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

editor:

Peter Witzgall

IOBC wprs Bulletin
Bulletin OILB srop

Vol. 24 (2) 2001

International Organization for Biological and Integrated Control of Noxious Animals and Plants
Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles
West Palaearctic Regional Section / Section Régionale Ouest Paléarctique

IOBC / WPRS

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

OILB/SROP

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

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

Proceedings of the Working Group Meeting

Hohenheim (Baden-Württemberg, Germany)
November 10-12, 1999

Edited by Peter Witzgall

IOBC wprs Bulletin
Bulletin OILB srop **Vol. 24 (2) 2001**

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Ingetrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Régionale Ouest Paléarctique (OILB/SROP)

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ISBN 92-9067-130-0

Preface

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

The development of new pest control methods is a necessity. The use of several well-established insecticides has been restricted or banned within the past few years. In addition, conventional insecticides meet increasing criticism and dissatisfaction, due to health hazards, environmental problems and insect resistance.

Mating disruption by pheromones. Insects use sex pheromones to communicate for mating. By permeating the atmosphere with synthetic pheromone, olfactory communication and mate-finding can be prevented. Pheromones are species-specific and non-toxic, beneficial arthropods are not adversely affected. The mating disruption technique is close to a commercial breakthrough, but needs to become more economic and more reliable, especially at high population densities. Most important work is done by growers and pest control experts who coordinate and survey the practical use of pheromones. Successful applications require basic knowledge of dispenser materials, active ingredients and their effect on insect behaviour, as well as the population dynamics of the pest species. Another crucial issue is the assessment of damage during the season.

Monitoring traps baited with sex pheromones. Pheromone traps are an efficient, inexpensive and specific tool to detect the presence of insects and to monitor their flight period. Pheromone traps are available for virtually all economically important lepidopteran species. Pheromone lures are commercially available from a number of companies - but these lures vary greatly in efficacy. This is due to a varying degree of purity of the starting materials as well as inadvertent changes in dispenser materials, lure composition and dose from one year to the other. The Working Group is engaged in the quality control of pheromone lures.

Other biological techniques. The mating disruption technique is available only for key species. Insect pathogens, parasites and predators are efficient antagonists for a range of insects, which complement and enhance the efficacy of pheromonal methods.

Development through continued research. In Europe, mating disruption is used on ca. 30 000 ha against grape berry moths (*Eupeocilia ambiguella, Lobesia botrana*) and on ca. 10 000 ha against codling moth (*Cydia pomonella*). These applications demonstrate the potential of the mating disruption technique for insect control. However, these orchards and vineyards in Germany, Italy and Switzerland represent only a fraction of the surface on which conventional insecticides are used. The practical use of pheromones is still lagging behind our expectations. Pheromone research, funded by the public hand over four decades, has provided the basic knowledge for the development of new pest control techniques. It is important to realize that only continued, goal-oriented research will lead to reliable and more widespread applications. The practical implementation of pheromonal methods is certainly a challenging task. It can only be achieved if communication and cooperation between research groups, chemical industries and extension organizations is improved.

Alnarp, December 1999

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Reproducibility and shelf-life of pheromone lures

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Abstract: Standards are needed to assure a continuous qualtiy of lures used in pheromone traps. A logical way to obtain comparable results with pheromone lures is the batch certification applyed by the Phero.Net organisation. Field tests were carried out in 1999 following the Phero.Net standard certification procedure to establish attractivity and specificity of lures of the majority of tested species. We also confirmed a high reproducibility of pheromone lures which were produced with the same chemical batch, but using a new batch of dispensers. Correct storage conditions which are essential for the performance of lures were studied in field tests. Based on these results, we assume that storage of lures in the original package at ambient temperatures for short-term storage by the grower is safe, whereas for insects using doubly-unsaturated compounds, and long-term storage in general, we recommend low temperatures.

Keywords: pheromone lures, reproducibility, batch certification, quality control, shelf life,

Introduction

Pheromone lures in combination with sticky traps are useful and inexpensive tools to detect harmful insect species in a crop. This information can be used to predict infestations at an early stage and to apply insecticides at the right moment. In some cases, low trap catches indicate that the population level of a pest is too low to result in an economical damage and that no treatment is needed.

However, users of pheromone traps often complain that lures from different suppliers and years of manufacture, even if prepared with the best quality control, may differ from others in attractiveness to target and non-target species.

Batch certification

This problem càn be resolved with a batch certification proposed by the Phero.Net organisation (Bengtsson et al. 1999) applying the following rules:

1. A quantity of lure is prepared, set aside under appropriate conditions and given an identification code which carries chemical information. This batch should last for several seasons (Arn et al. 1997).

2. The batch is formulated on dispensers and field-tested to verify if the lure is attractive for the target species and which non-target species are caught.
3. Applied rules for the field tests: a) to establish a distinciton between an effective and an ineffective lure, we used at least 3 replicates; b) quantitative differences between lures were based on tests with 5 to 10 replicates; c) trap catches of less than 10 specimens per trap and season were considered insufficient to claim efficacy (Arn et al. 1979; 1986).

The above procedure defaults to step one on two occasions: a) if the results of the field tests are not satisfactory or b) as soon as the stock is close to depletion.

Reproducibility of Pheromone Lures

The reproducibility in the preparation of pheromone lures was studied in 1999 in various crops and different regions of Switzerland. Fresh lures (Phero.Net 99) were compared with lures of a previous production, made with the same chemicals but with a new batch of dispensers (Table 1).

The obtained data confirm a high reproducibility in attractiveness for the majority of the tested lures with no significant changes in various habitats. As an exception, the *G. lobarzewskii* caps of 99 caught some *C. pomonella,* possibly due to contamination. As a result of this test a considerable number of lures could be certified for the Phero.Net organisation.

Shelf Life of Pheromone Lures

Correct storage conditions are essential to assure the performance of pheromone lures. Phero.Net lures are formulated on gray rubber caps which are mounted on holders and individually packaged in sealed PVDC-laminated bags to reduce risk of deterioration and contamination during storage and handling.

The shelf life was verified in a field test with lures kept for one or two years either at 4°C or ambient. As standard we used lures which were stocked at -18°C (Table 2).

Only the lures of *Lobesia botrana* lost attractiveness even after one year outside the freezer. The other lures were not affected by the storage at ambient conditions.

Based on this, we assume that storage of lures in the original package at ambient temperature by the grower is quite safe for most species. For the pheromones of *Lobesia botrana* and possibly other insects requiring doubly-unsaturated compounds, such as *Cydia pomonella*, and long term storage in general, low temperatures are recommended.

Table 1. Reproducibility of pheromone lures

Species	Number of males caught					
	1st flight		2nd flight			
	Phero.Net cap 99	previous year	Phero.Net cap 99	previous year	No. traps	Location
Adoxophyes orana	12	12	40	30	10	Eschenbach LU
	-	-	653	485	10	Martigny VS
Cydia pomonella	116	68	92	74	10	Maur ZH
	-	-	97	171	10	Martigny VS
Eupoecilia ambiguella	-	-	871	610	10	Giornico TI
	117	93	737	679	10	Maienfeld GR
	157	158	533	465	10	Winterthur ZH
	735	777	3776	3289	10	Aigle VD
Grapholita funebrana	25	22	113	75	5	Mezzana TI
	144	133	243	186	5	Porza TI
	101	94	156	77	10	Münsterlingen TG
Grapholita lobarzewskii	42	37			10	Dietenwil SG
Cydia pomonella	48	0				
Grapholita lobarzewskii	42	37				Wädenswil ZH
Cydia pomonella	35	0				
Lobesia botrana	-	-	112	105	10	Giornico TI
	394	246	321	118	10	Maienfeld GR
	66	40	106	118	10	Fläsch GR
	-	-	3032	2682	10	Vétroz VS
Pammene rhediella	0	1			5	Mezzana TI
	7	11			5	Iragna TI
	517	495			5	Entetschwil TG
	13	15			5	Dietenwil SG
Pandemis heparana	84	41			10	Zizers GR
Sparganothis pilleriana	102	189			10	Leytron VS
Synanthedon myopaeformis	53	37			8	Hitzkirch LU
Zeuzera pyrtina	1	-			5	S. Antonino TI
	28	-			5	Fully VS
	20	-			2	Remigen AG
	25	-			3	Roggwil TG

Table 2. Effect of storeage on attracitivity of pheromone lures

	ageing conditions	1995 males caught[a] caps aged 1 year	1996 males caught[a] caps aged 2 years
Adoxophyes orana	- 18° C	322 a	388 a
	+ 4° C	217 a	340 a
	ambient	383 a	363 a
Cydia pomonella	- 18° C	78 a	
	+ 4° C	85 a	
	ambient	90 a	
Eupoecilia ambiguella	- 18° C	74 a	282 a
	+ 4° C	85 a	240 a
	ambient	90 a	263 a
Lobesia botrana	- 18° C	1162 a	809 a
	+ 4° C	449 b	902 a
	ambient	454 b	306 a

[a]Numbers followed by the same letters are not significantly different at P=0.05 (ANOVA, followed by Duncan test)

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Identification of *Cameraria ohridella* sex pheromone and its possible use in horse chestnut protection

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Abstract: The major component of sex attractant released by the virgin female of the horse-chestnut leafminer Cameraria ohridella Deschka et Dimiæ (Lepidoptera: Gracillariidae), which devastates horse chestnut trees in Europe, was identified in picogram quantities as (8E,10Z)-tetradeca-8,10-dienal. The spectral methods were not used, the identification relied entirely on alternative analytical methods like 1) gas chromatography with electroantennographic detection (GC-EAD), 2) calculation of Kovats' indices of the active principle on different GC phases, and 3) construction of EAG response profiles to C12 and C14 saturated and unsaturated standards with different functional groups. The synthetic pheromone was prepared by a stereospecific synthesis and shown to be highly active for conspecific males and was proved to be fully comparable to the natural substance in all respects. The potential use of the pheromone to protect horse chestnut trees in Europe is discussed.

Key words: sex pheromone, identification, horse-chestnut leafminer, *Cameraria ohridella*, Lepidoptera, Gracillariidae, EAG, GC-EAD, wind tunnel, field test, delta traps

Introduction

The horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimiæ (Lepidoptera: Gracillariidae) is presently the most dangerous pest of horse-chestnut, *Aesculus hippocastanum* L., in Southern and Central Europe. This pest gradually radiated from the original place of occurrence in Macedonia (Deschka & Dimiæ 1986) to Austria, Hungary, Germany, Slovakia and the Czech Republic (Skuhravy 1999). *C. ohridella* can have up to four generations a year and the infested trees are usually completely defoliated before the end of the season. When trees are precociously defoliated for several consecutive years they can eventually die, which greatly affects the environment in urban areas.

Current literature reports bionomical data of the species, until now chemical communication has not been studied. At present, the possibilities to control this pest are rather limited (raking of the damaged leaves, spraying with insecticides). The identified sex pheromone can represent a basis for an alternative - designing an integrated pest management (IPM) program.

Materials and methods

Insects

Insects were collected either as adults from naturally occurring populations in Prague (park Stromovka and Royal Garden of Prague Castle) in the morning hours from 22 July to 28

September, 1998 or infested chestnut leaflets were taken to the laboratory, immersed in water and kept in fine fabric-netted cages (0.4 x 0.4 x 0.4 m) at room temperature. Alternatively insect emerged from pupae overwintering in collected dried fallen leaves outdoor. Emerged adults were periodically removed to prevent mating. Males and females were kept separately in glass containers with perforated polyethylene stoppers at LD 14:10 and 24 °C. Females were observed during photophase to determine their calling period. Males were maintained at 5 °C until used in behavioural and electrophysiological (EAG) experiments.

Sample Preparation and Extraction

Calling virgin females (1 to 2 days old) were cooled to –20 °C for 5 min. The abdomen of a female was squeezed with a pair of forceps under a binocular microscope and the extruded abdominal tip was excised between the 7^{th} and 8^{th} abdominal segments, extracted with hexane (ca 10 μl per abdominal tip) and the solution was stored at –20 °C.

Chemicals

High purity grade hexane (Fluka) was used for extractions of calling females and a sample preparation for GC analyses. Series of dodecen-1-yl, tetradecen-1-yl acetate, dodecen-1-ol and tetradecen-1-ol monoenes were obtained from IPO-DLO (Wageningen). Dodecenals and tetradecenals series were prepared from the corresponding alcohols with PCC oxidation (Svatos at al. 1999). The geometric isomers of the 7,9-; 8,10-; and 9,11-tetradecadienals were prepared in our laboratory (Svatos at al. 1999, Hoskovec & Svatos 2000).

EAG Recordings

A male to be used in EAG recordings (Hoskovec et al. 1996) was placed in the tip of a disposable pipette (200 μl, Eppendorf) with a cut end. The exposed head and antennae were fixed in place by melted wax. Electrical activity from the fixed antenna was recorded by using glass Ag/AgCl microelectrodes filled with insect haemolymph saline. Dissected female glands or extracts were applied to a filter paper disc (10 mm) inserted into a Pasteur pipette. Stimuli were delivered onto the prepared antennae by air pulses blown through the pipette.

Wind tunnel bioassay

C. ohridella males (3–4 days old) were flown in a wind-tunnel (Hoskovec et al. 1996). Air velocity was maintained at 0.4 m/s. The experiments were performed from 2 to 3 h after the beginning of photophase. Males were released from the middle part of the tunnel into an odour plume which was created by pinning a filter paper disc (7 mm, Whatman No. 2) loaded with the test stimulus onto a holder placed centrally near the upwind end.

Field tests

The field tests were performed in the upper part of the Royal Garden of Prague Castle from 22 July to 28 September, 1998 an from 1 May to 10 September 1999 in park Stromovka (Prague). Delta traps (25 x 10 cm), baited either with three virgin females or with three males housed in

small metallic cages (2 x 1.5 cm) and fitted with sticky inserts (Tanglefoot glue) were suspended in the lower part of the horse-chestnut canopy ca 2.5–3 m above ground level. The traps were checked daily and trapped males were identified and counted. Cages without lure were used as control.

Results

Traps with virgin females caught a substantial number of conspecific males when compared to the control traps (Table 1). Traps baited with males were not attractive. The insects caught in control traps were either different microlepidopteran species or mixtures of *C. ohridella* males and females. When a series of 12:Ac and 14:Ac monoenes and their mixtures were tested in the field, none of those chemicals selectively attracted *C. ohridella* males, nevertheless, several different related insect species were trapped (Svatos et al. 1999a). Interestingly, some of the tested compounds showed notable EAG potentials (Z10-14:OAc >>E6-12:OAc).

Table 1. Catches of *C. ohridella* males in Delta traps, Royal garden of the Prague castle, 1998.

Lure:	3 Females	3 Males	Control
Date	Moths caught: *C. ohridella* males (other species)		
30.7.[1]	113 (27)	nd	5 (1)
10.9. - 12.9.[2]	56 (14)	1(0)	1 (0)
21.9. - 28.9.[2]	18 (3)	0 (0)	0 (0)

nd: not determined, [1] third generation, [2] fourth generation.

When virgin females were observed at the beginning of photophase, more then 80% showed a typical calling position as was described by Mozuraitis et al. (1997). Females were calling from the first post-emergence day for ca 3 h, with a maximum frequency at 1.5 h of photophase period. Females ceased calling after the 4th day of their life. One dissected female abdomenal tip, presumably containing the pheromone gland, was highly attractive for *C. ohridella* males in wind tunnel experiments. Large numbers (90%) of tested males took off and flew in the odour plume, 20% reached the odour source and made copulatory attempts (N = 20). When a similar preparation (from three females) was inserted into a glass Pasteur pipette and blown over male antennae, significantly higher EAG potentials (0.28 ± 0.04 mV, N = 55) were measured than for the control preparation (0.02 ± 0.05 mV, N = 55). Hexane extracts of three female abdominal tips loaded on a paper disc was very attractive for *C. ohridella* males in the wind tunnel bioassay, and the attractiveness was comparable to that elicited with the abdominal tip preparation. The extract (from one tip) elicited an EAG response comparable to excised abdominal tips.

The GC-EAD (male antenna was used as a biological detector, Struble & Arn 1984) examinations of pheromone gland extracts showed pronounced antennal activity on the EAD trace, but no corresponding GC peak was detected by FID detector (Figure 1A). Furthermore, when ~ 100 female equivalents (FE) were injected on a GC/MS (ion trap) instrument no reliable mass spectrum was obtained from the EAD-active area. Clearly, the only analytical tools available were: 1) retention behavior of the EAD active peak on different GC phases, 2) an examination of antennal specificity to libraries of pheromone-like synthetic compounds (EAG response profiles), and 3) micro-derivatizations of gland extracts combined with EAG. Kovats' indices (KIs) of the EAD peak (Figure 1A) were determined using several GC phases of increasing polarity and the measured values were compared with KIs of straight-chain aldehydes, alcohols and acetates (Table 2).

Table 2. A comparison of Kovats' indices[a] of the EAD active peak in *C. ohridella* female extracts with some synthetic compounds

GC phase[b]	Female extract[c]	Compounds			
	ca 5 FE	12:Ac	14:Ald	14:OH	8E,10Z-14:Al[c]
DB-1[d]	1623.3	-	-	-	1623.9
DB-5[d]	1674.4	1605.9	1610.8	1675.4	1674.3
DB-WAX[e]	2031.2	-	-	-	2031.8

[a] based on saturated hydrocarbons; [b] J & W Scientific, 30m × 0.25 mm, film thickness 0.25 mm; [c] for the EAD trace; [d] 170 °C; [e] 140 - 240 °C @ 5 °C / min

Based on these measurements, a series of all geometric isomers of dodecen-1-yl acetates, tetradecen-1-ols and tetradecenals and their saturated congerers (1 μg) were tested on the EAG preparation. Both KIs and the EAG profiles obtained clearly showed that the pheromone should bear the aldehyde functionality and that unsaturation must be situated near the C-9 atom. The aldehydic nature of the pheromone was confirmed by micro-derivatization experiments where the hexane extracts, treated with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride in methanol solution (Svatos et al. 1999b) were tested both on EAG and in a wind tunnel. The pheromonal activity diminished after this derivatization. However, (*E*)-tetradec-9-enal which had the highest EAG potential (from EAG profiles) showed low behavioral activity (wind tunnel bioassay) and a different KI (1601.8 on DB-5) in comparison to the natural extract. Therefore, it was not considered to be the sex pheromone.

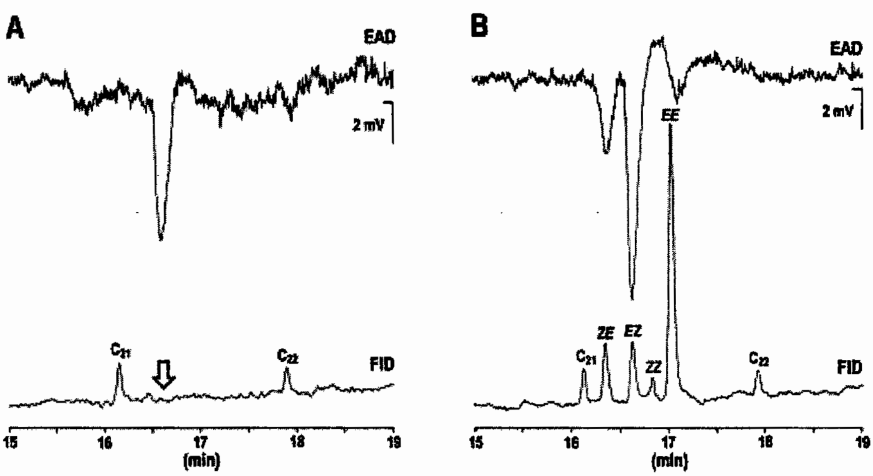

Figure 1 Sections of GC-FID-EAD traces; **A**: a hexane extract of *C. ohridella* calling females (~ 5 FE); **B**: a synthetic mixture of 8,10-tetradecadienal (7) isomers (100 ng) on DB-WAX phase, both co-injected with hydrocarbon standards (C_{21}, 5 ng).

From comparison of KIs on DB-1 and DB-5 phases it seems reasonable to speculate that the pheromone should have more double bonds. Its KI on the DB-5 phase is much higher than the respective KI on a DB-1 phase, which usually points towards conjugation (Attygalle & Morgan 1988). Based on this consideration, and on the measured EAG profiles we prepared mixtures of all geometric isomers of 7,9-, 8,10-, and 9,11-tetradecadienals (Svatos et al. 1999b).

The EAG examination of the mixtures of geometric isomers showed that only an isomeric mixture of 8,10-tetradecadienal isomers (8,10-14:Al) displays the highest antennal activity. Geometric isomers of the 8,10-14:Al were reasonably separated on GC capillary columns and we were able to obtain GC-EAD of the individual isomers (Svatos et al. 1999b). Although males' antennae were, to some extent, sensitive to more than one geometric isomer in the mixture we could clearly eliminate the Z8,E10-14:Al and E8,E10-14:Al isomers. The E8,Z10-14:Al isomer showed higher EAD activity than Z8,Z10-14:Al isomer (Figure 1B). When E8,Z10-14:Al was measured on GC-EAD using several GC phases the corresponding EAG activity showed identical retention behavior (at sub-ng amounts) to hexane-extracted female abdomens (Table 2).

In wind tunnel behavior assay 1 - 0.1 pg of the E8,Z10-14:Al isomer displayed high attractiveness, which was comparable to 3 FE of gland extract (Figure 2). In contrast, the pure Z8,Z10-14:Al displayed a different KI to the natural extract and its behavioral activity was ne-

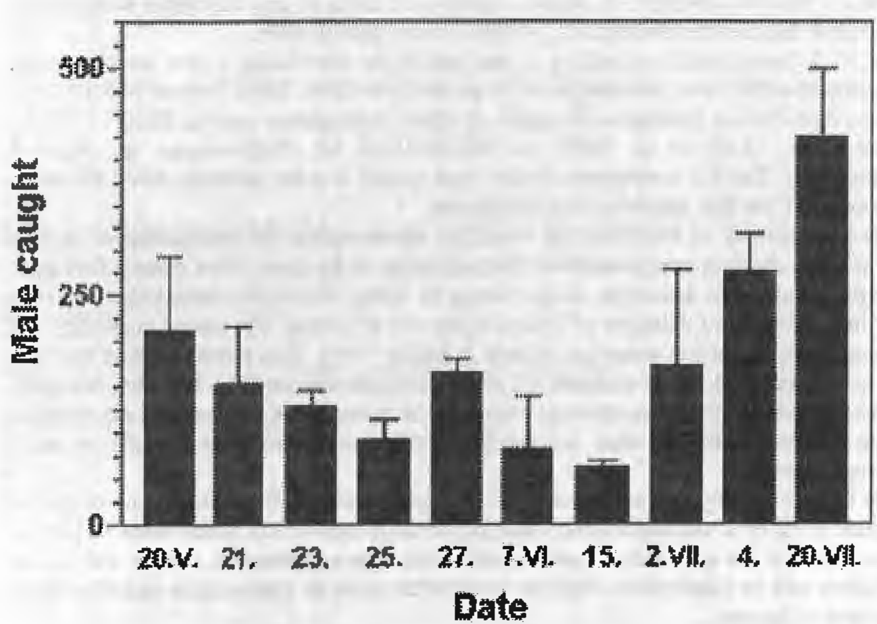

Figure 2 Catches of Δ-traps baited with synthetic sex pheromone (sticky inserts, rubber septum, N = 3). Each column represents number of *C. ohridella* males caught per day and trap. The bars represent S.E.

gligible. In preliminary field experiments sticky traps baited with 5 ng of E8,Z10-14:Al isomer (loaded on BBL Taxo paper disc, 1/2 inch dia) were, similarly attractive for *C. ohridella* males as virgin females. All the presented data confirm that E8,Z10-14:Al isomer is the main component of *C. ohridella* sex pheromone.

First field experiments with Delta-traps baited with a rubber septa lured with synthetic pheromone showed high attractiveness and selectivity of the pheromone. Preliminary results are presented in Figure 2.

Discussion

So far sex pheromones were identified for three *Phyllonorycter* species, which are related to *Cameraria*. Females of the tentiform leafminer, *P. mespilella* Hübner, produce E10-12:OAc accompanied by trace amounts of E4E10-12:OAc (Gries et al. 1993). For the tentiform leafminer moth, *P. ulmifoliella* Hübner, Z10-14:OAc was identified as the sex pheromone (Mozuraitis et al. 1997). Similarly for the apple leafminer, *P. ringoniella* Matsumura, where the pheromone is a mixture of E4Z10-14:OAc and Z10-14:OAc in the 6/4 ratio (Boo & Jung 1996). Structural similarities of sex pheromones in related species lead us to the assumption that *C. ohridella* can, in part, employ similar chemicals. But field tests showed that none of the tested compounds (isomeric dodecenols, tetradecenols and the corresponding acetates) is selectively attractive for *C. ohridella* males (Svatos et al. 1999a). Similar results were independently obtained by other research groups (G. Szöcs personal communication). Based on those results reported attempts of several commercial firms to use dispensers attractive for *Phylonorycter* spp. for monitoring of *C. ohridella* seem questionable.

(8*E*,10*Z*)-Tetradeca-8,10-dienal is, to the best of our knowledge, a new sex pheromone and the first identified sex pheromone in the genus *Cameraria*. Other isomers of 8,10-14:Al have been described as attractants for males of other Lepidopteran species; 8E10E-14:Al for *Acrocercops* sp, (Ando et al. 1980) and 8Z10Z-14:Al for *Phyllonorycter* sp. (Reed & Chisholm 1985). The E,Z conjugated double bond system is quite common, found for example in bombykol, the first identified sex pheromone.

The high activity of 8E10Z-14:Al would be advantageous for establishing of an IPM system of horse chestnut trees protection. Determination of the onset of the miner's first generation will be helpful to determine proper timing for using of selective insecticides and additionally in forecasting of damages of foliage at the end of season. The second possibility will be to adopt male confusion technique (Cardé & Minks 1995). This control method has been proved to be successful e.g. in orchards (*C. pomonella*) and vineyards (*L. botrana*), the usability in the case of isolated horse chestnut trees must be proved. The third method can be based on using pheromone-baited traps contaminated with insecticide (trap-and-kill) or insect growth regulators.

The high selectivity and attractiveness of the pheromone will possibly allow us to find the original place of *C. ohridella* occurrence and to study mechanism which keeps its population low there. In the case that a larval paratization, entomopathogens, viruses and similar biotic factors will be localized we shall try to introduce them to Europe as an agent for biological control of the pest.

Acknowledgements

We are highly indebted to Mrs. J. Titzenthalerová and to Mr. O. Blasek for skillful technical assistance in insect handling and to the staff of the Royal Garden of the Prague Castle for help in trapping tests and to Dr. J. Liska for identification of insects. Financial supports from the Ministry of Environment of the Czech Republic and our Institute are acknowledged with pleasure.

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Mating disruption in orchards

Pheromones for Insect Control in Orchards and Vineyards
IOBC wprs Bulletin Vol. 24(2) 2001
pp. 13-22

Four years experiences with tortricid control in apple orchards by mating disruption in the region of Lake Constance: Is combination with mating disruption a new chance for other biological methods?

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Abstract: During the years 1996-1999 mating disruption for the control of codling moth *(Cydia pomonella* L.) and summer fruit tortrix moth *(Adoxophyes orana* F.v.R.) was introduced intro practice in the Lake Constance area. In the central areas of codling moth mating disruption average results were satisfying and better than those of "conventional" treatments. The border areas were more problematic. However, nevertheless results can be compared with those of "conventional" treatments. Due to unreliable results, mating disruption is not recommended for the control of *A. orana* any more. The long term approach of preventive population control on wide areas of the mating disruption method should lead to a reconsideration if other biological methods as additive or complementary tools. Granulosisvirus, for instance, has a much higher potential in long term population control than in damage control. Results from commercial orchards are presented and the efficacy and the economic viability of different combinations is discussed.

Keywords: codling moth, summer fruit tortrix moth, mating disruption, granulosis virus

Introduction

The codling moth *Cydia pomonella* L. (CM) and the summerfruit tortrix moth *Adoxophyes orana* F.v.R. (STM) are key pests in apple orchards in the region of Lake Constance, Germany. Since a reduction of the sensitivity of codling moth to IGR's was observed mating disruption was introduced in 1996 in practice as part of a resistance management strategy.

In 1996, 61 growers started with an area of 260 ha. Since one of the main problems was the higher cost of this method the Ministery of Baden-Wuerttemberg supported the use of mating disruption with DM 210,- per ha in the following year. This corresponds to the additional costs of the method compared to conventional insecticides. Consequently the area with mating disruption increased to 830 ha in the next year and to 950 ha in 1999. Due to the augmentation of the regulatory restrictions of conventional insecticides it is expected to increase further on. Since 1996 the efficacy of mating disruption against codling moth and summerfruit tortrix moth was monitored bei E. Lange and M. Trautmann from Plant Protection Service at the ALLB Markdorf, Überlingen.

With growing importance of mating disruption method, the strategy of pest control is changing (Tab. 1). The short term damage control strategy is replaced by a long term population management approach on wide areas. Insecticides used as additive treatments have to meet the requirements of this new strategy und must be reevaluated under this new aspect.

Biological control methods such as Codling moth Granulosis Virus (CpGV) were up to now considered as rather moderate products for short term damage control in rather small areas as most single orchards in Southern Germany. To reach this aim frequent treatments with rather high dosages are necessary which decreases the economic viability. For this reason GpGV was not widely used in integrated fruit production whereas it is a standard treatment in organic fruit production.

Table 1: Comparison of mating disruption with "conventional" IPM-strategy

	"Conventional" IPM strategy	Mating disruption method
Basic principle	Control of the actual population and intervention if the population is so high that damage is to be expected	The aim of interventions is to keep the population so low that damage is not to be expected
Threshold(s)	Comparison of cost of the treatment and probable damage without treatment	A population density lower than a certain threshold is necessary for the effectiveness of the method
Aim	Damage control (short term strategy)	Population control (long term strategy)
Conditions	The products are very effective and reliable "killing agents"	Large areas are covered Good monitoring of population development
Approach	Curative	Preventive

Considering its effect as a long term population control agent as reported by Huber and Dickler (1976) and Charmillot and Pasquier (1998) CpGV could be very interesting as one part of a combined strategy for long term population control on wide areas.

In the year 1999, several orchards with high population density of CM in the same mating disruption area and different additive treatments (GpGV, phosphoric esters (PE)) were monitored to evaluate the costs and the practical impact of these strategies on long term (next generation). population control. The main question was the effect of both treatments on the population performing a second generation.

The long term approach of preventive population control on wide areas may be also a new chance for biological methods of STM control. The efficacy of the commercial STM-granulosis-virus product CAPEX 2 is usually considered to be lower than that of "conventional" insecticides. However, after application of CAPEX 2 most larval parasitoids are still able to hatch from the infected larvae (Andermatt 1989; Kienzle 1995). For the approach of long term population control on wide areas the ratio of surviving pest adults and parasitoids maybe more important than a high efficacy of the product. If the efficacy of CAPEX 2 could be enhanced and/or the dosage (and so the cost) could be reduced by combination with another biological product without modification of the survival of the parasitoids this strategy could be very interesting either as additive treatment in STM mating disruption areas or as concomitant wide area strategy in areas with CM mating disruption. To demostrate the possible potential of such combinations the results of a first field trial on the effect of a combination of CAPEX 2 and NeemAzal-T/S (a commercial product based on plant oils and an extract of the Neem tree *Azadirachta indica*), on the survival rate of pest and parasitoids are presented.

Material and methods

In the region of Lake Constance CM normally has one or two generations. In the ordinary, there is a partial second generation. In the year 1996 CM had high populations in the region of Lake Constance whereas STM population density was of no economic importance . In 1997 the populations of both pests were rather low, however in autumn , there were some outbreaks o f STM. In 1998 the infestation of CM was high and, due to the high temperatures in summer there was a strong second generation. In 1999 the infestation of CM was also rather high, the second generation was late and not so high. STM was of no importance in 1999.

The mating disruption systems used were RAK 3+4 (BASF) and Checkmate CM (Spiess). 1996 the complete area was treated with RAK 3+4, 1997 RAK 3+4 was applied on 77 % of the treated area, 1998 on 80 % and 1999 on 85 %. In the remaining area, Checkmate CM was applied. Checkmate CM is a combined system were until June treatments with insecticides are used whereas afterwards CM is controlled by mating disruption. In the orchards where RAK 3+4 was applied, in some cases a treatment against *Grapholita lobarzewskii* with fenoxycarb took place at the end of May or the first decade of June. Sometimes also in July fenoxycarb was applied to control STM. Both measures are expected to have a side-effect of additive control of CM.

During the season a careful monitoring is performed by the Plant Protection Service. The data presented in this paper, however, are due to the visual control of 1000 fruits per orchard shortly before harvest time (CM, fruit damage of STM) and of 300 shoots per orchard in summer (population control of STM). In total, 364 orchards for CM and 178 orchards for STM were controlled.

The orchards for the observation of the long term effects of CpGV and phosphoric esters were situated in the same mating disruption area with similar infestation rates. Both orchards were rather small (about 1 ha). The orchard with CpGV is managed according to the guidelines of organic farming since five years. Thus, CpGV was used for five years successively. The application dates are described in Figure 1. CpGV was used in 1/10 and 1/3 of the regular concentration, phosphoric esters with standard concentration.

The fruit damage (number of damaged fruits per tree) was assessed on 9.7. and 26.7. 99. Injured fruits were taken to the laboratory and stored until larvae emerged. Two kinds of fruit damage were distinguished: "active" damage, when a living larva emerged and "stopped" damage when the larva could not be found and the gallery did not reach the core.

Card bends were applied around 130 - 150 trees per orchard. The aim was to monitor the number of CM larvae of the first generation that may result in (or might lead to) a second generation. With the purpose to allow a better comparison of the results, the number of injured fruits or larvae in the card-bends per tree were multiplicated with the respective number of trees per ha in each orchard. In early summer 1999 temperatures were rather low. This led to a small percentage of larvae pupating for a second flight.

For this reason the card bends were monitored two times: 1) 26.7.99 at 622 degree days (the degree days are calculated as daily mean of the hourly means of the values measured each 12 minutes) shortly before the hatching of the first adults of second generation 2) 10.8.99 at 782 degree days.This last date was chosen in order to gather the larvae that in a year with a hot early summer could have potentially pupated to perform a second generation.

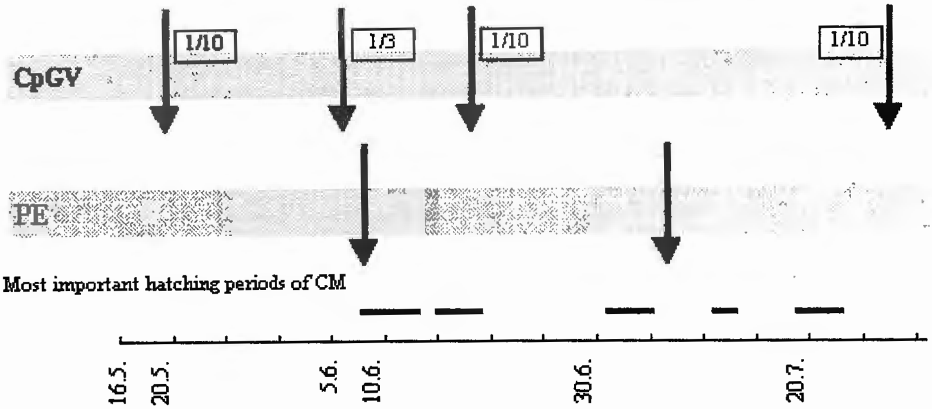

Figure 1. Application dates and most important hatching periods of CM in the investigated interval in the two orchards in comparison for additive treatments at Lake Constance

Two orchards of about 1 ha with the same apple variety situated at opposite of each other were chosen to evaluate possible effects of combining CAPEX 2 and NeemAzal-T/S on the survival of adults of STM and its parasitoids. One plot was treated with CAPEX 2 standard concentration at red bud stage and about twenty days afterwards with half concentration. Due to problems of the sprayer of the fruitgrower, the treatments were applicated some days later than normally recommended. In the other plot the standard concentration (3 l/ha) of NeemAzal-T/S was added to the first CAPEX-treatment. Some days after the application the webs of around 80 larvae per plot were covered directly on the infested shoot in the field with nets so that the development of individual larvae could be monitored. The hatched adults of STM and the parasitoids were determined.

Results

The average fruit damage by codling moth in the orchards in the four year period was 0,9 % in the central area of the mating disruption. 11 %. of the orchards showed fruit damage higher than 2 %. In the border areas the results were different: the average damage was 2,2 % with 34 % of the orchards showing a fruit damage higher than 2 % was found.

Looking at the average of three years results the efficacy of mating disruption of STM with 0,9 % of fruit damage in the central aerea and 0,6 % in the border aerea seems satisfying. However, these results have to be discussed in a broader context: The fruit damage not always shows the real abundance of STM and the infestation of STM was rather moderate - with one exception:

In 1996 the population of STM was very low, the more surprising was its sudden appearance in the following year.

In the central area of the two largest mating disruption areas in the region the infestation on shoots and fruits was higher than in the border area. In July 1/3 resp. 1/5 of the orchards showed an infestation rate on the shoots higher than 5 %.

Table 2. Codling moth mating disruption (RAK 3+4/CheckMate CM): Assessment before harvest (1000 fruits per orchard) Four-year-results, region of Lake Constance

Location	Year	Number of orchards	% Orchards with damage < 1%	1 - 2 %	> 2 %	Average damage in %
Central area	`96	40	53	32	15	1,5
	`97	74	73	16	11	0,7
	`98	39	55	33	8	1,1
	`99	26	58	27	15	0,9
Average	*96-99*		*64*	*25*	*11*	*0,9*
Border area	`96	32	22	28	50	3,5
	`97	51	63	27	10	0,9
	`98	49	31	18	51	2,5
	`99	31	39	29	32	1,7
Average	*96-99*		*41*	*25*	*34*	*2,2*

A comparison of the orchards in the central area of mating disruption with orchards nearby treated with "conventional" insecticides shows noticeably worse results for the "conventional" method. The efficacy of mating disruption in the border area was similar to the "conventional" treatment (Tab. 3).

Table 3. Comparison of Codling moth mating disruption (RAK 3+4/CheckMate CM) and "conventional" treatments (common insecticides); Assessment before harvest (1.000 fruits per orchard); Results of `98-`99, Lake Constance

Location	Years	Number of Orchards	% Orchards with damage < 1%	1 - 2 %	> 2 %	Average damage in %
Central area	`98 - `99	65	58	31	11	1,0
Border area	`98 - `99	80	34	22	44	2,2
Conventional treatment	`98 - `99	22	32	32	36	2,6

Conclusively fruit damage was to be expected and when the fruit damage was monitored shortly before harvest the percentage of orchards with fruit damage higher than 1 % was 32 in the aerea "Kippenhausen" and. 53 in the aerea "Oberdorf". 1998 most fruit growers stopped the use of mating disruption for the control of STM in both mating disruption areas.

Table 4: Summerfruit tortrix moth *(Adoxophyes orana)* mating disruption (RAK 3+4), Assessment before harvest (1.000 fruits per orchard), three year results, Lake Constance

Location	Year	Number of Orchards	% Orchards with damage < 1%	1 - 2 %	> 2 %	Average fruit damage in %
Central area	`96	40	80	12	8	0,5
	`97	47	72	9	19	1,2
	`98	11	91	9	--	0,5
Average	*96-97*		*79*	*9*	*12*	*0,9*
Border area	`96	32	91	6	3	0,4
	`97	34	68	26	6	0,9
	`98	14	86	7	7	0,6
Average	*96-98*		*80*	*15*	*5*	*0,6*

Table 5: Summerfruit tortrix moth *(A. orana)* mating disruption (RAK 3+4), Assessment in summer (300 shoots per orchard) and before harvest (1.000 apples per orchard), results of 1997, Lake Constance

Location	Nr. of orchards shoot-/fruit assessment	% Orchards with infested shoots		% Orchards with fruit damage			Fruit damage in %	
		< 5 %	> 5 %	< 1%	1 - 2 %	> 2 %	Average	Max
K'hausen	6 / 22	67	33	68	18	14	1,0	3,0
Oberdorf	14 / 17	79	21	47	29	24	1,4	3,8

The comparison of additive treatments to Codling Moth Mating disruption gave rather clear results. At the first assessment date the fruit damage in both treatments was nearly the same. "Active" damage was only found in the orchard treated with phosphoric esters. On 26.7.99 the fruit damage in the CpGV-plot was slightly higher but the active damage was much lower. The monitoring of the card bends gave similar results: In the CpGV-plot only at the second monitoring date 0,02 larvae per tree could be observed whereas at both monitoring dates in the "conventional" plot about 0,1 larva per tree were found,. However, the virus treated larvae died soon in the laboratory whereas the other larvae survived.

The results of the combination of CAPEX 2 and NeemAzal-T/S on the survival of STM and its parasitoids are shown in figure 3. The main parasitoid was the Ichneumonid *Teleutaea striata* Grav., a larval parasitoid hatching at the end of larval development. The Braconid *Meteorus ictericus* Nees was only facultatively found. When NeemAzal-T/S was added, the ratio of parasitoids hatched and surviving STM was modified in favour of the parasitoids. The absolute mortality of STM increased, too.

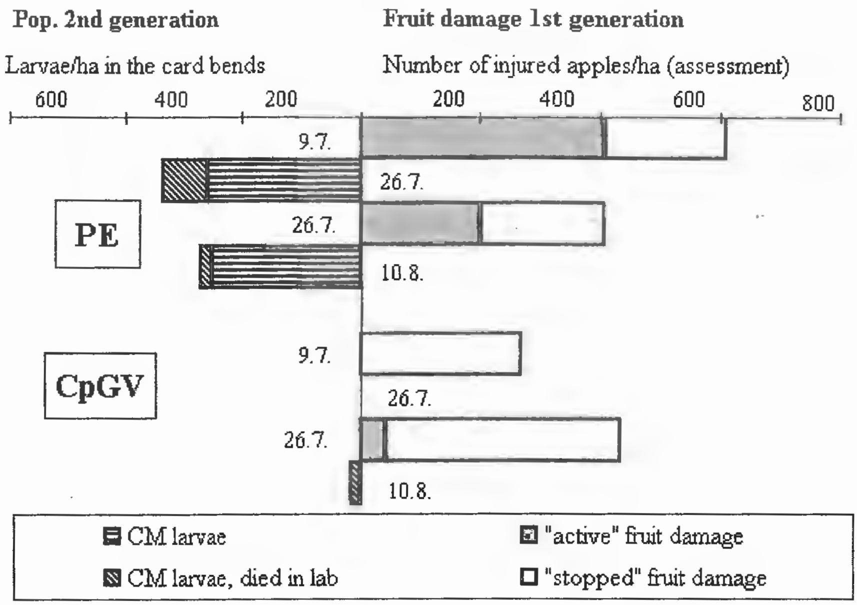

Figure 2. Effects on fruit damage and on population control of the following generation of additive treatments with phosphoric esters (PE) and CpGV.

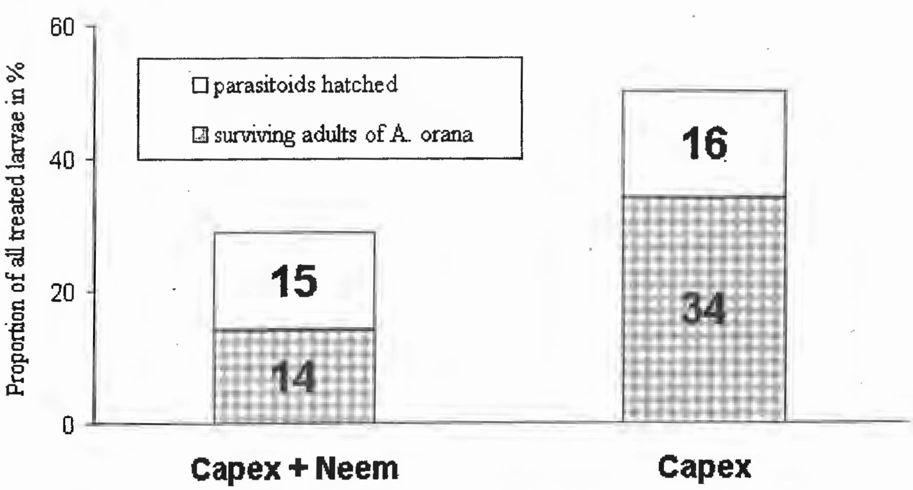

Figure 3. Ratio of surviving adults of *A. orana* and its parasitoids from larvae treated with Capex 2 or with Capex 2 + NeemAzal-T/S

Discussion

Four years experience with mating disruption have shown the potential but also the problems of this method. In the central areas of mating disruption average results are satisfying and better than those of "conventional" treatments. The border areas are more problematic, however, nevertheless results can be compared with those of "conventional" treatments. Summarizing, mating disruption is a valuable instrument for codling moth control. However, a careful monitoring by visual control and pheromone traps during the whole season is indispensable. This is the only method to avoid unpleasant surprises. In the border areas, the strategy must be improved. Regular additive insecticide treatments will be recommended for each year in border areas and in orchards with infestation > 1 % in the previous year

Due to a really drastical change of the situation of registration of insecticides in Germany with IGR's possibly nomore available in the near future, mating disruption will be the key instrument and the basic strategy for codling moth control whereever possible i.e. on estimated 4.000 to 5.000 ha in the Lake Constance area).

Consequently, insecticides will be estimated more and more from the point of view of their suitability as additive treatments in combination with mating disruption.The key factors for this purpose are: (1) Ecological and legal suitability (compatibility with the increasing restrictions for environmental impact; and compatibility with the requirements for additive treatments if mating disruption is supported by the ministery. [In Baden Wuerttemberg, in aereas where codling moth mating disruption is supported, for additive treatments against codling moth only products registered in Annex II of the council regulation (EEC) Nr. 2092/91 can be used.]. (2) Economic viability (low costs of the treatments; few treatments necessary). (3) Reliable efficacy as population control agent

The good efficacy of CpGV as population control agent has been already shown by Huber and Dickler (1976) and Charmillot & Pasquier (1998). The results presented here provide further evidence for the pratical importance of these method . Furthermore, these results have been observed not in experimental orchards but in orchards managed by the growers themselves. Thus, also the economic factors were considered in the spraying programs of both orchards in comparison. In the respective area the intensity of infestation of CM is usually very high. However, in both orchards the interval between the applications were longer than it would have been advisabale in a control program without mating disruption. Especially in the orchard treated with CpGV a period of four weeks passed without application.

As a consequence, in both orchards a certain level of fruit damage could be observed. During the "untreated" period in the CpGV plot fruit damage reached similar levels as in the PE-plot. However, since most larvae died before cocooning and the two cocooned larvae died later on, the effect of the strategy for population control of first generation was eccellent. In contrary, in the PE-treated orchard, a second flight had to be expected. Since the fruit damage of the first generation can be for the most part neglected, altogether, CpGV was the more suitable additive treatment regarding the efficacy for population control. The product cost of all treatments in the investigated period was nearly equal. The product cost of all treatments in the investigated period was nearly equal although. Since the farmer in these cases sprayed anyway for scab control this was of no significance for him. Usually, the spraying dates were oriented mainly on scab control and not on CM hatching dates, CpGV was added in case of need.

Looking at these results - corresponding to the pratical experience of several years - the use of CpGV with lower dosage and larger distances between the applications than generally recommended for damage control in a combined strategy with mating disruption seems a realistic approach. Further research should be done to evaluate the possibilities to reduce dosage and number of treatments. In doing so the first regard should not be the perfect damage control in orchards with normal infestation of CM as it was mostly done up to now but the best strategy for the reduction of CM population on wide areas.

For this approach many single orchards with different owners must work together in the same strategy. In the present situation not all farmers will agree to accept the new "unknown biological product". Others will have problems to accept the population control approach and the possibility of fruit damage even if it generally is of no economic relevance..

As a proposal, the solution for this problem might be that those farmers use normal insecticides for damage control and additionally CpGV to ensure the long term control of the remaining population. This solution would be economically viable, if very low doses of CpGV (1/10 of the normal CpGV dosage) are used and no "extra treament" for the CpGV is necessary but the CpGV is added each time they spray for other reasons. This could be a low cost strategy to ensure long term population control in those orchards, too. IN this way, wide areas could be covered with CpGV.

However, first results of our working group indicate that the strategy proposed may not be successful. It has to be considered that the good experiences with 1/10 of the normal dosage for population control and the present results come from orchards where CpGV has been used for several years. This might lead to a certain accumulation of infective agents in those orchards. In orchards where CpGV never has been applied before, possibly the dosage of 1/10 will not ensure population control. In this cases, a first treatment with a higher dosage might be advisable. On the other hand this would increase the costs so that the farmers might not accept this solution. Further research must answer these questions.

At the actual point of knowledge the combination of mating disruption and CpGV as an economically sustainable strategy for the use of CpGV for population control cannot be reccommended for wide use in practice. These strategies have to be tested and optimized in pilot projects carefully followed by technicians. If more experience is gained the strategy may prove to be a useful solution for CM control in many areas.

For STM it was astonishing that mating disruption results were worse in the central area. The sudden outbreak in 1997 in spite of the low infestation level in 1996 is difficult to explain. Thus, for the control of STM the mating disruption system RAK 4 actually marketed by BASF is not recommended any more because results were too unreliable. The use of the method in practice is strongly decreasing. A complementary strategy of long term population control of STM to CM mating disruption might be the "preventive" use of granulosis virus products, as for instance CAPEX 2.

For the long term population control of STM, a pest that causes fruit damage only at high populations densities a different approach should be taken, since a high efficacy in "killing" seems not as important as a long term persistance (some effect also on following generations) and the survival of parasitoids.

Furthermore, the results of the first field tests seem to indicate that it is possible to enhance the efficacy of CAPEX 2 using NeemAzal-T/S for aphid control before blossom whereas the survival of the parasitoids and the persistance of the virus seem not to be affected by this combination.

Further research must be done on the possibilities to reduce the dosage, especially when the product is used for several years and in combination with NeemAzal-T/S. First promising experiences in practice indicate that an economically suitable strategy of STM control might be possible with reduced dosages of CAPEX 2 (Triloff, 1998).

STM mating disruption may not be effective as exclusive control method for this pest. However, it may have a chance as a tool in a biological strategy of wide area long term population control of STM comparable to the proposed strategy for codling moth mentioned above. This possibility should be considered as a more realistic aim than the approach of trying to control STM exclusively by mating disruption.

In this case research should focus not so much on the highest possible efficacy of the single method buton a good complementary effect and a cost that is low enough to ensure an economic viability of the combination strategy.

Acknowledgements

The team of the University of Hohenheim thanks Dr. E. Lange and M. Trautmann for valuable advice and fruitful discussions. Furthermore, we are very much obliged to Harald Wetzler from PBO, Peter Triloff from MaBo, the members of the Beratungsdienst Ökologischer Obstbau e.V. and Mr. Brandt from LWA Ludwigsburg for help and advice. We thank the ecological and integrated fruitgrowers for the possibility to work in their orchards. Moreover, we thank the Volkswagen-Stiftung, Hannover, for the financial support of the procject.

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Pheromones for Insect Control in Orchards and Vineyards
IOBC wprs Bulletin Vol. 24(2) 2001
pp. 23-30

Ten years implementing codling moth mating disruption in the orchards of Washington and British Columbia: starting right and managing for success!

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Abstract: In 1991, Isomate C (Pacific Biocontrol Corp., Ridgefield, WA.) became the first commercial product registered by the United States EPA for control of codling moth. Currently, there are 5 products available including Isomate C+, CheckMate CM (Consep, Inc., Bend, OR), NoMate CM (Scentry, Inc., Billings, MT) and Disrupt CM (Hercon, Inc., Emigsville, PA). In Washington State in 1991, codling moth mating disruption (CMMD) was used on approximately 600 hectares. By 1999, use of CMMD had increased to approximately 25,000 hectares. In British Columbia, the efficacy of CMMD was first demonstrated in organic orchards in 1990. In 1999, Isomate C+ (currently the only registered product in Canada) was used on approximately 700 hectares. Research into new CMMD technologies including sprayable pheromones and large aerosol dispensers is underway in both countries. Due to increased regulatory restrictions of conventional insecticides and other factors, a large increase in the commercial use of CMMD is forecast for both countries. The careful selection of orchards and the adoption of a pheromone-based IPM approach can minimize the risk and control problems associated with CMMD. Most importantly in orchards with high resident populations, the application of supplemental insecticides and/or intensive sanitation is essential to achieve commercially acceptable levels of control. CMMD works best in orchards where the physical characteristics and environmental conditions ensure a uniform distribution of pheromone concentration. Dispensers should be deployed within 1 meter of the top of the canopy prior to spring emergence. Borders of pheromone-treated orchards are susceptible to high levels of codling moth infestation and growers generally treat the border areas with supplemental insecticides or an increased application rate of pheromone. Border problems can also be reduced by the area-wide application of CMMD technology. Monitoring codling moth adult activity in orchards treated with CMMD is difficult. Capture of moths in pheromone traps baited with 1 mg of codlemone is an unreliable indicator of efficacy. The sensitivity of pheromone traps can be improved by using traps baited with 10 mg lures and locating them in the upper part of the canopy. False negatives (no or low captures of moths in orchard sustaining economic damage) do occur even with the deployment of 10 mg-baited traps and it is important to interpret trap capture data carefully. Fruit should be inspected on a regular basis to ensure that economically acceptable levels of control are achieved. Economic studies show that the selective application of CMMD in conjunction with the judicious use of insecticides can be more cost effective than conventional programs. Other advantages of CMMD include enhanced levels of biological control, reduced costs associated with worker protection and labour management, and decreased potential for the development of insecticide resistance. Disadvantages of CMMD include the perceived high cost of the technology and its application, concerns over efficacy, the need for intensive monitoring and outbreaks of secondary insect pests. Continued research is required to identify the most effective pheromone blend relative to the delivery system. Greater knowledge of moth flight and mating behaviour relative to the dynamics of pheromone concentration within orchard canopies is essential. Increased understanding of the mechanisms of CMMD will aid in the design of more cost effective technology. The development of improved monitoring techniques in conjunction with the use of economic thresholds to determine the need for and timing of supplemental controls is essential. The continued adoption of CMMD will depend on how well it meets grower concerns about risk, efficacy and cost.

Keywords: *Cydia pomonella*, mating disruption

Introduction

Codling moth, *Cydia pomonella* L., is the key pest of pome fruit in Washington State (Beers & Brunner 1992) and British Columbia (Judd *et al.* 1997) and until recently has been primarily controlled by one or more applications of an organophosphate insecticide (Gut & Brunner 1998). Issues associated with the widespread use of organophosphates, including insecticide resistance (Varela *et al.* 1993), toxicity to natural enemies (Gut & Brunner 1998), worker safety and food residues (Brunner 1994), have provided the impetus to research and develop alternative control technologies. Research on the use of pheromone-mediated mating disruption technology for control of codling moth (CM) has shown considerable promise in various pome fruit production areas around the world (Rothschild 1980; Charmillot 1990; Thomson et al. 1999; Barnes & Blomefield 1997, Waldner 1997).

History of use in Washington and British Columbia

In Washington State, research into the use of pheromones to control CMbegan in 1987 (Howell *et al.* 1992). In small plots, levels of fruit damage in pheromone-treated plots relative to untreated controls were reduced by 80 to 90%. However, the authors cautioned that CMMD would not work well as a stand alone technology when CM populations were high and supplemental applications of insecticides would be necessary to ensure commercially acceptable control. In 1991, Isomate CTM (Pacific Biocontrol Corp., Ridgefield, WA.) became the first mating disruption product to be registered for control of codling moth in Washington State. In 1991, approximately 600 hectares of pome fruit in Washington State were treated with codling moth mating disruption (CMMD) (Thomson 1997). By 1999, the use of CMMD had increased to approximately 25,000 hectares and a substantial increase in use is forecast for 2000 (J. Brunner, P. communication). Currently, commercial products are available from Pacific Biocontrol, Ridgefield, WA (Isomate C+TM, Isomate CM/LRTM), Consep, Inc., Bend OR (CheckMate CMTM), Scentry, Inc., Billings, MT (NoMate CMTM) and Hercon, Inc., Emigsville, PA (Disrupt CMTM). In Washington State, (Alway 1998) surveyed 153 growers farming 4,655 hectares and found that92% of the growers used Isomate C+TM.

In British Columbia, research into the use of CMMD began in 1987 (Judd *et al.* 1992). The efficacy of CMMD was first demonstrated in commercial organic orchards in 1990 (Judd *et al.* 1997). Damage in 4 organic orchards was reduced from an average of 25% in 1989 to a mean of 0.7% during the 3 year period from 1990 to 1992. The 3 year mean % damage in an untreated control orchard during the same period was 48% (Judd *et al.* 1997). In 1991, 75 growers, representing 140 hectares of pome fruit, participated in a large-scale evaluation of CMMD. The median level of damage in pheromone-treated blocks was 0.42% and 0.88% in apple and pear orchards, respectively (Judd & Gardiner 1992). Isomate C+ was registered in Canada in 1994. In 1994 and 1999, CMMD (Isomate C+ is the only registered product in Canada) was used on approximately 100 and 700 hectares, respectively (G. Judd, P. communication). Use is forecast to increase to approximately 2,000 hectares in 2000. The large increase is due to the recommended use of CMMD in conjunction with insecticides by the Sterile Insect Release (SIR) program in British Columbia to help reduce regional CM populations (G. Judd, P. communication).

Selecting an orchard

The careful selection of orchards can minimize the risk and control problems associated with CMMD. High resident populations are the most important limitation to the successful use of CMMD In orchards with high resident CM populations, the application of supplemental insecticides (Gut & Brunner 1992) and/or intensive sanitation (Judd *et al.* 1997) is essential to reduce populations to levels low enough to achieve commercially acceptable control. In 1997 in Washington State, 153 growers farming 4,655 hectares were surveyed on the particulars of how they used CMMD (Alway 1998). In the first year, 95% of the growers applied at least 1 insecticide to supplement CMMD. In the second and third year , the percentage of growers applying at least 1 supplemental insecticide was reduced to 83% and 76%, respectively. Azinphos-methyl was the most frequently used supplemental insecticide, used by 90% of the growers. Growers in British Columbia have also used pre- and post-harvest fruit removal and tree banding to successfully lower CM populations to ensure the efficacy of CMMD (Judd *et al.* 1997). In addition, careful consideration should be given to the topography (slope), wind exposure and size and structure of the canopy (Thomson *et al.* 1999, Gut & Brunner 1994).

Applying dispensers

Pheromone dispensers should be deployed within 1 meter of the top of the canopy just prior to the start of CM flight (Gut & Brunner 1994). Research has shown that most CM mating activity occurs in the upper part of the canopy. For example, Riedl *et al.* (1979) showed that higher numbers of male CM were caught in lure-baited pheromone traps when traps were placed in the upper canopy. Weissling & Knight (1995) using a variety of techniques, including tethered virgin females, unbaited sticky traps and blacklight observation of released moths, showed that males and unmated females are mostly distributed in the upper canopy. Further, they demonstrated that when pheromone dispensers were placed at 2 meters, there was still mating of tethered females occurring at 4 meters. The placement of dispensers at 4 meters further reduced the incidence of mating. The results strongly suggest that dispensers should be applied in the upper canopy to optimize the efficacy of CMMD.

Treating borders

Borders of pheromone-treated orchards are susceptible to high levels of codling moth infestation. In a 3 year study of the use of CMMD in 6 Washington apple orchards, Gut & Brunner (1998) found that approximately two-thirds of the CM damage occurred within 30 meters of the border. Knight *et al.* (1995) showed similar results. In a study of an orchard treated with CMMD, 65% of the fruit damage occurred along the border. Factors contributing to border infestations include the immigration of mated females from adjacent untreated orchards and reduced concentrations of pheromone in the border zone (Gut & Brunner 1998). Milli *et al.* (1997) showed that wind reduced pheromone concentrations for up to 15 meters from the edge of a CMMD treated orchard. This resulted in a border zone with potentially insufficient concentrations of pheromone to disrupt mating. Recommendations to reduce border infestations include increasing the application rate of dispensers in border areas and/or spraying the borders with insecticides (Gut & Brunner 1996). Given the cost of pheromone dispensers and questions regarding the effectiveness of higher application rates especially when CM populations are high (Gut & Brunner 1998), most growers in eastern Washington and British Columbia apply insecticides or remove infested as supplemental border treatments. Based on the work of Milli *et al.* (1997), supplemental controls should be applied to areas up to 15 meters from the edge. Border problems can also be reduced by treating adjacent orchards or wind breaks with pheromone dispensers and/or by the area-wide application of CMMD technology (Kogan 1994).

Area-wide use

The Codling Moth Areawide Management Program (CAMP) was initiated in 1993 by university and United State Department of Agriculture (USDA) scientists (Kogan 1994). The program was a joint effort between the University of California, Berkeley, California, Oregon State University, Corvallis, Oregon, Washington State University, Wenatchee, Washington and the United States Department of Agriculture/Agricultural Research Service, Wapato, Washington. The objective of the 5 year program was to implement an area-wide and multi-tactic pest management strategy that would effectively control CM while reducing the negative impacts of pesticide use on natural enemies of secondary pests, the environment and the farm workers (Kogan 1994). The major tactic for control of CM was CMMD. CM was chosen as it is the "key pest" of pome fruit orchards in western North America. Five area-wide projects comprising a total of 1,294 hectares in 3 western states were initiated in 1995 (Kogan 1996). By 1998, CAMP had expanded to over 6,000 hectares involving 17 sites (Alway 1998). CAMP has been very successful in demonstrating the effectiveness of the CMMD technique when used in an area-wide approach. Recently, the SIR Program in British Columbia recommended the use of CMMD in conjunction with insecticides to reduce moth populations to levels where the technique of releasing sterile insects can be effective. The area-wide use of CMMD and insecticides is expected over a large region comprising 2,000 hectares(G. Judd, P. communication).

Monitoring CMMD-treated orchards

Monitoring codling moth adult activity in orchards treated with CMMD is difficult. Pheromone traps should be uniformly distributed in the orchard at a density of 1 trap per hectare. Additional traps should be placed to monitored the borders and known "hot spots". Traps should be inspected at least once a week. Lures should be changed every 2 to 3 weeks or according to the manufacturer's recommendations. Capture of moths in pheromone traps baited with 1 mg of codlemone is frequently an unreliable indicator of efficacy (Thomson *et al.* 1999). Charmillot (1990) showed that the sensitivity of pheromone traps can be improved by using traps baited with 10 mg lures and locating them in the upper part of the canopy. False negatives (no or low captures of moths in orchards sustaining economic damage) do occur even with the deployment of 10 mg-baited traps and it is important to interpret trap capture data carefully. Welter *et al.* (1997) demonstrated that the effective active space (area of attraction) of lure-baited pheromone traps in CMMD treated orchards is less than 0.5 hectares. Therefore, it is essential that fruit be inspected on a regular basis to ensure that economically acceptable levels of control are achieved.

Economics of CMMD

A study of the economics of CMMD conducted in Washington State during 1991 and 1992, found that the cost of Isomate C technology when adjusted for materials, labour and machinery costs was $133.38 per hectare higher than a conventional insecticide program (Williamson *et al.* 1996). In the years following the registration of Isomate C, the higher costs provided a strong disincentive to the adoption of CMMD technology in Washington and British Columbia. However, the use of CMMD at reduced rates in conjunction with the judicious use of insecticides has made the economics more attractive to growers. In his survey, (Alway 1998) found that 73% of growers (153 growers farming 4,655 hectares) surveyed in Washington State deployed Isomate C+ at reduced rates. Connor (1999), in a study conducted between 1995 and 1998, compared the economics of Isomate C+ applied at reduced rates with conventional insecticide programs in Pacific Northwest apple and pear orchards. He found that the cost competitiveness of Isomate C+ was a function of CM population densities. Where CM populations were low (growers normally apply 2.5 insecticides or less for CM), he found that the use of Isomate C+ was not cost effective even when dispensers were applied at half the labelled rate of 500 dispensers per hectare. However, if CM populations were high (normally greater than 5 insecticides applied for CM), the use of Isomate C+ was more cost effective compared to conventional insecticide programs after 2 years. Growers implementing CMMD in orchards with moderate CM populations, would expect to net savings by years 3 and 4. He concluded that the decision by growers to adopt CMMD was equivalent to an investment decision in that the additional costs in the first years following the deployment of CMMD would be off-set by future savings (Connor 1999). Economic advantages of CMMD not factored in the above studies include reduced costs associated with worker protection and labour management in orchards and decreased potential for the development of insecticide resistance. Economic disadvantages not factored into the study include the need for intensive monitoring and outbreaks of secondary insect pests other than leafrollers.

Pheromone blends

Roelofs (1978) in his "Threshold Hypothesis" suggested that the efficacy of mating disruption would be enhanced by dispensing the complete blend. CM pheromone was first identified as (E,E)-8, 10-dodecadien-1-ol (Roelofs *et al.* 1971). Evidence for the presence and behavioural role of secondary components has been reported by many authors (Arn 1985; Bartell & Bellas 1981; El-Sayed *et al.* 1999; Rothchild *et al.* 1988; Witzgall *et al.* 1999). However, only one commercially available CMMD product incorporates the secondary components into the formulation (Thomson *etal.* 1999). Witzgall *et al.* (1999) suggested that the lack of efficacy of CMMD at high populations was due in part to the failure to dispense the complete blend of CM pheromone . Continued research is required to determine the importance of secondary components to the efficacy of CMMD. In addition, the importance of secondary components relative to the delivery system needs to be better understood . Research into new CMMD technologies including sprayable pheromones (G. Judd, P. communication) and large aerosol dispensers (Shorey & Gerber 1996) is underway in both Washington State and British Columbia.

Concluding remarks

Due to increased regulatory restrictions of conventional insecticides and other factors, a large increase in the commercial use of CMMD is forecast for both Washington State and British Columbia. The continued adoption of CMMD will depend on how well it meets grower concerns about risk, efficacy and cost. Greater knowledge of moth flight and mating behaviour relative to the dynamics of pheromone concentration within orchard canopies is essential and will lead to an increased understanding of the mechanisms of CMMD. This will aid in the design of more cost-effective CMMD technology. Extensive research is still needed to improve monitoring techniques and to develop economic thresholds to ensure commercially acceptable levels of control. The development and registration of new formulations will provide growers with more alternatives and the resulting competition should lower the price to the grower thus encouraging even greater adoption.

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Pheromones for Insect Control in Orchards and Vineyards
IOBC wprs Bulletin Vol. 24(2) 2001
pp. 31-35

The use of the pheromone mating disruption method against fruit moths in private allotments

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Abstract: The availability of pesticides in private gardens is limited by local laws in the State of Baden-Württemberg (Germany). Only 26 insecticides, fungicides and molluscicides are permitted. Hence, only pesticides such as *Bacillus thuringiensis*, granulosis virus, pyrethrum or mating disruption by pheromone (MD) are permitted for moth control in apple, plum and other fruit trees. Beneficials like *Trichogramma* sp. or *Chrysoperla carnea* can also be used. MD had previously not been investigated in allotments. In the experiments carried out in 1998 and 1999, MD was compared with the use of the egg parasites, *Trichogramma dendrolimi* and *Trichogramma cacoeciae*. The observations were done in 102 allotments, each of about 300 m^2. In 1997, attacks of codling moth were generally low throughout southern Germany. This was reflected in the allotments where there was less than 1% fruit damage with treatment by MD or egg parasites the following year. During 1999, the attacks were generally much higher, resulting in a higher use of insecticides in commercial German orchards. In the allotments, about 6 to 8% of fruits were damaged by codling moth in both experimental treatments. The results indicate that MD can be successful in allotments, and is equally effective as the use of egg-parasites. However, further experiments are required.

Keywords: private gardens, allotments, *Cydia pomonella*, codling moth, *Adoxophyes orana*, summerfruit tortrix moth, *Grapholitha funebrana*, plum fruit moth, pheromone, mating disruption technique, biological control, *Trichogramma dendrolimi*, *Trichogramma cacoeciae*.

Introduction

Allotments are important for recreation, especially for people who live in densely populated areas of south-west Germany. The availability of pesticides in these private gardens is limited by the law (GBI, p. 426: "Gesetz über die Einschränkung der Anwendung von Pflanzenschutzmitteln"; State of Baden-Württemberg, 17 December 1990). Hence, only pesticides such as *Bacillus thuringiensis*, granulosis virus, pyrethrum or pheromone mating disruption method (MD) are permitted for moth control in apples, plums and other fruit trees.

MD in orchards is widely used in some areas of southern Europe (Waldner 1996, 1997), South Africa (Barnes & Blomefield 1997) and North America (Thomson 1997) and is progressing from the experimental phase into commercial practice in Baden-Württemberg (Galli 1998). However, MD is a completely new method for private gardens.

In the two years of experiments, MD was compared with the use of the egg parasites, *Trichogramma dendrolimi* and *Trichogramma cacoeciae* to see if these methods would work in allotment gardens ("Kleingarten, Schrebergarten"), and which should be used in the future.

Material and methods

In the first year, pest species and beneficials were monitored by pheromone traps and the direct observation of damage. In the second year, the whole allotment area was divided into three sections for (1) application of MD, (2) application of granulosis virus and (3) use of the beneficials *Trichogramma dendrolimi* and *T. cacoeciae*. In the third year, the experiments were repeated, but MD was used in the granulosis virus plot. In each section, 1 to 3 pheromone traps were installed for each moth species. Traps for codling moth, *Cydia pomonella*, and summerfruit tortrix, *Adoxophyes orana,* were installed on 4 May 1998 and for plum fruit moth *Grapholitha funebrana*, on 8 May. All traps were inspected weekly from 11 May to 28 of September. In 1999, the *Cydia pomonella* and *Grapholitha funebrana* traps were installed from 16 April to 6 May, and the *Adoxophyes orana* traps from 10-14 May. Inspections were done weekly up to 17 September.

The observations were done in 102 allotments which suffered from codling moth and plum fruit moth. Allotments were each about 300 m^2, comprising about 4.5 ha. About one third of the allotments area is used for vegetable-production, one third for fruit culture (mostly apple, but also plum, peach, pear, yellow plum and apricot) and one third for ornamentals and lawn. Thus on an area of 4.5 ha there is dispersed fruit culture on only about one hectare.

Section treatments were as follows: (1) Granulosis virus: 0.3 to 0.5 l/ha (only once). (2) *Trichogramma*: altogether 1260 cards (1998) and 1240 cards (1999). In plum, there were six applications from 20 May to 10 August, in 1998 and in 1999, there were also six applications from 12 May to 12 August. In apple, there were five applications from 20 May to 10 August (1998) and 12 August (1999). (3) MD: 12 dispensers (codling moth and summer fruit tortrix moth (RAK 3+4) and 15 dispensers (plum fruit moth (RAK 5) per garden and 297 in the surroundings of the allotment area. In all, 1512 dispensers (applied on 11 May) were used in 1998 and 1917 dispensers (applied on 17 May) in 1999.

Results

In 1997, attacks of codling moth were generally low throughout the southern Germany. This was also the situation in the allotment gardens (Table 1).This low attack rate was reflected in the catches of the pheromone traps in 1998. In the Rosenweg and Mondscheinweg, where granulosis virus and *Trichogramma* had been used, the catches were relatively low with 1.39 and 2.93 moths per trap per week (Table 2). However, where MD was used in the single gardens and the Gulaschgasse, codling moth trap captures were between 0.08 and 0.31 males per trap per week. On the other hand, the attack of the plum fruit moth in 1997 was high (100% plum attack). The 1998 trap catches of this species were subsequently high with up to 24.28 moths per trap per week.

Table 1. Estimation of moth infestation in apple and plum in the allotment gardens (Tapach, 1997).

Date	*C. pomonella*	*G. funebrana*
14 July 1997	1%	100%
8 September 1997	4%	-

Table 2. Mean number of moths per pheromone trap per week, 1998

Section	Method	*C. pomonella*	*A. orana*	*G. funebrana*
Single gardens	MD	0.31	1.0	0.67
Gulaschgasse	MD	0.08	0.0	0.81
Rosenweg	*Trichogramma*	1.39	2.0	4.0
Mondscheinweg	granulosis virus (once) and *Trichogramma*	2.93	5.2	24.28

These very low codling moth catches are in line with very low fruit damage. In summer and autumn 1998 there was less than one per cent fruit damage on apples by MD and the egg parasites in the allotments (Table 2). The other two moth species (*A. orana* and fruitlet mining tortrix, *Pammene rhediella*) were unimportant in the gardens. The high catches of the plum fruit moth in the pheromone traps corresponded to a relatively high infestation of the plums (Table 4), but this is low compared with the attack in 1997.

Table 3. Estimation of moth infestation on apple in the allotment gardens (Tapach, September 1998).

Section	Method	*C. pomonella*	*A. orana*	*P. rhediella*
Single gardens	MD	0.9	0.05	0.1
Gulaschgasse	MD	0.76	0.06	0.06
Rosenweg	*Trichogramma*	0.55	0.06	0.06
Mondscheinweg	granulosis virus (once) and *Trichogramma*	0.58	0.08	0.08
Mondschein-weg	untreated	4.0		

During 1999, the catches of the pheromone traps were much higher in the area where the beneficials were used than in 1998, whereas in the MD area, the catches were low (Table 5). This was reflected by apple infestation. In the allotments, more than 6% of fruits were damaged by the moths in both experimental treatments (Table 6). In that year, the attacks of the fruit moths were generally much higher, resulting in a higher use of insecticides in commercial orchards in South-western Germany.

Table 4. Estimation of moth infestation on plum in the allotment gardens (Tapach, July 1998, and July 1999).

Section	Method	*G. funebrana*	
		1998	1999
Single gardens	MD	3.67	-
Gulaschgasse	MD	2.75	6.00
Rosenweg	*Trichogramma*	0	3.00
Mondscheinweg	granulosis virus (once) and *Trichogramma*	10	5.3
Average		3.6	4.5

Table 5. Mean number of moths per pheromone trap per week (1999)

Section	Method	*C. pomonella*	*A. orana*	*G. funebrana*
Single gardens	MD	0.13	0.74	0.05
Gulaschgasse	MD	0.1	0.38	0.1
Rosenweg	MD	0.24	1.12	0.48
Mondscheinweg	*Trichogramma*	3.96	6.43	27.9
Cultivation of stonefruits	untreated	6.65		

Table 6. Estimation of moth infestation on apple in the allotment gardens (Tapach, September 1999).

Section	Method	*C. pomonella*	*A. orana*	*G. funebrana*
Einzelgärten	MD	5.78	0	0
Gulaschgasse	MD	5.95	0.05	0.05
Rosenweg	MD	7.49	0	0
Mondscheinweg	Trichogramma	6.08	0.13	0.064
Tree in nearby meadow	untreated	32.00		

The approximate costs of the different methods are shown in Table 7. The cheapest method is the application of the granulosis virus. When MD was used against all three important moth species, the price is more than 30 DM. The price for the parasitoids is intermediate at about 24 DM.

Discussion

The granulosis virus is directed only against the codling moth. Additional methods have to be applied for the control of the summer fruit tortrix moth and the plum fruit moth. The practical application of the granulosis virus is sometimes difficult. In particular, the correct timing of application is a problem. When the treatment was planned for one section of the gardens (Mondscheinweg), rainfall and wind made an application often impossible. There is also a problem with the drift of the pesticide to adjacent plants. Thus granulosis virus application is a task for the individual gardener rather than a task for the club.

Table 7. Costs of the different methods per garden

MD		*Trichogramma dendrolimi,* *T. cacoeciae*	Granulosis Virus
RAK 3+4	15 DM	24 DM	10 DM
RAK 5	15 DM		

MD has been quite successful but the mating disruption of the plum fruit moth has not yet been registered in Germany. Hence, other methods have to be employed against plum fruit moth. For example, the use of pheromone mating disruption against the codling moth could for example be combined with the use of *T. cacoeciae* on plums.

For both MD and egg parasites, the rate of fruit infestation was less than 1 % in 1998 and ca. 6 % to 8 % in 1999. In 1999, the rate was also much higher than normal in commercial orchards. Thus it seems that the pheromone mating disruption can be successful in allotment gardens and is as effective as the use of egg-parasites. However, further investigations are needed with both methods.

Acknowledgements

I thank Les Wardlow, Ashford, Great Britain for linguistic corrections

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Combination of pheromone and an additive for the control of codling moth, *Cydia pomonella*

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Abstract: BASF AG recently developed a modification of the codling moth mating disruption dispensers RAK 3. In addition to the pheromone used in dispenser RAK 3, RAK 3R contains citral as an additional additive filled in a separate chamber in the dispenser, designed to enhance mating disruption. Semi-field and laboratory experiments were conducted to study the effectiveness of citral. Mating rates in cages with different moth densities placed in an apple orchard treated either with RAK 3R, RAK 3 or untreated were not significantly different. In small cages in the RAK 3 treatment, were mating differences significant. However, mating at low moth densities per cage were significantly lower in the treatments (RAK 3R, RAK 3) compared to the control. Experiments in a windtunnel revealed a significantly reduced attractivity of the pheromone if combined with Citral. In a closed system of glasscylinders (3,3 l) mating could be prevented when Citral was present at concentrations exceeding 2000 mg/l.

Key words: mating disruption, *Cydia pomonella*, codling moth, Citral

Introduction

Mating disruption using the main pheromone component 'codlemone', against codling moth has been used in apple production for several years. However, this control method does not always work reliable enough and is more expensive than insecticide applications (Waldner 1997, Cardé & Minks 1995). Different non-pheromonal compounds have been investigated for their effectiveness on mating disruption (Hathaway *et al.* 1979, Witzgall *et al.* 1997). In windtunnel experiments with *Lobesia botrana* Den. & Schiff. the monoterpene Citral - a secondary plant component - showed an impact on pheromone perception (Meiwald 1995). Therefore the BASF AG has developed a new type of dispenser that contains pheromone plus Citral (RAK 3R) aiming the improvement of mating disruption success. First field trials were carried out with a density of 125 dispensers per hectare as recommended by BASF. In these studies semi-field and laboratory experiments were conducted to study the effectiveness of the RAK 3R-dispensers in comparison to traditional RAK 3-dispensers and of Citral alone.

Material and methods

Semi-field experiments

Different moth densities. For testing the dependence of mating disruption success on moth density, cages (2 m × 2 m × 2 m) were placed between rows of an apple orchard treated either with RAK 3R (125 dispensers/ha), RAK 3 (500 dispensers/ha) or no dispensers as control.

Either 6, 20 or 50 pairs of moths were released in each cage and two days later the females investigated for successful mating by assessing spermatophores. Densities at 50 pairs of moths were repeated only twice, 20 pairs of moths four times and 6 pairs of moths five times, therefore, only the last two densities were analysed statistically.

Small cages. Small cages (30 cm high, Ø 30 cm) were placed in an apple orchard treated either with RAK 3R (125 dispensers/ha), RAK 3 (500 dispensers/ha) or no dispensers as control. One pair of one day old moths were put in each cage and two days later the females were investigated for successful mating by assessing spermatophores.

Low moth-density. To examine the control effect of the two different types of dispensers at a low moth density, one fixed female and three male moths were put into cages (2 m × 2 m × 2 m) placed between apple tree rows. RAK 3R and RAK 3 dispensers were placed outside the cages at appropriate distances according to the dispenser density per hectare. The next day females were investigated for successful mating by assessing spermatophores.

Laboratory experiments

To estimate the mode of action of Citral, experiments in a windtunnel and in glasscylinders were carried out.

Windtunnel. Windtunnel trials were made to test if Citral has an impact on pheromone attractivity for moths. Four minitraps were baited with different lures and the recaptures observed over a two days period. Each minitrap consisted of a plastic syringe with two longish pieces of filterpaper placed next to each other and soaked in the according solution. This method was taken from Meiwald (1995). A constant air flow through the syringes (100 ml/min) was guaranteed by a pump. Temperature in the windtunnel was 20-24 °C and the relative humidity about 40%. The experiment was repeated five times.

Traps were baited either with: (1) control (silicon oil), (2) pheromone, (E,E)-8,10-dodecadien-1-ol solved in silicon oil, at a dilution of 10^{-3}, (3) bait 2 plus Citral solved in silicon oil at a dilution of 10^{-1} (4) bait 2 plus Citral non-diluted

Glasscylinder. Based on the results obtained from the windtunnel experiments, the question arose whether Citral is able to prevent copulation in a closed system (glascylinders 3,3 l). One pair of one day old moths per cylinder were exposed to different concentrations of Citral solved in hexane. Two days later, females were investigated for successful mating by assessing spermatophores.

Results

Semi-field experiments

Different moth densities. No significant differences were observed between mating rates in the three plots of 20 and 6 pairs per cage (Fig.1).

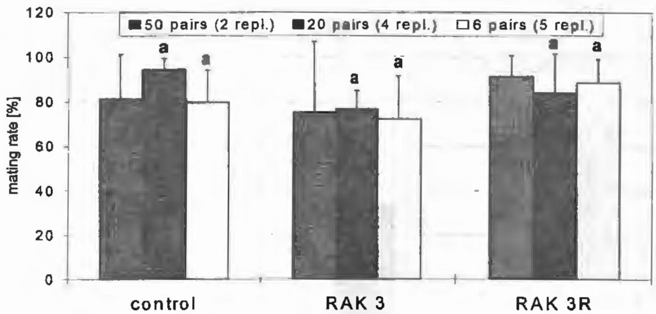

Figure 1. Mating rate in cages with different moth densities (Wilcoxon-test, p<0,05, same letters mean no significant difference within treatments)

Small cages. Only RAK 3 treatment was able to reduce mating significantly (Fig.2).

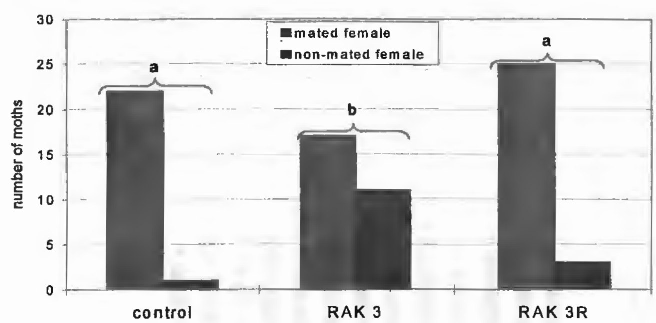

Figure 2. Mated and non-mated moths in small cages (χ2-test, Ryan, p<0,05, same letters mean no significant difference within all treatments)

Low moth density. In this experiment both dispensers RAK 3R and RAK 3 could prevent mating to a sufficient extent (Fig.3).

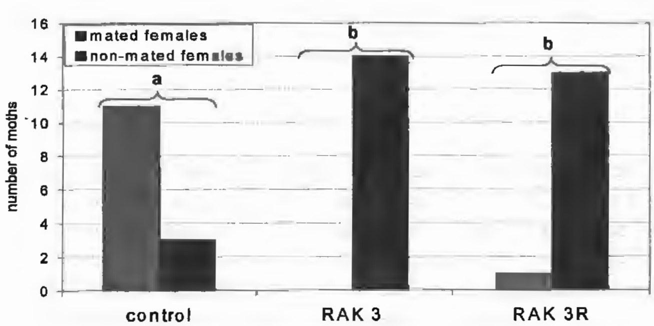

Figure 3. Number of mated and non-mated females in cages (χ2-test, Ryan, p<0,05, same letters mean no significant difference within all treatments)

Laboratory experiments

Windtunnel. The windtunnel experiments showed a significant reduced attractivity of the pheromone (Ph) if combined with Citral (C) (Fig.4).

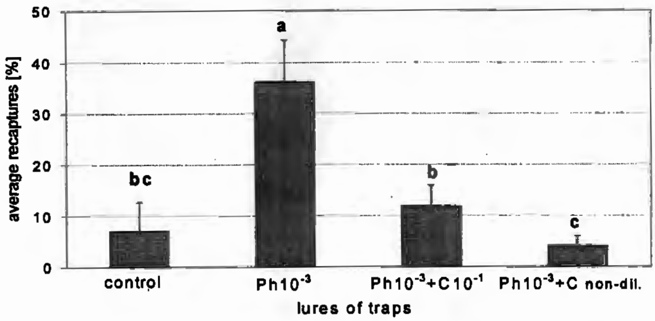

Figure 4. Recaptures of moths in a windtunnel baited with different traps (Tukey-test, p<0,05, same letters mean no significant difference within all treatments)

Glasscylinder. Mating is prevented if the concentration of Citral solved in hexane exceeds 2000 mg/l (Fig.5)

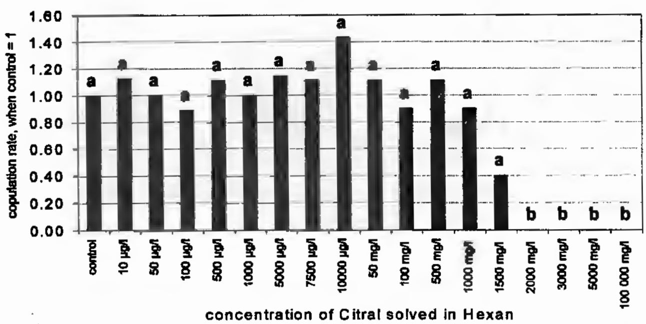

Figure 5. Copulation rate in glasscylinders with different concentrations of Citral (χ2-test, Ryan, p<0,05, same letters mean no significant difference within all treatments)

Discussion

In trials testing the dependence of mating disruption success on moth density there were no obvious effects of either Citral or RAK 3 treatment on mating of *C. pomonella*. However, the lowest density of six pairs of moths in a space of 8 m^2 is still high and would correspond to approximately 25000 pairs of moths per hectare. It is known that at high population densities the mating disruption technique does not work well (Cardé & Minks 1995, Neumann 1997, Casagrande & Jones 1997), because of increased chance meetings. However, matings in small cages in the RAK 3 treated area were reduced compared to RAK 3R treated area and control. A possible explanation could be the larger mesh size of the gauze of the small cages compared to the big cages and therefore better wind and pheromone transmission. At low moth densities with one female per big cage, matings were prevented by both RAK 3R and RAK 3 dispenser types. However, the contribution of Citral towards mating disruption efficiency is still not clear. In field experiments, RAK 3R showed good results at low moth densities. At high moth densities both RAK 3R and RAK 3 failed (Kirchert *et al.* 2000).

Laboratory windtunnel experiments showed that Citral could reduce the attractivity of the pheromone significantly. Meiwald (1995) obtained similar results with *L. botrana.* Moreover Citral was able to prevent copulation in a closed system if the concentration exceeded 2000 mg/l.

Little research has been done about fumigant effects of Citral on insects. It showed some insecticidal properties against Diptera and Coleoptera by fumigant treatment (Rice & Coats 1994). According to Ryan & Byrne (1988) Citral works as a competitive inhibitor of acetyl-cholinesterase and, thus, has a toxic effect on the sensory nervous system in insect antennae. But the interaction of Citral and pheromone is still not clear and requires further investigation.

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IOBC wprs Bulletin Vol. 24(2) 2001
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Early studies on mating disruption technique of codling moth, *Cydia pomonella*, in the Aegean Region, Turkey

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Abstract-*Cydia pomonella* is the key pest in apple orchards in Turkey. The studies concerning the mating disruption technique were carried out in an 3 ha isolated apple orchard between 1996-1999 years in Aegean region. The experimental orchard was divided up three blocks. The dispensers were applied in mating disruption block before the first appearance of the spring moth only once throughout the season. Chemical treatments were applied in the second block according to forecasting and warning system. Relatively smaller the third block left as control. Weekly moth catches were checked in the pheromone traps placed in each block. Assessments were based on the numbers of fruit damaged fallen down during the vegetation and the number of infested fruits at the harvest. As for the result of four year studies, infestation rates were found to be 7,32%, 35,01%, 1,46% and 11,92% in the years of 1996-1999 respectively.

Key words : Sex pheromone, mating disruption, apples, codling moth, *Cydia pomonella*

Introduction

Codling moth (*Cydia pomonella* (L.)) is the major pest in the apple growing areas of Turkey and causes 40-60 % crop losses under inadequate control measures. Its control has been based on Forecasting and Warning system since 1984 and by utilising this technique the pest can be suppressed with 2-4 sprayings yearly. However, undesired side effects of the chemical control on environment and natural balance have led the researchers to seek the alternate control methods. Mating disruption (MD) technique which seems to be the most promising biotechnical aspect of codling moth has been investigated by many researchers up to date (Rama,1997; Charmillot,1997; Waldner, 1997). This technique was first applied against the codling moth in Turkey.

Materials and Methods

The experiment was conducted in a 3 ha apple orchard in Balikesir-Dursunbey in Aegean Region within the years of 1996-1999. Apple varieties were Golden Delicious and Starking. MD was conducted on 215 trees while the number of trees were 312 and 43 in chemically treated and control parcels respectively. Pheromone traps (Pherocon) were utilised to maintain the population trend of the pest. The dispensers used were Isomate - C-Plus (Shin - Etsu Chemical Co., Tokyo), containing 52.9% E,E-8,10-Dodecadien-1-ol, 29.7% Dodecanol, 6.0% Tetradecanol and 11.4% inert ingredients in 165 mg/dispenser. The dispensers were applied according to recommendations at 1000 dispenser/ha, before the first appearance of the spring moth and once during the season. The trees at the edge of the orchard were applied as double. Assessments were based on the numbers of fruits which fell down during the vegetation (weekly) and the number of infested fruits at the harvest. Weekly moth catches were also taken into consideration. In 1999, dispensers were weighed in order to determine the amount of pheromone left inside.

Results and discussion

During the years of the study conducted, codling moth flights were observed between the period of late april- early may and early september (Fig. 1).

The pest was suppressed with 2 and 3 sprayings in the chemically treated parcel in the years of 1996-1998 and 1999 respectively.

As for the result of 4-year mating disruption applications, the lowest infestation rate was found to be 1.46% in 1998 (Tab.1); by the time the lowest population density was also observed. On the other hand the highest infestation rate was found to be 35.01% when the population density was highest in 1997 (Tab.1,Fig.1). However, dispensers used in that year were manufactured in 1995. Consequently, by considering some loses on the effectiveness of the dispensers with time, these results were not taken into consideration. Besides the results of 1997, the efficiency of mating disruption technique was supposed to be higher in subsequent years. However, the population trend seemed high and the infestation rate was found to be 11.98%, as was not expected in 1999 (Tab.1, Fig.1).

Table 1. Fruit infestation rates of the MD experiments conducted in Balikesir Dursunbey in 1996-1999

Year	Fruit infestation (%)		
	MD block	Sprayed	Control
1996	7.32	1.00	18.03
1997	35.01	1.83	10.50
1998	1.46	2.21	13.91
1999	11.98	2.99	17.42

Witzgall et al (1997) has reported that some male moths were also attracted from nearby untreated orchards. The author has also stated that partly copulation was a possibility on the top of the trees at the edge of the orchards. Parallel to these findings, although there was no capture in the traps, there was a high infestation rate in MD parcels in 1999.

Charmillot et al (1997) has studied on the weight and gas-chromotographyic analysis of the Isomate-C-Plus dispensers and has found that approximately 50% of the pheromone inside the dispensers was left for the next season. Thus density of 500 dispenser/ha give the same result in subsequent years. In our studies, the weights showed that the pheromone of the dispensers completely diffused in a single season (Fig. 2).

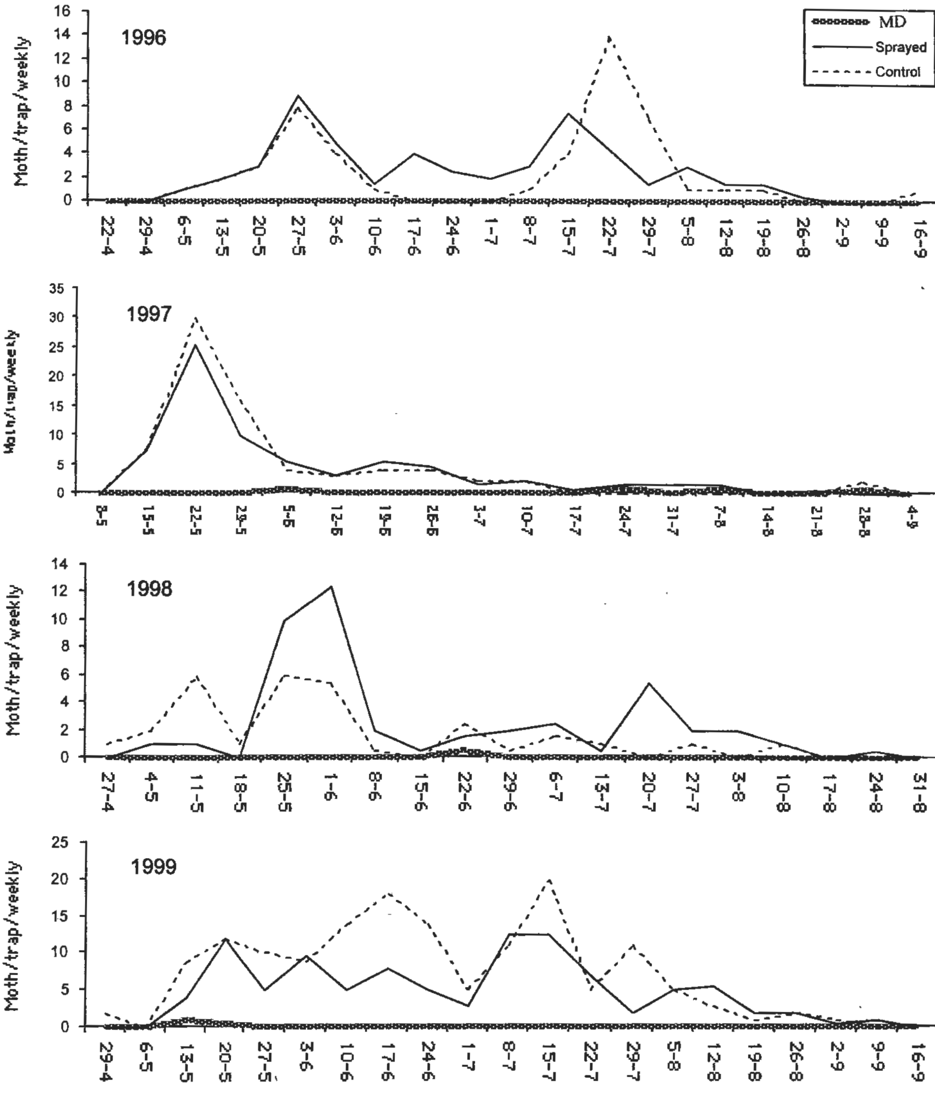

Figure 1. Pheromone trap catches of the MD, sprayed and control plots in Balikesir-Dursunbey in 1996-1999

Figure 1 shows the comparison between the flight period of codling moth in 1999 and diffusion rate of the dispensers in 1999. The flight period of codling moth lasted until the mid-september whereas the dispensers lost their effectiveness by August 13th. This probably was the result of the high infestation rates due to the damage occurred at that period.

Witzgall et al (1997) and Charmillot (1997) stated that mating disruption is a promising and widely used technique for the codling moth being an environmentally- friendly approach and getting ecological safe yield. Between the years of 1996 and 1999, no chemical application was needed against spider mites in mating disruption parcel. Spider mites were suppressed by predatory mites in mating disruption parcel. Our studies have also showed that the efficiency of this technique may be increased by using dispensers containing enough

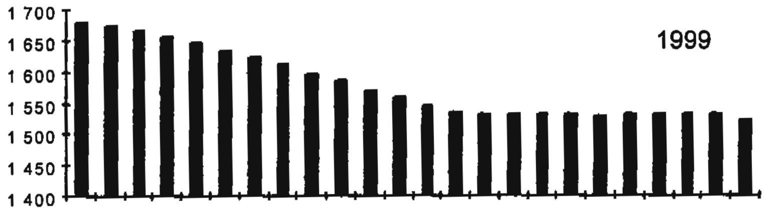

Figure 2. The weight of the Isomate-C-Plus dispensers (average 5 dispensers/week)

amount of pheromone to match our ecological conditions. On the other hand, this technique can be combined with an appropriate insecticide application when the population density of codling moth is higher and flying period is longer. The future of the ecological agriculture shall be based on the implementation of these kinds of alternate control methods.

Acknowledgements

We gratefully acknowledge the ideas given by Dr. Pierre-Joseph Charmillot, Nyon and the support by Shin-Etsu, Japan.

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IOBC wprs Bulletin Vol.24(2) 2001
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Can additives to pheromone enhance their efficiency in mating disruption of codling moth?

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Abstract: In 1998 and 1999, mating disruption efficiency of a new dispenser RAK 3R, containing pheromone plus Citral, a monoterpen, was compared with RAK 3 dispenser, which has been registered in Germany since 1997 for codling moth control. Dispensers were placed in two commercial orchards with different population densities of *Cydia pomonella.* To evaluate mating disruption success, pheromone traps with synthetic lures and pheromone traps with virgin females were used and, every fortnight fruits were checked for codling moth infestation. In Orchard A, both mating disruption methods were successful over a two -year test period. There were no differences between RAK 3R and RAK 3 treated orchards concerning apple damage and catches in the pheromone traps using synthetic lures. In Orchard B, mating disruption failed in 1999. Apple damage exceeded the economic threshold of 2and the trial had to be stopped by mid July. Infestation and catches by the pheromone traps with synthetic lures were much higher in the RAK 3R-plot than in the RAK 3-plot. Pheromone traps with virgin females caught more males in the RAK 3R-plot in both orchards.

Key words: mating disruption, codling moth, *Cydia pomonella*, Citral

Introduction

Codling moth, *Cydia pomonella* L., is the most important pest world-wide in pome fruit production. In the past, it was controlled primarily by the use of pesticides. Due to pesticide resistance (Riedl & Zelger 1994), the mating disruption technique has played an increasingly important role. Furthermore, some pesticides lost their registration in 1999 because of government restrictions (Koch & Schietinger 1999).

However the mating disruption method does not always give reliable control and is more expensive than chemical insecticide applications (Waldner 1997). In this study, a new form of mating disruption was tested. Meiwald (1995) revealed an impact of the monoterpen Citral on pheromone perception of *Lobesia botrana* Den. & Chiff. Therefore, BASF developed a new control method för *C. pomonella* using this compound. Citral, filled in dispensers (RAK 3R) should, together with the pheromone component, improve mating disruption success.

In field experiments, the new RAK 3R-dispensers were compared against RAK 3-dispensers in two commercial orchards.

Material and methods

The two orchards were situated in the Rhine Valley near Heidelberg. Because of the mild climate there were two generations of *C. pomonella*.

Orchard A had a size of 2 ha. Trials were made over the two successive years, 1998 and 1999. Infestation of apples was low in 1998 (< 2%). Orchard B had a size of 4 ha. Apple damage in 1998 varied from low to high values (1-9.9%) in different parts of the field. Trials with the two mating disruption methods were done in 1999 only. Each orchard was divided into a RAK 3R- and RAK 3-plot. In Orchard B, a small area of 88 trees was left as a control plot.

The different dispensers were placed in orchard A in mid May of 1998 and in both orchards in the beginning of May in 1999. The RAK 3R-dispenser contained Citral in one chamber and codlemone in the other. RAK 3 contained only codlemone. 125 RAK 3R-dispensers/ ha and 500 RAK 3-dispensers/ ha were hung, as recommended by BASF, on shady places on the trees. At the border, dispenser density was doubled.

Pheromone traps with synthetic lures and pheromone traps with virgin females were used to evaluate mating disruption success. The latter trap contained a small cage with two calling females positioned in the middle of the sticky insert instead of a rubber septum. The females were changed every 3-5 days. Every fortnight damage controls on apples were done to record codling moth infestation. 3000 apples per plot (1000 apples per cultivar and the border row) were checked at each control date.

Results

Orchard A

In 1998 the infestation rate remained below 1% till the end of August. Shortly before harvest the damage slightly increased and reached values up to 2,3%. There were no obvious differences between the two plots (Fig. 1).

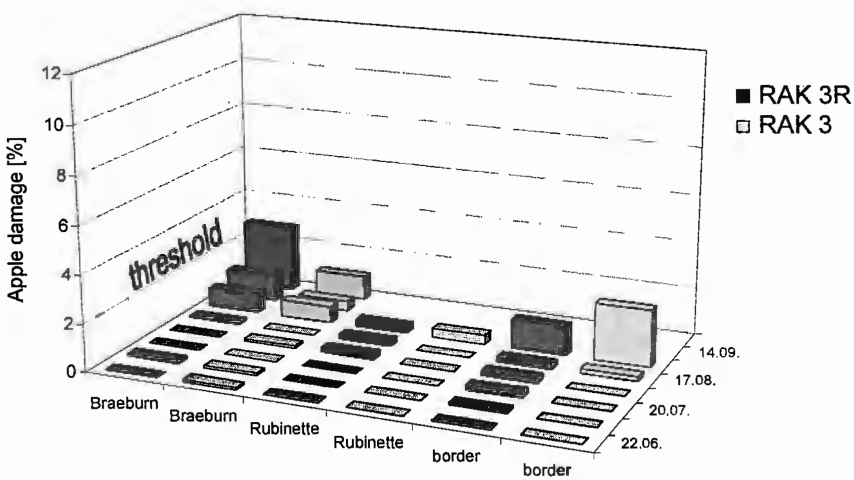

Figure 1. Apple damage Orchard A 1998

Pheromone trap catches were low (0-4 males per week). High trap catches in August might be explained by the change of lures in the beginning of that month (Fig. 2). Both mating disruption methods appeared to work sufficiently in 1998.

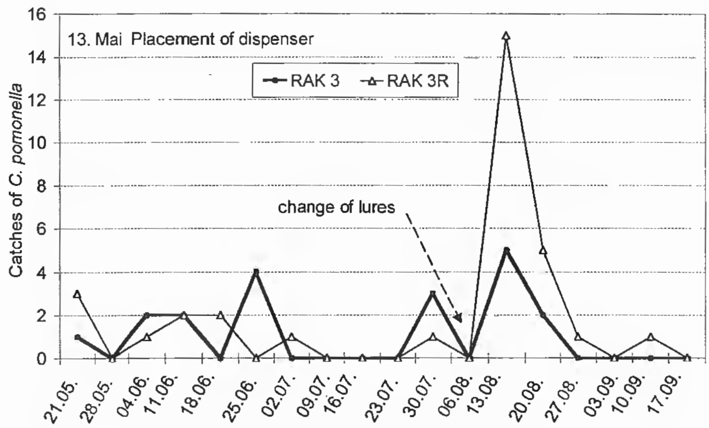

Figure 2. Pheromone trap catches (synthetic lures) Orchard A 1998

In 1999, the situation was almost similar with an infestation rate below the economic threshold. Shortly before harvest the damage increased slightly and reached values up to 3,6%. Infestation rates higher than 2% were found only at the orchard border. There were no obvious differences between the two plots as seen in previous year trial (Fig. 3).

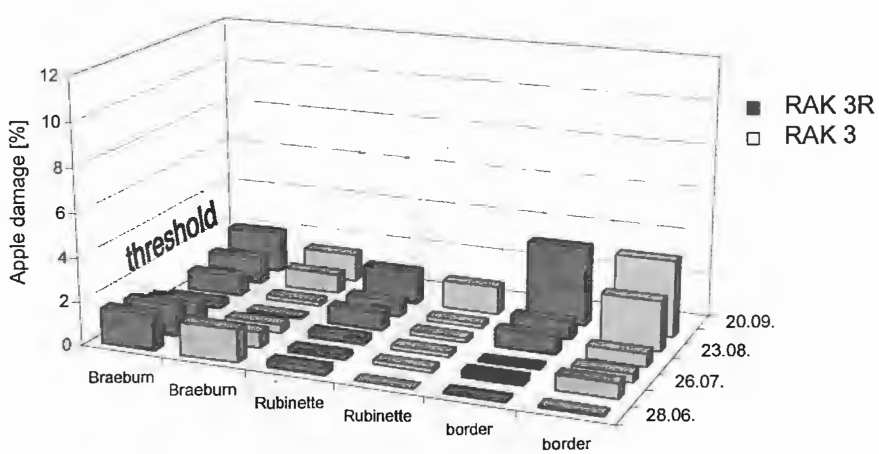

Figure 3. Apple damage Orchard A 1999

Pheromone traps caught 5 males in the RAK 3R- and 7 males in the RAK 3-plot. Compared to 1998, trap catches were lower (Fig. 4).

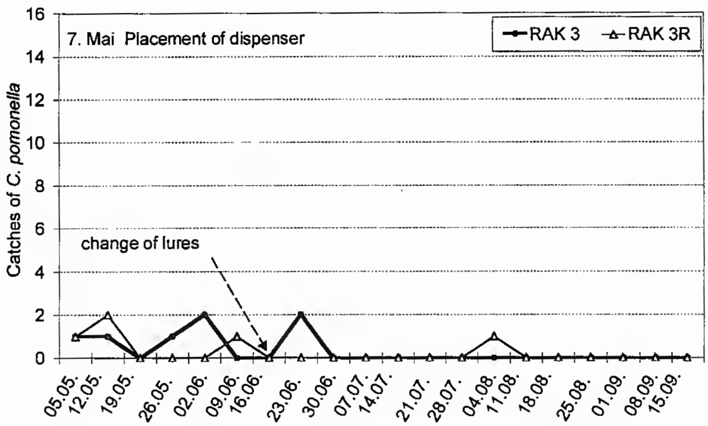

Figure 4. Pheromone trap catches (synthetic lures) Orchard A 1999

In 1999, pheromone traps with virgin females were also used. These traps caught 26 males in the RAK 3R- and 5 males in the RAK 3-plot. However, these results do not correspond with the infestation rate (Fig. 5).

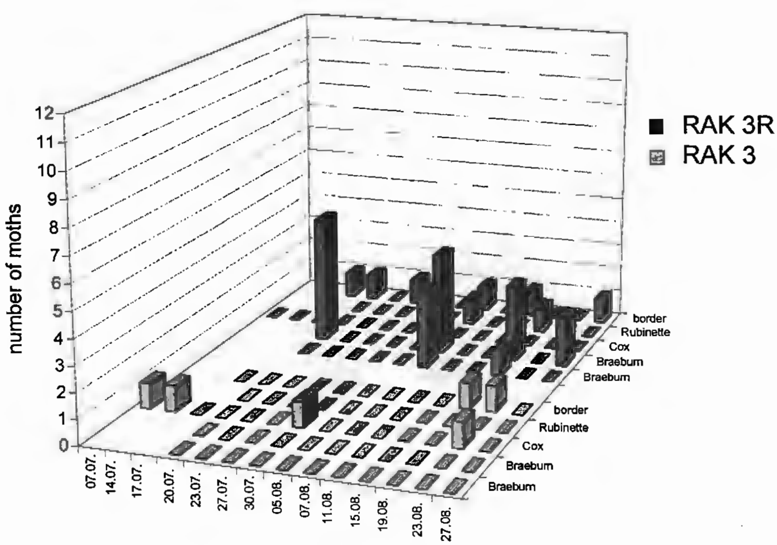

Figure 5. Catches of the pheromone traps with virgin females in Orchard A 1999

Orchard B

At the second damage control date, infestation was already beyond the 2%-threshold. Thus, the experiment was stopped by mid July and the orchard treated with insecticides (Fig. 6).

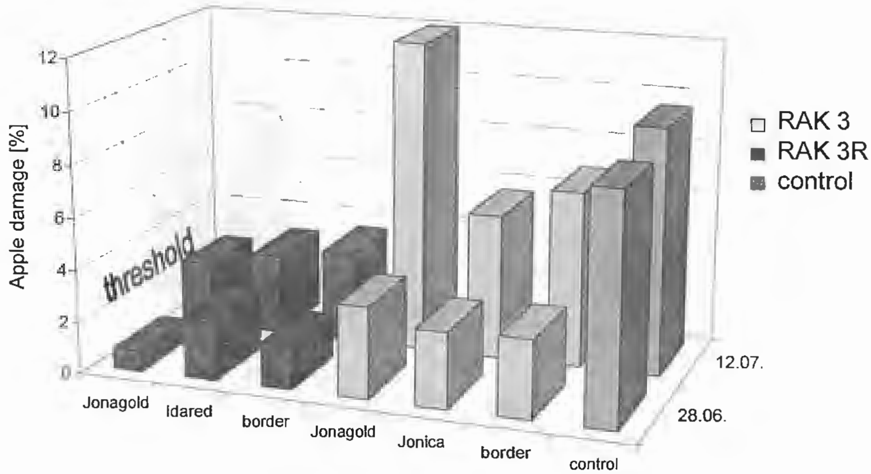

Figure 6. Apple damage Orchard B 1999

Apple damage in the RAK 3R-plot was much higher than in the RAK 3-plot. This situation might be explained by a higher codling moth infestation during the previous year in the RAK 3R-plot than in the RAK 3-plot. Pheromone traps caught 34 males in the RAK 3R-plot, 12 males in the RAK 3-plot and 31 males in the control-plot (Fig. 7).

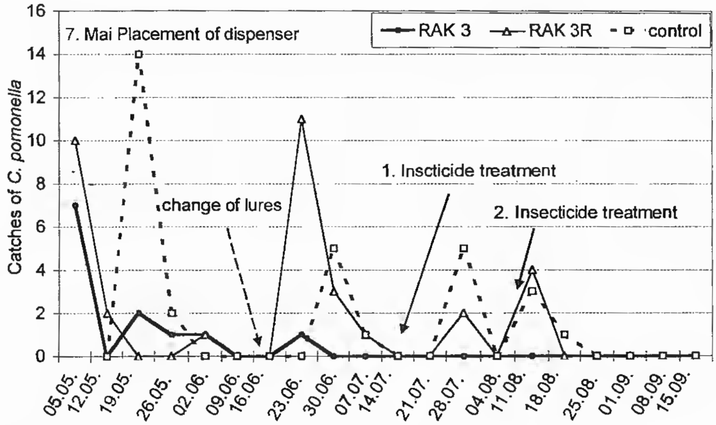

Figure 7. Pheromone trap catches Orchard B 1999

Traps with virgin females caught in total 92 males in the RAK 3R- and 5 males in the RAK 3-plot (Fig. 8).

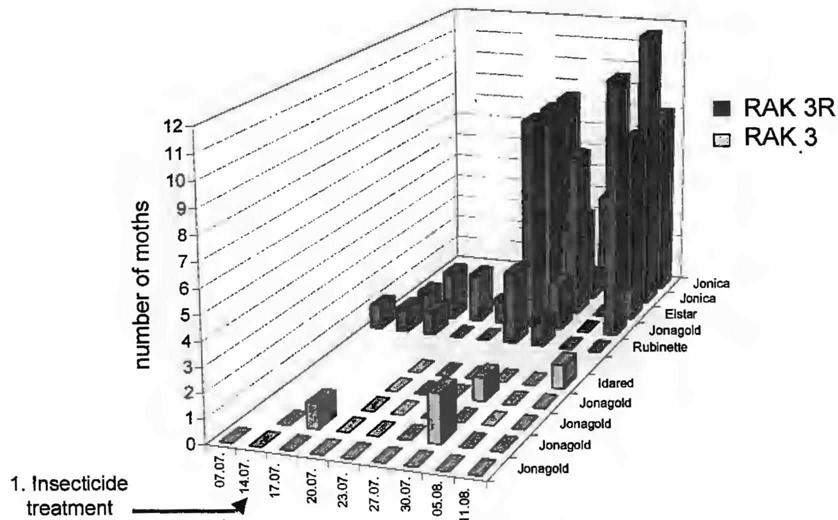

Figure 8. Catchcs of the traps with virgin females in Orchard B 1999

Mating disruption failed in orchard B. Population densities in 1998 were too high in some parts of the field to control codling moth successfully with this method the following year.

Discussion

Mating disruption with the RAK 3R-dispensers was successful in Orchard A which had low population densities as was mating disruption with RAK 3-dispensers. Only in the border rows infestations were beyond the 2% economic threshold. Damage at the border is often higher than in the centre of a mating disruption plot because wind blowing in from an adjacent untreated field can reduce pheromone concentration in that area (Milli & Koch 1997). Furthermore, migration of gravid females causes higher infestation at the border (Neumann 1997). Pheromone trap catches with synthetic lures showed no difference between the two plots, whereas the traps with virgin females caught slightly more males in the RAK 3R-plot than in the RAK 3-plot.

In Orchard B both mating disruption methods failed. Pre-infestation in 1998 was too high in some parts of the orchard to control codling moth by mating disruption only. It is known that mating disruption does not work sufficient enough at high population densities (Casagrande & Jones 1997). Before starting this trial insecticide sprayings would have been useful to reduce codling moth population (Neumann 1997). Pheromone traps with synthetic lures in the RAK 3R-plot caught more moths than in the RAK 3-plot. The same results showed the pheromone traps with virgin females, even with a greater difference between number of trap catches in both plots. Moreover, these traps caught many more males than standard pheromone traps in both orchards, so the natural pheromone blend of the females seems to be more attractive than the synthetic one. Contrary to that Barrett (1995) found no difference between traps with calling females and traps loaded with 1 mg pheromone lures.

In trials testing the new mating disruption technique on seven farms near Lake of Constance, carried out by Lange and Trautmann, the infestation rate in all cases did not reach the threshold of 2 %. The standard pheromone plot as a comparison, showed the same results. For further improvements in the new mating disruption method more research has to be done. In particular the mode of action of Citral and the combination of Citral and the pheromone requires detailed investigation. First trials were done by Hapke *et al.* (2000).

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EAG-Measurement of pheromone concentrations in apple orchards treated for mating disruption of *Cydia pomonella*

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Abstract: Mating disruption in apple orchards may be greatly improved by improving our knowledge on the distribution of the pheromone concentration in the orchard. We have measured pheromone concentrations with our EAG-system, which offers short measurement cycles and fast readout. An absolute calibration had been provided by simultaneous air sampling experiments. We have investigated the pheromone density profile at the upwind edge of a pheromone-treated orchard, the change of pheromone density with treee height, and the concentrations resulting from different dispenser types in apple orchards in Southern Sweden.

Key words: mating disruption, pheromone concentration, field EAG measurements, *Cydia pomonella*

Introduction

Measurements of airborne pheromone concentrations can make an important contribution to the further development of the mating disruption technique. Investigations of wind effects, plant-surface interactions and concentration profiles in vertical and horizontal dimensions all require the acquisition of multiple measurements during time intervals which can be considered short in comparison to the time course of changes in temperature or wind.

Methods using air sampling and subsequent chemical analysis (*e.g.* Flint et al. 1993) offer absolute average concentration values, but they are not fast enough to yield a sufficient number of measurements in the required time.

Field EAG measurements of pheromone concentrations yield reproducible concentration values on a relative scale. The field EAG measurement system developed in Kaiserslautern has been used to measure pheromone concentrations in vineyards (Milli 1990; Sauer 1991; Karg 1992; Karg et al. 1990, 1995; Koch et al. 1992, 1995; Färbert 1995; Karg & Sauer 1995), in cotton fields (Cardé et al. 1993; Färbert & Koch 1993; Färbert 1995; Färbert et al. 1996), in pea fields (Bengtsson et al. 1994) and in apple orchards (Milli 1993; Karg et al. 1994; Suckling et al. 1994; Milli et al. 1996). The newer models of our system make use of a signal superposition technique to suppress the influence of plant odors and other non-pheromonal airborne stimuli on the pheromone concentration measurements.

Methods

The field EAG system has been described in detail by Färbert et al. (1996a, b). Briefly, it consists of an excised antenna of *Cydia pomonella* (for measurements of airborne codlemone) placed in a special antenna holder. The holder is attached to the bottom of a vertical tube in which a steady current of air (14 ml/s) is maintained using a suction pump. A charcoal filter is placed at the tube upper entrance to remove all stimulating odors from the incoming air.

Three calibration sources (glass syringes, containing a vial with a pheromone-oil mixture; Sauer 1991) 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, and cannot be used as a measure for pheromone concentration. While the filter remains open, additional calibration pulses are released. The additional response of the EAG signal to the superimposed calibration puffs is used to measure the airborne pheromone. The calculation of the pheromone concentration is based on the dose response curve as measured continuously, and a basic model of the mixing of the different airstreams and their effective pheromone concentrations at the antenna.

The EAG measurement system including pumps, calibration syringes and associated step motor drives is mounted on a compact probe which is fully remote controlled and can be operated on a pole up to 5.5 m high. Wind velocity and direction are recorded in 40 ms intervals by two vector anemometers, one mounted on the EAG probe, the other at 5.7 m height.

Results

The relative pheromone concentration units used in the following figures 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. In calibration measurements involving air sampling and subsequent GC analysis (Bäckman, 1997), the equivalent absolute concentration was determined as: 1.0 x 10^{-6} relative units or 1.85 * 0.3 ng/m^{3}.

In the following graphs, the measurement data points carry two different indicators for the confidence interval: the thin lined bars with caps indicate the confidence interval for each individual measurement, caused by noise in the antenna, and the uncertainties in determining the amplitudes of EAG responses to each stimulation. The thick black bars without cap respresent the confidence interval due to the averaging of several measurement values. If the signal fluctuates strongly, *e.g.* due to wind influence, the thick bar may become larger than the individual measurement error.

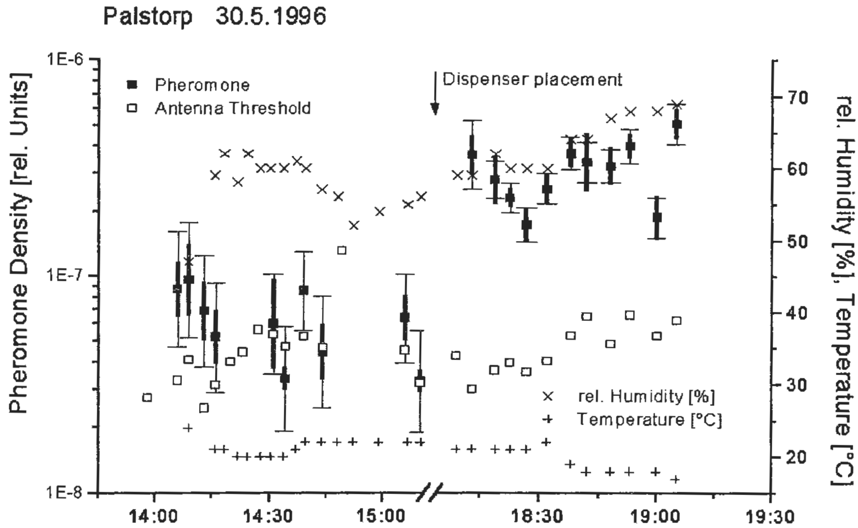

Figure 1 Pheromone concentration before and after dispenser placement

Background and dispenser installation

Figure 1 shows results from the installation of the dispensers on May 30, 1996 in an orchard at Pålstorp, 50 km north of Malmö. Between 13:45 and 15:00, the pheromone concentration was measured in the orchard. There were no dispensers exept a small number of Shin-Etsu dispensers from last year's treatment that had been left on the trees. These may have given rise to the small amount of pheromone concentration which is above the threshold concentration (white squares) at the beginning of the measurement. Later on, the threshold rose steadily, and thus occluded the detection of pheromone signals of such low densities. At 17:00, all dispensers had been placed in the orchard. The concentration values measured now were clearly above threshold and showed a steady increase, after a short decline. We attribute these changes in concentration to the changes in wind speed.

Effects of dispenser type and density

The measurements presented in Figure 2 were taken on June 11, 1996 in the same orchard at Pålstorp, on a transect parallel to the rows. The orchard had been treated differently in three sections: in the first section, Ecopom (Isagro) dispensers loaded with 250 mg of codlemone had been applied at a density of 400/ha. In the middle section, Ecopom-dispensers loaded with 250 mg of (codlemone) and 250 mg of (E,E)-8,10-dodecadienyl acetate (codlemone acetate) had been applied at a density of 400/ha; in the third section, the dispensers of the middle section had been applied at a density of 1000/ha. The results show a clear dependence of the recorded signal on the type and density of pheromone dispensers: in the first section, we recorded only concentration values near threshold, whereas in the middle section, there were much stronger concentration signals. In the third section, the concentration values are between 3 and 4 times higher than in section 2.

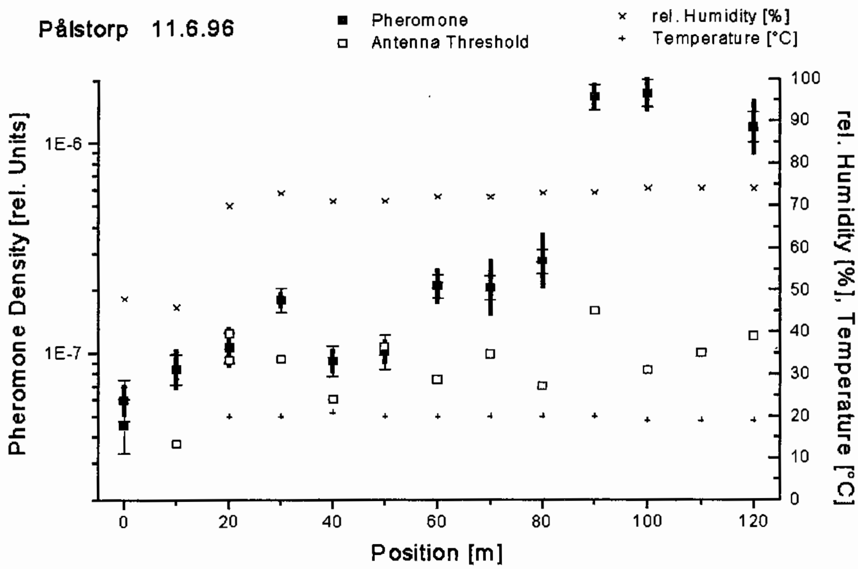

Figure 2. Pheromone concentration in 3 sections of the Pålstorp orchard. Borders between zones of different treatments are at 40 and 80 m.

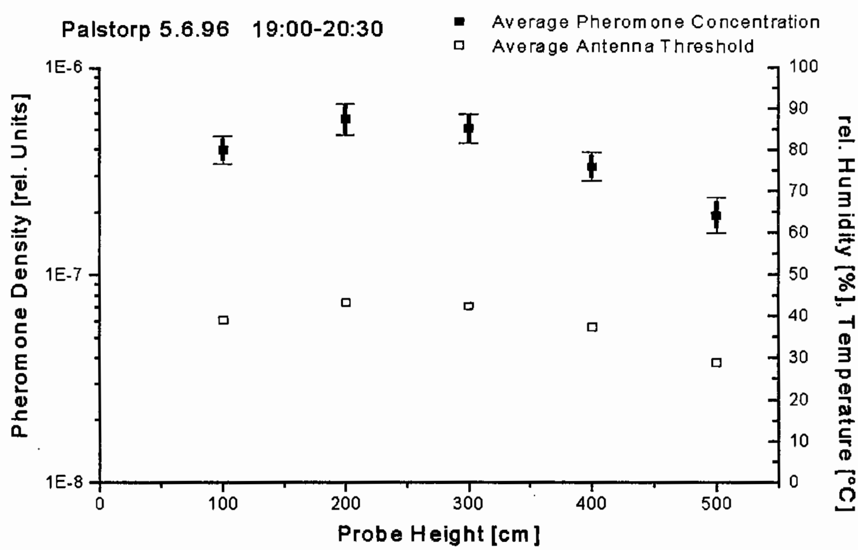

Figure 3. Averaged pheromone concentration plotted versus height of the measurement probe.

Height profile

Figure 3 shows pheromone concentrations measured at different probe heights in the third section of the Pålstorp orchard. The concentration increases slightly from the values found at 1 m probe height to a flat maximum about 2 m probe height; this is the height at which the dispensers were fixed in the trees. The concentration then decreases slightly at a probe height of 3 m and decays further at probe height of 4 m and 5 m. The height of the canopy was around 3.50 m. These height profiles were measured on different occasions. They always showed a similar pattern.

Concentration profile at an upwind orchard edge

The following measurement was made to asess the pheromone distribution at the upwind edge of an apple orchard. The measurements were made at Fjelje, an orchard 20 km north of Malmö. The tree spacing was 1.5 m, the distance between rows was 2 m and the canopy height was 2.20 m. The concentration measurements were taken along a transect parallel to the tree rows. The wind direction was parallel to the transect; the measurement was performed starting at the upwind edge of the orchard (0 m).

Figure 4 shows the concentration values found along the transect: At 5 m upwind from the orchard, there is no pheromone signal detectable above treshold. At the orchard edge, the pheromone signal fluctuates strongly and is barely above threshold. As the measurements proceed along the transect into the orchard, the pheromone concentration rises further. A stable level of the pheromone concentation is only reached after proceeding 20 m from the upwind edge into the orchard.

When looking at the wind speeds recorded at the different positions along the transect, it can be seen vey clearly that the wind speed at 1 m probe height decreases markedly while the probe moves further into the orchard, wheras the windspeed at the top of the measurement pole remains the same. This illustrates how the wind is attenuated by the foliage further inside the orchard.

Discussion

While EAG measurements in apple orchards have been reported repeatedly (Milli 1993; Karg et al. 1994; Suckling et al. 1994; Milli et al. 1996), the measurements described here are the first which have a concentration scale that can be traced to absolute concentrations gained by air sampling and GC analysis. The details of these parallel measurements can be found in Koch et al (1997) and Bäckman (1997). Further details on the orchards and the dispenser applications can be found in Witzgall et al. (1999).

Some nonlinear interactions between plant volatile background and pheromone reactions in the EAG signal had been reported by Rumbo et al (1995) as disturbing quantitative measurements of pheromone concentrations with the field EAG in *Cydia pomonella*. Since in our evaluation system, the superposition of external field pheromone, plant volatiles and additional pheromone calibration stimuli was modeled adequately, the remaining nonlinearities were small compared to other measurement errors. Therefore, the concentration values recorded were not significantly affected by the replacement of an antenna or by changes in the concentration of background volatiles.

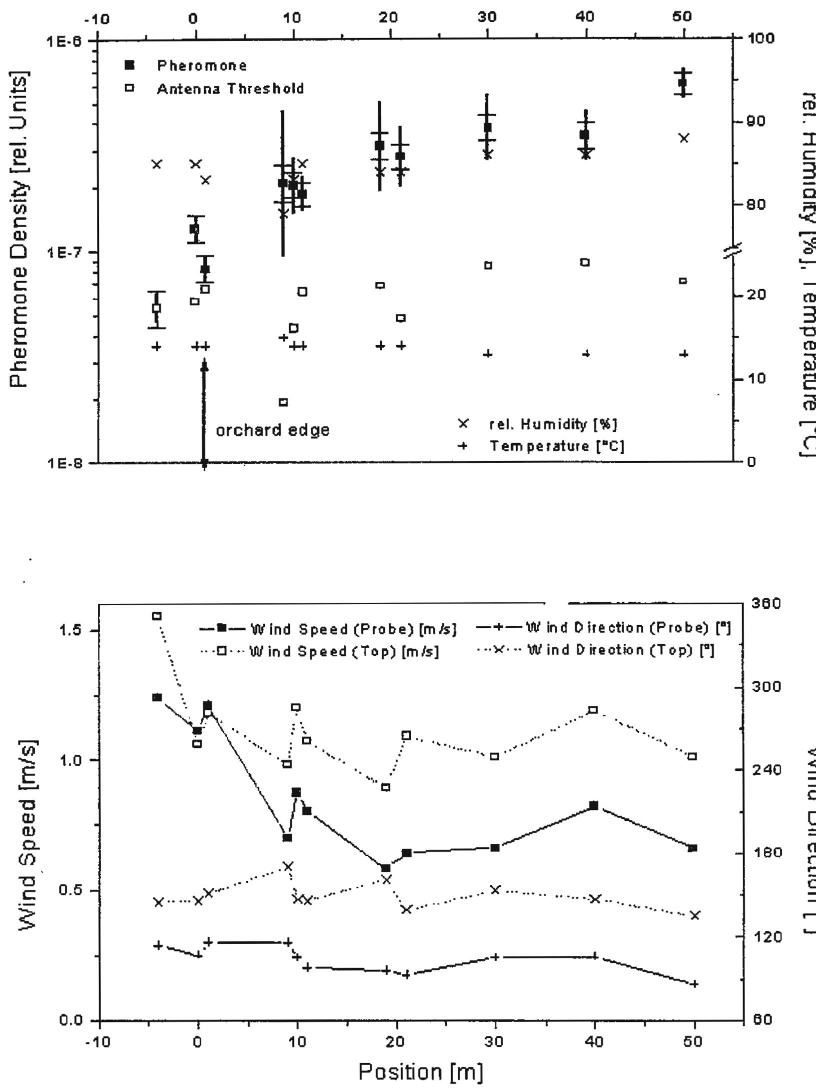

Figure 4. Pheromone concentration and wind data plotted versus distance from the upwind edge (0 m) of an orchard at Fjelje. Note the wide zone of pheromone depletion at the orchard edge.

While Karg et al. (1994) and Suckling et al. (1994) measured pheromones in orchards treated against *Epiphyas postvittana*, Milli (1993) and Milli et al. (1996) measured pheromone concentrations in apple orchards treated against *Cydia pomonella*. Our measurements of the pheromone distribution at the upwind orchard edge confirm the results of Milli et al., who had described an area of pheromone depletion in a zone of 15 m width at the upwind edge of an apple orchard. This reduced pheromone concentration may explain in part why the success of mating disruption is always reduced at the borders of a treated area. In order to have the same pheromone density in all parts of an orchard, one would need a border treatment over a zone with a width considerably wider than hitherto recommended.

Apart from further investigations on pheromone distributions in different types of plant arrangements and climate situations, the field EAG with proper calibration and evaluation systems have been successfully used to determine the lifetime of sprayable pheromone formulations (Koch, in prep.).

Acknowledgements

We are indebted to Wolfgang Lüder, Liliana Cichòn, Stephan Clemenz, Christoph Klos, Georg Haas and Christian Trautwein who took part in our measurements and contributed their work force and ideas. Special thanks are due to Anna-Carin Bäckman for the collaboration in the air sampling work. This work was made possible by a grant from the Stiftung Rheinland-Pfalz für Innovation. Special help was also provided by the University of Kaiserslautern central mechanics and central electronics workshops.

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Essai préliminaire de lutte par confusion contre la cochylis *Eupoecilia ambiguella* et le carpocapse *Cydia pomonella* au moyen des microcapsules 3M

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Abstract: *Preliminary trials to control grape berry moth Eupoecilia ambiguella and codling moth Cydia pomonella by means of 3M microencapsulated pheromones.*
3M microencapsulated pheromones were applied once or twice during the first flight of grape berry moth, *Eupoecilia ambiguella.* During the 12 days following the treatments, catches were reduced by 87%-98%. However, the pressure of the pest in all the region being extremely high, the reduction of grape bunches damaged in the trial was not significant compared with the untreated check. In a trial to control codling moth, *C. pomonella,* by means of 5 applications of microencapsulated pheromones at 28-35 days interval, reduction of catches in sex traps of natural and released coloured males reached 60%-80%. Reduction of mating by tethered females in the trial was about 80%. However, fruit damage reached 1.83% at harvest and the overwintering population of codling moth, estimated by catches of diapausing larvae in corrugated paper bands increased compared to the previous year.

Key words: mating disruption, microencapsulated pheromones, *Eupoecilia ambiguella, Cydia pomonella*

Introduction

Des essais préliminaires de lutte par confusion au moyen de microcapsules de la firme 3M, Canada, ont été réalisés en 1999, d'une part en vignoble contre la première génération de cochylis *Eupoecilia ambiguella*, et d'autre part en verger contre les deux générations du carpocapse *Cydia pomonella.*

Matériel et méthode

Lutte contre cochylis E. ambiguella

L'essai a été réalisé à Aigle dans un vignoble où la pression du ravageur est très importante. Dans une parcelle de 7 ha (secteur A), les microcapsules 3M contenant 20% d'attractif sexuel Z9-12Ac ont été appliquées une première fois immédiatement après le début du premier vol, à 100 ml p.f/ha (20 g m.a./ha), puis le traitement au même dosage a été répété vers le milieu du vol. Dans une autre parcelle de 0.8 ha (secteur B) un seul traitement à 125 ml p.f/ha (25 g m.a./ha) a été appliqué immédiatement après le début du premier vol.

Lutte contre le carpocapse Cydia pomonella
Un essai de lutte a été réalisé à Begnins dans une parcelle de 1,6 ha où la pression du ravageur est modérée. Les microcapsules 3M contenant 25% d'attractif sexuel E8,E10-12OH ont été appliquées 5 fois durant la saison à 400 ml p.f/ha (100 g m.a./ha), en mélange avec les fongicides. Le premier traitement a été effectué au début du premier vol, les suivants à des intervalles de 21 à 35 jours. Dans la parcelle d'essai ainsi que dans un verger de référence, des papillons mâles colorés ont été lâchés et des femelles vierges y ont été attachées pour déterminer le nombre d'accouplements par la recherche des spermatophores.

Résultats et discussion

Essai en vignoble
Par rapport au témoin, les microcapsules ont provoqué une réduction importante des captures de *E. ambiguella* aux pièges sexuels. Durant les 12 jours qui suivent le premier traitement, la réduction atteint 87% dans le secteur A et 98% dans le secteur B. La réduction des captures est de 78% dans le secteur A du moment de la répétition du traitement jusqu'à la fin du vol, alors qu'elle est de 60% pour la même période dans le secteur B qui n'a été traité qu'une fois.

Dans les témoins non traités, le taux de grappes attaquées en première génération varie selon les parcelles entre 38.5% et 64.4%. L'attaque moyenne est de 36.8% dans le secteur A traité 2 fois et de 22.0% dans le secteur B traité une seule fois. Signalons toutefois que depuis quelques années, la pression de cochylis est tellement forte dans la région d'Aigle que ni la lutte classique, ni la confusion, n'ont permis de ramener ce ravageur en dessous du seuil de tolérance.

Essai en verger
A la suite de 10 lâchers de mâles colorés de carpocapse *C. pomonella*, totalisant 330 individus, 26 papillons ont été capturés dans l'essai, soit 7.9%, alors que dans le verger de référence le taux de capture des mâles colorés atteignait 23.2%.

Dans le témoin, 7.4% des femelles attachées étaient accouplées après 3 à 4 jours d'exposition en verger alors que 1.92% étaient accouplées dans l'essai. L'attaque sur fruits a progressé régulièrement durant la saison dans la parcelle d'essai, pour atteindre 1.83% avant la récolte. La population hivernante estimé par les captures dans les bandes-pièges a passé de 0.05 larve/arbre en 1998 à 0.78 en 1999.

Conclusion

Les microcapsules 3M ont indubitablement perturbé le comportement des papillons de cochylis et du carpocapse, les captures aux pièges sexuels ayant été fortement réduites par rapport aux témoins. Dans le vignoble d'Aigle où la pression de cochylis était très forte, les microcapsules n'ont pas permis de réduire significativement le taux d'attaque. Dans le verger de Begnins, les 5 traitements appliqués à raison de 100 g d'attractif par ha n'ont toutefois pas pu empêcher une augmentation de l'attaque et des populations du ravageur par rapport à l'année précédente. Il est par conséquent nécessaire de poursuivre les recherches pour améliorer l'efficacité des microcapsules.

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Mating disruption in Switzerland

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Abstract: Since registration of RAK 2 (against *Lobesia botrana*) and RAK 1+2 (against *Eupoecilia ambiguella* and *L. botrana*) in 1996, mating disruption has become an important technique for controlling the main pests in viticulture. The three products RAK 1+2, RAK 2 and Bocep Viti (against *E. ambiguella*) were applied in 1999 on 4500 ha or 30 % of the total vineyard surface in Switzerland. The success of mating disruption in vineyards is mainly based on the efficacy of the technique and the ecology trend in agriculture. The main problem we had in getting the technique established was the large number of vineyard owners that had to be convinced. Precise instructions for application and control of the two generations during the season are very important and help give the users confidence in the technique. Isomate-C Plus is registered since 1996 for orchard use against *Cydia pomonella* . The surface treated with mating disruption has since grown to over 400 ha, which amounts to about 10 % of the Swiss orchard surface. The main factors hindering its spread are the relatively high price as compared to chemical treatments, and additional pests such as *Grapholita lobarzevskii* and *Adoxophyes orana* that are partially controlled by chemical treatments against *C. pomonella* but not by Isomate-C Plus. Isomate-M Plus or Isomate-OFM Rosso are mating disruption products normally used against *Cydia molesta*. In Switzerland they are used against *G. lobarzevskii* as a complement to Isomate-C Plus in apples or against *Grapholita funebrana* in plums. The efficacy on plums is rather limited due to little surfaces and high populations of *G. funebrana*. It is only used in organic farming.

Key words: *Eupoecilia ambiguella, Lobesia botrana, Cydia pomonella, Grapholita lobarzewskii, Adoxophyes orana, Grapholita funebrana*, mating disruption, pheromones, dispensers

Introduction

In Switzerland the mating disruption technique is being applied on 4500 ha in vineyards and on 400 ha in orchards. The surface treated with pheromone dispensers has increased rapidly within the last 4 years and the technique has become – especially in vineyards - one of the most important instruments for controlling the main pest. The following presentation shall give an overview of the development and success of mating disruption in Switzerland.

Mating disruption in vineyards

The grape berry moths *Eupoecilia ambiguella* and *Lobesia botrana* are the main pests in Swiss vineyards. In the southern part of the country *Lobesia,* is especially abundant, whereas in the eastern part there is mainly *Eupoecilia*. In most of the vineyards, though, both species are present.

There are only few places where no treatment against the grape berry moths is necessary. In most vineyards insecticide treatments had been carried out once or twice a year before the mating disruption technique was applied.

Development

In the seventies first trials were made in Switzerland with the *Eupoecilia* Pheromone. A double chamber dispenser was developed and registered in 1986 by BASF containing 340 mg pheromone. This modern technique of grape moth control was subsidised in the following years. The treated area did not increase very much, though, because *Lobesia* spread especially over the warmer parts in the west and south of Switzerland but also north of the Swiss Alps. With the registration of RAK 2 against *Lobesia* and RAK 1+2 against both species in 1996 the products needed to control both grape berry moth species became available. The mating disruption surface grew from 500 ha in 1996 to 4500 ha in 1999. This represents about 30 % of the whole Swiss vineyard area. Nowadays about 60 % of this surface is being treated with RAK 1+2 and about 20 % each with one of the other two products.

Efficacy in 1999

Under our conditions, the mating disruption technique is applied in the last week of April. This protects the crop throughout the season against both generations of the two grape berry moths.

This year we had very special weather conditions that favoured grape berry moth development. The flight period lasted very long, until mid August, and especially the population of the second generation was very strong compared to previous years. In extreme situations the flight was up to 6 times stronger than in the past year.

Three percent of the surface treated with the mating disruption technique reached an infestation rate in the first grape berry moth generation of over 10 % for *Eupoecilia* or over 5 % for *Lobesia*. These are considered threshold infestation rates for the two species. A supplementary insecticide treatment in the second generation becomes necessary to bring down the grape berry moth population sufficiently to be able to successfully continue with mating disruption.

During the second generation, though, about 13 % or 600 ha of the mating disruption surface had an infestation rate of over 5 %. This is much more than in previous years in which only very few vineyards had an infestation rate surpassing the 5 % threshold. The 5 % infestation threshold of the second generation is the upper limit for a successful use of the mating disruption technique in the following year. Higher infestation rates require a preventive insecticide treatment to reduce population size. Normally the infestation of the second generation is not a problem, because the intervention threshold for the first generation sufficiently reduces the population. Under normal conditions, there is a reduction in population size from the first to the second generation of grape berry moths in pheromone treated surfaces. This was not the case this year. This was probably due to the long flight period of the second moth generation and to varying warm weather conditions with no temperature peaks over 30 °C. Consequently, the conditions for flight were good and egg and young larvae mortality was low.

Vineyards that had been treated with pheromones for years did not have any problems, but newly treated vineyards with high initial populations or small and unfavourable surfaces were severely hit in 1999.

To check the efficacy of mating disruption, Dr. P.-J. Charmillot of the Federal Research Institute in Changins compared the technique with insecticide treatments in the field. In nearly all cases the pheromone method showed a better efficacy than the insecticide treatments.

There are several reasons for this. First of all, the long flight period made it difficult to time an insecticide treatment. Secondly, the warm and wet weather favoured berry and foliage increase. This led to a dilution of the insecticide on the grapes. The foliage covering the grapes hindered the insecticide application.

Please note, though, that the diagram does not show an insufficient efficacy of the insecticide treatments, simply a higher efficacy of the mating disruption technique!

Surface composition and organisation

The composition of the surfaces on which the pheromone technique is being used is very variable. The smallest vineyards are only 0.5 ha, whereas the biggest connected areas are around 450 ha. The surface per owner is also very variable. In the Wallis in the south of Switzerland, we have an average surface of 0.2 ha per owner, whereas in the canton of Geneva you can find owners with up to 50 ha and the average is around 5 ha.

Especially for big vineyards with different producers a well organised concept is needed to get the mating disruption technique established. One possibility is dividing the whole area into sectors of 10-15 ha, with one person in each sector responsible for applying the technique together with the different producers in that sector. This organisation group can also control the infestation of the first and second generation. For the application of the dispensers 1.5 – 3 hours are normally needed, depending on the topography.

For the promotion of the mating disruption technique about 30 % of the surface treated is subsidised (50 % of the costs). Furthermore, the official viticulture technicians support the technique by organising application of the dispensers and the control of infestation. Our aim is to provide as much information on the technique as possible. We therefore organise meetings to present technical information and results. The controls of the first and second generation infestation that we carry out in as many vineyards as possible are very much appreciated by the producers. These results are published once a year in viticulture journals.

Mating disruption – pro and contra

Many people are surprised by the how important mating disruption has become in Switzerland, especially as the price is higher than for insecticides.

One of the most important reasons for the success of the technique is the difficulty in timing an insecticide treatment, whereas with mating disruption – once installed – there is no timing problem. It is e application date is even more difficult to determine the right application date when both species of grape berry moths are around, or when big differences in exposition and altitude exist.

Changing consumer demands make alternatives to insecticide applications economical. Several regions made good publicity for their wine by cultivating their vineyards without the use of insecticides and by emphasising this fact. This then led to an expansion of mating disruption to nearby vineyards, because the latter realised that wine sales were more difficult without.

To cut prices many producers reduce the number of dispensers per ha from 500 to 300 after one or two years of successful introduction of the mating disruption technique. Resistance of grape berry moths against insecticides is not yet a real argument.

The organisation needed for mating disruption is sometimes considered a negative aspect, especially if you have people voting against the technique. There is e.g. an area in the Wallis with 387 different producers on 16 ha. It is a real challenge to get all these producers to use the pheromone technique!

Mating disruption in orchards

The situation for mating disruption in orchards is very different from that in vineyards. There are some large coherent orchard areas, especially in the western part of Switzerland, but more often the orchards are small and isolated with a single producer. The mating disruption technique can thus be established by a producer even if his neighbour does not participate.

Yet orchard areas have their own particular problems: There are often old tall trees near the orchards that are an infestation source for the codling moth and on which no plant protection is undertaken. In mating disruption supplementary treatments against additional pests are often needed whereas the unspecific insecticide treatments eliminate these pests at the same time. Furthermore, insecticides are cheaper than the mating disruption technique.

To date only few cases of codling moth resistance to insecticides exist in Switzerland. As long as insecticides guarantee a sufficient efficacy many fruit growers will not change to the more expensive technique.

Efficacy of Isomate-C Plus against the codling moth

In orchards with a low initial codling moth population there was no pest control problem with Isomate-C Plus. Only about 20 ha or 5 % of the surface had an infestation rate over 2 %. Many orchards have hale protection nets which are favourable to codling moth control with pheromones as there is less migration and the pheromone cloud is more stable.

The pheromone components in Isomate-M Plus/OFM Rosso dispensers are normally used to control the oriental fruit moth, but the pheromones are also effective against *Grapholitha lobarzewskii* and the plum fruit moth.

Grapholita lobarzewskii has become an important pest in orchards in which mating disruption against thecodling moth is being carried out. This year's results show a good efficacy with infestations below 2 %.

For the plum fruit moth the trials carried out, showed that the efficacy of the technique is limited by high populations and small surfaces. In Switzerland most plum orchards are less than 1 ha. The contradicting results this year for the different pests show the limits of the application. Isomate-M Plus/OFM Rosso is consequently only used in organic farming and here only due to lack of an alternative.

Sale of the combined product Isomate-C Special against the codling moth and the summer fruit tortrix has been stopped because of its rather limited efficacy against the summer fruit tortrix. Trials will be carried out next year with Isomate-C Super. These pheromone compounds should be more effective against the summer fruit tortrix, *Adoxophyes orana*.

A summary of all the results made with mating disruption in Switzerland shows a very positive balance. The efficacy is normally very good and the method is appreciated by the producers as well as by the consumers. A critical point is always the relatively high price. New alternatives like sprayable pheromones or cheaper pheromone dispensers are needed to make the mating disruption technique even more attractive in the future.

Acknowledgements

Many thanks to Dr. P.-J. Charmillot from the Swiss Federal Research Station for Plant Protection, CH-1260 Nyon, who supported the whole investigations. We also thank all the Cantonal Offices for Plant Protection for their technical support.

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Mating disruption in vineyards

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Twelve years of practical experience using mating disruption against *Eupoecilia ambiguella* and *Lobesia botrana* in vineyards of the Wuerttemberg region, Germany

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Abstract: Starting with first trials in 1985, the use of mating disruption against *Eupoecilia ambiguella* and *Lobesia botrana* in the Wuerttemberg region continuously increased. In 1999 75 % of the vineyards were treated with pheromones. The overall rate of successful treatments was better than in vineyards treated with insecticides. The use of pheromones in viticulture seems to be more a social problem than a technical problem.

Key words: *Eupoecilia ambiguella, Lobesia botrana*, mating disruption, pheromone, Wuerttemberg

Introduction

Main pests in Southern Germany vineyards are the grape and the vine moth (*Eupoecilia ambiguella* and *Lobesia botrana*). While *Eupoecilia* represents the most common species in the vineyards, *Lobesia* exists in some sites only. The typically wine-farm is very small (0.8 ha) and most of the vineyards are cultivated as a side-job and grapes are processed in co-operatives. After first successful trials in 1985, the use of mating disruption rapidly increased to now more than 75 % of the total vinegrowing area in the region Wuerttemberg (Figure).

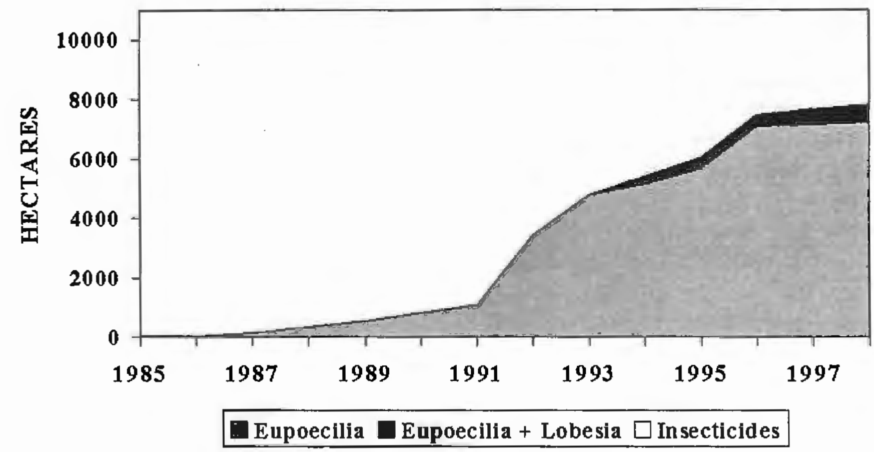
Problems and solutions

One of the biggest problems was to organize treatments over a large area The larger the covered area is, the better mating disruption works. In most cases, a lot of winegrowers had to work together. Basic structures as co-operatives and reparcelling organizations proved to be very helpful in this case. The dispensers were suspended generally in a joint action, which was processed as a social event.

Public pressure against the use of chemical insecticides and the fact, that most vineyards in the region are located extremely close to residential areas, were also very helpful. It proved to be of importance, that everybody could realize the use of the new system against pests. Celebrating the suspension as a social event, mating disruption was best reported in the local newspapers and winegrowers had an excellent chance to cultivate a better image.

One of the biggest problems was the higher cost of the new technique. Mating disruption costs about 240 DM/ha for one species and 340 DM/ha for two species. Additional costs of about 50 to 100 DM/ha were needed for the suspension of the dispensers (2 – 3 man-hours/ha) and organization. In contrast, insecticides rated from 30 to 180 DM/ha for 1 or 2 sprays. A holistic approach had to calculate additional costs for sprays against mites caused by the reduction of beneficial arthropods especially *Thyphlodromus pyri*. The reduction of the beneficials by insecticides required at least one additional spray against spider mites. One spray costs 180 DM/ha. The holistic approach concluded, that mating disruption is cost-neutral. The use of mating disruption was supported by the government of Baden-Wuerttemberg starting with 80 DM/ha in 1987 up to 200 DM/ha in 1998.

Use of Pheromones in Wuerttemberg Vineyards



Furthermore a control system was established. First control (1st generation) was performed during bloom. Where larval attack exceeded 10, the winegrowers were informed that there might be a risk of higher attack during the 2nd generation. In this case, the vineyards were controlled very intensively in the second generation especially during the end of the oviposition period. In some cases additional use of insecticides was proposed. A control of the second generation was carried out using data of pheromone traps from outside the treated area. Controls were performed 7 and 14 days after the first peak.

Conclusion

The mating disruption technique proved to be successful. In more than 98% of all cases, the attack did not exceed the damage threshold of 5% grape attack. A higher rate of attack was found in some cases along the border line of the treated area and in some hot spots, with an extremely high population density. Soon the winegrowers learned to handle these problems. The overall rate of successful treatments was better than in vineyards treated with insecticides. The rate of success increased if the method was used for more than three years. Relevant changes in the abundance of other pests were not observed. In the first and second year in some cases the leaf hopper population (*Empoasca vitis*) increased but did not exceed the damage threshold. Mite problems decreased very quickly because of the higher number of beneficial arthropods.

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Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards with very high population densities

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Abstract: Two tortricids, *Lobesia botrana* and *Eupoecilia ambiguella*, are the most important insect pests in German viticulture. Both species are bivoltine and the second generation is the most damaging to grapes. In many German viticultural regions, populations of *Lobesia botrana* have been increasing over the past years, while *Eupoecilia ambiguella* populations have been decreasing. Today, both species can be controlled by mating disruption. Although the mating disruption technique can be used to effectively control *L. botrana* at low population densities, the technique is not as effective at high population densities. An evaluation of the potential for using a combination of insecticide and mating disruption to control *L. botrana* at high densities was begun in Neustadt/Weinstraße during 1999. The objective of the two-year project was to reduce *L. botrana* populations within the trial site to a level where mating disruption could be exclusively used for control. The results obtained in 1999 demonstrated that moth captures in pheromone-baited traps as well as larval densities were significantly reduced, compared to previous years. However, density was not reduced to a level where mating disruption could be used alone for control of the first generation of 2000. Therefore in 2000, a combined treatment of pheromone plus insecticides has to be repeated, in an attempt to reduce population densities to a lower level where mating disruption will perform effectively.

Keywords: sex pheromone, mating disruption, viticulture, *Lobesia botrana*, *Eupoecilia ambiguella*, Lepidoptera, Tortricidae

Introduction

In Germany, viticultural areas cover about 100.000 ha. The main pests are two tortricid species, *Lobesia botrana* Schiff. and *Eupoecilia ambiguella* Hbn. In many of the German vine growing areas a striking shift of the two species has been recorded: *L. botrana* populations increased while *E. ambiguella* populations have been decreasing over the past years. In some areas, for example the Palatinate, there are regions where now only *L. botrana* occurs. This species shows a greater flight activity and reproduction potential, with resultant greater injury to grape clusters than in the case of *E. ambiguella*.

In 1986, the mating disruption technique was officially registered for *E. ambiguella* in Germany. Mating disruption against this species was then used on up to 16.000 ha in some German states, also due to a financial support of the government. In 1994, mating disruption was registered for control of *L. botrana*. Mating disruption is currently used to control both *L. botrana* and *E. ambiguella* on about 4000 ha of German vineyards, where these species occur in sympatry.

Since 1996, the total area treated with pheromone for control of *E. ambiguella* and *L. botrana* has not increased above 20.000 ha, or 20% of the total German vineyard production area. One reason for the lack of increase in the use of this technology could be the high price of the pheromones and therefore the low acceptance of the vinegrowers. Another reason could be the fact that the mating disruption technique is not been efficient in some vineyards with high tortricid densities. Feldhege (1993) found that mating disruption to control *L. botrana* did not work successfully in vineyards with a moth density of more than 4000 females and males per ha.

In 1999, we initiated a two-year investigation to determine if mating disruption could be used in combination with insecticide to control large populations of *L. botrana* and reduce populations to levels where mating disruption alone could be used for control. An additional objective of our work was to demonstrate to grape growers that mating disruption could be used to control large populations of *L. botrana.*

Material and Methods

The 45-ha trial site (site 2) was situated next to the southeastern border of an area of about 60 ha of vineyards (site 1), which had been treated very successfully with pheromones against *L. botrana* since 1992. Between 1992 and 1998, site 2 was used as an untreated control plot for the 60-ha pheromone site and had an extremely high population of *L. botrana*. Average density of first-generation larvae was 50 - 100 individuals per 100 inflorescences. Especially the infestation of the second larval generation caused severe problems in the past: The density of second generation larvae was even greater with from 200 to more than 600 larvae per 100 clusters.

In April 1999, before the beginning of moth flight, the entire site 2 was treated with pheromone dispensers (RAK 1+2; (E)-9-dodecenylacetate and (E,Z)-7,9-dodecadienyl acetate) at the rate of 500 dispensers per ha. In addition, at site 2, the first and second generation of larvae was treated using conventional insecticides (*e.g. Bacillus thuringiensis*, Parathion-ethyl, Parathion-methyl). The efficacy of the pheromone treatment was evaluated by establishing nine control plots distributed at site 2, each consisting of 72 vines which were not treated with insecticide. A total of 9 pheromone traps, one in each control plot, was installed to monitor the flight activity of male moths. Three pheromone traps were deployed within control vineyards located about 2 km from site 2. A hailstorm in June damaged 100% of the crop in this control vineyard. Therefore, the untreated control plots had to be moved to other vineyards situated at the same distance. To compare the efficacy of the pheromone treatment in the trial site with the efficacy within the 60-ha pheromone-treatment, 3 pheromone traps were put into the 60-ha pheromone treatment to monitor the flight activity.

Results and Discussion

Monitoring the flight activity

An average of 2251 males/trap were captured during the first generation within the control plots. The pheromone treatment reduced trap capture in site 2 by about 94%, compared to trap capture in the control plots. At site 1, captures of *L. botrana* males were reduced to about 99%, compared to trap capture I

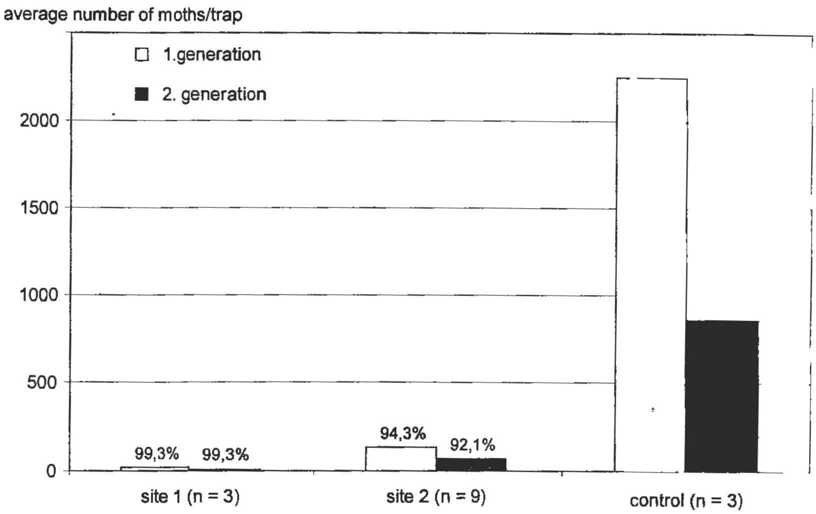

Figure 1. Effect of pheromone treatment on the capture of *Lobesia botrana* males in pheromone traps (Neustadt/Weinstraße, 1999). Reduction of captures compared to control is shown above columns.

The flight activity of the second generation within the new control plots reached an average of 863 male moths/trap. Trap captures were 99% in site 1 and 92% in site 2 when captures were compared to those in the new control plots (Figure 1).

Compared to pheromone treatments in previous years, the larval infestation of the second generation in site 1 was very high (Figure 2). The pheromone treatment reduced larval density by 96% in site 1 and by 39% in site 2. The negative results in site 1 were probably induced by the weather situations mentioned above. The results of mating disruption obtained in the "high density area" of site 2 were not satisfying as well, because the damage threshold for the second generation should not be exceed 5 to 10%. In comparison to the results of the years before in 1999, a significant reduction could be noticed in site 2, even if the population pressure in general was high (Figure 3).

The investigations in 1999 have demonstrated that in 2000 another combined use of pheromone technique and insecticide applications must be applied at least for the first generation of *L. botrana*. Evaluations of the first generation will show if there are the conditions for exclusive use of the mating disruption against the second generation of *L. botrana*.

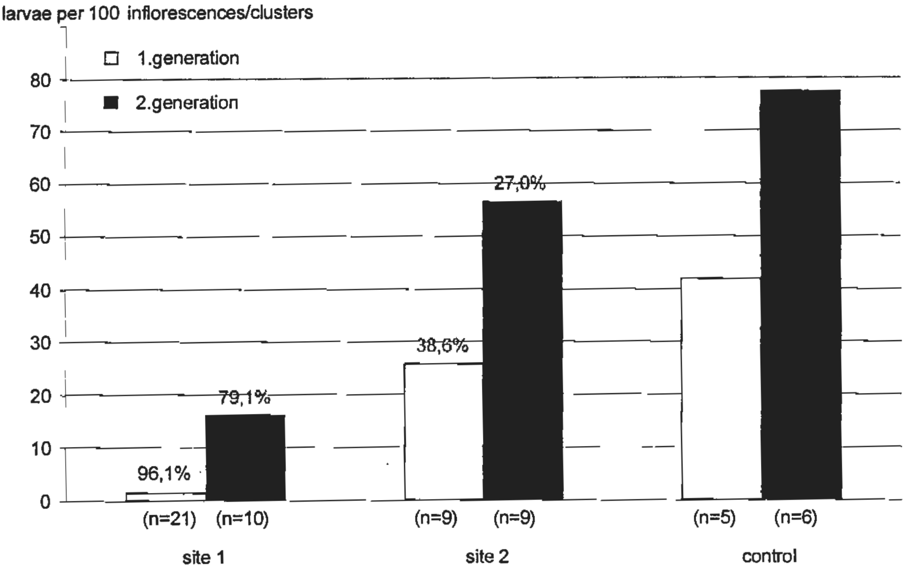

Figure 2. Mating disruption against *Lobesia botrana*. Evaluation of the larval infestation (Neustadt/Weinstraße, 1999). Reduction of larval infestation compared to control is shown above columns.

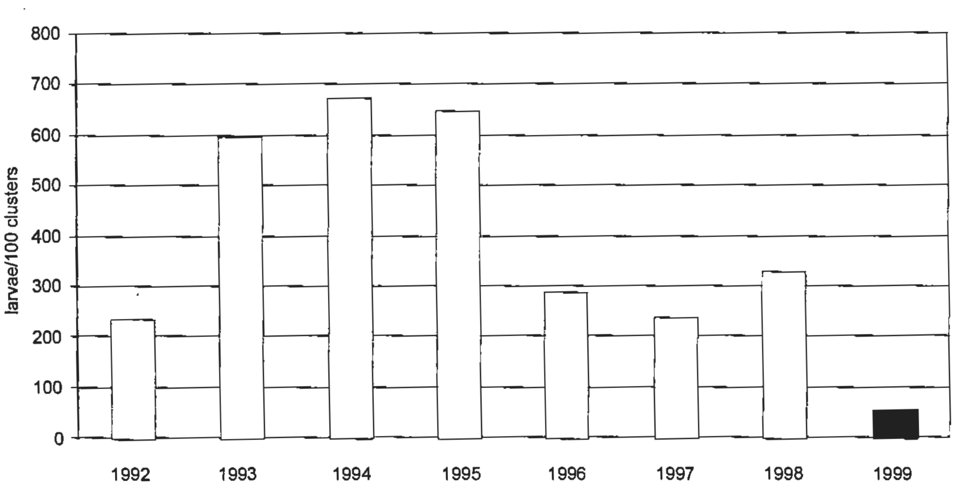

Figure 3. *Lobesia botrana* infestation rates (second generation) in site 2, untreated control plots 1992 to 1998, and first use of pheromone 1999 (RAK 1+2, black column)

Acknowledgements

Investigations were made possible by financial support of the Ministry of Economy, Traffic, Agriculture and Viticulture Rheinland-Pfalz, Mainz, Germany and the BASF AG, Germany providing us with the pheromone dispensers. Special thanks go to the vinegrowers for their cooperation.

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IOBC wprs Bulletin Vol. 24(2) 2001
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Experience with mating disruption technique to control grape berry moth, *Lobesia botrana*, in Trentino

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Abstract: The efficacy of mating disruption for controlling grape berry moth, *Lobesia botrana*, was evaluated from 1991 to 1999 in Trentino. Tests began on a surface of 14 ha in 1991 and reached 1537 ha in 1999. This surface is divided in 6 different areas, ranging from 10 ha to 1100 ha. The results are quite promising, and during these 10 years we collected, especially in the large area of 1100 ha, a lot of experience about different controls, reduction of pheromone-dose/ha, behaviour of the population, new pests, organisation of the vine-growers, contacts with cooperatives and the Province of Trento.

Key words: grape moth, *Lobesia botrana*, mating disruption

Introduction

During the last decades research has provided a very promising method of control of the grape moth, the mating disruption technique. Experiences with this technique are being increasingly gained all over Europe. In Italy, about 2000 ha are subjected to mating disruption and the province with the largest area is the Province of Trentino with about 1550 ha, equivalent to about 20% of the total wine producing area.

The area subjected to mating disruption increased between 1991 and 1999 from 14 to 1537 ha and during those years a considerable amount of information could be gathered. The technique proved to be very efficient particularly in the case of the following two basic conditions: (1) large areas involved, (2) low pest population density.

During all those years different behaviours and consequences as a result of the application of the mating disruption technique were observed and we consider it interesting pointing out some of the results. The farming world starts to get acquainted to the mating disruption technique and the affected areas are increasing because the growers themselves are getting convinced of the advantages.

Modern viticulture requires an increasingly constant presence of the grower in the field for an increasing number of manual operations. The growers themselves which are enabled to work in a cleaner ecosystem are therefore the first beneficiaries of the mating disruption technique.

Materials and methods

Mating disruption and area involved

As already mentioned, the area of mating disruption expanded from the initial 14 ha of the "Sottodossi di Mezzocorona" to most of the viticultural area of the "Piana Rotaliana" and of Lavis. Further areas are the hills around Trento, Dro and Arco in the Sarco valley, Ala and

Rovereto in Vallagarina and Telve in Valsugana. Most of the observations refer to the "Piana Rotaliana", where in 1999, 1164 ha (76,1% of the total area of mating disruption) were located.

Mating disruption and number of growers involved

Viticulture in Trentino is characterized by a high degree of fragmentation of the farm units, and this aspect would constitute a severe limitation to the applicability of mating disruption, if there were not the co-ordinating activity of the growers' co-operatives.

Table 1. Area and number of growers involved in mating disruption in viticulture

Year	Area (ha)	Number of Growers
1991	14	36
1992	75	158
1993	179	276
1994	217	341
1995	137	254
1996	177	321
1997	233	386
1998	653	749
1999	1537	1447

Table 1 shows the area and the number of growers involved in mating disruption. In 1999 the area of 1537 ha comprised 1447 different growers. The most important duties of the growers are the timely and correct placement of the dispensers and the avoiding of sprays against the grape moth unless under specific authorisation.

Dispensers

Until 1995 ampoules (RAK 2) provided by the German producer BASF were used. From 1996 onwards, we have used the "spaghetti" of the Japanese company Shin-Etsu.

About 500 dispensers are placed per ha and one "spaghetto" therefore serves about 20 m^2. The timing of the placement coincides with the beginning of the flight of the first generation and needs to be as regular as possible over the whole area. In the border areas a higher degree of protection is required. This is achieved either by increasing the density of the dispensers in the border area of 15-20 m, or by extending the protected area for further 15-20 m towards the exterior. The dispensers are subjected to a series of quality controls for the assessment of the correct release of the pheromones into the vineyard.

Mating disruption and evaluations

In order to assess the efficacy of this method, an accurate control of a series of traps inside the "disruption"-zone was carried out. Absence or presence of flight are fundamental parameters to be taken into account: if the method works, there shouldn't be captures in the traps. During the 9 years, increasing the area, there has never been a capture in the traps inside the "disrupted" zone; a few captures, however, took place in the border areas.

In addition to this evaluation, visual controls were carried out to estimate the incidence of damage of the first generation and the presence or absence of second generation eggs. In a final assessment the real efficacy of the method was evaluated. The assessment of oviposition and final damage were carried out in distinct areas separating the border areas from the central areas. The assessment of the oviposition of the second generation is decisive for the achievement of an efficient control. According to this assessment, the growers will be advised on the necessity of carrying out a further treatment.

With increasing areas, the assessment of oviposition of the second generation in the whole area within a short period of time becomes difficult. For this reason since 1998 the method proposed by Dr. Charmillot of the Federal Agricultural Research Station in Changins in Switzerland has been applied.

The controls are being carried out on the first generation: at least 100 grapes are examined in different areas and the population quantified; in case the incidence of the grape moth exceeds 5-8% of grapes with nests control measures on the second generation will become necessary.

Mating disruption and evaluations on the first generation

In 1998, on the basis of the evaluation of the first generation about 75 ha were sprayed with a single treatment against the second generation. This represents 12% of the total area of 656 ha of mating disruption. These 75 ha were under mating disruption for the first year and have always been areas of high population densities of the grape moth.

One of these treated areas was "Vizinia" at Mezzocorona; the only varieties grown there were Chardonnay and Pinot Gris, the most susceptible varieties in our environments, and the evaluation on the first generation revealed on average an incidence of 5.6% of grapes with larvae, with the highest values reaching 39.5%.

Nevertheless, the effect of mating disruption was already visible during the first year also in this area. Table 2 shows the first generation population outside the disruption area (34.7% of grapes with nests), in the border area (13.1% of grapes with nests), in the disruption area next to the border (30 m: 9,1% of grapes with nests) and in the centre of the disruption area (2.5% of grapes with nests). In 1999 about 30 ha were sprayed (1,9% of the area covered by mating disruption).

Table 2. Evaluation of first generation in the area "Vizinia" at Mezzocorona

% of grapes with nests of grape moth			
outside disruption area	border area (0 - 10 metres)	area next to border (30 metres)	centre
34,7	13,1	9,1	2,5

Results and discussion

The results were generally promising, showing a very low incidence of damage. Table 3 shows the mean damage (% of grapes with larvae) at harvest in the area "Piana Rotaliana". The figures in the table speak for themselves and don't require much comment.

Mating disruption in areas of high population density

In two areas of high population density where the control was carried without chemical means using only mating disruption the evolution of damage was monitored.

Table 3. Final evaluation - % of grapes with larvae in the area "Piana Rotaliana"

Year	% grapes with larvae
1991	0,9
1992	4,4
1993	3,2
1994	1,3
1995	1,8
1996	0,9
1997	1,2
1998	0,9
1999	1,0

Area "Camorzi" at Mezzocorona. This area is covered by mating disruption since 1993. The incidence of damage near the inhabited area was initially high (up to 16,3% at the border). During the years this incidence got considerably reduced resulting in a very low population density.

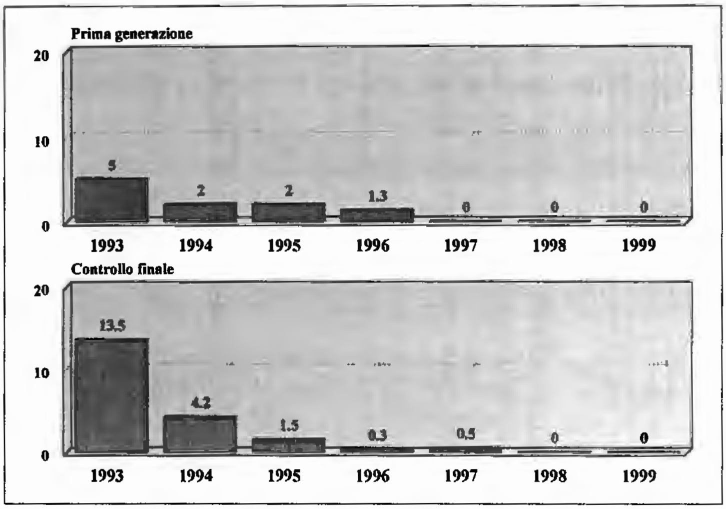

Figure 1. Percent grapes with larvae of first and second generation in the area Camorzi at Mezzocorona from 1993 to 1999.

Area S. Michele a/A. On the estate of the Istituto Agrario di S. Michele all'Adige, mating disruption is being applied from 1997 on ca. 21 ha of vineyards. In 1997, the results were promising (on average 3.6% of grapes with larvae) with the exception of some areas bordering to forests (*e.g.* the alluvial fan "Rauti") with a very high incidence of damage at

harvest. The damage in those areas was distributed as follows: border area 22.2%, centre 4,5%. The mean value was 9.8% of grapes with larvae of the grape moth.
In 1998 and in 1999, the situation changed considerably: the mean damage on the estate was 0.05% and 0.21%, respectively, but also in the area with high incidence of damage in 1997 (alluvial fan "Rauti") there was a reduction of the damage with the following distribution: (1) border 0.5% and centre 0% in 1998, (2) border 1.7 % and centre 0.5% in 1999.

Table 4. Final evaluation “alluvial fan Rauti” S.Michele a/A. Percent grapes with larvae

Year	Border (0 - 10 metres)	Centre	mean damage
1997	22.2	4.5	9.8
1998	0.5	0	0.15
1999	1.7	0.5	0.9

The varieties grown in this area are Sauvignon Blanc, Rheinriesling and Pinot Gris, which are all extremely susceptible varieties; the obtained results therefore assume even further significance. This confirms that problems would be more likely during the first year of application with a reduction to follow thereafter.

What happens when mating disruption is halted?

From 1992 to 1994, mating disruption was increasingly applied on an area cultivated with "Teroldego" grapes between the urban centres of Mezzocorona and Mezzolombardo. From 1995, the application has continued in the area of Mezzocorona, whereas it was interrupted in the municipality of Mezzolombardo. Thus it was possible to compare the pest populations during the following years performing evaluations on the first generation. The results show that an interruption of mating disruption is automatically followed by an increase of the pest population.

It can be observed that at Mezzocoraona with continued mating disruption the population is constantly kept at very low levels with a trend to further decline, whereas at Mezzolombardo with the interruption of the application the population resumes to increase.

This underlines the general validity of the mating disruption technique, which shows the best results after application over a number of years, as could be confirmed on various occasions; in fact it is so far the only known method able to reduce the population in the long term. From 1999 mating disruption has been applied again in the area of Mezzolombardo.

Mating disruption and new pests

With the application of this strategy of control and the reduction of the use of chemical pesticides new problems may arise in the respective areas. This is what happened in 1998. In some areas located between 350 m and 450 m on the hill of Faedo damages caused by *Argyrotaenia pulchellana* were detected, reaching a maximum of 8% in some vineyards.

This insect is very polyphagous and completes three generations a year. Normally it is the second generation which can cause damages on the vines in restricted areas. The larvae feed on the berries and on the stalks of the grapes with common erosions and an abundant production of silk as compared to the grape moth. In general, the sprays against the second generation of the grape moth control also this pest.

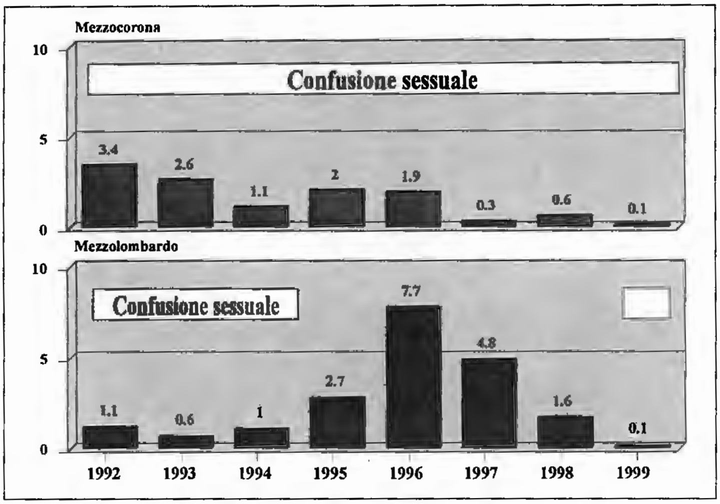

Figure 2. Effect of mating disruption on the population of *Lobesia botrana*. Percent of grapes with first generation larvae

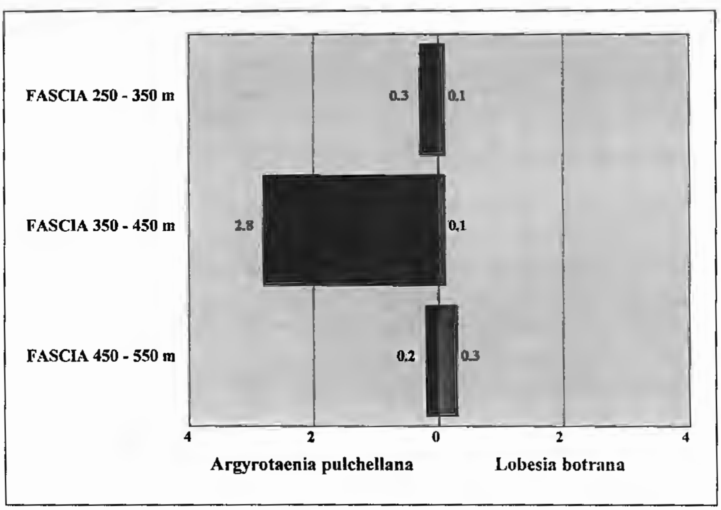

Figure 3. Hill of Faedo. Percent of grapes with larvae of *Argyrotaenia pulchellana* and *Lobesia botrana* at harvest in 1998.

Since the beginning of the mating disruption programme (1991) *Argyrotaenia pulchellana* appeared for the first time in 1998. In 1999 this pest appeared in some restricted areas, but with lower intensities than in 1998. This shows us that a certain level of attention should always be maintained, not only in order to control the grape moth, but also in order to prevent possible new problems.

Mating disruption in future. This interesting method requires some efforts in the next future. The first point is, that on the basis of the achieved results, the area of applied mating disruption can be considerably extended leading to a viticulture with reduced impact on the environment in general and on the human population and the growers in particular.

A further possibility derives from the cooperation with Dr. Charmillot in Switzerland, who has worked for some years with reduced dosages of pheromones (reducing the number of dispensers up to 1/3 of the full number) on areas with very low pest population densities or on areas covered by mating disruption for several years.

From 1998 reduced mating disruption (250 dispensers/ha instead of 500) has been applied on an area of 47.5 ha at Mezzocorona, which had been covered by mating disruption for the previous 7-8 years and which showed very low pest population levels. The results were very promising both in 1998 and 1999. The long term effect however still needs to be evaluated.

This confirms that after determining the validity of a method there is never an end to the quest for improvement, and the cooperation with foreign partners in research is a useful way for achieving this purpose.

Table 5. Total number of taxa (taxa is a parameter used for summarising the diversity of the fauna; it becomes thus possible to concentrate in a single concept various taxonomic levels, including order, family, subfamily, genus, species). Comparison of 3 orchards with different control strategies (1991 to 1994).

	harmful species	useful species	indifferent species
organic orchard	36	28	11
pilot orchard	32	21	10
conventional orchard	30	14	6

Conclusions

These last 9 years of research on mating disruption in viticulture have yielded valuable results for the control of the grape moth. The work is intense, but also satisfactory, and in future we will even require a stronger participation of the growers during the evaluation of the first generation.

With an increasing extension of the areas covered by mating disruption some general aspects need to be further investigated. The observations carried out in horticulture from 1991 to 1994 revealed that the application of mating disruption (pilot orchard) together with the reduction of the amount and spectrum of fungicides used (only Ziram, Delan and IBS) leads to an increase of the biodiversity of the agro-ecosystem particularly in favour of the "useful" organisms. The impoverishment of the ecosystem in conventional horticulture affects mainly the "useful" species. The more a system is complex, the more it becomes stable in time.

Much more research at various levels is still necessary, however an increasing interest of the cooperatives and the growers can be mentioned as a positive aspect, which will facilitate the acquisition of further knowledge.

The control of *Empoasca vitis* still remains to be improved, after which the "problem of the phytophagous organisms of vines (grape moth, red spider mite and vine leafhopper)" will be fully managed through an integrated approach.

Maybe this is too big a statement for the moment, but who approaches the problem with enthusiasm, conviction and the spirit to create deeds rather than words still believes in an integrated control based on values and principles and not on chatter. Time is always an inexorable judge!

Acknowledgements

We express our thanks to: The colleagues of the technical advisory service ESAT (Ente Sviluppo Agricoltura Trentina) who have assisted the mating disruption programmes in different areas of the Trentino-Province: Mescalchin Enzo, Margoni Michele, Michelotti Franco, Fellin Francesco, Pellegrini Ferruccio, Piva Umberto, Ribolli Francesco; Claudio Gottardi for the constant monitoring of the grape moth traps; the team 3P of Roverè della Luna for their invaluable assistance in the application of mating disruption in their area; and the "Famiglia Cooperativa" of Mezzocorona (Emporio Verde) for providing the dispensers to all the interested growers in the area of the "Piana Rotaliana".

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IOBC wprs Bulletin Vol. 24(2) 2001
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Successful control of *Sparganothis pilleriana* (Lepidoptera: Tortricidae) by mating disruption - Conclusions from a three-year study

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Abstract: Mating disruption using sex pheromones was tested for control of *Sparganothis pilleriana* Schiff., a tortricid moth causing occasional but serious damage to grapevines. Chemical control is often inefficient due to the particular life habits of the larvae. Disruption of sexual communication in *S. pilleriana* was investigated as alternative, environmental friendly control method. Based on the mixture of the main pheromone compound of *S. pilleriana* (E9-12Ac) and of *Eupoecilia ambiguella* (Z9-12Ac), four blend compositions were tested in the vineyard. A blend of E9-12Ac, Z9-12Ac, Z11-14Ac, E9-12OH, Z9-12OH was most effective and reduced the larval infestation rate of *S. pilleriana* by 80 to 98%. Infestation of *E. ambiguella* was decreased by 40 and 50%. A combined control of these two grape tortricids is discussed.

Keywords: mating disruption, sex pheromone, viticulture, *Sparganothis pilleriana*, *Eupoecilia ambiguella*, Tortricidae, Lepidoptera

Introduction

Increasing population densities of *Sparganothis pilleriana* Schiff., a tortricid grapevine pest of worldwide distribution, has been observed in the South Palatinate (Germany) since the early 1990's (Schirra & Louis 1995). The larvae may cause high economic damage by feeding on shoot tips, leaves, inflorescences and grape bunches. The effectiveness of common insecticides in reducing the infestation rate is often unsatisfactory. The poor performance of insecticides is likely due to the life habits of this tortricid. For example, the overwintering first instar larvae migrate over a period of six weeks from under the bark of the vine in spring. In addition, the larvae hide in shoot tips and build feeding shelters out of leaves. These habits make it extremely difficult to accurately time applications of insecticide and for insecticides to contact the larvae.

In 1996, a research project was launched at the Federal Education and Research Institute Neustadt/Weinstraße, Germany, with the goal of finding an effective and environmental safe method to control *S. pilleriana*. A major objective was to examine the potential of mating disruption using sex pheromones against this pest. Saglio *et al.* (1977), Roehrich (1977) and Guerin *et al.* (1986) demonstrated that E-9-dodecenyl acetate (E9-12Ac), E-9-dodecen-1-ol (E9-12OH), E-11-tetradecenyl acetate (E11-14Ac) and Z-11-tetradecenylacetat (Z11-14Ac) play a role in sexual communication of *S. pilleriana* moths. Another objective was to determine if *Eupoecilia ambiguella* Hbn., another important vine pest, could be concurrently controlled by mating disruption. For this reason we added the main sex pheromone component of this tortricid, Z-9-dodecenyl acetate (Z9-12Ac) (Arn *et al.* 1976), to the mixtures of components, we tested for mating disruption of *S. pilleriana.*

Materials and Methods

The experiments conducted from 1996 and 1997 and the experimental design used in 1998 are described by Schmidt-Tiedemann *et al.* (1999a, b). In 1998 two 5 ha sites were treated with a 70:70:60:4:1 blend of E9-12Ac, Z9-12Ac, Z11-14Ac, E9-12OH and Z9-12OH (Blend 1, relative amounts). The blend was formulated in polyethylene tube dispensers (Shin-Etsu Chemical Company, Ltd. Tokyo, Japan) with 85 mg of the blend per dispenser. The plots were treated with 1000 dispensers/ha without an additional border treatment. The effectiveness of the treatment was monitored with pheromone-baited traps (for *S. pilleriana* and *E. ambiguella*) and traps baited with a virgin female (for *S. pilleriana*) (Table 1 and 2). The traps and lures used for *S. pilleriana* are described in Schmidt-Tiedemann *et al.* (1999b). Tetra traps from Hoechst AG, Frankfurt, Germany, were used for *E. ambiguella.* The pheromone-baited traps for *S. pilleriana* were arranged in groups of three within the pheromone plot (9 traps at site 1; 12 traps at site 2) and within untreated plots (9 traps at site 1; 9 traps at control site 2) which were about 75 and 180 meters from the pheromone-treated area. The traps with the virgin females (pheromone-treated area: 3 traps at each site; untreated control plot: 3 traps at each site) and the traps for *E. ambiguella* (pheromone-treated-area: 3 traps at site 1; 4 traps at site 2; untreated control plots: 3 traps at both sites) were adjacent to the groups of *S. pilleriana* pheromone-baited traps.

Moreover, squares of ca. 150 m^2 containing 72 vines were established in each pheromone-treated (3 at site 1, 4 at site 2) and control plots (3 at site 1 and). The squares did not receive insecticide treatment and the centered 20 vines of each square were used to estimate egg density in 1998, and larval density in 1999.

The pheromone-treated plots were enlarged to 8,5 ha (site 1) and 11,5 ha (site 2) in 1999. Two additional groups of *S. pilleriana* traps were arranged within the pheromone-treated areas and one additional group in a new control plot in site 2. The number of traps used for checking the moth flight and squares for investigating the number of eggmasses per vine is shown in table 2.

Results

Sparganothis pilleriana

In 1998, treatment with blend 1 reduced moth catch rate in traps baited with synthetic pheromone and virgin females in both sites between 98 and 100% in comparison to the untreated control plots (Table 1). The density of eggmasses was reduced by 99% (site 1) and 84% (site 2) in the pheromone-treated plots (Table 1). Larval infestation rate was reduced by 98% (site 1) and 80% (site 2) after pheromone treatment. During 1999, the catch rate in pheromone traps was decreased by 97% at site 1 and by 99% at site 2. The number of eggmasses per vine was reduced by 91% (site 1) and 83% (site 2). The larval infestation rate will be examined in spring 2000.

Table 1. Effectiveness of a 70:70:60:4:1-blend of E9-12Ac, Z9-12Ac, Z11-14Ac, E9-12OH and Z9-12OH (blend 1) on the different life stages of *S. pilleriana* in 1998/1999

	Treatments			
	Site 1		Site 2	
	Blend 1	Control	Blend 1	Control
Plot size (ha) 1998	5		5	
No. pheromone traps	9	9	12	9
Average catch (± SD) per pheromone trap	0.2 ± 0.4	49.2 ± 16.4	0.9 ± 0.9	90.7 ± 19.1
No. virgin female traps	3	3	3	3
Average catch (± SD) in virgin female trap	0.3 ± 0.6	33.0 ± 21.1	0.3 ± 0.6	49.7 ± 18.5
No. vines examined for eggmasses	60	60	80	60
Average no. eggmasses (± SD) per vine	0.02 ± 0.1	1.6 ± 1.4	0.8 ± 1.1	5.1 ± 4.3
No. vines examined for larvae (1999)	60	60	80	60
Average no. larvae (± SD) per vine	0.1 ± 0.35	4.8 ± 3.14	2.8 ± 2.9	14.1 ± 10.2
Plot size (ha) 1999	8.5		11.5	
No. pheromone traps	15	9	18	12
Average catch per pheromone trap	1.1	35.6	0.8	151.3
No. vines examined for eggmasses	100	60	120	80
Average no. eggmasses per vine	0.1	1.1	0.5	3.0

Eupoecilia ambiguella

Treatment with blend 1 reduced the capture of moths by 99 - 100% in pheromone traps compared to the untreated control plots in both years at both sites. In 1998, larvae were not found in grapes. This may depend on the high temperature of 40°C which could be measured at some days during summer in the South Palatinate. In 1999, 40% (site 1) and 50% (site 2) fewer larvae were found in the pheromone-treated than in the control plots (Table 2).

Discussion

The results of 1996 showed that a mixture of the main pheromone components of *S. pilleriana* (E9-12Ac) and *E. ambiguella* (Z9-12Ac) resulted in a good catch reduction (83%) of *S. pilleriana* moths in pheromone traps compared with untreated control plots (Schmidt-Tiedemann *et al.* 1999b). The addition of Z9-12Ac did not decrease the effectiveness of the E9-12Ac (86%) alone.

Different blends of this binary blend were tested in 1997 for their potential as mating disruptants of *S. pilleriana,* but blend 1 (E9-12Ac, Z9-12Ac, Z11-14Ac, E9-12OH, Z9-12OH) produced the best efficacy in reducing the larval infestation rate of the following generation. Three factors may have influenced these results. First, blend 1 was the most complete pheromone blend; second, blend 1 was tested in a 5-ha plot whereas blends 2, 3 and 4 were tested in 1 ha plots and third, the environmental conditions (e.g. predators and parasitoids) were different at both sites.

Excellent results with blend 1 in controlling *S. pilleriana* were obtained in 1998 (trap catch, eggmasses and larvae) and in 1999 (trap catch and eggmasses). The lower effectiveness of the pheromone treatment in site 2 (concerning eggmasses and larval infestation rate) can be explained by the fact that the moth flight was already more advanced in this location when the dispensers were applied in the vineyard.

Table 2. Effectiveness of blend 1 on the different life stages of *E. ambiguella* 1998/1999

	Treatments			
	Site 1		Site 2	
	Blend 1	Control	Blend 1	Control
Plot size (ha) 1998	5		5	
No. pheromone traps	3	3	4	3
Average catch per pheromone trap	2	205	1	439
No. grapes examined for larvae	90	90	120	90
Average no. larvae per grape	0	0	0	0
Plot size (ha) 1999	8.5		11.5	
No. pheromone traps	3	3	3	3
Average catch per pheromone trap	4	607	8	825
No. grapes examined for larvae	125	75	150	100
Average no. larvae per 25 grapes	0.8	1.3	1.5	3.0

Moreover, the pheromone treatment for *S. pilleriana* control appeared to be successful in small plots whereas good control of *E. ambiguella* was not obtained in small plots. The minimum area recommended for treatment with pheromone for this tortricid is 20 ha and we treated only 8,5 and 11,5 ha respectively. This may explain the relatively low efficacy of blend 1 in reducing the larvae of *E. ambiguella* of 40 and 50%. Another point which has to be considered for the interpretation of the *E. ambiguella* data is the time when the dispensers were fixed in the vineyard. *S. pilleriana* was the target insect for this study, and therefore, the experimental design was optimized for this species. By contrast, *E. ambiguella* had begun to fly before pheromone was applied. Better control of *E. ambiguella* would be obtained if the pheromone treatment would be applied earlier. Moreover, E9-12Ac is an attraction inhibitor for *E. ambiguella* (Arn *et al.* 1979) and the blend of both isomers have an repellent effect, as described for *Cydia nigricana* (Bengtsson *et al.* 1994).

This study demonstrates that *S. pilleriana* can be successfully controlled by mating disruption with sex pheromone. There is a good chance that both *S. pilleriana* and *E. ambiguella* can be controlled with a blend containing a blend of E9-12Ac and Z9-12Ac. This method is not registered yet.

Acknowledgements

This study was supported by the Ministry of Economy, Traffic, Agriculture and Viticulture Rheinland-Pfalz (Mainz, Germany). Shin-Etsu Chemical Company Co., Ltd. (Tokyo, Japan) and BASF AG (Ludwigshafen, Germany) provided the dispenser material. Special thanks go to the vinegrowers for their cooperation.

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Other topics

Pheromones for Insect Control in Orchards and Vineyards
IOBC wprs Bulletin Vol. 24(2) 2001
pp. 95-99

Appeal: efficacy and mode of action of attract and kill for codling moth control

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Abstract: Appeal is a novel attracticide product for the control of the codling moth, *Cydia pomonella*. It is a viscous formulation based on castor oil containing the pyrethroid cyfluthrin and (E,E)-8,10-dodecadienol, the main component of the codling moth sex pheromone. The attract and kill efficacy and the pheromone release rate of field aged samples of the formulation were tested. Both factors remained relatively stable over a period of up to six weeks. Field trials in Poland showed that this semiochemical based strategy can compete with conventional spray applications of insect growth regulators and is therefore a good alternative for codling moth control in integrated fruit growing.

Key words: Attract & kill, codling moth, *Cydia pomonella*

Introduction

The codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is a major pest of fruit orchards worldwide (Barnes, 1991). Currently, codling moth control relies primarily on conventional spray applications, predominantly of organo-phosphates or insect growth regulators (IGR). The development of resistance against the latter group of insecticides makes it necessary to develop novel control methods compatible with the aims of integrated pest management (Croft & Riedl, 1991). In this context, the mating disruption technique has become a widespread method for controlling codling moths (Waldner, 1994) despite a number of problems associated with this strategy, such as the large size of the treated plots and the high costs of the pheromone active ingredients (Charmillot, 1990; Minks, 1997). Another semiochemical based approach is the attract and kill strategy, involving the combination of a semiochemical lure with an insecticidal effector (Howse *et al.*, 1998). The principle has already been successfully applied to the control of the codling moth (Hofer & Brassel, 1992; Charmillot *et al.*, 1996, 1997; Dickler *et al.*, 1998; Lösel *et al.*, 1998a, b, 2000). Appeal is a novel attracticide product for the control of the codling moth utilising the main component of the *C. pomonella* sex pheromone E8, E10-dodecadienol (Codlemone) as the lure to draw male moths to 100µl sized drops of a sticky formulation containing the contact insecticide cyfluthrin. The castor oil based formulation has a sticky consistency for the efficient uptake of the insecticide and was optimised for the controlled release of the attractant over a period encompassing a complete codling moth generation. Appeal is applied to branches in the upper parts of the tree crown with the aid of a hand operated pump-type dispenser.

The aim of work described in this paper was to investigate the longevity of the protective effect against male codling moths in the field. To this end field-aged samples of the formulation were tested using a simple attract and kill bioassay in the greenhouse and using analytical procedures to examine pheromone release characteristics. In addition, field trials were conducted in commercial apple orchards in Poland to assess the feasibility of the attract and kill strategy.

Material and methods

Laboratory and greenhouse experiments. Drops of the formulation were applied to plastic strips (3 x 20 cm) which were attached to a wire fence surrounding a test orchard in the Agricultural Research Centre, Monheim, at the beginning of May, shortly before the start of the codling moth flight period. Four strips were removed for the purposes of bioassay and analytical testing at biweekly intervals. Bioassays to test the attract and kill efficacy were conducted with two strips in the greenhouse as described by Lösel *et al.* (2000). Ten male moths (2-3 d old) were kept in a cage (40 x 40 x 40 cm) together with one plastic strip for three days after which mortality was recorded. Each strip was tested twice. For the analytical testing, volatiles released from each of two samples of the formulation headed to 40°C in a 100 ml glass gas washing flask were collected on Tenax-filters. A stream of nitrogen gas was continuously flushed through the apparatus. After thermal desorption, samples were analysed quantitatively with GC-MS using internal standards.

Field experiments. Experiments were conducted in a mature orchard (± 1 ha, 0.25 ha per treatment) in Drabovice, Poland, during the 1998 and 1999 growing seasons. The plot used for experiments in both years of the study was identical. It consisted of about 2000 trees (cv. Elstar) planted at a spacing of 2 m between trees and 4 m between rows. The product was applied to branches in the upper half of the tree crown at the end of May and six weeks thereafter. The first application was carried out immediately after the onset of male moth catches in monitoring traps. The treatments consisted of 2000, 4000 or 6000 drops/ha for each application. For comparison, one plot was treated with one spray application of Alsystin 480 SC (0.4 l/ha) and one plot remained untreated. Damage was assessed using 1000 hand-picked and 200-400 windfall fruits from selected trees in the centre of the various test plots.

Results and discussion

Laboratory and greenhouse experiments. The attract and kill efficacy of the product remained relatively stable over a period of up to six weeks, causing around 90% mortality in the cage experiment, after which it dropped visibly (Figure 1). A similar trend was observed for the codlemone release rate. Although the insecticidal effect is known to decrease over a six week period the 4% insecticide dose in the fresh formulation ensures that there is sufficient reserve for the treatment period. The overall decline in efficacy measured here, is therefore most likely to be linked to a decline in the release rate of the attractant. Unlike the insecticide dose, that of the attractant cannot be increased without affecting its release rate. Lösel *et al.* (2000) showed that increases in the codlemone concentration of the formulation had a deleterious effect on its attractiveness to male codling moths. Maintaining attracticide activity of Appeal for a period of time spanning a complete codling moth generation is essential for the success of the strategy since the attract and kill source must compete effectively throughout this period with the natural sex pheromone sources: the calling female moths in the orchard. The longevity of the treatment offers the grower a further major advantage, in that the timing of applications based on first catches in pheromone baited traps is much easier to achieve than are those for products targeting the period of oviposition or hatching of larvae.

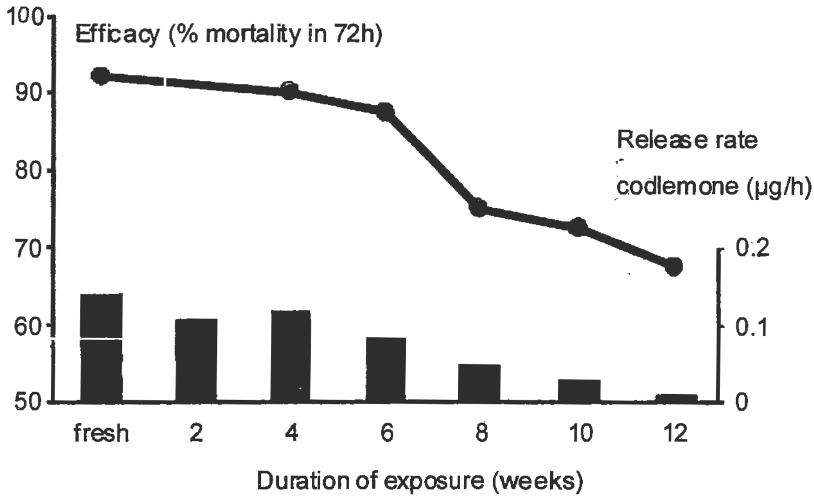

Figure 1. Efficacy of Appeal against male codling moths in a cage experiment (line; n = 4) and codlemone release rate (bars; n = 2) after different periods of weathering under field conditions.

Field experiments. Two applications of 4000-6000 drops/ha gave a level of control comparable to that achieved with the Alsystin treatment, demonstrating0 that this semiochemical based strategy can compete with conventional spray applications of IGR products which are currently the main stay of codling moth control for integrated fruit growers. The results with Appeal in the Drabovice field trial confirm early field data with the prototype of Appeal (Dickler et al., 1998; Lösel et al., 1998a, b) and broadly agree also with findings in studies employing other attract and kill products for codling moth control (Hofer & Brassel, 1992; Charmillot et al., 1996, 1997).

The stochastic principles underlying all sex pheromone based trapping and attracticide strategies dictate that the success of the treatment depends upon the chances of the target organisms, here the male moths, encountering an attract and kill point source are considerably greater than are their chances of encountering a mate. The greater the density of the attract and kill sources, the greater theoretically should be the success of the treatment, i.e. the smaller the number of successful matings. The albeit limited data from field experiments in the current study bear this out, with the level of damage decreasing with increasing drop density in the range tested (Figure 2) and agree with results of earlier work (Dickler *et al.*, 1998). From a practical point of view however increasing the number of manually applied attract and kill sources affects the economic viability of the approach because of increases in labour and material costs incurred. An optimisation of drop density based on reliable data regarding the population density of female moths in the orchard would therefore be needed to predict which treatment density would be required at a particular site.

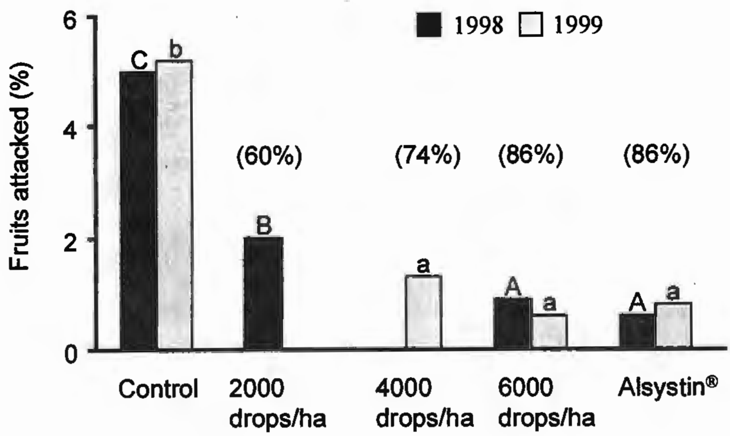

Figure 2. Efficacy of Appeal applied at different densities compared with a standard insecticide in an apple orchard near Dabrowice, Poland, in two seasons. Abbott values are given in parentheses. Different letters (upper case - 1998; lower case - 1999) indicate significant differences ($P < 0.05$; Duncan's *t*-test).

Conclusions

Attract and kill is a highly efficaceous method of controlling codling moths in integrated fruit growing. The specificity of the sex-pheromone employed ensures that only the target species is affected, avoiding deleterious effects on beneficial and other non-target organisms often associated with broadcast spray-applications of conventional insecticides.

This study demonstrates that not only the longevity of the product but also its spatial distribution significantly affect the efficacy of the strategy. The stable pheromone release characteristics displayed by Appeal ensure that when 4000 or more sources per ha are applied, male moths are effectively controlled for a period of up to six weeks. According to the length of the codling moth's flight activity two or more applications of Appeal give season long protection.

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IOBC wprs Bulletin Vol. 24(2) 2001
pp. 101-106

Development of a sprayable slow-release formulation for the sex pheromone of the Mediterranean Corn Borer, *Sesamia nonagroides*

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Abstract: In the FAIR project "Pheromaize", CT96-1302, the main objective is to provide European growers with a reliable, cost effective and environmentally friendly technology based on pest mating disruption. The project is mainly focused on Mediterranean Corn Borer (MCB), *Sesamia nonagroides*, the k-pest of maize grown under Mediterranean conditions. TNO has developed a sprayable formulation consisting of a biodegradable matrix in which the pheromone is dissolved, together with an UV-stabilizer, an antioxidant, a surfactant and a sticker material. During outdoor exposure experiments release of pheromone was found to be high enough for more than 30 days. This formulation has been tested in large scale field experiments by helicopter spraying on 5 ha maize by field partners in Spain, Greece and France.

Key words: slow-release formulation, sprayable, pheromones, mating disruption, *Sesamia nonagroides*

Introduction

Use of pheromones and other semiochemicals for pest control in Europe is practically restricted nowadays to the control of moth pests in vineyards and in less extent, in orchards. The development of pheromone-based technology more adapted to field crops and the improvement of procedures to scale up the synthesis and the sprayable slow-release formulation of blends should allow to enlarge acreage treated with this non chemical methodology.

In the FAIR project "Pheromaize", CT96-1302, the main objective is to provide European growers with a reliable, cost effective and environmentally friendly technology based on pest mating disruption. The project is mainly focused on Mediterranean Corn Borer (MCB), *Sesamia nonagroides*, the k-pest of maize grown under Mediterranean conditions (that is the European maize grown below 45°N parallel, an estimated maize surface of at least 600,000 ha). The mating disruption technique has been aimed at the second generation of the MCB that emerges normally at the beginning of July.

Two general goals are enviseaged in the present project. First, to provide European growers with a reliable, cost effective and environmentally friendly technology based on pest mating disruption, and second, to improve European pheromone production and marketing. Use of pheromones and other semiochemicals for pest control in Europe is practically restricted nowadays to the control of moth pests in vineyards and, in less extent, in orchards. The development of pheromone-based technology more adapted to field crops and the improvement of procedures to scale up the synthesis and formulation of blends, should allow to enlarge acreage treated with this non chemical methodology.

Materials and methods

Microsphere production

Three approaches of making sprayable pheromone formulation have been examined: (a) An emulsion in water is made from a 10% pheromone/Bionolle 3001 solution in dichloromethane. After evaporating of the dichloromethane a dispersion is formed of round Bionolle/pheromone particles. Particle size can be adjusted accurately by the speed of stirring during emulsifying. After spraying of the resulting particles, the pheromone showed a release of more than 25 days during outdoor exposure; (b) granules can be made by mixing Bionolle and pheromone in a twin screw extruder. After cryogenic milling of the string, particles with a predicted size distribution were obtained in a laboratory scale experiment. These particles can then be dispersed in water for spraying application. Release was always found to be somewhat higher than from the particles made with method (a); (c) controlled hydrolysis of the Bionolle to lower molecular weight material. This way the melting point of Bionolle can be decreased to 60°C. Pheromone can be dissolved in the Bionolle and the Bionolle/pheromone can be emulsified in water at temperatures above 60°C. By lowering the temperature a dispersion of solid polymer particles with pheromone can be obtained; (d) a different matrix material, consisting of one of more polymeric compounds, can be chosen with an overall melting behaviour below 100°C. This alternative polymer/pheromone combination can then be emulsified and dispersed in water as above. In these last two methods the pheromone is dissolved in the matrix material together with an UV stabilisor and an antioxidant. This mixture is then emulsified using a surfactant using temperature above 80°C. After cooling down sticker material(s) is/are added to the solution.

The first two methods have their disadvantages. The evaporating step of dichloromethane is quite critical. Fast irreversible coagulation can occur if conditions are not controlled carefully. Even after optimising this method still alrge quantities of dichloromethane are needed for dissolving the Bionolle polymer. This makes upscaling of this method for industrial application difficult. During upscaling of the second method for field trial application milling has to be done cryogenically on a semi-technical scale. It was found that this way the particles had a flake structure and therefore a relative large surface area and consequently a release which is too fast in practice. By using method c. it was impossible to obtain a good particle size distribution in a controlled way. The only method that could reproducibly produce microspheres and could be easily up-scaled proved to be method d. In fact, apparature can easily be adapted for (continuous) production of formulation at kg (based on a.i.) scale.

Trial batches were made by varying the rotation speed, the number and concentrations of adjuvants and the concentration of pheromone. First trials were made by using Z11-C14Ac instead of the pheromone mixture Z11-C16Ac/Z11-C16OH because of price and availability. Final formulations were tested with the pheromone mixture both in the laboratory as in outdoor experiments.

Release of pheromone from microspheres was followed from formulations that were sprayed onto aluminium plates. These plates were hung in a field and collected every week for analysis by GC of residual content. Release studies in the laboratory were conducted by hanging the same aluminium plates in a ventilated oven and collecting plates regularly for analysis.

Mating disruption

For mating disruption purposes, a two component blend (90/10, Z11-C16Ac/ Z11-C16OH) has been in field trials in every country (Mazomenos, 1989). In Spain, France and Greece 2 fields of each 5 ha has been sprayed by helicopter using the microsphere formulation containing 80 g pheromone per hectare. Isolated plots has been used in the field trials. The blend has been applied just before the second adult flight (Frérot *et al*, 1997) . The final date of application was set during the first week with no catches in pheromone traps. Equipment and dosage for applications varied between 5-25 l/ha. Evaluation of every trial has been done by the field partners. Experimental fields has been submitted to the same conditions (cultivar, sowing date, soil insecticide treatment, no insecticide treatment on foliage).

Results and discussion

The release from the hydrolysed Bionolle samples in a ventilated over at 30°C (particles existing of clusters of primary particles of 100 µm) was found to be longer than 40 days, see Figure 1.

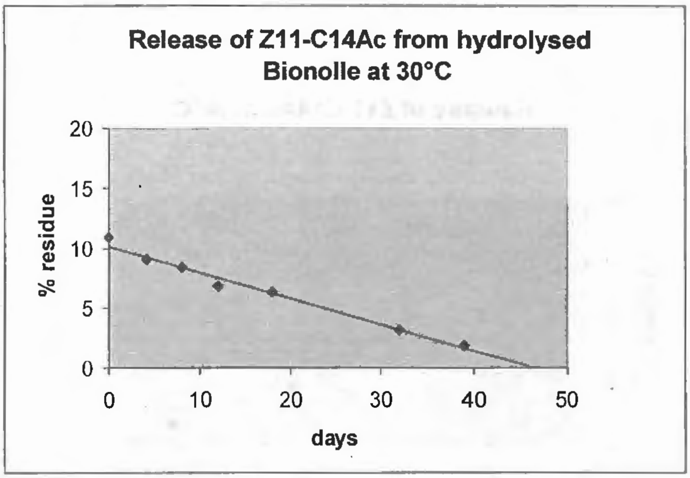

Figure 1

However, with the new method of making microspheres (method d) using the new matrix material the average particle size could be adjusted by varying the stirring speed, see Table 1, with A, B and C being different samples made from the same formulation.

The effect upon release of temperature seemed to be much higher than of particle size. At 20°C the halflife of a formulation is 30 days and at 30°C the halflife has dropped to 15 days. However with some earlier formulations the release from particles of approximately 100 µm could be as long as 40 days while particles of 50 µm had a release not longer than 20 days at 30°C. Later formulations had release times in the laboratory always much longer than 40 days and halflife times of approximately 25 days, see Figure 2. However, the release in outdoor experiments is always much faster than in the laboratory, see Figures 3 and 4. In the formulations tested in these experiments the 90/10 pheromone blend Z11-16Ac/Z11-C16OH has been used.

Table 1. Effect of stirring speed on average particle size

Sample	Stirring speed (rpm)	Average particle size (µm)
A	250	200
B	750	100
C	1500	50

Ten formulations in total have been tested using this pheromone blend, the variations being the polymer matrix and the concentrations of the different ingredients. In addition, from most formulatins different particle sizes have been produced in order to measure the effect upon release of pheromone. Formulations have also been tested for rain-fastness using different concentrations of adjuvants.

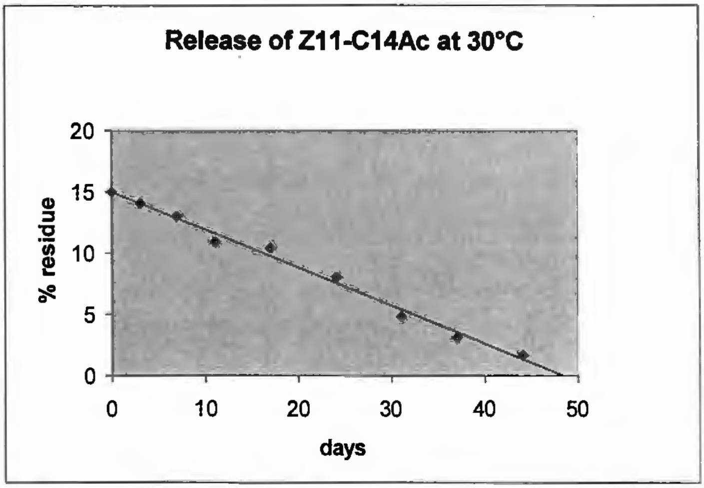

Figure 2

The best formulation in outdoor experiments, release is shown in Figure 4, contained 15% of the pheromone blend and had a particle size of about 100 µm. This formulation could be produced in a reproducible way and was finally up-scaled and produced for the field experiments in 1999.

The objective of this research has been to make a biodegradable sprayable formulation with a two component blend of Z11-C16Ac/Z11-C16OH=90/10 with a release of more than 30 days in the field. In the laboratory as well as in outdoor experiments it has been shown that such a long release from a sprayable formulation is possible. During the first year of the project a very nice formulation could be produced but this proved very difficult to up-scale, the second year formulations for the field trials were made by extrusion, milling and dispersing but these did not show the longevity found before. A different approach was used in the third year by dissolving the pheromone in the polymer melt and then make a dispersion in water. This proved to be an approach whereby release of more than 30 days could be observed, at least in small scale outdoor experiments. This technology was found to be versatile, cheap and easy to produce and up-scale.

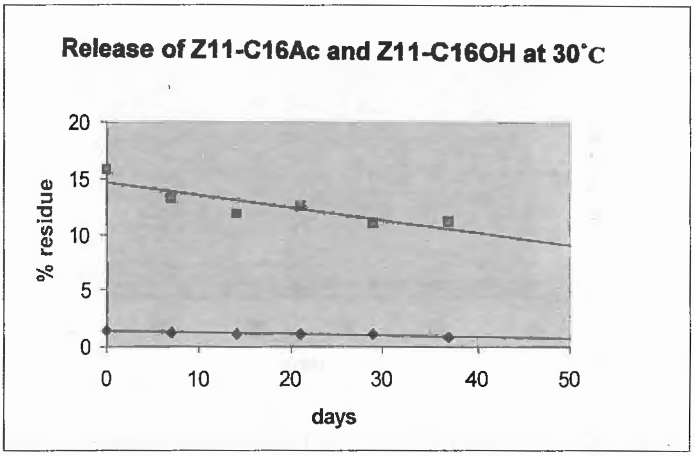

Figure 3

After spraying with pheromone in the field trials it was found that in the first week after spraying the percentage of mated females were lower in the treated field than in the control field. Two weeks after the aerial treatment traps placed in the treated fields catch significantly less males than traps in control fields. The effect of mating disruption can also be found when looking at the increase of plant attack, see Table 2.

Table 2. Increase of plant attack (in percentage) of *Sesamia nonagroides* between the first and second generation and between the second and third generation.

	Population increase (% attacked plants) of *Sesamia nonagroides*		
	TNO 1	TNO 2	Control
2nd - 1st	1.8	3.8	25.8
3rd – 2nd	4.4	1.5	1.9

Although in the second sampling of attacked plants (after spraying) the attack in TNO2 has been significantly higher than in the other fields, the percentage of attacked plants increased only by

3.8% between the first and second sampling while in the control the percentage of attacked plants had increased with 25.8%

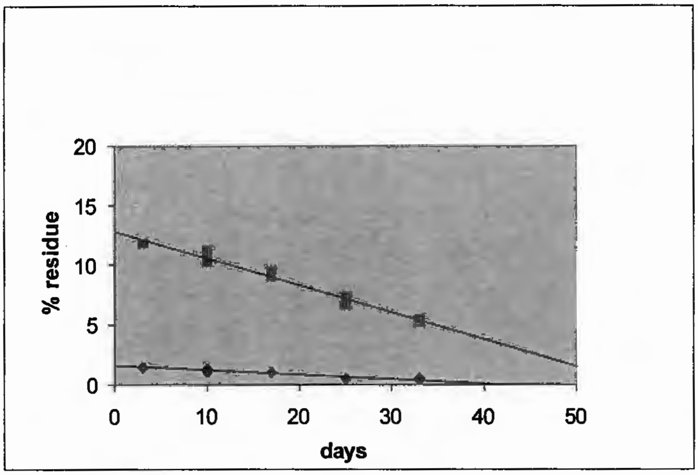

Figure 4. Release of Z11-C16Ac and Z11-C16OH during outdoor exposure in The Netherlands

Acknowlegdements

This work was supported by the EU programme FAIR CT96-1302 "Application of pheromones and other semiochemicals for pest control in maize. Coordinator of this programme is Prof. Dr. R. Albajes of the University of Lleida and Institut de Recerca i Tecnologia in Spain. The author acknowlegdes further the support of Jacco Eversdijk, Henk Rendering and Theo Kock who contributed their work force and ideas.

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IOBC wprs Bulletin Vol. 24(2) 2001
pp. 107-113

Influence of temperature and relative air humidity on the oxidative destruction of pheromones

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Abstract: In this study the oxidative reaction of pheromones with ozone is investigated. Additionally the influence of some environmental factors like temperature and air humidity is examined. A kinetic model was developed in order to describe the differences in the decrease of the amounts of pheromone when fumigated with ozone or charcoal-filtered air respectively. From these data the influence of the examined environmental factors on the ozone-induced pheromone transformation is evaluated. The obtained rate constants are compared with data from other publications and possible consequences for the use of pheromones in pest management are discussed.

Key words: pheromone destruction, ozone, oxidation, rate constant, environmental factors

Introduction

Pheromones play an important role in pest management and are used either in monitoring traps, for mass trapping or in mating disruption (Jutsum & Gordon 1989). For the success of each method it is necessary to guarantee a definite pheromone concentration under various weather conditions and during a long period of use. Besides several factors ranging from the dispenser type and their release rate (Bradley et al. 1995, McDonough 1997, Suckling et al. 1997) to the number of these pheromone sources in the field many environmental factors influence the effective concentration of pheromones in the air. Some of these factors are oxidative compounds of the atmosphere of which ozone is an important one.

Since pheromone structures such as the sex pheromones of *Cydia pomonella* L., *Adoxophyes orana* F.v.R., *Eupoecilia ambiguella* Hb. and *Grapholita funebrana* Tr. can in principle be destroyed by ozone (Arndt 1995, Lorenz 1998, Lorenz & Arndt 1997, Veit & Lorenz 1999), we tried to estimate this oxidative transformation taking into account temperature and relative humidity. By determining rate constants for the observed decrease of pheromone due to ozone it is possible to estimate the effect of this oxidant on the ambient pheromone concentration.

As an example for several lepidopteran sex pheromones we examined *trans*8,*trans*10-dodecadienol (E8,E10-12:OH) (fig. 1). This unsaturated long-chain alcohol serves as a sex pheromone for *Cydia pomonella* (Arn 1991) and is used e.g. in mating disruption. The results of our investigations can be transferred to many other pheromone components with a similiar structure (cf. Mayer & Mc Laughlin 1991, Arn 1991).

Material and methods

Pheromone fumigation. 10 µl of a solution of the pure pheromone in hexane [1µg/µl] were dropped on small glass fibre filters, the solvent was allowed to evaporate and the samples were then transferred into fumigation chambers. A constant stream of charcoal filtered air flowed through these chambers. In one chamber the charcoal filtered air was mixed with ozone in such a manner that it was possible to maintain a definite ozone concentration. Besides the ozone concentration and the flow rate temperature and relative humidity were regulated and controlled throughout the fumigation.

After definite periods of time several samples were taken out of the chambers, the remaining amount of pheromone was eluated from the filters with hexane, a standard (Dodecanol) was added and the amount of pheromone was determined by means of gas chromatography.

Kinetic model. In order to separate the processes evaporation and chemical reaction we suggest the following kinetic model which turned out to agree with our experimental results. This model provides the two different rate constants k_D and k_O for these two processes. For the desorption of the pheromone from the glass fibre filters we assumed a first order process (1). k_D in (1) can be obtained from the decrease of pheromone adsorbed to the glass fibre filters and fumigated with charcoal filtered air. The oxidation of the pheromone due to ozone can be described as a bimolecular reaction (2). Using k_D the rate constant k_O of this chemical reaction can be obtained from the decrease of pheromone in the ozone fumigated chamber.

$$\frac{dn}{dt} = -k_D \cdot n \qquad (1)$$

$$\frac{dn}{dt} = -k_D \cdot n - k_O \cdot n \cdot c(O_3) \qquad (2)$$

$$\ln(n_t) = \ln(n_{t=0}) - k_D \cdot t \qquad (3)$$

$$\ln(n_t) = \ln(n_{t=0}) - (k_D + k_O \cdot c(O_3)) \cdot t \qquad (4)$$

n_t: mean amount of pheromone at time t
$c(O_3)$: mean ozone concentration
k_D: rate constant which describes the evaporation of the pheromone
k_O: rate constant which describes the oxidation of the adsorbed pheromone

Results

The sex pheromone of *Cydia pomonella* easily reacts with ozone (fig. 1) as it could be expected due to its chemical structure. The effect of ozone on the transformation of the pheromone is significant at a concentration of 80 µg/m^3 already. At 240 µg/m^3 the difference between the release of pheromone treated with charcoal filtered air and pheromone fumigated with ozone is obviously higher than at 80 µg/m^3.

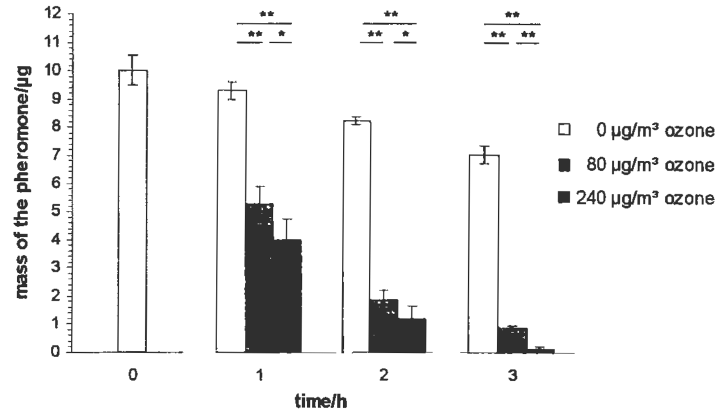

Figure 1. Decrease of E8,E10-12:OH due to ozone at concentrations of 80 $\mu g/m^3$ and 240 $\mu g/m^3$, 25°C and 40% relative air humidity [means and standard deviation; * p>95%, ** p>99,9% (t-test)].

Using our kinetic model we can separate the effects of evaporation and chemical transformation. Figure 2 shows an example of the determination of the two rate constants.

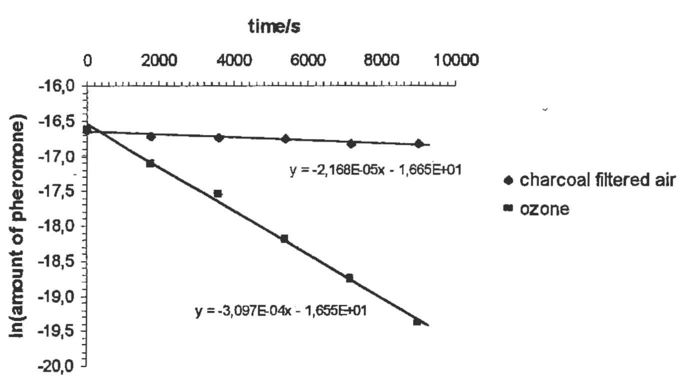

Figure 2. Determination of the rate constants k_D and k_O for E8,E10-12:OH at an ozone concentration of 240 $\mu g/m^3$, 25°C and 40% relative humidity:
$k_D = 21{,}68 \cdot 10^{-6}\ s^{-1}$; $k_D + k_O \cdot c(O_3) = 309 \cdot 10^{-6}\ s^{-1} \rightarrow k_O = 5{,}74 \cdot 10^4\ l\ mol^{-1} s^{-1}$.

The influence of the parameters ozone concentration, temperature, relative humidity and amount of pheromone could be assessed by determining the rate constants k_O at different experimental conditions (Tab. 1).

These data show that rising temperatures as well as lower relative air humidities increase the oxidative pheromone transformation. In addition the transformation of these chemical messengers appears to be faster when using smaller amounts of pheromone. The term $k_O \cdot c(O_3)$ (rate of chemical transformation) increases at higher ozone concentrations as expected whereas the rate constant k_O decreases slightly.

Table 1: Determined rate constants of the oxidation of E8,E10-12:OH at different experimental conditions.

mass of pheromone/µg	ozone /µg·m^{-3}	temperature /°C	relative air humidity/%	k_D /10^{-6} s^{-1}	k_O /10^4 l mol^{-1} s^{-1}	k_O c(O_3) 10^{-6} s^{-1}
10	80	25	40	35,8	9,34	158
10	240	25	40	24,7	5,62	285
10	360	25	40	61,1	4,12	307
10	240	25	10	54,2	5,89	301
10	80	25	80	59,4	4,88	84,3
10	80	35	40	17,2	10,2	177
10	240	35	40	16,9	7,28	372
50	80	25	40	8,23	3,15	53,4

Discussion

The present study shows that the oxidation of pheromones by tropospheric ozone is an important chemical reaction which decreases pheromone concentrations in the field. Especially the concentration of pheromones containing double bonds may be governed by this reaction. The data in table 1 clearly show that the rate of pheromone transformation ($k_O \cdot c(O_3)$) is rising with an increasing ozone concentration. Enhanced ozone concentrations (Umweltbundesamt 1997), which have been frequently observed during the summer months within the last years, may therefore severely disturb insect communication.

Due to the extremely low pheromone concentrations the exact course of the ozone oxidation reaction of pheromones in the environment requires further investigation. It seems to be likely though that the oxidation of the pheromone in the field may well be described using the reaction mechanisms published by Criegee (1975) and Dodge & Arnts (1979).

Whereas most determinations of rate constants k_O are performed in the gas phase (Atkinson et al. 1997) we performed our experiments with pheromones adsorbed to glass fibre filters. The results of such experiments appear to be interesting also for the assessment of the influence of enhanced ozone concentrations on the use of dispensers in pest control and on the transformation of pheromones adsorbed to various surfaces like plants etc. (cf. Suckling et al. 1996).

The data compiled in table 1 show the possible effects of different reaction conditions on the variation of k_O. As expected the rate of transformation increases with higher temperatures.

The decrease of k_O with increasing relative humidity, increasing ozone concentration and an increasing amount of pheromone appears to be caused by our experimental setup which affords rather heterogeneous reaction conditions. The pheromones are adsorbed to glass fibre filters and therefore, the reaction is restricted to occur within a limited volume. Before any transformation reaction can proceed the ozone molecules have to enter into this volume. The rate of this process is very fast but still limited.

The data in table 1 show that k_O decreases with increasing relative humidity. At the moment we can give three possible explanations: a) increasing relativ humidity causes an increase of the thickness of the water film on the surface of the glass fibres filters. Therefore, the free space between the fibres decreases and the rate of diffusion of ozone into the glass fibre filters decreases. The apparent effect is a decrease of k_O. b) The pheromones can be dissolved in the thicker water film on the glass fibres filters. As the ozone concentration in the water film is lower than in the adjacent gas phase (Ostwaldsche Löslichkeit) the apparent k_O decreases with increasing relative humidity. c) If the pheromone is surface active it may be enriched in the surface of the water film. The orientation of the pheromone in the surface of the water film depends on the specific properties of the pheromone molecule. If such a process is active in consequence the apparent k_O can either decrease as the results of the present study show or increase as observed in other experiments using the sex pheromone of *Tortrix viridana* L. This pheromone (Z11-14:Ac) also contains an unsaturated chain of hydrocarbons, but the functional group is an acetate and not an alcohol (Arn et al. 1979, Arn 1991). Z11-14:Ac reacts faster at higher air humidities which can also be proved by the increasing amount of a reaction product (Bauer et al. 1999).

The decrease of k_O with an increasing ozone concentration can result from the limited rate at which ozone can enter into the glass fibre filters. Therefore, an increased ozone concentration outside of the filters does not result in an equivalent increase of ozone concentration within the filters. In consequence the apparent k_O drops. A similar situation can occur when the amount of pheromone is increased. The higher amount of pheromone within the glass fibre filters causes the ozone concentration within the filters to drop below the (measured) concentration outside of the filters. Again in consequence the apparent k_O decreases with an increasing amount of pheromone. But this effect can be neglected with smaller amounts of pheromone like those used in pest control.

A consequence from these considerations is that the rate constants determined in the laboratory at high amounts of pheromone are lower than the ones which can occur at lower – environmental – amounts. These will most probably exceed the values determined in the laboratory. All in all the obtained data for the rate constants correspond to other published data (Atkinson & Carter 1984).

Our investigations suggest that different ozone concentrations, temperatures and relative air humidities influence the present pheromone concentration. This can have consequences for the success of pest management using pheromones in mating disruption, monitoring or mass trapping. Other factors like heat and UV radiation may also influence the pheromone destruction (Ideses et al. 1982) as may different adsorption processes on leaves (Suckling et al. 1996) and the soil. The best arrangement density and the amounts of pheromone to be used in the dispensers were determined from many years of experience. Calculations of the arrangement of dispensers and its effects have already been done (Suckling et al. 1999). But in order to guarantee the success of pheromone-based insect control systems the respective environmental factors of the area to be treated should also be taken into consideration. It would therefore be extremely useful to calculate the effective pheromone concentration in the air taking into account the results obtained in our examination.

Acknowledgements

We thank the Landesgraduiertenförderung Baden-Württemberg, that supports these examinations with a personal scholarship to Uwe Veit.

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Pheromones for Insect Control in Orchards and Vineyards
IOBC wprs Bulletin Vol. 24(2) 2001
pp. 115-123

Pheromones - future techniques for insect control?

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Abstract: Four decades of pheromone research have provided the tools for environmentally safe control of insects through manipulation of male sexual behaviours. In Europe, disruption of mating by aerial dissemination of synthetic pheromones is used to control several economically important tortricids in orchards and vineyards on ca. 50 000 ha. In addition, attracticide lures have been developed for population control. The importance of pheromone-based methods is accentuated in view of increasing problems associated with the use of conventional insecticides. However, for a more widespread use of pheromones, application techniques must become more reliable and more economic. The key to further development is closer communication and collaboration between academic research institutions, plant protection industry and extension services.

Key words: sex pheromone, monitoring, mating disruption, attracticide

Introduction

"There are many reasons for using pheromones. One is that they are elegant." (Arn 1990). Pheromones are indeed elegant and safe tools for insect control. The fascination of being able to control insect populations through species-specific manipulation of sexual communication, without adversely affecting other, beneficial organisms, has been a driving force for research on insect pheromones during four decades.

The work done by chemists, biologists and plant protection experts has provided us with a solid knowledge of the chemistry and biology of the sex pheromones of many economically important insects. This know-how has been successfully used in some species, where people have gone beyond laboratory research and experimental trials and have established practical real-life applications which outcompete conventional insecticides.

The most important application technique for lepidopteran species is to permeate the atmosphere with synthetic pheromone, and to thereby prevent olfactory communication and mate-finding. Successful, area-wide applications of the mating disruption technique in Europe concern three lepidopteran insects from horticultural crops, codling moth *Cydia pomonella*, and the grape berry moths *Eupoecilia ambiguella* and *Lobesia botrana* (Ridgway et al. 1990, Witzgall and Arn 1997).

Will the use of pheromones expand in the future? Will established methods be used on a wider scale and will methods be developed against further insect species? An early and most successful development has been achieved with pink bollworm *Pectinophora gossypiella* - but the availability of Bt-transgenic cotton has led to a dramatic decrease in the use of pheromones (Jenkins 2002). In contrast, the surface treated by mating disruption against codling moth in the north-western United States increased from ca. 1.000 ha in 1991 to 9.000 ha in 1996 (Thomson 1997). What looked like a rather linear increase in 1996 then turned into an almost exponential increase - the area under mating disruption expanded to 45.000 ha in 2000 (Doerr and Brunner 2001, Brunner et al. 2002).

There is data in support of both pessimistic and optimistic projections into the future. The use of pheromone will continue to depend on cost and efficacy of pheromones vs. other methods. Failures of mating disruption at high population densities, elevated cost, and the occurrence of other insects which can only be controlled with insecticides are serious obstacles.

Only continued goal-oriented research will overcome these obstacles and lead to more reliable and more widespread applications. It is important to realize that all sucessful applications have been achieved through a combined effort made by researchers from universities, government institutions, extension services and the chemical industry. However, time is up for pheromone research at most academic institutions and the backup provided by research funded by the public hand will rapidly diminish. The future will tell whether chemical industries, plant protection sector and growers' associations are willing and able to continue the struggle.

Chances for success

Restrictions of insecticide use

Detrimental health effects, environmental issues, insect resistance, or marketing opportunities for organically-produced food are well-known arguments against the use of insecticides.

Increasing restrictions in the use of conventional insecticides in Europe further emphasize the need for new, environmentally safe methods of pest control. The "Environmental Action Programme" adopted by the European Union for the year 2000 aims at "*the significant reduction in pesticide use per unit of land under production, and conversion to methods of integrated pest control, at least in areas of importance for nature conservation*". The five-year-programs and resolutions adopted by European authorities are not always entirely realistic, but it remains a fact that a number of neuroactive insecticides have been or are being deregulated in several countries. In Germany, for example, the number of insecticides available for control of fruit insects has been decreasing steadily over the past decade, from 40 compounds in 1988 to 24 compounds in 1998 (Koch and Schietinger 1999).

Resistance against the available insecticides continues to be an important issue. In codling moth, for example, insecticide resistance is increasing all over Europe (Sauphanor & Bouvier 1995, Charmillot et al. 1999). In addition, it is a well-known fact that the overuse of insecticides induces outbreaks of secondary pests such as phytophagous mites, which are difficult to control with pesticides (Knisely and Swift 1972, Tanigoshi et al. 1983). Replacing insecticide with pheromone treatment against codling moth renders treatments against phytophagous mites superfluous, which, for example in South Tyrolian orchards, compensates for the cost of the pheromone treatment (Waldner 1997).

Insecticides do not work as well as often believed, and a long-term population decrease has never been achieved, not in any species. In comparison, a well-implemented mating disruption programme may clearly be more efficient than insecticides in some species. An observation shared by many working with mating disruption is that its long-term use results in continuously decreasing populations of the target species. This may be due to recovery of the beneficial insect fauna and an overall increasing stability of the orchard ecosystem.

Insecticide sprays harm the terrestric and aquatic fauna. This is true even for chitin synthesis inhibitors, which have been long thought to be environmentally rather safe. In Scania (southern Sweden), the contamination of ground water resources with agrochemicals has risen to such a pitch that protected areas need to be established, where all use of insecticides is prohibited in order to conserve drinking water resources. In such areas, farmers are forced by law to turn to organic production methods from one year to another.

It then is plain to see what we know, even though we are not always aware of it - that environmentally safe control methods are available only against a precious few insect species, and that biological control methods other than pheromones are often even more expensive or less reliable than pheromones. It then also becomes apparent that there is great potential in pheromone-based methods, once the "tools" have been developed properly. The tools are the knowledge of the pheromone chemistry and biology of a given species, economic synthesis, appropriate dispensers and the knowledge and experience of how to use them in the field. It was possible to elaborate pheromone-based methods against several species, and with the database on insect pheromones which is available today, it will certainly be possible to extend the use of pheromones to other species.

Economic aspects

The current conflict in Scania between cheap and rather efficient plant protection by insecticides and contamination of water resources by the same insecticides offers the trivial, but nevertheless important insight that the *price* of insecticides or pheromones should not be confounded with *economy of use*. Simple equations such as "insecticides are less expensive than pheromones - therefore, insecticide use is more economic" are no longer valid.

The economic importance of horticultural production in Scania is ever decreasing. More precisely: the revenue from the produced commodities is on the decline. As a result, the area covered by orchards has significantly decreased in the past decade. However, fruit production is, beyond fruit sales, of great significance for the attractivity of this region. Tourism is an important source of income, as this is the case in other European fruit- and vine-growing regions. The farmer is becoming a landscape gardener and "landscape" is turning into "viewscape". A pleasant environment will not only attract tourists, but also new enterprises and investors, and people who wish to inhabitate this land. The character of the Scanian fruit-growing region will change and most likely suffer if the orchards are replaced with agricultural crops. This could accentuate already existing structural problems in this area.

Today, both the growers' economy and Scanian orchards are threatened by cheap fruit imports. At the same time, the consumer demand for unsprayed fruit has steadily increased over the past decade but cannot be met by the local production. A leading Swedish supermarket chain has based its marketing campaign on a green image, and all attempts to cut down on pesticide use receive much attention in local and national newsmedia. Employment of safe insect control techniques will improve fruit quality. This will be rewarded by the consumers and strengthen the competitiveness of local fruit production. The farmers' efforts to establish sustainable production methods will thus contribute to rural development.

Reasons for lack of success

Motivation

The main applications of the mating disruption technique in Europe are against codling moth on >10.000 ha and the grape berry moths on >30.000 ha (Arn and Louis 1996, Waldner 1997, Kast 2001, Zingg 2001). This clearly demonstrates the potential of the mating disruption technique for insect control and the figures are encouraging, until we take a closer look and realize that the area under mating disruption represents only a fraction of the several millions of hectares of European orchards and vineyards on which conventional insecticides are being used.

One reason for the comparatively limited use of mating disruption is that the technology currently in use must become more reliable. The most urgent shortcomings have been outlined in detail by many authors, and will be shortly mentionned below. However, one most important factor is certainly motivation and determination. A growers' association in Northern Italy has been determined to implement mating disruption against codling moth and has been successful (Waldner 1997). The conditions in Northern Italy for using pheromones are as favourable or poor as in most other European growing areas, but the main difference is that growers were determined to cut down insecticide use.

A similar situation is encountered with the grape berry moths. A significant part of German and Swiss vineyards are being treated with pheromones since many years (Kast 2001, Zingg 2001), but the river Rhine in Germany and the Jura mountains in Switzerland are natural borders for pheromone use. Admittedly, the climatic, economic and faunistic conditions may be less favourable for mating disruption in Southern European vineyards. However, pheromone use in grapes is rapidly increasing - in Northern Italy (Varner et al. 2001).

Motivation as a main factor for pheromone vs. insecticide use obviously concerns not only the farmers, but also researchers and chemical industries. Pheromone research, funded by the public hand over four decades, has provided the basic knowledge for the development of new pest control techniques. It is important to realize that only goal-oriented research will lead to reliable and more widespread applications. However, for many researchers at academic institutions it may instead be more rewarding to explore new directions and to point out the potential, future use of knowledge yet to be acquired. Some companies in the agrochemical sector may have used pheromones mainly as a public relation tool, whereas other chemical companies have made a most significant contribution to pheromone synthesis and dispenser systems. Work done by chemical companies was again driven by individuals motivated and determined to make pheromones work.

Integrated research

For future development, we must put stronger emphasis on a multidisciplinary approach. Clearly, there is a need for intensifying communication and collaboration, and for coordinating activities between academic research institutions, chemical industries and extension organizations. Insecticides are at least as complex chemicals as pheromones and they are difficult to formulate, market, and difficult to apply. However, in contrast to pheromones, the entire development, from research to application, is carefully planned and has often been done under one roof.

Already existing knowledge on pheromone technology can be contributed from various disciplines to achieve improvements or to rapidly establish new methods. Unfortunately, lab people all too often lack understanding for the difficulties associated with field implementation work. Chemical companies sometimes tend to believe that they "know all about pheromones" and that access to published information is sufficient. And farmers' organizations rarely manage to establish a dialogue with people from the academic circuit.

Summerfruit tortrix *Adoxophyes orana* is the most important leafroller moth in European orchards under mating disruption for codling moth and provides a good example of how people from different disciplines view the same problem and how lack of integration can become an important obstacle. A detailed account of female-produced compounds and their effect on male behaviours was published in 1986 by Guerin and coworkers, showing that the main pheromone compound of summerfruit tortrix is (Z)-9-tetradecenyl acetate and that it must be blended with additional minor pheromone compounds to attract males. Nonetheless, dispensers for summerfruit tortrix control were formulated to contain (Z)-11-tetradecenyl acetate (*e.g.* Neumann 1997, Rama 1997) and it comes to no surprise that satisfactory control was not achieved.

This explanation is brought forward by the author of this paper, who knows summerfruit tortrix only from lab rearings and wind tunnels. It takes field experience to realize that elevated population densities may be another cause for failures. Farmers tolerate high population levels of summerfruit tortrix, before populations further increase and start to cause damage. It may be impossible to control this species with pheromones once a certain population level has been attained, disregarding which pheromone blend is used (Charmillot *pers. comm.*, Neumann 1997).

The summerfruit tortrix reminds us that population biology rarely receives sufficient attention in mating disruption studies. Models on insect population dynamics have been elaborated during the seventies and eighties, and should always be incorporated into mating disruption studies. As long as population fluctuations are badly known, it is probably impossible to answer the question whether a full, partial or off-blend of pheromone chemicals is appropriate for summerfruit trotrix mating disruption.

Last not least, pheromone companies need to compete with the price of insecticides, and the amount of active ingredient or the number of compounds formulated in mating disruption dispensers is therefore critical. (Z)-11-tetradecenyl acetate is a pheromone component of many other leafrollers which are important especially in North America (Arn et al. 2000, Walker and Welter 2001) and this compound is easier to synthesize than (Z)-9-tetradecenyl acetate. It is obviously more economic to use one formulation all over and to include single compounds rather than complex blends into dispenser formulations.

Field implementation

Field implementation is the focal point of mating disruption research. All available knowledge of an insect's biology, its pheromone chemistry and biology, and dispenser technology needs to be integrated. Lack of success is often equivalent to lack of know-how, or the lack of knowledge transfer.

Heinrich Arn, the former convenor of the Pheromone Working Group, emphasized the need for field measurements on insect behaviour and pheromone dispersal as he coined the title of the the Neustadt meeting: "*Mating Disruption - The Behaviour of Moths and Molecules*" (Arn 1987). This meeting reflected the optimism that the necessary tools would soon be at hand. A quantum leap was then achieved by Uwe Koch and his working group, who managed to measure ambient pheromone concentrations using an insect antenna (Koch et al. 1992, 1997), and several behavioural studies, including work in field wind tunnels (Cardé et al. 1993, 1997), made a significant contribution to our understanding of insect behaviour in a pheromone-permeated atmosphere .

The optimistic attitude of the late eighties that collaboration between academic research and chemical industries would inevitably lead to the design of state-of-the-art mating disruption technology cooled off during the nineties. The 1994 meeting of our Working Group ran the provocative title "*The Wormy Apple Brainshop*", illustrating the general feeling that the methods available to study the behaviour of moths and molecules were all too often not taken into account when designing practical applications. Obvioulsy, progress with optimizing dispenser density, dispenser load or the composition of the disruptant blend will be painstakingly slow as long as the "wormy apple" is our only bioassay. It goes without saying that a great many factors contribute to insect attack, many of which are not directly related to the efficacy of the mating disruption method.

A pessimistic and possibly somewhat arrogant point of view is that implementation of mating disruption relies even today largely on an empirical trial-and-error approach. Roger Bartell manifested in 1982 the need to study the "behavioural mechanisms underlying mating disruption". Despite numerous discussions and scientific publications devoted to this topic we still cannot claim of having succeeded in deciphering these mechanisms or that we are taking advantage of the knowledge acquired. Let alone the fact that the study of insect behaviour in the field, including migration and dispersal, may simply be beyond our means, one may also argue that distinct mechanisms do not suffice to explain or describe the extremely heterogenous and complex field situation (Bengtsson et al. 1994).

False trail following, i.e. male upwind orientation requires structured plumes and a pheromone blend which is attractive to the males. Camouflage of female-released plumes requires a homogenous and sufficiently concentrated pheromone cloud. Sensory overload requires that males are exposed long enough to large enough amounts of pheromone. All of these conditions may be met at the same time, in the same pheromone-treated orchard: structured plumes in the tree tops and along the borders, a thick and homogenous pheromone cloud within tree canopies in the centre of the orchard, and males with long-term exposure to pheromone vs. newly emerging or migrating, sensorily largely unaffected males. And, the situation will change as soon as dispenser density, dispenser load or the pheromone blend are modified.

Dispenser technology

The discussion above tacitly assumes dispenser technology as a constant. Pheromone synthesis, dispensers and dispenser application dictate the cost of mating disruption. Discussions of behavioural mechanisms may turn out to be futile if only we were able to afford or to achieve sufficiently high aerial pheromone concentrations.

Major achievements have been made with large-scale industrial synthesis of insect pheromones (e.g. Yamamoto and Ogawa 1989). It is important to realize that commercial use of codling moth mating disruption became possible only after economic synthesis of codlemone became available. The price for this compound has been diminished by a factor of 50 to 100 since the eighties.

A major flaw of currently used commercial pheromone dispensers is that they are "passive" and that pheromone release depends on ambient temperature. In Swedish apple orchards, ca. 90% of pheromone applied is released outside codling moth diel flight period, mainly during daytime at peak ambient temperatures (Witzgall et al. 1999). In addition, dispensers must be applied early in season when population densities are still low, while their release rates decrease during the season, as population densities start to increase (Ogawa 1997). These problems are circumvented by using active pheromone "puffers", releasing large amounts of pheromone when the insects are active (Shorey et al. 1996, Baker et al. 1997). However, their commercial use is still limited.

Hand-application of dispensers does not pose a problem in labour-intensive horticultural crops, but precludes the use of mating disruption in most field crops. The formulation of sprayable pheromone dispensers has been attempted in the past (Hall et al. 1982, Weatherston 1990), and is subject of recent efforts (Polavarapu et al. 2001).

Outlook

More reliable and economic applications of communication and mating disruption by pheromone can probably be achieved, and a more widespread use of this technology is not unlikely. Most technical difficulties can certainly be overcome, if progress with mating disruption is deemed useful and desirable, and if a joint effort is made by people from different disciplines and organizations.

A most important obstacle, however, will probably remain. Laboratory assays to predict the behavioural effect of crop treatments with a particular dispenser formulation are not at hand. Development of mating disruption technology still relies on repeated field trials and will therefore remain to be costly and slow. In addition, the focus of research in chemical ecology is turning away from lepidopteran pheromones and future improvements will increasingly depend on work done by commercial companies, extension services and farmers' associations.

A clear drawback of pheromone-based methods is that only male moth behaviours can be manipulated. This, and the considerable effort and cost associated with development of mating disruption stimulates current work on attracticides (Charmillot and Hofer 1997; Suckling et al. 1999). Attractide lures can be optimized in laboratory windtunnel assays - a more attractive lure results will show a better effect. Formulation of experimental dispensers requires comparatively small amounts of chemicals and can be done in any laboratory. Attracticide lures make is also possible to use attractants for egg-laying females, which are expected to become a most important complement to mating disruption. A volatile compound from pear has recently been shown to attract codling moth males and females (Light et al. 2001).

Last not least, more emphasis should be placed on the integration of different biological control methods. Microbial pesticides and resistant plants can be used to reinforce the effect of behaviour-modifying chemicals. All biological methods are species-specific to varying degree and do not cover all insects associated with a crop. In addition, biological insect control methods produce rather subtle effects compared to conventional insecticides, which are lethal upon contact. Single biological control methods can therefore rarely replace insecticide treatments and the available biological tools should be developed as components of an integrated crop management programme, rather than as sole agents.

Acknowledgements

The work on practical application of pheromones at SLU, Alnarp is financed by the Foundation for Strategic Environmental Research (MISTRA), the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), the Carl Trygger Foundation and the Norwegian Departement of Agriculture.

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The IOBC/WPRS Bulletin is published by the International Organization for Biological and Ingetrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Régionale Ouest Paléarctique (OILB/SROP)

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ISBN 92-9067-130-0