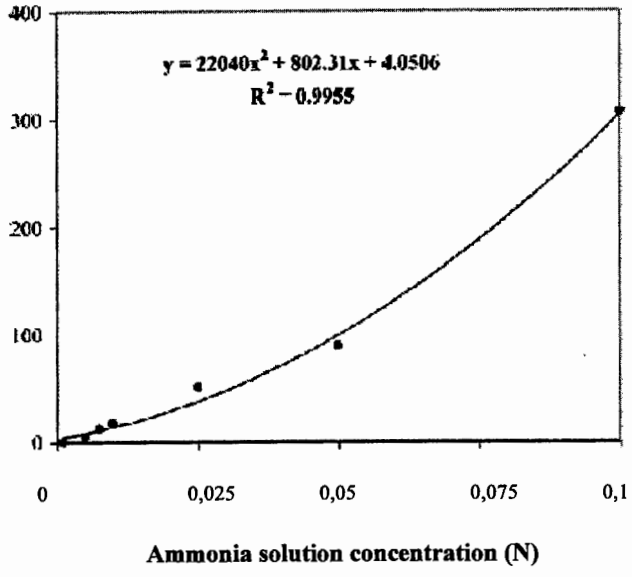

Figure 2. The rate of release of ammonia from increasing concentrations of ammonia solution. (n=12).

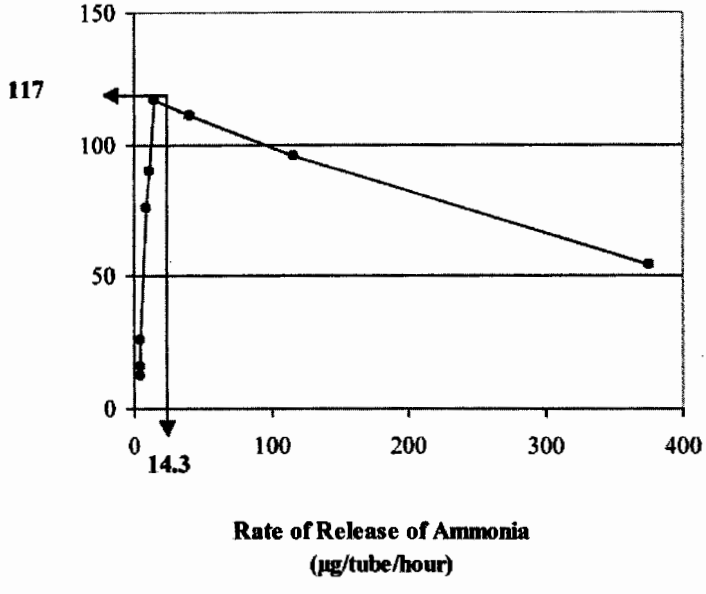

Figure 3. Female catches in relation to calculated increasing rates of release of ammonia. (n=16).

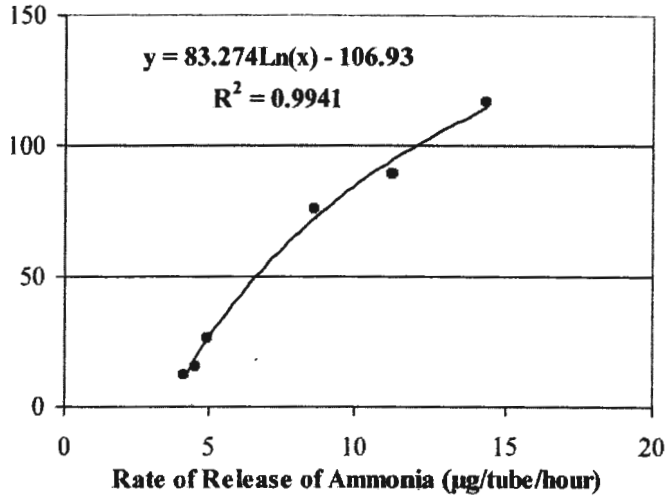

Figure 4. The relationship between female catches and the calculated rate of release between the lowest and the highest points of attraction. (n=16).

The relationship between the rate of ammonia release and attraction of females was tested with the medfly, but since most, if not all fruit flies adult females share the same need for external supply of protein for egg production and survival (Tsipsipis, 1989; Jacome *et al.*, 1999), such studies for other fruit fly species may contribute to the development and improvement of more efficient baits for them.

The next step of this study is to translate its results into practical use. A device that will release ammonia at the desired optimal rate of release that will be fitted for application in traps and use in poison bait sprays will be developed. The requirements needed for trap application are different from those needed for poison-bait spray. Bait for trap should be only attractive, while bait for poison-bait spray should be also phagostimulative. Another difference is the application phase which is liquid for sprays, whereas a solid phase is much more convenient in trap application.

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Epilogue

Use of pheromones and other semiochemicals in integrated production
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Mating disruption and working group in retrospect

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Abstract: Almost immediately after the establishment in 1975 of the IOBC/WPRS Working Group 'Use of Pheromones in Integrated Control', mating disruption became a major topic in most of its meetings. Research and development of mating disruption with all its ups and downs are clearly reflected in the Working Group's activity reports.With the help of the proceedings of the various Working Group meetings I will describe how mating disruption developed to a full-grown insect control method during the 25 years of the Working Groups' existence.

Key words: dispensers, formulations, IOBC/WPRS, IPM, meeting place, semiochemicals, sex pheromones

Introduction

Some months ago I paid a visit to the BBA Institute at Dossenheim near Heidelberg in Germany. During summertime Heidelberg is full of tourists and all hotels appeared to be full. My colleagues were able to find a room for me at a 'Winzerei' (vinery), located in the hills just outside the town. The vineyard was completely surrounded by mixed forest and quite isolated. Every evening I drank a glass of wine with the grower and he told me that since 10 years his vineyard was successfully treated with pheromone mating disruption to control the leafrollers. I was really impressed by the grower's enthusiasm for this 'wonderful' method. He liked mating disruption as he was able to protect his crop by means of an environmentally benign method without detrimental effects on his financial outcome. He further told me that most of his colleagues in the region also used mating disruption to their great satisfaction and that the use of pheromones in vine-grapes had increased to approximately 20,000 ha in 1999. As I had not closely followed the developments since 1997 it came as a surprise to me that mating disruption became such a big success in the Palatinate and other regions of southwest Germany.

Inwillingly my mind wandered back to the meeting of this Working Group held in 1986 at Neustadt an der Weinstrasse in Germany, in the middle of the wine-growing region, not far from Heidelberg. I remembered the stimulating atmosphere at that meeting where it was clearly demonstrated in extensive trials that effective mating disruption of grape moths was feasible. But I also remembered that the atmosphere in the successive Working Group meetings had its ups and downs, which I believe was strongly influenced by the prospects of pheromone application, particularly of mating disruption. I think that this festive silver-jubilee meeting provides the right opportunity to review the status of mating disruption in the subsequent working group meetings. Before doing so I like to present a brief summary of the Working Group's activities and a short general introduction into mating disruption.

The Working Group

The major aim of the Working Group is to bring together people interested in the use of pheromones and other semiochemicals for environmentally safe control of noxious insects in agriculture, and to collect information about this field.

How did it all start? The idea to create a special working group on pheromones was born at a meeting of another IOBC/WPRS Working Group, held at Wädenswil (Switzerland) in November 1973: The programme dealt with IPM in fruit orchards and at least 30% of the contributions presented at the meeting was devoted to pheromones. The pheromone researchers assembled there began to realize the great significance of their own area of interest and found it appropriate to have 'their own' meeting place. So, after approval of the IOBC/WPRS Council the Working Group 'Use of Pheromones in Integrated Control', later renamed as 'Use of Pheromones and Other Semiochemicals in Integrated Control' was established. Its first meeting was held in 1975 at Wageningen (The Netherlands) with 32 participants from 8 different countries.

Table 1. Meetings of the IOBC/WPRS Working Group on Semiochemicals since 1975 (update from Minks & Voerman, 1996)

Convenor: Albert Minks (1975-1985)			
Wageningen	NL	1975	Introductory meeting, various topics
Harpenden	UK	1977	Chemistry and biological activity of pheromones
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 of moths and molecules
Avignon	F	1988	Insect monitoring and attractants
Granada	E	1990	Pheromones in Mediterranean pest management
San Michele all'Adige	I	1992	Mating disruption
Chatham	UK	1993	Insect pheromones
St.Peters Insel	CH	1994	Use of mating disruption in practice
Convenor: Peter Witzgall (since 1995)			
Montpellier	F	1996	Technology transfer in mating disruption
Budapest	H	1997	Pheromone lures for detection and monitoring
Dachau	D	1998	Scents in orchards
Stuttgart/Hohenheim	D	1999	Pheromones for insect control in orchards and vineyards
Samos	GR	2000	25th Anniversary Reunion

Since then the Working Group has met at 15 other occasions, including the 25th anniversary meeting at Samos (Greece) in September 2000 (Table 1). It has traditionally served as a meeting place for basic and applied research workers on semiochemicals. This research area has an outspoken multidisciplinary character and to make progress in application it is essential to bring colleagues together from academic and governmental

research institutions, plant protection industry and extension services, who have knowledge of insect behaviour and sensory physiology, chem-istry and formulation technology, and of applied entomology and integrated pest management. The Working Group meetings turned out to be extremely useful to European specialists in the first place, but were often attended by colleagues from outside the WPRS-region, such as the USA, Canada, Australia and Japan.

Mating disruption: general aspects

Minks & Kirsch (1998) characterized mating disruption as permeation of the air over the crop to be protected with synthetic pheromone. Male moths are then unable to locate their female mates when using their own pheromone system and mating is therefore reduced or even eliminated.

They also stressed the point that slow-release formulations are absolutely essential for pheromones used in mating disruption. They prolong the release and efficacy of the highly volatile pheromone compounds and provide in-field stabilization of pheromone remaining in the formulation. While formulations can be sprayable (e.g. microcapsules), at present most commercial formulations are designed for hand application by clipping, hanging or twisting them around stems or branches of the crop. Table 2 shows a list of the best known pheromone formulations.

Table 2. Pheromone formulations used commercially in recent years (update from list in Minks & Kirsch, 1998)

Laminate flakes (Hercon, USA)
Twin-ampullae dispensers (BASF Doppelampullen, Germany)
Twist-tie polyethylene dispensers (Biocontrol/Shin-Etsu, Japan)
Polymer dispensers (TNO, the Netherlands)
Isagro cellulose fibre dispensers (Donegani, Italy)
Consep membranes (Consep, USA)
Biosys polymer dispensers (Biosys, USA)
Scentry micro-fibres (Ecogen, USA)
Microcapsules (3M, USA)
Metered Semiochemical Timed Release System (MSTRS, USA)
Paraffin emulsion dispensers (USA)

Mating Disruption and Working Group Meetings

WG meeting at Nyon in 1982

At the first three meetings mating disruption was discussed in various sessions, but major attention was aimed at other areas such as chemical identification, biological activity, and pheromone trapping. But the fourth meeting, held at Nyon, was exclusively devoted to the technique of mating disruption in fruit orchards and vineyards. Approximately one year before the meeting the Hercon laminate flakes (Table 2) came on the market. Several reports were presented at that meeting on effectivity tests of the flakes, but also of self-made

dispensers, such as rubber tubing made from bicycle-tyres. The main conclusion was that 'despite intensive research efforts, progress has been slow and practical application is still far away. Prospects in wine growing are better than in fruit orchards, probably due to a much simpler pest situation in grapes. More attention to basic studies on behaviour, formulation techniques and on damage assessment methods is recommended'.

WG meeting at Neustadt in 1986

I earlier mentioned that the atmosphere at this meeting was full of expectations. This was stimulated by promising results obtained with mating disruption of the grape berry moth, *Eupoecilia ambiguella,* in trials over an area of more than 100 ha of grapes by the colleagues at Neustadt, and on another 30 ha by others working in the region along the river Moselle. Both groups used laminate flakes or the newly introduced twin-ampullae in their tests. As the outcome looked sufficiently reliable over a period of 5 years, the synthetic pheromone of *E. ambiguella* could be registered in Germany for control of this moth by mating disruption, initially only against the second generation, later also against the first generation (it took another 8 years before the pheromone of the grape vine moth, *Lobesia botrana* was registered!). Heinrich Arn, the new convenor of the Working Group called this 'a breakthrough where many pheromone researchers in Europe are hoping for and perhaps the signal for developing other applications'.

But in other areas, for instance, fruit growing, research on mating disruption appeared to proceed much slower with a significant percentage of failures, often for unknown reasons. Prospects for codling moth looked better than for leafrollers and in 1986 the Swiss authorities accepted a provisional registration of the pheromone of the codling moth, *Cydia pomonella*, primarily based on an extensive 10-year testing programme in that country.

Despite these positive developments, one can conclude from the proceedings that a better knowledge of the mechanisms of disruption is still urgently needed, so that growers or advisors can be told when the method will or will not work, and what and how much the dispensers should contain and when and where in the treated area they ought to be placed for best results.

WG Meeting at Granada in 1990

This meeting successfully linked the research activities in North- and Central Europe with applications in the Mediterranean region. In various countries mating disruption tests were set up: in Israel as well as in Spain for the pink bollworm, *Pectinophora gossypiella* and the armyworm, *Heliothis armigera* in cotton, in Italy and Spain for the peach moths, *Grapholita molesta* and *Anarsia lineatella,* in Italy also for codling moth and apple leafrollers, and in Spain for the grape vine moth, *Lobesia botrana.* All these trials were in the experimental phase and executed on plots of a few ha at most. A significant improvement was the introduction of the twist-tie polyethylene dispenser (see Table 2) into most of the tests.

The only large-scale commercial application of pheromone mating disruption was reported from Egypt against the pink bollworm on several hundreds of ha of cotton. However, our colleagues involved pointed out that, although it had considerable success and proven advantages over broad-spectrum insecticides, pheromones have yet to be generally accepted or applied on a broader basis in Egypt. A fully integrated IPM system for control of cotton pests in Egypt needs to be developed first, before control by pheromones can really become effective.

WG Meeting at San Michele all'Adige in 1992

I remember in the first place that the wine and food has never been so good as at this meeting: the arrangements of our Italian collegues were outstanding! But Heinrich Arn wrote in his report for the General Assembly of IOBC/WPRS when referring to this meeting 'that mating disruption, the most successful of the control techniques employed with semiochemicals, is in a crisis'. He was right. Most studies on mating disruption presented at this meeting showed the same trend: mainly positive results, but some failures. This created a lot of frustration among the participants, the more as most of these failures could not be properly explained. Inadequate performance of the dispensers could be the cause, although the two mostly used dispensers, the twisted-tie ropes and the twin-ampullae were experienced as an improvement. Also high populations densities or migration were suggested as the problem, but it was all speculative. It came back to the old problem of a lack of knowledge of mechanisms and the actual conditions for successful control, such as the composition and protection of the active ingredient, its dosage and dispersal in the field.

So the major recommendations of this meeting were: 1) Continue with implementing mating disruption at its current technological state in integrated production, 2) Study the mechanisms of mating disruption in order to optimize active ingredients and application techniques, and 3) Improve the reliability of controlled release formulations.

In connection to this it is worth mentioning that a portable EAG apparatus for the measurement of pheromone concentrations and dispersion in the field was demonstrated at this meeting by Koch and co-workers: an important breakthrough that will enable us to optimize the formulation of the active ingredient in the dispensers.

WG Meeting at Chatham in 1993

This was a large meeting with 134 participants from 30 different countries, that brought together colleagues from both the 'developed' and the 'developing' countries with the aim of reviewing the current state of pheromone technology in Europe and whether and how this technique might be transferred to developing countries. The presentations covered the whole field from basic science to field applications with special attention devoted to Eastern Europe, Africa, Asia and South America. In addition to Europa experimental studies on mating disruption were reported from the Ivory Coast, Pakistan, Egypt, and China. Large-scale commercial application of the pink bollworm sex pheromone in Egypt was successfully continued in 1992 over more than 20, 000 ha of cotton. Two formulations were used: the twist-tie dispensers and microcapsules in combination with one or two treatments of conventional pesticides, if necessary. Worth mentioning are also 1) the mating disruption project against the rice stem borer, *Chilo suppressalis*, in Spain on 4000 ha, using self-made hand-applied dispensers, and 2) the area-wide application of Oriental fruit moth mating disruption in the Tulbagh valley in South Africa over approximately 2000 ha..

Basic research presented were among others: analysis of pheromone plume structure in the field by means of single sensillum recording, evaluation of disruption mechanisms in field wind tunnels, and laboratory studies on insect behaviour. Of a more applied character were the tests of two new dispensers: the TNO polymer dispenser and the Consep membrane (Table 1). A strategic study was devoted to the registration of semiochemicals as pest control agents.

WG Meeting at St. Peters Insel in 1994

This was a special meeting for which a small group of pheromone experts was invited in a courageous attempt to set up practical guidelines for a better, more effective use of the mating disruption technique. Unfortunately the meeting stranded in endless discussions, for instance, on the definitions of the various terms and standards to be used in this area. The guidelines were never finished.

WG Meeting at Montpellier in 1996

Only 4 years after the crisis meeting at San Michele the atmosphere had changed to moderately positive at Montpellier. The new convener, Peter Witzgall, wrote in his preface: "the proceedings show evidence that the mating disruption technology has reached maturity", may be at first sight a surprising change in opinion, indeed! But it was a fact that the number as well as the size of successful mating disruption projects had grown significantly in those years. Minks & Kirsch estimated that the world-wide application of commercial mating disruption products increased to ≅ 300,000 ha in 1996. More than 80% of this acreage was on cotton against *P. gossypiella* in Egypt, the south-western states of the USA and in Mexico, and ≅ 5% respectively on pome fruit against *C. pomonella* in the north-west of the USA and in N. Italy and on grapes against the grape moths *E. ambiguella* and *L. botrana* in south-west Germany. And is also true that in several cases mating disruption has become an integral part of pest control programmes, and may even appear to be more (cost-)effective than conventional pesticides.

It is also undeniable that in past years considerable progress has been made with the industrial synthesis of pheromones, controlled release technology and the in-field measurement of airborne pheromones. And with the increasing importance of mating disruption growers and advisory organisations have acquired more knowledge in how to apply the technique.

Our convener rightly observed that, now we have arrived at this point "we must consolidate the recent achievements and establish mating disruption as a reliable and cost-effective technique. Our success will determine the public interest in further research in the field of olfactory communication and chemical ecology.

WG Meetings at Dachau in 1998 and at Hohenheim in 1999

These meetings showed a steady increase of the major commercial mating disruption operation in Europe: in grapes in the Palatinate region on ≅ 20, 000 ha, to which should be added several thousands of ha from other regions in Germany, 4500 ha from Switzerland and ≅ 1500 ha from the Trentino region in Italy. However, in fruit there was an unfortunate set-back, as the project in South Tyrol in Italy almost disappeared, mainly because of a lack of financial support. But in the western USA the codling moth area-wide management programme was extremely successful and grew further to 24,000 ha in 1999 and to probably 40,000 in the 2000 season. Also cotton control by pheromones continued to increase.

Conclusions

My conclusions can be brief. Although the development of pheromone mating disruption has progressed much slower than was anticipated in the 70's and the early 80's, we can only point

to a limited number of successful introductions, I still think that it is the best insect control method that one can imagine. Because of its environmentally friendly properties it ideally fits in integrated and biological control programmes, for which there is an increasing public demand. So I believe it is well worth to continue or even to intensify our efforts to work on our case and to make pheromones ready not only for mating disruption, but also for monitoring, mass trapping or attract and kill, depending on the pest and the crop situation.. In doing so I really hope that besides the practical work my younger colleagues will get the opportunity to proceed with basic and strategic research, as this is indispensible for a further successful development of mating disruption. For the moment, though, I am not really worried as I am pleased to see in the programmes of the Working Group Meetings that more than half of the contributions deal with basic studies.

Undoubtedly our Working Group will continue to play an crucial role in these developments, just as it has done in the past 25 years. It has been and still is an important meeting place for all who feel themselves involved in the field insect semiochemicals. Long may the Working Group live!

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