Session 8B

Semiochemicals in Practice

Chairman & Session Organiser:	Professor David Hall *Natural Resources Institute,* *University of Greenwich, UK*
Platform Papers:	8B-1 to 8B-4
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Pheromones come of age

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It is now almost 50 years since the first insect pheromone was isolated and identified. Once considered by many as a niche technology with only peripheral importance, pheromone-based insect control systems are increasingly taking a central role in pest management. Initially used for insect monitoring, they have also been applied to control mainly through the mating disruption technique. With the notable exception of the government-funded Gypsy moth programme in the U.S.A. their use has been focussed on high value crops such as top fruit, vines and increasingly on vegetables. With the drivers of increasing demands for lower residues on crops, restricted availability of traditional insecticides due to changing regulation and the ever present spectre of resistance, pheromones are now starting to take centre stage as serious mainstream pest management tools.

World market for pheromones

Based on published data and unpublished information which has been made available to the author, pheromone based products were estimated in 2005 to have world-wide sales in the US$ 150-170 million range at the manufacturers' level. While this constitutes less than 2% of the US$ 9 billion worldwide insecticide market, the industry is making important impacts in important high value markets (Table 1).

Table 1. Crop areas treated in 2005 with pheromone based products (Shin Etsu Company data published in *Agrow* 504, September 2006)

Crops	Pest Species	Area treated (ha)
Forestry	*Lymantria dispar*	200,000
Apples	*Cydia pomonella*	155,000
Stone Fruits	*Grapholitha molesta*	58,000
Grapes	*Lobesia and Eupoecilia*	106,000
Cotton	*Pectinophora gossypiella*	55,000
Others	Various spp	67,000
Total		**641,000**

Pheromone-based products have found markets all over the world, with no great concentration on any one continent. The producers of these products, however, tend to be concentrated in the USA, Europe and Japan and over 60% of the sales are achieved by about a dozen companies. The sales of traps and lures for monitoring insect pests account for nearly 30% of the pheromone market, while the bulk of the remaining sales come from mating disruption products for moth pests.

Changing strategies

Initially viewed as a radical and fringe alternative to conventional insecticides, pheromones are now recognised as an essential complement to these. Consumer pressure, resistance and regulation have reduced the options available to growers forcing a change of approach. For instance, for the control of codling moth in pome fruits mating disruption has now become the central pillar in the control strategy. Insecticides are used as needed to complement and reinforce the pheromone treatment. The pheromone is known to be most effective when populations are kept low. Depending on location a typical strategy is to apply the pheromones before the insect emergence, possibly apply an insecticide or two in the first generation to lower the population as well as take care of other pests such as leaf rollers. For the rest of the season further conventional insecticides or virus products when there is perceived risk. This strategy offers good pest control with much reduced insecticide input.

Technology in evolution

The challenge with pheromone-based pest control was to develop formulations capable of protecting the active ingredient over extended periods and releasing it at a controlled rate over that time. These formulations initially came in the form of hand-applied dispensers lasting up to 3 months and applied at rates up to 1000 per hectare. Over time the technologies have improved so that now there are formulations that last up to 6 months and can be applied at much lower rates. Selibate CS for the control of *Chilo suppressalis* in rice is now used at a rate down to 30 dispensers per hectare.

New technologies have started to appear in recent years. Advances in controlled release technologies have led to the development of sprayable micro-encapsulated pheromone formulations. The best of these can now offer up to 30 days field efficacy. With the increasing scarcity of labour at critical times of the year when hand held dispensers are applied, the sprayables offer the grower much greater flexibility in the use of pheromones.

An alternative approach has been the development of the "Puffer" technology. These are mechanical devices containing the pheromone in an aerosol can. The device delivers metered quantities of pheromone at specific times of the day. Puffers offer season long control at the rate of 2.5 devices per hectare. At this low number installation in the field is quick and inexpensive.

More recent innovations are looking at reducing the amount of pheromone necessary by using false trail or disorientation approaches. These use a large number of smaller, low dose dispensers. Effective control has been achieved with as little as 10g pheromone per hectare where current mating disruption systems use over 100g. These promise more efficient use of still very expensive active ingredients if the application issues can be resolved.

While the use of pheromones has grown dramatically in recent year, their application is still restricted to a limited number of pests (mainly moths) in selected crops. The challenge is now to expand this by looking at alternative ways to use pheromones. One area of research combines pheromones with or without other attractants with insecticides to form Attract & Kill systems. This shows great promise for some pests where the classical mating disruption has not worked.

The future is fragrant….

Sprayable microencapsulated pheromone products for MD have an advantage in easy application with standard spray equipment and compatibility with most insecticides and fungicides. The first Australian field trials of a sprayable pheromone formulation, MEC-OFM phase V for MD, were established in 2002 and continued in 2003-05. Trials were conducted on randomly selected 2 ha blocks of peaches and pears in orchards in Victoria. Initial trials used sprayable pheromone, applied monthly by conventional spray equipment. Later trials demonstrated that fortnightly spray intervals provided control equivalent to the performance of standard hand-applied MD dispensers and were more effective in MD control than monthly applications. Sprayable pheromone MD products have good potential in pheromone based IPM and require further study.

New multi-species hand-applied MD dispensers were evaluated in counter seasonal replicated trials in Victoria (Australia) and Michigan (USA) over three consecutive seasons. Our results demonstrated that dual hand-applied MD dispensers, designed to disrupt both OFM and codling moth (CM), were as effective as individual dispensers for these species. Combined control of both pests in pears using dual MD dispensers will be more economical than use of individual species dispensers, because the price and application cost of the dual dispenser is about half that for individual species dispensers.

Sex pheromone based IPM programs in orchards are designed to target male moths, when the real goal is to reduce the female's ability to reproduce. Unfortunately, conventional sex pheromone traps are not very reliable for monitoring of males in orchards treated with MD. Attracting females, and mated females in particular, to synthetic host-plant volatiles may enable growers to control females and their unlaid eggs and to monitor pest life cycles precisely in orchards treated with MD.

New host-plant attractants (new traps) for OFM and CM were tested to improve pest monitoring in orchards treated with MD. Sex pheromones and new female attractants were used for detailed daily monitoring of CM in apples and pears, and OFM in peaches, pears and apples early in the growing season to identify the first catches of males, virgin and mated females. The results demonstrated that OFM males and females emerged in pear blocks earlier than in their preferred host peach. First catches of OFM males and females in new traps in apples were recorded approximately one month later than in pears. Also, OFM males were caught in sex pheromone and new traps two to three weeks earlier than females in new traps. The first mated OFM females were caught in the new traps four to 10 days later than the first virgin OFM females. Virgin and mated CM females were caught in the new traps with female attractants in pears at similar dates, but about a week later than first males in sex pheromone traps. The results of the daily monitoring of OFM and CM males and females helped to estimate Biofix dates for different fruit varieties more correctly and were used in Day-Degree Models.

Improved monitoring and possible mass trapping of major orchard pest mated females using new host-plant attractants could reduce damage without the need for extensive insecticide sprays, maintaining the 'clean and green' image for Australian fruit. Semiochemical based IPM and selective area-wide MD programs are the key elements in development of cost-effective strategies for pest control, while protecting the environment by reducing pesticide pressure in orchards.

Development of an attract-and-kill strategy for the potato tuber moth complex *Phthorimaea operculella* Zeller and *Symmetrischema tangolias* (Gyen) in Peru

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Introduction

The common potato tuber moth (PTM, *P. operculella*) and the Andean potato tuber moth (APTM, *S. tangolias*) are important pests of potato (*Solanum* sp.) and other Solanaceae crops. PTM has a worldwide distribution and is considered the most damaging potato pest in the developing world. It had been a key pest in Andean potato production but was replaced in many areas by the APTM. The damaging life-stage of both species is when larvae attack leaves, stems and tubers. Sex pheromones, identified for both moths and formulated in lures, have been widely used in IPM for monitoring the flight activity of PTM/APTM males. Further, they have been tested for mass trapping. We tested the hypothesis that an attract-and-kill strategy consisting of pheromone and contact insecticide is effective in controlling both pests. The objective of our preliminary studies was to evaluate different insecticide concentrations, the stability of the formulation and to monitor the effect on the male population under field conditions.

Material and methods

Male moths of PTM and APTM used in this study were obtained from a laboratory colony maintained at CIP, Lima. Newly hatched males were kept for 48 h prior to their use in the experiments, to reach full sexual maturity. Pure PTM/APTM pheromones (Pherobank, the Netherlands) and the insecticide Baythroid TM 525 SL (Cyfluthrin 25 g/l; Bayer Crop Science, Chile) were the active ingredients employed in all experiments. The pheromones were used in one concentration (0.05%) and the insecticide was tested at 0.03, 0.06, 0.125, 0.25 and 0.5%. The active ingredients were formulated with castor oil (50%) and ultraviolet absorbers (UVINIL M40 and UVINIL 539T; BASF, Germany, both at 7.5%) stabilised by the nonionic emulsifying agent Cremophor CO 40 (BASF, Germany, at 7.5%) and Aerosil 200 V (Degusaa Pharma, at 7.0%). Wooden cages (75 x 50 x 60 cm) covered with nylon gauze were installed at temperatures of 20ºC. The pheromone formulation was dropped on to a Petri dish (100-µl droplet) placed in one corner of the cage. In each of the cages 20 males of the same age were released. The experiments for PTM and APTM were carried out at different times except when pheromones of both species were tested in one formulation. The formulation stability was tested by releasing a new set of males when all previous 20 males had been killed. Survival of males was monitored every day until all moths in all treatments died. All experiments were repeated three times. The field experiment for PTM was carried out on CIP's experimental station La Molina (300 m a.s.l), February-April 2007. Potato (var. Unica) was planted on 10 plots of 225 m^2 surrounded by maize (*Zea mays* L.) with at least 50 m between them. The efficacy for APTM was tested in 12 potato fields (from 600 to 3,500 m^2) in the Rio Mantaro valley (3,200 m a.s.l.). In each plot, pheromone-baited water traps were employed to monitor the flight activity of males after the treatment. The formulation consisted of 0.5% cyfluthrin and was tested in droplet densities of one drop (100 µl) per 1, 2, 4 and 8 m^2, equal to 10,000, 5,000, 2,500 and 1,250 droplets ha^{-1}. Treatments were repeated after 10 days with a total of two or three applications. The experiments were replicated twice for PTM and three times for APTM.

Application of pheromones in an area-wide IPM in horticulture

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Pheromone mediated mating disruption (MD) is now a major tool of semiochemical based and sustainable Integrated Pest Management (IPM) systems in Australian horticulture. One of the first successful applications of MD for pest control was demonstrated for *Trichoplusia ni* (Hubner) over 30 years ago, and five years later MD was successfully applied for oriental fruit moth (*Grapholita molesta* Busck.) control in Australian orchards. Since then, it has been demonstrated for many insect species that the release of large quantities of sex pheromone into a target crop could disrupt mate location and prevent or delay mating, reducing egg fertilisation and pest damage.

The most successful use of MD over the last 20 years has been for control of Lepidopteran pests. In Australian orchards, MD techniques were successful for long-term sustainable control of oriental fruit moth (OFM), codling and light brown apple moth. Initially the general approach was to treat individual orchard blocks and only known host-plants with MD. The incidence of OFM damage on the borders of MD treated blocks stimulated an area-wide application of MD for better crop protection. An area-wide MD program, with more than 1,100 ha of 40 contiguous orchards covered with MD dispensers, applied to all fruit trees in the Cobram region of northern Victoria, Australia, substantially improved protection against OFM damage. Later, local growers took the initiative to continue this area-wide MD program as a self-sufficient community approach and achieved re-establishment of MD across the whole Cobram region. This example of close collaboration within the grower community to control OFM with MD at an area-wide level quickly spread to Greater Shepparton and Invergordon regions of northern Victoria. The area-wide MD strategy helped growers to reduce insecticide interventions substantially, to protect the orchard environment for beneficial insects and to produce 'clean and green' fruit for domestic and international markets.

Although successful, the area-wide MD program was an expensive approach. To reduce the cost of area-wide MD program, trials were conducted where only infested blocks and border areas were treated with MD. Such a selective approach was successful for control of localised pest outbreaks and areas of increased infestation. To make MD more cost-effective, trials were also conducted to investigate the use of a reduced MD rate, barrier MD treatments and new MD products, including sprayable microencapsulated and multi-species MD dispensers.

For example, MD barrier treatment applied to 54-60 meters of neighbouring pears adjacent to MD treated peaches was able to reduce both the number of OFM caught in traps and damage to shoot tips and fruit, thus giving similar results to MD treatment of the whole neighbouring pear block. Extending the MD treated area for 54-60 meters into the neighbouring pear block significantly reduced the edge damage in MD treated peaches in the first season and almost eliminated OFM damage in the second season.

Results

Cyfluthrin at 0.5% resulted in the highest and fastest kill of both moth species after 24 and 48 h (data not shown), killing all the males of both moth species after three and four days, respectively. Mixtures of the two species also gave equal control, indicating no interference of the pheromones in one formulation (Table 1). At 20 °C and over a period of 28 days no reduction in the efficacy of the attract-and-kill formulation could be observed (data not shown). In the field experiments, compared to the untreated control, droplet densities of one drop per 1 and 2 m^2 were almost equally effective, reducing the daily PTM male catches by 89.4 and 83.8%, respectively (Table 2). Densities of one drop per 4 and 8 m^2 still reduced the PTM male catches by 64.5 and 51.8%. The efficacy for APTM was lower, with a mean daily reduction of 73.7%, 55.1% and 54.7% for the three densities tested. While the PTM experiment was carried out under high PTM population with mean daily trap catches of 122 males, the mean number of APTM males was lower, with only 16 males per day.

Table 1. Mortality of tuber moth males over time in cages after the application of one drop of the test formulation containing 0.05% of the sex pheromone and 0.5% cyfluthrin.

Pheromone/	Accumulated mortality (%) after days										
species tested	01	02	03	04	05	06	07	08	09	10	13
PTM	70	85	95	100							
Control	0	0	5	5	10	10	10	10	15	25	100
APTM	70	90	100								
Control	5	5	5	5	15	15	15	15	15	30	100
PTM and APTM	68	88	95	100							
Control	0	10	10	10	18	18	25	33	55	80	100

Table 2. Efficacy of attract-and-kill in four droplet densities to reduce tuber moth male population compared to untreated fields as monitored by pheromone-baited water traps.

No. of Application (10-day interval)	Mean daily reduction of males (%) in traps						
	Phthorimaea operculella				*Symmetrischema tangolias*		
	1 drop (100 µl) per						
	1 m^2	2 m^2	4 m^2	8 m^2	1 m^2	2 m^2	4 m^2
1st	87.8	76.6	55.8	45.0	84.5	56.9	52.6
2nd	90.0	85.8	64.8	48.7	62.8	53.3	56.8
3rd	91.4	88.9	73.0	61.8	-	-	-
Average	89.4	83.8	64.5	51.8	73.7	55.1	54.7

Discussion

In comparison to the disorientation approach that requires large quantities of pheromone, the attract-and-kill approach does not rely on a stable pheromone cloud; it kills and hence removes males from the population. Experiences with other Lepidopteran pests (e.g. codling moth, *Cydia pomonella* L.) suggested efficient control at small plot sizes and higher pest densities, reflecting the situation of potato farming in developing countries. Our results indicate a higher efficacy at higher pest densities. We still need to determine if a reduction of males by more than 80% will reduce female reproductive capacity sufficiently and hence leaf and tuber damage at harvest. We will evaluate this in different environments and pest densities and apply modeling tools. Further, the formulation could be optimised by testing reduced pheromone and/or increased cyfluthrin concentrations to speed up the mortality and hence improve the field efficacy of the attract-and-kill approach.

UK Pesticides Safety Directorate efficacy data requirements and guidance on trials design for mating disruption pheromone products (and other semiochemicals)

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Mating disruption is a highly specialized technique and as such presents particular challenges in efficacy trials design. Further, the regulatory efficacy data requirements in some areas can be addressed in ways other than generating data. Guidance covering these aspects has been produced by the UK Pesticides Safety Directorate (PSD) to assist applicants ('Data requirements and trials design for mating disruption pheromone products', Efficacy Guideline 220, available at www.pesticides.gov.uk). Many of the aspects discussed are also relevant to other semiochemical techniques designed for plant protection purposes. Applicants are actively encouraged to discuss their trials programmes and how to address data requirements as early as possible in the product development. Semiochemicals are considered under the UK biopesticides scheme, and as such are subject to reduced registration fees (full details on the 'Biopesticides' page of the PSD website).

The efficacy data requirements as specified under 91/414/EEC directive can be broadly divided into those relating to effectiveness (including dose justification), and those relating to crop safety (both direct and indirect effects). For mating disruption pheromones (and other semiochemicals) the primary need is to address the effectiveness of the product. Data should demonstrate a measurable benefit (for example, reduction in crop damage and perhaps longer term suppression of pest population), and support the product label claims and directions for use. Given their specialised nature, the latter are particularly important in assisting growers to get optimum benefits from the product (for example the placement of dispensers, monitoring techniques, when and how to use in IPM programmes). Crop safety aspects can usually be addressed by reasoned argument, supported for the formulated product by visual assessments in the effectiveness trials.

It is essential when addressing the data requirements to provide a detailed explanation of how the product works. Sources of information may include published papers (reviews of techniques are particularly useful), laboratory and small scale studies. The UK is also content to accept non-UK data, provided there is a clear and justified case for its relevance. This information, alongside preliminary data, can explain key aspects such as mode of action, species-specificity, pheromone composition, effects of environmental conditions, and comparison of emitted with natural levels. Initial studies with different formulations and/or types of dispenser can further support the proposed use. Information on pest biology must also be submitted and is vital in gaining both an understanding of the technique, and also the appropriateness of the trials design. The extent and quality of available preliminary studies and published data will also influence the subsequent number of field trials required to support an approval. The information and reasoned arguments provided are also relevant to other areas of the risk assessment. The OECD guidance on the registration requirements for pheromones and other semiochemicals (OECD series on pesticides, number 12) illustrates how this approach can be applied across areas of the risk assessment.

Trials design is a particular challenge for mating disruption (and other semiochemical) techniques and the biology of the pest will have a major influence. Behavioural factors will affect plot size and include movement distances of adults, mating and egg laying sites, spatial distribution of population within crop. Other factors to consider include the sex ratio, whether unmated females lay viable eggs, whether males are polygamous, and how many matings a male can achieve. Immigration of mated females for example will affect where treatment dispensers and monitoring devices are placed, as well as the need for any border treatments with conventional insecticides. Mating disruption techniques result in potential treatment interference between plots. Often a trials site cannot be split into a conventional, replicated small-plot design, and any untreated or comparison treatment areas may need to be separated some distance away. Plot size for the pheromone treatments may also need to be of significant size in order to achieve reliable performance. All these factors make comparisons of damage and population levels between treated areas less accurate.

Site location needs to consider the above factors along with local geographical features that may influence pest migration. Ideally a single large site containing the various treatments with appropriate spatial separation is preferred, but in practice this may not be possible, particularly for pests with longer migration distances. It is especially important, therefore, where plots are spatially separated to gain information on pest history and damage levels from previous seasons at the chosen site. This can be supplemented by information on local area pest density and damage during the course of the season. Drafting a site map can also be useful to compare treatment sites and determine likely areas of immigration, and identify the placement of monitoring and treatment dispensers. It can also assist in interpreting results from the site, particularly where damage occurs in localised areas.

Monitoring of pest populations has two purposes: firstly to determine the timing of treatments, and secondly as an indicator of effectiveness by estimating pest populations. However the latter is difficult as monitoring techniques often involve sex pheromones as the lure. The traps can suffer from interference ('trap shutdown') from the mating disruption dispensers. This can potentially be overcome by increasing the concentration of sex pheromone on the lure. Alternatively, different semiochemicals (for example kairomones, attractants based on fruit extracts) can be used as the lure. Assessing effectiveness can be difficult because it is not reliant on direct control. Estimates of population levels can give an indication but is, as discussed, subject to many factors which can make interpretation of results difficult. The main assessment is based around reductions in crop damage, and also comparing with other local sites (as well as damage in previous years). These assessments can also be very usefully supported by other techniques (for example, using tethered females to indicate mating levels, or assessing the number of over-wintering larvae and comparing with previous seasons). It is accepted that mating disruption techniques are less successful when population numbers are high and often the technique may be combined with other control methods, including chemical insecticides. It is important that where such recommendations are on the product label, some of the trials should include use in integrated programmes.

In summary, whilst mating disruption techniques may not give robust levels of control in all situations, especially for high population levels, they can provide a valuable contribution to reducing damage. The label needs to provide clear information on directions for use and the level of effect that can be expected. Trials design can be complex and the type of monitoring and assessments used must be carefully considered. It is essential that the product is used in some trials as directed on the label, and that the applicant fully considers and explains how the data supports the wording and claims on the product label.

Odour-baited traps for control of the pubescent rose chafer, *Tropinota squalida* Scop (Coleoptera: Scarabeidae)

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Introduction

The pubescent rose chafer, *Tropinota squalida* Scop adult is an important pest in newly reclaimed areas in Egypt and causes serious problems to fruit crops during winter and early spring. Farmers in Egypt are using different types of dung for manuring and amending the new reclaimed lands, and El-sayed & Homam (2005) concluded that any one of the tested dungs (cattle, horse, poultry and their mixture) were essential for *T. squalida* to develop. In recent years efforts have been concentrated on achieving acceptable crop protection with no need for chemical pesticides. The aim of this work was to confirm and quantify earlier observations by Homam (1994) that odour plays a significant role in attraction of *T. squalida* adults. Field experiments were carried out to screen eight fragrance oils to determine the most suitable for use in traps to control *T. squalida* adults.

Materials and methods

The eight fragrance oils tested were rose, pink, vanilla, Arabian jasmine, mint, jasmine, apple and peach. Each fragrance oil (20 ml) was blended with 96% ethanol (10 ml) as a carrier to increase the release rate. The trap consisted of a plastic dish (25 cm diam. and 7 cm deep) filled with water (1000 ml) and suspended 0.5-0.8 m above ground level on a rope or wire. The odour-baited traps were fixed above the main trunk of apricot trees to ensure the presence of beetles feeding on flowers. The study showed that traps fixed inside the tree were more efficient than traps placed between apricot trees. The treatments were distributed in randomised blocks with six replicates. Each trap was located between approximately four trees and the distances between traps were 12-16 m. The baits were renewed every four days during the period of flowering of the apricots. Beetles were collected from the traps every four days and frozen until they were sexed and counted.

Adults of *T. squalida* were also collected by hand and transferred to the laboratory for sexing and counting. Males were distinguished by a median, longitudinal cleavage in the second, third and fourth abdominal segments.

Catches were subjected to analysis of variance and differences between means were tested for significance by Duncan's multiple range test.

Results

Pink oil was significantly more attractive to *T. squalida* adults than any of the other oils, although traps baited with Arabian jasmine or rose oils also caught more beetles than unbaited traps (Table 1). The highest captures (63.5%) in the pink-baited traps were recorded as 90% of the flowers had set. A decrease in flowering caused an increase in effectiveness of the trap. The pink-baited trap recorded sex ratio 0.88:1 female:male, while the hand collection recorded 0.63:1. The six traps baited with pink oil collected 4,832 beetles, while hand collection by three workers collected 3,579 beetles in seven sessions.

Table 1. Mean numbers of *T. squalida* adults captured by odour-baited traps

Bait oil	Mean number of *T. squalida**			Sex ratio F/M
	Female	Male	Total	
Pink	75.3 a	85.8 a	161.1 a	0.88 a
Arabian jasmine	30.5 b	40.5 b	71.0 b	0.75 a
Rose	17.9 c	31.6 b	49.5 b	0.57 a
Apple	8.4 c	16.4 c	24.8 c	0.51 a
Mint	7.3 c	9.2 c	16.5 c	0.80a
Jasmine	7.1 c	7.7 c	14.8 c	0.93 a
Vanilla	7.0 c	5.7 c	12.7 c	1.23 a
Peach	3.1 d	3.7 c	6.2 c	1.02 a
Unbaited	1.2 d	1.0 c	2.2 c	1.20 a
L.S.D.	12.0	14.4	25.8	0.56
P	0.0001	0.0001	0.0001	0.119

*means in same column followed by the same letter not significantly different (P>0.05)

Discussion

These finding agree with those of Homam (1994) who first found that not only the colour of flowers governs the attraction of *T. squalida* but also the odour. Traps baited with pink oil were most effective in capturing beetles of pubescent rose chafer. The traps were successful in apricot trees where the flowering time coincided with warm weather (22°-27ºC). Warm weather increases the diffusion of volatile odours and also the activity of beetles. In contrast, traps hung in apple trees gave less-promising results because apple flowers at a time of lower temperature.

Hence 18 trap/feddan will collect approximately four times the hand collection of three workers for seven sessions during the period of flowering. The cost of 18 traps during the apricot flowering is about $17, while the cost of hand collection of three workers in five sessions is $60 during the flowering phase of apricot. Thus traps baited with pink oil combined with hand collection provide an effective and safe method for controlling the adults of *T. squalida* in apricot trees. Advantages of the traps are (1) they are safe; (2) they are easy to apply; (3) they are low cost; (4) they catch a higher proportion of females relative to hand collection; (5) the traps can be used as protection barrier in or from neighbouring plants or trees.

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Use of a decision tree approach to human risk assessment of semiochemicals

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There is considerable demand for use of alternatives in crop protection to conventional synthetic broad-spectrum pesticides which have a predominantly toxic mode of action on the target pest. Since semiochemicals act as signals regulating behaviour or development of the pest (or crop), rather than by toxic or other direct physiological effects, effective pest control methods based on their use may be more acceptable than synthetic conventional pesticides. However, naturally derived semiochemicals that are applied as 'plant protection products' still require registration under EC Directive 91/414/EEC. The main objective of this research project was to use literature information on three specific examples of semiochemicals to assess whether there was sufficient data for regulatory submission.

Methods
The three natural semiochemicals chosen were hop β-acids, methyl salicylate and *cis*-jasmone. As a first step the literature and databases were searched for all available data on these substances. A wide selection of databases was searched for information on toxicity using a selection of key words and alternative names for the three semiochemicals. Key studies were summarised and presented in the dossier format required under 91/414/EEC. A use scenario for each active substance was used as the basis for a data gap analysis and an estimate was made of the cost of conducting studies needed to fill the data gaps. Finally, alternative approaches to satisfying regulatory requirements were considered.

Results
More published toxicology data were found for methyl salicylate, as a result of it being used as a flavouring material and pharmaceutical, than for hop β-acids and *cis*-jasmone. Although *cis*-jasmone has been used in perfumery and hop β-acids in beer for many years there was very little published toxicity data. Only two of the studies found were GLP compliant (a requirement for laboratory studies submitted to support registration under the EU regulatory Directive 91/414/EEC) and the standard of data varied considerably. In some cases no experimental methodology was published and, in general, little of the information required in a dossier to Directive 91/414/EEC standards e.g. strain, sex, body weight was reported. Most studies were conducted over non-standard periods of time and it was often necessary to include bottom line data with no experimental information at all, as that was all available. For none of the three semiochemicals was there enough data of sufficient quality for Annex I inclusion as is likely with most semiochemicals. Also, it is unlikely that the commercial value of semiochemicals in the European plant protection market would be enough to bear the cost of generating new data for endpoints e.g. carcinogenicity or reproductive toxicology. So, it is unlikely that an adequate dossier could be produced by using a combination of literature data plus a small expenditure on new studies.

Alternative approaches to providing assurance that the active substances would provide an acceptable risk were considered. The OECD Guidance for Registration Requirements for Pheromones and Other Semiochemicals (OECD, 2002) provided a rationale for considering reduced data requirements but some of the assumptions used to justify a reduction in data do not necessarily apply to all semiochemicals. The use of the concept of a threshold of toxicological concern was also considered as a way of minimising the generation of new data. The threshold of toxicological concern (TTC) is a value below which there is a very low probability of appreciable risk to human health. The principle of the TTC is that a threshold can be applied to many substances in the absence of a full toxicological database. The concept of the TTC was developed as a result of a review of toxicity data of chemicals which were divided into three structural classes (Munro *et al.*, 1999). This showed that a TTC could be assigned to each structural class. The TTC concept is used by various regulatory authorities including the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to evaluate flavouring agents.

JECFA use the TTC as part of a decision tree and a decision tree approach has also been recommended recently by an expert group convened by the International Life Sciences Institute. A simple decision tree was therefore devised that could be used to decide whether adequate data were available in the literature and when new studies would be required. The first stage is to estimate the dietary and operator exposure under the proposed conditions of use of the semiochemical product or Good Agricultural Practice (GAP), and compare these values with an estimate of the natural exposure from dietary sources. It is important to have a detailed understanding of the proposed use of the semiochemical at this stage as this helps to understand the potential hazards and also to estimate dietary and operator exposure on a realistic worst-case basis. If dietary/operator exposure < natural exposure no further toxicology studies should be required on the active substance. If dietary/operator exposure > natural exposure, the structural class of the semiochemical should be identified and a TTC assigned. Again if dietary/operator exposure < TTC no further work should be required but, if this is not the case, an assessment should be made of what studies are needed and a literature search should be conducted. Only if the required data are not available from the literature or are not of acceptable standard should new studies be needed.

Conclusion

A decision tree approach involving the use of exposure assessments, the threshold of toxicological concern and a literature search could be used to facilitate the registration of semiochemicals under EC Directive 91/414/EEC. This could reduce animal usage and the costs could make registration of such products more commercially viable.

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Control of codling moth, *Cydia pomonella* L., in apple orchards of Bulgaria by use of new technology: preliminary results

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Introduction

Codling moth, *Cydia pomonella* L. (CM), is the most important pest of apple worldwide (Dorn *et al*., 1999). In spite of the relatively large number of chemical insecticides available for control of this species, it continues to pose a serious threat, due to development of resistance to various groups of insecticides (Pasquier & Charmillot, 2003). On the other hand, CM was found to be a model for application of novel, non-chemical control methods, in particular mating disruption (Quarles, 2000). The objective of this study was to test mating disruption with Ecodian CP dispensers of the Isagro company under conditions of Bulgaria.

Material and methods

The trial was set up in a commercial orchard near Burgas, consisting of two plots, A of 2.50 ha and B of 1.25 ha. The orchard contained cvs Idared, Mutsu, Golden Delicious, Melrose, Mollie's Delicious and Granny Smith. In 2005 the CM pressure was relatively high, as indicated by about 3-5% damaged fruits at harvest. The Ecodian-CP dispensers (Isagro, Novara, Italy) are small blue hooks, impregnated, with 10 mg of codlemone. On 27-28 April 2006 and 23-24 April 2007, 10,000 dispensers were placed on both plots, corresponding to 2667 units per ha. A second set of dispensers was applied each year at the end of June – on 23-24 June 2006 with 11,000 dispensers on the two plots, corresponding to 2933 units per ha, and on 29-30 June 2007, with 10,000 dispensers, corresponding to 2667 units per ha. The applications required 4½-5 man-hours per ha. Complementary insecticide treatments were applied in July 2006, with thiacloprid, because leaf rollers appeared during the season. As a reference, conventionally treated plot, another 10-ha commercial orchard was used, located 56-165 m North from the trial orchard. In 2006, nineteen chemical treatments (with 31 different a. i.) were applied there to control CM, leaf miners, leaf rollers, aphids and mites. Seventeen of them (with 27 a. i.) were aimed against CM. Many treatments were applied at a double dosage. In 2007 seven (8 a. i) treatments was made only against the first CM generation. Prior to the start of CM flight, four CM pheromone traps were installed in the trial plots and two in the reference orchard. In both seasons, fruit damage was evaluated on samples of 1,000-2,000 fruits from every plot and from untreated rows, located inside the conventionally treated orchard. Preharvest evaluation was carried out in 2006 on 7-8 September, on 2000 fruits from both trial plots, on reference plot and in untreated rows. In June, 40 corrugated cardboard bands were placed in both trial plots, as well as 20 bands in the treated reference plot and 20 bands in an untreated orchard near Burgas. They were recovered in autumn and the hibernating CM larvae were counted.

Results

In the reference orchard the first flight of CM began on 3 May in 2006 and on 27 April in 2007. It successively intensified, to reach its maximum by the third decade of May. In 2006 the flight of the second genaration, overlapping the first, started at the end of June, reached its maximum in the 1st or 2nd decade of July, then decreased till mid-August. In 2006 a few moths were caught even in September. In the Ecodian-treated plots a single moth was caught in 2006 (on 3 May). In 2007 no moths of the first generation were caught. In the reference orchard the first moth of the second generation appeared on 2 July. The fruit damage rate in the Ecodian treated plot A was 0.35% in the 2nd decade of June 2006, at the time of the second application of dispensers. Then it increased to 0.6% and then progressively decreased to 0.2% before harvest. In the plot B, damage reached 0.1% at the end of June, decreased to 0.05% by mid-August and stabilised at the same level at harvest. In the reference orchard, where 19 insecticide treatments were applied during the season, damage progressed from 1.84% on 23-24 June up to 5.3% at harvest. In untreated rows, damage was 4.6% at the end of the first generation and rose progressively to 87% at harvest. In autumn 2006 the overwintering population in Ecodian-treated plots was 0.325 larvae/tree in plot A and 0.225 in plot B. In the reference orchard, where CM population was suspected to be resistant, it reached 3.7 larvae/tree and in the untreated rows 7.6 larvae/tree. In 2007, the fruit damage from the first generation was 0.1% in plot A and 0.05% in plot B, i.e. less than in 2006. Fruit damage in the reference orchard was 2.1% and in untreated rows 18.6% at the same time.

Discussion and conclusions

The Ecodian dispensers were tested during the seasons 2006-2007 in the orchard near Burgas, where the CM population density was 3-5%. Under these conditions, fruit damage in plots treated with Ecodian stayed at a low level during the whole 2006 season. In 2007, during the 1st CM generation, it was even lower. The overwintering population, checked in autumn 2006, was also very low in Ecodian plots, in contrast to the reference orchard, treated 19 times with chemical pesticides. The latter indicates that the CM population in the commercial orchards of the region is probably resistant to insecticides. The positive results obtained in our trials have shown that the mating disruption method may work perfectly under conditions of Bulgaria and that Ecodian dispensers may be effective in that respect. Use of these products may result in limiting the use of chemical insecticides and thus in reduction of environmental pollution, improvement of food quality and finally in favouring a better human health condition.

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Choristoneura rosaceana (Harris) and *Pandemis pyrusana* Kearfott (Lepidoptera: Tortricidae) male control in Washington State (USA) apple orchards treated with different source densities of several attracticide formulations

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Introduction

Attracticides have been reported for several Tortricidae. Formulations include the specific sex pheromone to attract male moths and an insecticide to kill them after contact. Attracticides are an alternative to conventional pesticides, and reduce risks for applicators, the environment, and beneficial arthropods. The objective of this study was to evaluate attracticides containing either 1.6 or 16% of the pheromone, with or without permethrin, using different source densities, to suppress male populations of *C. rosaceana* or *P. pyrusana*, two increasingly important apple pests in the State of Washington, USA, under field conditions.

Materials and methods

The attracticide used was a grease formulation that also included an ultraviolet stabiliser, a thickener, and a sticker. For *C. rosaceana* the attracticide was loaded with 95.5% Z11-14Ac, 2% E11-14Ac, 1.5% Z11-14OH, and 1% Z11-14Ald. For *P. pyrusana* the attracticide was 94% Z11-14Ac and 6% Z9-14Ac. The pheromone components were added directly to the matrix (grease) to prepare mixtures containing either 1.6% or 16% of the pheromone blend by weight, then mechanically stirred while in a warm-bath for 5 min, and finally poured into a 250 mL bottle. Technical permethrin was also added at 6%. Batches not loaded with insecticide, but containing pheromones, were used as blank attracticides for each species.

Field trials were established in apple orchards cvs. Red Delicious and Fuji, in central Washington, USA. The orchards were infested with *C. rosaceana* and/or *P. pyrusana* and were not sprayed with insecticides during the season. Drops (50 µl) of attracticide were distributed evenly around the canopy of apple trees. The attracticide was applied only once in each orchard. Delta traps were placed in the centre of each replicate and there was at least 25 m between traps. Traps were baited with regular lures. Monitoring started a few days before the treatments were applied in the field. Traps were examined every two to three days for about one month for counting moths, liner replacement, and cleaning.

A completely randomised block design with three replicates (at least 0.1 ha each) was used. The impact of treatments on males was evaluated by recording capture of moths in traps. Proportions of cumulative captures within blocks were arcsin square-root transformed, analyzed by ANOVA and Tukey test before and after applications for each orchard.

Results and discussion

Similar trends among field trials were observed for both species, *C. rosaceana* and *P. pyrusana* (Tables 1 and 2). The average cumulative male captures before attracticide applications were relatively low and no statistical differences were observed between plots assigned to the different treatments. After applications, the captures in all attracticide treated plots (except 1.6% pheromone with permethrin using 1200 droplets/ha) were significantly lower than the untreated check. The attracticide treatments reduced the ability of males of both species to find pheromone sources. Either the males were killed by contact with the attracticide or they were inhibited from locating the trap because of some male disruption effect. Both explanations are possible and have been proposed previously to explain the results of attracticide treatments in other field studies, targeting other species. Based on previous reports, it is expected males will be strongly attracted to attracticide sources with the higher pheromone concentrations, that is, more males would be eliminated or at least removed from the reproductive population after the contact with those attracticide formulations. However, the results reported here indicate that in all cases there were no statistical differences in favour of using a high concentration, 16% pheromone attracticide with or without insecticide, suggesting that the lethal agent was not necessary to affect moth populations. In some experiments higher numbers of moths were captured in the attracticide treatment that included the insecticide. Thus it is more likely that disruption or competitive attraction is the main factor reducing the approach of males to traps. If so, the addition of an insecticide to the attracticide formulation may not be necessary. Further research is necessary to either confirm or deny this possible explanation. If a disruptive effect turns out to be the actual mechanism explaining the results shown here, these insecticide-free formulations should be evaluated as disruptive treatments for both species where mating disruption with other formulations have shown erratic results.

Table 1. Catches of *Choristoneura rosaceana* in an orchard treated with different attracticide source densities, with/without permethrin, and two pheromone concentrations, Washington, USA (17 - 19 Aug pre-treatments, 20 Aug - 18 Sep post–treatment)

Pheromone		Permethrin		Source density	Mean males/trap/day ± SEM	
%	gm/ha	%	Gm/ha	(droplets ha^{-1})	Pre	Post
1.6	1.0	0	0	1,200	0.7 ± 0.34 a	0.04 ± 0.000 b
1.6	2.9	0	0	3,600	1.0 ± 0.43 a	0.05 ± 0.002 b
1.6	1.0	6	3.6	1,200	0.7 ± 0.34 a	0.06 ± 0.006 a
1.6	2.9	6	10.8	3,600	0.7 ± 0.34 a	0.02 ± 0.001 b
16	9.6	0	0	1,200	0.5 ± 0.14 a	0.00 ± 0.000 b
16	28.8	0	0	3,600	0.5 ± 0.14 a	0.00 ± 0.000 b
16	9.6	6	3.6	1,200	0.7 ± 0.34 a	0.01 ± 0.000 b
16	28.8	6	10.8	3,600	0.8 ± 0.67 a	0.00 ± 0.000 b
0	0	0	0	0	1.0 ± 1.01 a	0.51 ± 0.155 a

Table 2. Catches of *Pandemis pyrusana*, in an orchard treated with different attracticide source densities, with/without permethrin, using two pheromone concentrations, Washington, USA (23-27 Aug pre-treatments, 28 Aug - 3 Oct post-treatments)

Pheromone		Permethrin		Source density	Mean males/trap/day ± SEM	
%	gm/ha	%	gm/ha	(droplets ha^{-1})	Pre	Post
1.6		6		1,000	0.42 ± 0.17 a	0.02 ± 0.02 b
1.6		6		3,000	0.50 ± 0.38 a	0.00 ± 0.00 b
16		6		1,000	0.33 ± 0.33 a	0.02 ± 0.02 b
16		6		3,000	0.33 ± 0.17 a	0.00 ± 0.00 b
0		0		0	0.42 ± 0.30 a	0.19 ± 0.09 a

Pheromone release behaviour of the female carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), under laboratory and field conditions

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Objectives

Synthetic sex pheromones of Lepidoptera have been widely used for monitoring and timing of spray and control methods. Chemical control of carob moth, *Ectomyelois ceratoniae,* is not practiced, and currently non-chemical methods of control are used and recommended. Therefore this research was conducted to study the calling behaviour of virgin female carob moth under three constant temperature conditions in the laboratory and under variable field conditions.

Methods

Moths were obtained from pomegranate orchards in the Gherdefaramarz region of Yazd province, Iran in 2006. Pupae were separated by sex and females were individually placed in plastic transparent cages and then held at 29±1°C for the duration of the pupal period. Upon emergence, virgin females were provided with a 10% sugar solution and then separated into three groups with one group set up in each temperature regime (20°, 25°, 30°C). In all experiments females were observed each night at 15 min intervals throughout the scotophase, using a torch covered with two layers of tissue paper and a red filter. Two series of field experiments were conducted in pomegranate orchards in early August and early September 2006.

Results

The mean age of calling for the first time was not affected by temperature under laboratory conditions (F=0.214, d.f. =2, P>0.05), with females at 30ºC calling for the first time at a not significantly older age than those at the lower temperatures. This parameter did not vary between two field tests and all females initiated calling from the eclosion day in both early August and early September.

The mean onset time of calling occurred significantly earlier at successive days of calling, and a significant temperature x age interaction resulted from the earlier onset of calling of females during forth day and later onset on seventh day of calling (Table 1).

The mean time spent calling increased significantly on successive days of calling, resulting more from an increase in the duration of individual calling bouts than from an increase in the number of bouts (data not shown). There was also a significant effect of temperature on the mean time spent calling, with females at 25ºC calling more than those at lower and upper temperatures (Table 1).

Table 1. Analysis of variance of the mean onset time of calling and mean time spent calling by virgin *Ectomyelois ceratoniae* females as a function of calling age under different constant temperature conditions in the laboratory.

	Effect	d.f.	*F*-value	*P*
Mean onset time of calling	Block	2	3.1784	-
	Temperature	2	5.5461	0.0702
	Age	7	10.8806	0.0000
	Temperature × age	14	0.0446	0.0045
Mean time spent calling	Block	2	3.9097	0.1145
	Temperature	2	20.0665	0.0082
	Age	7	6.4354	0.0001
	Temperature × age	14	2.6920	0.0125

Females placed in the field in early August (mean daytime temperature: 26.2°C) called for the first time at the same age (0 day) that the other cohort started 29 days later, when the mean daily temperature was 23.8°C. All females initiated calling from eclosion day at both early August and early September. The age-related changes in the mean onset time of calling and the mean time spent calling seen in the laboratory were less evident under the field conditions. This was due to marked day-to- day variations associated with changing climatic conditions, and is reflected by significant cohort × age interactions (Table 2).

Table 2. Analysis of variance of the mean onset time of calling and mean time spent calling by virgin *Ectomyelois ceratoniae* females as a function of calling age under field conditions at two different periods in summer 2006.

	Effect	d.f.	*F*-value	*P*
Mean onset time of calling	Cohort	1	1.9907	0.1774
	Age	7	13.2064	0.0000
	Cohort x age	7	4.5256	0.0002
Mean time spent calling	Cohort	1	0.5656	-
	Age	7	2.0594	0.0538
	Cohort x age	7	4.8896	0.0001

Conclusions

E. ceratoniae has been considered a crepuscular and nocturnal moth. Our data indicate that late night is a typical pheromone release period for carob moth. However, there was only one peak of calling, declining to zero at beginning of the photophase, contrary to some other moth species, e.g. the sesiids *Paranthrene tabaniformis kungesana* (Rott.) and *Synanthedon tipuliformis* (Cl.). Our results clearly indicate that patterns of calling in *E. ceratoniae* vary with age. The age at which females initiate calling for the first time is not temperature dependent. This finding is in agreement with the patterns generally reported for Lepidoptera species. Calling did not weaken with age and became intensive on the first to third day from the emergence and more intensive on the forth to sixth day and most extensive on seventh to eighth day, contrary to the patterns generally reported for most pyralids. Once pheromone emission has been initiated, both mean onset time of calling and mean time spent calling of *E. ceratoniae* changes as a function of age at all temperatures, although the differences are more pronounced under laboratory conditions than under variable field conditions. These changes, which result in an increasingly greater calling window over successive days of pheromone emission, have been reported in a large number of Lepidoptera.

Kovats retention indexes of mono- and di-unsaturated C18 alcohols, acetates and aldehydes found in Lepidopteran pheromone blends

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Introduction
Many Lepidopteran pheromones consist of blends of mono- and di-unsaturated alcohols, aldehydes and acetates. These compounds are obtained in only nanogram quantities from pheromone gland extracts, so that the use of Kovats indexes, in combination with mass spectrometry can provide substantial information about the structures. This paper reports Kovats Retention Indices of 49 mono- and di-unsaturated alcohols, aldehydes, and acetates with 18 carbon chains, measured on non polar (EC-5) and polar (Wax) stationary phases.

Materials and methods
The compounds were obtained from the PheroBank. Mixtures of the each compound were co-injected with C7-C27 hydrocarbons and analysed by GC-MS (HP 5890 GC and HP 5973 mass selective detector) in EI mode (70 eV). The GC-MS was equipped with a EC-5 or AT-Wax column (30 m x 0.25 mm ID x 0.25 um film thickness), temperature programme 100°C for one minute, then at 10°C/min to 250°C and hold for five minutes and helium as carrier gas.

Results
Retention Indices (KI) were calculated using temperature programmed GC. The mono-unsaturated alcohols (C18:OH) and acetates (C18:OAc) show different order of elution. On non polar EC-5 the mono-unsaturated alcohols eluted before the acetates (Tables 1 and 2). On AT-Wax the elution order is acetate before alcohol.

Table 1. KI Values for octadecenols on AT-Wax and EC-5 columns

Compound	KI AT-Wax	KI EC-5
E11:18OH	2118.3	2072.8
Z11:18OH	2121.0	2070.1
E13:18OH	2124.9	2076.0
Z13:18OH	2134.0	2078.0
E15:18OH	2134.0	2081.2
Z15:18OH	2150.3	2087.4
E9:18OH	2114.2	2068.5
Z9:18OH	2115.7	2066.4
Z2:18OH	2144.6	2085.9
Z3:18OH	2115.5	2073.3
E3:18OH	2089.3	2065.4
E2:18OH	2146.4	2078.0

Table 2. KI Values for octadecenyl acetates on AT-Wax and EC-5 columns

Compound	KI AT-Wax	KI EC-5
E11:18OAc	2037.8	2196.7
Z11:18OAc	2041.0	2193.4
E13:18OAc	2045.3	2200.0
Z13:18OAc	2054.0	2200.0
E15:18OAc	2054.0	2196.8
Z15:18OAc	2070.3	2212.4
E9:18OAc	2032.8	2191.8
Z9:18OAc	2031.5	2188.5
Z2:18OAc	2036.1	2200.0
Z3:18OAc	2030.3	2190.1
E3:18OAc	2026.8	2191.8
E2:18OAc	2065.3	2206.6

Di-unsaturated alcohol 2,13-C18:OH eluted before the 3,13-(C18:OH) in both columns (Table 3). On EC-5 column the isomers EZ-2,13:18OH and ZE-2,13:18OH show the same KI values (2076.4), and similarly ZE-3,13:18OH and ZZ-3,13:18OH (2065.4) (Table 3). Some di-unsaturated aldehydes 2,13-C18:AL and 3,13-C18:AL isomers show overlapping KI values (Table 4).

Table 3. KI Values for octadecadienols on AT-Wax and EC-5 columns

Compound	KI AT-Wax	KI EC-5
EE-2,13:18OH	2181.4	2074.9
EZ-2,13:18OH	2191.1	2076.4
ZE-2,13:18OH	2186.0	2076.4
ZZ-2,13:18OH	2192.3	2078.0
EE-3,13:18OH	2124.9	2055.9
EZ-3,13:18OH	2133.6	2057.5
ZE-3,13:18OH	2151.6	2065.4
ZZ-3,13:18OH	2161.3	2065.4

Table 4. KI Values for octadecadienals on AT-Wax and EC-5 columns

Compound	KI AT-Wwax	KI EC-5
EE-2,13:18AL	2056.5	2077.7
EZ-2,13:18AL	2065.3	2080.9
ZE-2,13:18AL	2056.5	2078.0
ZZ-2,13:18AL	2070.3	2082.2
EE-3,13:18AL	2053.5	2000.0
ZZ-3,13:18AL	2064.0	2000.0
ZE-313:18AL	2052.8	2000.0
EZ-3,13:18AL	2065.3	2000.0