# Analysis of the Sex Pheromones of
*Symmetrischema tangolias* and *Scrobipalpuloides absoluta*

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# Analysis of the Sex Pheromones of *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*

(with a summary in English)

(met een samenvatting in het Nederlands)

(con un resumen en Español)

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
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**Wageningen**

The research described in this thesis was part of the research program of the DLO Research Institute for Plant Protection (IPO-DLO), Wageningen. The work was a concerted effort of IPO-DLO, the Department of Organic Chemistry of the Wageningen Agricultural University (OC-WAU) and the International Potato Center (CIP), Lima, Peru. The project was financially supported by the Netherlands' Minister for Development Co-operation (DGIS).

# Stellingen

1. De DMDS-methode is geschikter voor het identificeren van seksferomonen dan de partiële reductie methode.

   Attygalle, A.B., Jham, G.N., Svatoš, A., Frighetto, R.T.S., Meinwald, J., Vilela, E.F., Ferrara, F.A. and Uchôa-Fernandes, M.A. 1995. *Tetrahedron Lett.*, 36, 5471-5474.
   Svatoš, A., Attygale, A.B., Jham, G.N., Frighetto, R.T.S., Vilela, E.F., Šaman, D. and Meinwald, J. 1996. *J. Chem. Ecol.*, 22, 787-800.

2. Het is onwaarschijnlijk dat de ratio van geëmitteerde seksferomooncomponenten gedurende het 'roepen' van het vrouwtje, waarbij er sprake is van een actief transport van deze verbindingen veranderd, als gevolg van het onderlinge verschil in vluchtigheid van die seksferomooncomponenten zoals Hunt *et al.* suggereert.

   Hunt, R.E. and Haynes, K.F. 1990. *J. Insect. Physiol.*, 36, 769-774.

3. Bij het onderling vergelijken van verschillende verbindingen in windtunnels en bij het maken van EAG's wordt er onvoldoende rekening gehouden met het verschil in vluchtigheid van deze verbindingen.

4. Dat het injecteren van intacte seksferomoonklieren via een 'solid-phase' GC-injector de levensduur van de kolom ten goede zal komen, zoals Attygalle *et al.* beweert, mag betwijfeld worden.

   Attygalle, A.B., Herrig, M., Vostrowsky, O. and Bestmann, H.J. 1987. *J. Chem. Ecol.*, 13, 1299-1311.

5. Het bepalen van de effectiviteit van een verwarringstechniek aan de hand van het aantal gevangen insecten in vallen met hetzelfde seksferomoon dat gebruikt wordt om te verwarren, kan leiden tot verkeerde conclusies.

   Tsai, R.S. and Chow, Y.S. 1992. *J. of the Agriculture Association of China, New Series No. 157*, 76-80.

6. Povolný concludeert ten onrechte dat *Scrobipalpuloides absoluta* een insect is dat hoog in de bergen leeft.

   Povolný, D. 1975. *Acta Univ. Agric. (Brno)*, II, 379-393.

7. Je moet ook van een mot geen olifant maken.

   Rasmussen, L.E.L., Lee, T.D., Roelofs, W.L., Zhang, A. and Daves Jr, G.D. 1996, *Nature*, 379, 684.

8. Goed kunnen tellen, is een vereiste bij het ontwikkelen van seksferomoonsyntheseroutes voor lepidoptera.

9. Het stoppen van de kinderbijslag is beter voor het milieu dan de verhoging van de brandstofaccijns.

10. Omdat de uitslag van een referendum in kan gaan tegen het beleid van de democratisch gekozen regering, kan men zich afvragen of een referendum wel zo democratisch is.

11. De verkeersveiligheid zou er bij gebaat zijn om naast de te-hard rijders ook te-langzaam rijders te bestraffen.

12. Vegetarisme is een luxe.

13. Jim Davis geeft in zijn strip Garfield een goede karakterisering van het verschil tussen honden en katten.

14. Dankzij het broeikaseffect zitten we momenteel niet in een nieuwe ijstijd.

15. Zoals het CPU'tje thuis tikt, tikt het nergens.

Stellingen behorende bij het proefschrift
"**Analysis of the Sex Pheromones of**
***Symmetrischema tangolias* and *Scrobipalpuloides absoluta***"

Wageningen, 6 november 1996
Frans C. Griepink

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## Voorwoord

Het boekje dat voor u ligt, is het uiteindelijke resultaat van ruim vier jaar werk, uitgevoerd op de vakgroep voor Organische Chemie van de Landbouwuniversiteit, Wageningen (OC-WAU), het Instituut voor Planteziektenkundig Onderzoek, Wageningen (IPO-DLO) en het International Potato Center, Lima, Peru (CIP).

Het beschreven onderzoek aan insectenferomonen is een combinatie van chemie enerzijds, en biologie anderzijds. Het samenbrengen van deze twee totaal verschillende expertises was een bijna op zichzelf staand deel van dit project, dat overigens niet alleen plaatsvond op het onderzoeksvlak maar eveneens op het organisatorische en overlegtechnische vlak. Om u een indruk te geven van de initiële benadering van het op te lossen probleem door de beide betrokken Wageningse groepen het volgende.
Vakgroep Organische Chemie: "Dan gaan we dus wat van die beestjes uitknijpen om vervolgens uitgebreid te gaan kijken naar de toe te passen chemische analysemethoden om de structuur van de seksferomonen op te helderen. Nadat we ook de chemische aspecten en de synthese ervan grondig hebben bekeken, kunnen we (oh ja) ook nog 'eventjes' kijken of het werkt in het veld."
Het IPO-DLO dacht er aanvankelijk het volgende van: "Na uitvoerig onderzoek aan de biologie en fysiologie van de beestjes zullen we met wat technieken de structuur van de seksferomonen 'eventjes' ophelderen. Die seksferomonen zullen we dan 'eventjes' gaan maken om ze vervolgens te kunnen gebruiken in uitgebreid gedragsonderzoek en veldwerk."
CIP: "Waar blijven die feromonen nou toch?"
Bovenstaande voorstelling is te simplistisch weergegeven. Desalniettemin geeft ze een globaal beeld van de verschillende invalshoeken waarmee diverse onderzoeksdisciplines een probleem kunnen beoordelen. De vier jaar die dit project duurde, hebben geleerd dat er geen 'eventjes' bestaat in onderzoek en dat slechts door nauwe samenwerking tussen de genoemde groepen het complexe en disciplineoverschrijdende vraagstuk zoals beschreven in de titel van dit proefschrift, kan worden opgelost.

Het gebruik van het woord 'groepen' in het voorgaande impliceert al dat dit onderzoek niet het resultaat is geweest van de inspanningen van een enkel individu maar dat van een groter gezelschap van mensen van wie ik er een aantal graag speciaal zou willen bedanken.
Prof. dr. Aede de Groot, dr. Teris van Beek, dr. Hans Visser en drs. Simon Voerman, voor de mogelijkheid die jullie me hebben gegeven om dit onderzoek te kunnen doen. Teris en Hans, vanwege jullie directe betrokkenheid bij het onderzoek en de daarbij behorende discussies hebben jullie in belangrijke mate bijgedragen aan het behaalde resultaat.
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Frank Claassen voor het synthetiseren van de eerste (en nog steeds de lastigst te synthetiseren) feromooncomponent.
Sander Houweling voor de dappere poging die hij gedaan heeft om de concentraties van feromonen in de lucht te meten.
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Dr. Romano Orru voor zijn hulp bij het synthetiseren van die ene lastige feromooncomponent waarbij hij mocht ondervinden dat die op het oog simpele seksferomonen soms toch lastig in elkaar te knutselen zijn.
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Dr. Stefan Schulz and Prof. dr. Wittko Francke of the University of Hamburg who taught me to make dimethyldisulphide derivatives of sex pheromone compounds.
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En als laatste natuurlijk niet te vergeten de kornuiten en kornuitinnen met wie ik mij altijd goed kon onderhouden in het ons bekende etablissement onder het genot van een lekker glaasje.

Frans

Wijk bij Duurstede, 12 september 1996

*voor Marianne*

*voor mijn ouders, Els Verhaar en Henk Griepink*

# Chapter 1

## General introduction

### 1.1 Insects and pests

With more than a million species, insects are by far the most abundant form, in numbers, of animal life[1]. From the coldness of the polar snow caps to the heat of the deserts, and from the aridity of the salt lakes to the dampness of the jungle, insects survive everywhere. An ability to fly, a short life cycle, and a high reproductive rate are the foundations for them becoming the most abundant form of animal life known.

Many insect species are herbivorous and when they feed on plants meant for human purposes, they become potential threats. In those cases where a significant part of the harvest is lost, insects are considered a pest and must be destroyed, or at least controlled. Some common known pest species are, for example, the migratory locust (*Locusta migratoria*), and the Colorado potato beetle (*Leptinotarsa decemlineata*). These insects are pests because they actually eat the plants. Other insects are considered pests not because they eat the plant, but mainly because they, or their immature stages, are the vector for viruses, fungi or bacteria which do the actual damage. Examples are aphids and thrips, which transmit viruses, or certain species of bark beetles which employ the fungus *Ceratostomella ulmi* which is responsible for the Dutch Elm disease.

At present, mankind is incapable of controlling many pest insects in a way that is effective and also sustainable and compatible with the environment. Insects evolved long before higher animals and man appeared on earth. Whatever ended the era of the dinosaurs apparently did not stop the occurrence of the insects. Therefore, it is likely to assume that humanity can be considered more capable of destroying higher organisms and itself, before exterminating a single pest insect species.

### 1.2 Butterflies and moths

Butterflies and moths belong to the Class Insecta and to the Order Lepidoptera. Various species have been reported to be crop pests. Whereas most (coloured) butterflies fly

during the daytime, most (non-coloured) moths restrict their activities to the night. Because they live in the dark, there is no need for them to carry excessive colours. Instead, moths have evolved a remarkable way of recognising and locating each other. In moths females usually, but sometimes males, release a blend of volatile chemicals which is detected and recognised by conspecific members of the opposite sex. The partner is specifically attracted to this special volatile chemical blend. Through this mechanism, the probability of mating is highly increased, and thereby the existence of the species is secured.

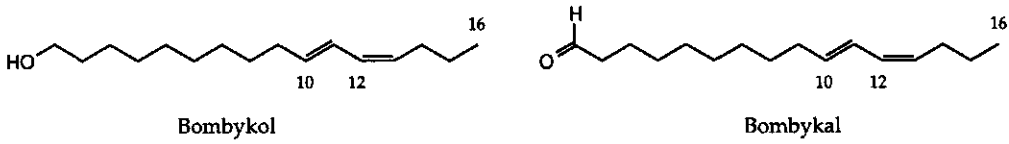
## 1.3 Lepidopteran sex pheromones

### 1.3.1 General

The word pheromone is a contraction of the Greek words *'pherein'*, which means to transfer and *'hormōn'*, which means to excite. Pheromones are defined as substances, which are secreted to the outside by an individual and when perceived by a second individual of the same species, they trigger a specific response[2]. Several types like alarm, trail and aggregation pheromones are known to exist for insects. When a *pheromone* is released with the intention of attracting members of the opposite sex for mating, it is called a *sex pheromone.* In moths, most sex pheromones are released by females to attract conspecific males. In some primitive moth species, males, or both the males and females release a sex pheromone[3].

To date, sex pheromones have been characterised for more than 400 species and subspecies of Lepidoptera[4,5]. In addition, for over 900 other species and subspecies, male sex attractants have been found. The latter are called sex attractants, because although they might be strongly attractive to the males of a particular species, there is no proof that the individual compounds are actually released by the females. In this thesis, a sex pheromone is defined as the mixture of chemical compounds, proven to be present in the females, which is the most attractive to conspecific males in the natural habitat of the insect. It is assumed that the female moth does not release other chemical compounds as part of the sex pheromone than those which are present in her sex pheromone gland.
The first insect sex pheromone was isolated and identified in 1959 by Butenandt[6]. He and his co-workers extracted and purified about 12 milligrams of a, to the males, highly attractive compound from 500,000 females of the oriental silk moth (*Bombyx mori*) . They identified this compound as (E,Z)-10,12-hexadecadienol (Bombykol) (figure 1.1). In these early pioneering years it was never considered that a sex pheromone might consist of

more than one compound. Later it became obvious that multiple component pheromones were more a rule than an exception. In 1978 it was discovered that sex pheromone gland extracts of *Bombyx mori* contained, in addition, the corresponding aldehyde of Bombykol, namely Bombykal (figure 1.1), which was part of the sex pheromone[7].




Bombykol Bombykal

***Fig. 1.1*** **Sex pheromone components of *Bombyx mori*, (E,Z)-10,12-hexadecadienol (Bombykol) and (E,Z)-10,12-hexadecadienal (Bombykal).**

Male moths are extremely sensitive to their sex pheromones. For example, amounts of less than 10 pg ($10^{-11}$ gram) of the sex pheromone of *Bombyx mori* when offered on a piece of filter paper to the males elicit a behavioural response[8]. Other research shows that male moths are able to detect and to respond to sex pheromone concentrations as low as picograms per litre of air. Experiments have been carried out with *Adoxophyes orana*, marked with radioactive $^{32}P$, to determine the distance over which these moths were able to locate a source with virgin females. It turned out that the males were able to locate the females over a distance of 75 metre in just one night. Measured over several nights, males were even capable of reaching sources that were several hundreds of metres away[9].

The individual components that occur in a sex pheromone are not necessarily chemically specific for a single moth species, however, in practice females of one species attract and mate only with males of the same species. One of the reasons is that the correct ratio between the different components of the sex pheromone blend is also an important factor for the attractiveness[10]. To show this, a comparison has been made of 34 species with the same two-component attractant/sex pheromone system, namely (Z)-9- and (Z)-11-tetradecenyl acetate (figure 1.2). A 3 : 1 mixture of (Z)-9-tetradecenyl acetate and (Z)-11-tetradecenyl acetate, for example, is attractive to *Adoxophyes orana* but not to *Clepsis spectrana*. If the ratio of these two components is inverted, the attractiveness for *Clepsis spectrana* and *Adoxophyes orana* is reversed as well[11]. Several species as indicated in figure 1.2 use the same, or almost the same, ratio of individual components. Because of this, and considering the fact that the ratio of sex pheromone components always shows variation from individual to individual[12-14], it could be expected that certain species respond to other species. In practice this does not happen because these species are separated geographically, or their activity differs in the time of the season or time of the day[15,16].

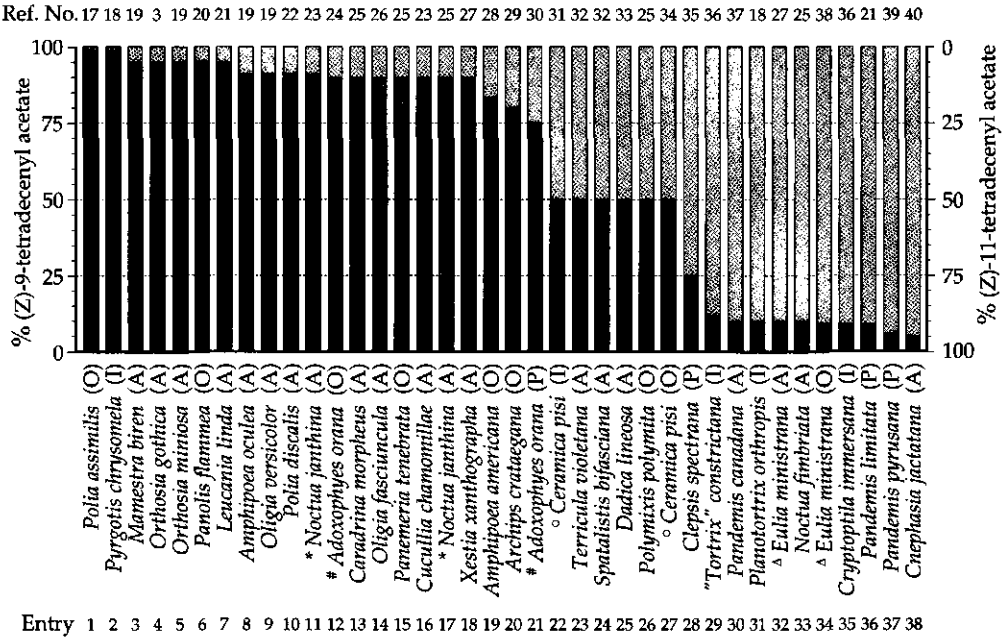



*Fig. 1.2* **The same two pheromone compounds in a two-component attractant/sex pheromone system for 34 species. (A) attractant, (I) chemical identification only, no behavioural tests, (O) optimised attractant, (P) sex pheromone.**

Some of the references which were used for figure 1.2 are rather old. It is possible that the sex pheromone contained more than just these two components, but due to the less sensitive analytical equipment, minute, but biologically important, components may have been overlooked (see for an example[41]). Moreover, the actual recognition and acceptance occurs at the moment when males and females approach each other very closely, and probably also by other means than just sex pheromone recognition. It can be seen from figure 1.2, that the identified sex pheromone composition in the insect sex pheromone gland is not inevitably the most attractive blend (for example, entry 12 and 21). A reason for this may be that a long-maintained laboratory colony has altered the sex pheromone composition slightly, but biologically significant when compared to the wild species[42]. Sex pheromone producing glands may probably contain antagonists as well, meant to repel other species, and precursors, which are not released as part of the sex pheromone blend. This must all be kept in mind when examining insects and their sex pheromones.

Up to now, all the identified sex pheromones and attractants for Lepidopteran are compounds with a linear carbon chain with lengths varying from 10 to 23 carbons. The

sex pheromones originate from the fatty acid biosynthesis. Therefore, most of them have an even number of carbon atoms in the chain, however, exceptions are known. For example the moths *Phthorimaea operculella*[43] and *Keiferia lycopersicella*[44], which both are closely related to *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*, have sex pheromones with chain lengths of 13 carbon atoms. The majority of lepidopteran sex pheromones have an acetate as terminal functional group, nevertheless alcohols, aldehydes, and occasionally formates, propionates, (iso)butyrates, and (iso)valerates have been found. In one insect, *Bucculatrix thurberiella*, a nitrate ester was identified as the terminal functional group[45]. The chain itself may contain zero to four double bonds, triple bonds, (chiral) methyl groups, ketones or (chiral) epoxides[4]. To date, only non-branched straight chain compounds with a length of 10 to 16 carbons, zero to three double bonds and an alcohol, acetate or aldehyde as functional groups, have been identified as sex pheromones or sex attractants in Gelechiidae (table 1.1)[4].

*Table 1.1* **All the sex pheromones, and related structures which have been identified for members of the Gelechiidae family[4]. (A) attractant, (I) chemical identification only, (O) optimised attractant, (C) possible attractant, (P) sex pheromone. (× no. of publications)**

| chain length | 10 | | 11 | 12 | 13 | 14 | 16 | | |
|---|---|---|---|---|---|---|---|---|---|
| functional group | OH | Ac | Ac | Ac | Ac | Ac | Ald | OH | Ac |
| saturated | | 1×P | | | | | | | |
| (E)-3- | | | | 2×P, 2×A | 1×P | 2×A | | | |
| (Z)-3- | | | | 1×P, 1×O, 1×A | | 1×O, 5×A | | | |
| (E)-4- | | 1×P | | | 1×P | | | | |
| (Z)-4- | | 1×P | | | 1×A | | | | |
| (E)-5- | 1×P | 1×P | | | | 1×A | | | |
| (Z)-5- | | | | 5×A | | 1×A | | | |
| (E)-7- | | | | 2×A | | 1×A | | | |
| (Z)-7- | | 3×A | | 2×A | | 1×A | | | 1×A |
| (E)-8- | | | | | 1×A | | | | |
| (Z)-8- | | | 1×A | | | | | | |
| (E)-9- | | | | | 3×A | 1×C, 1×A | | | |
| (Z)-9- | | | | | | 2×A | | | |
| (E)-10- | | | | 1×C | | | | | |
| (E)-11- | | | | | | 1×C, 1×A | | | 1×P |
| (Z)-11- | | | | | | 1×A | | | |
| (E,E)-3,5- | | 1×P | | | | | | | |
| (E,Z)-3,5- | | 1×P | | 1×A | | 1×A | | | |
| (Z,E)-3,5- | | | | 1×A | | | | | |
| (E,Z)-4,7- | | | | | 1×P | | | | |
| (E,Z,Z)-4,7,10- | | | | | 1×P | | | | |
| (Z,E)-7,11- | | | | | | | 1×O | 1×P | 2×P, 1×O, 3×A |
| (Z,Z)-7,11- | | | | | | | | 1×P | 1×P, 6×A |

### 1.3.2 Isolation techniques

There are two ways to collect sex pheromones from an insect: 1) by extracting (part of) the insect with a suitable solvent like hexane or dichloromethane, or 2) by collecting airborne volatile compounds from (part of) the insects onto a suitable adsorbent like Porapak Q, Tenax, activated charcoal, or directly onto the column of a gas chromatograph. The second approach gives less chance of degradation, however, the method is limited by the amount of sex pheromone that is released by individual insects. The latter is species dependent and varies from approximately 5 to 160 ng/hr[46,47]. The extraction of sex pheromone glands is easier to scale up, however, with this approach much non-relevant material is co-extracted and it is not always apparent which compound is actually part of the sex pheromone. Sex pheromone gland extracts are examined directly or can be subjected to purification first. Purification can be done by column chromatography, high pressure liquid chromatography (HPLC) or preparative gas chromatography. A more or less combined method involves the direct introduction of the sex pheromone gland into the gas chromatograph (GC). The intact sex pheromone gland is heated in the GC which causes the volatile compounds to evaporate. They are then focused at the start of the GC column (see for details about this approach, chapter 4).

### 1.3.3 Identification techniques

The GC is an excellent, sometimes underestimated, tool for the analysis of complex mixtures of volatile compounds, like insect sex pheromones in a sex pheromone gland extract. GC analysis is very sensitive. Amounts of less than one nanogram can be detected with the commonly used flame ionisation detector (FI detector or FID). The GC is able to separate complex mixtures into the individual components. The retention time for a particular compound depends on the type of column that is used. The retention times obtained are often converted into their retention indices (RI's) by comparing them to a standard range of alkanes thereby improving the accuracy[48]. The comparison of the calculated RI's of the sex pheromone compounds with those calculated for reference compounds on several columns, provides information about the length of the carbon chain, the presence and number of double bonds, sometimes even the position and configuration of double bonds, and the functional group present, like an alcohol, aldehyde, acetate, etc. If more than one double bond is present in the molecule, it is possible to determine whether some, or all, double bonds are conjugated. In case the sample is very complex, or contaminated, it is possible to resolve the individual peaks by using a two-dimensional GC (2D-GC) made up by two interconnected GC's. Instead of

the normally used FID, other detection or analytical techniques can be applied on-line with the GC, such as an electroantennographic detector (GC-EAD), a mass spectrometer (GC-MS) or a Fourier transform infrared spectrometer (GC-FT-IR).

Electroantennography (EAG) is a technique which relies upon the specificity and sensitivity of the olfactory system of the insect, the set of olfactory receptors on the antenna. In moths, the antenna is covered with thousands of sensory sensilla, each of which contains two or more sensory neurones, sensitive to particular compounds or to a group of chemically related compounds. A neurone recognises a particular molecule through its binding with a receptor protein in the dendritic membrane. The subsequent depolarisation, the receptor potential, causes the neurone to fire action potentials, which are transmitted to the brain. Part of the receptor potential leaks into the haemolymph of the antenna and it is thought that the sum of these leaking receptor potentials is measured with EAG[49]. EAG is restricted to the observation whether or not an insect is able to detect a particular compound and in what intensity. The effect of a perceived compound on the behaviour of the insect has to be determined by other methods. For an electroantennogram, the antenna from a male moth is cut off and usually connected to glass electrodes filled with electrolyte. The electrodes are connected to an amplifier and recording equipment[50]. A continuous air-flow is blown over the antenna to which a sample of an extract or a reference compound is added for a short moment. When the EAG technique is used as the detector of a GC, the retention times (or retention time intervals) are measured of the compounds which are EAG-active. These compounds are physiologically perceived by the insect and thus, are sex pheromone candidates. By duplicating the experimental conditions of the GC-EAD to the GC-MS, mass spectra are acquired of the compounds that have proven to be perceived by the insect. In this way, the molecular mass and elemental composition of the sex pheromone candidate are obtained. The configuration of the double bonds can be determined in various ways. When the sex pheromone candidate is only mono-unsaturated, the comparison of the RI's with those calculated for reference compounds is usually sufficient. However, if more than one double bond is present in the molecule, it becomes more difficult to determine the position and configuration just by comparison of RI's. If the double bonds are separated by at least two methylene groups, the EAG measurements of all mono-unsaturated reference compounds can provide useful information about the position and the configuration of the double bonds (see also chapter 3). If the double bonds in the sex pheromone are conjugated, or homo-conjugated (separated by zero or one methylene group), the EAG measurements give no unambiguous results (see also chapter 5). Another approach for the determination of the double bond positions is to derive the sex pheromone compounds with dimethyl disulphide (DMDS) and subsequent analyse the obtained derivatives with MS (chapter 2). It is also possible to partially reduce the sex

pheromone compound and analyse the obtained mono-unsaturated compounds[51-53]. The configuration of double bonds in a sex pheromone compound can also be deduced from Fourier transform gas phase infrared (FT-IR) spectroscopy[52,54,55]. This technique was not available for the research described in this thesis. If enough pure material is isolated, this can be examined with nuclear magnetic resonance (NMR)[56-58]. NMR is a powerful analytical technique which provides information about the status of the hydrogen atoms in the molecule. Because of the low sensitivity of the NMR equipment, this technique is not always useful for sex pheromone analysis (in practice, tens of micrograms of pure compound are needed). The most straightforward, and most labour-intensive, approach to determine the double bond configuration of the sex pheromone, is to synthesise all the possible structural candidates (see also chapter 5). The ultimate stage in the identification of the sex pheromone, is to determine whether the identified and synthesised molecules really are capable of attracting male moths. With the bioassays this, and the (optimal) ratio of the identified compounds is determined. For this research, the bioassays were carried out in the wind tunnel of the IPO-DLO (chapter 6) and in fields and storehouses in Peru.

### 1.3.4 Chemical synthesis

It is essential to confirm the analytical results by synthesis of the (tentatively) identified compounds. For this, so-called, analytical synthesis, only small amounts of products are needed. Normally in synthesis, stereoselective reactions are preferred which produce only one (E/Z) isomer per reaction step. Nevertheless, if the E/Z configuration of the target sex pheromone molecule is not yet clear, it might be advantageous to use a non-stereoselective step to produce both isomers in one step. Of course, the product mixtures should not exceed the level of complexity where the different components can no longer be separated. The cost of reagents does not have the first priority when synthesising on an analytical scale. This changes when the structure elucidation of the sex pheromone is completed. By that time there will be a demand for gram quantities of the sex pheromone, for example to start field tests or for sales. Then, the emphasis will be on cost control. Effective exploitation of human resources, the number of synthetic steps, and the price of reactants must be optimised in relation to the quality so that the product sales are most profitable. The greater part of the chemical reactions needed in the synthesis of sex pheromones are relatively simple and easy to scale up[59-61]. Unfortunately, most chemical reactions are not as stereoselective as we would wish, therefore always a few percent of undesired isomers will be present in the product. When it appears that the contaminations are deteriorating the effect of the sex pheromone, extensive purification of

the final product is necessary. This is usually done on a silver-loaded ion-exchange chromatographic column[62]. After such a purification step the final product could have an (isomeric) purity of more than 99%.

## 1.4 Insect control

### 1.4.1 Pesticides versus alternative control methods

Today, it has been recognised that the use of pesticides is not the all-comprehending answer to the problem of insect pests as it was once thought to be. Persistent pesticides accumulate in non-target animals higher in the food-chain. Insects seem to become resistant faster than new pesticides can be developed (this includes the time needed for registration procedures). In third-world countries, resistance develops faster compared to first-world countries, because of the thoughtless and improper use of agro-chemicals. For example, farmers using herbicides or fungicides against insect pests have been encountered in Peru. In contrast to the large scale tomato farming, the growing of potatoes in Peru is mostly restricted to farmers having one hectare of ground or often less. If a farmer is using pesticides for example and his neighbours are not, it turns out that the pests simply move to the neighbour's land. Surviving insects find there an untouched source of food to recover on. In tomato cultivations this problem exists less because this type of farming is done at a much larger scale. In these cases the large scale monoculture is the problem. Such cultures are known to promote the development of pests. The International Potato Center (CIP) in Peru has been working on alternative ways of controlling different insect pests, like developing plant resistance, pre- and post-harvest management for crop and seed-potatoes, biological control and the use of sex pheromones[63]. For the potato moth *Phthorimaea operculella*, an effective biological control has been developed by means of the *Phthorimaea Baculovirus* which is added to the stored potatoes[64]. When larvae eat the potatoes they get infected with the virus and will subsequently die. The disadvantage of this type of pest management is that infested larvae live for another 12 - 21 days and thus, still cause considerable damage to the stored potatoes. The same problem occurs when, for example, parasitoids are used as control agent. For *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*, one has tried to develop similar strategies. It seems, however, that neither of these moth species is very sensitive to the methods developed so far.

### 1.4.2 Sex pheromones in pest control

In contrast to pesticides, sex pheromones are substances that are produced and used by insects themselves. Therefore, it is unlikely that resistance against them will develop. When sex pheromones are chemically identified and available, they can be used in pest control in four different ways: (1) monitoring, (2) mass trapping, (3) mating disruption[65] and (4) the attraction and subsequent killing of the insects without trapping them, known as attract-and-kill.

Monitoring is the most common use of pheromones. As a monitoring tool, sex pheromones are used to attract exclusively the species of interest and, therefore, provides data about the presence and abundance of the insect pest. The appropriate time for pesticide application can be calculated, so that pesticides will only be used at the moment when they are most effective and needed.

The second way in which sex pheromones can be used is mass trapping. This method is not used very often, especially not in first-world countries. One reason for this is that mass trapping is less thorough than the application of pesticides. Another reason is that the application of sex pheromones for mass trapping is a rather time consuming way of controlling a pest because one needs a lot of traps which have to be installed and maintained. In first-world countries where labour is expensive, the use of sex pheromones in mass trapping is commercially conceivable only in few cases.

The third approach is mating disruption. Here, the sex pheromone is applied in such high concentrations onto the crop or in storehouses that the male pest insects are no longer able to locate the female insects. In this way, no copulation will occur and, as a consequence, no new offspring will develop. This method has advantages over mass trapping because it is relatively easy-to-use. In practice however, there are still few cases where mating disruption has shown to be of practical value in pest control[66]. Not all insects are sensitive to this method and insect sex pheromones are often too expensive for the application as mating disruptant. Another important cause is the commitment to register the sex pheromones in many countries before they may be applied for mating disruption[67], which is an expensive and time consuming procedure.

The fourth method which involves insect sex pheromones in the control of insect pests was developed as "Attract and Kill"[68]. The sex pheromone is formulated into a glue-like liquid UV-absorber (for light protection) with a small amount of a very potent insecticide. It is applied in droplets onto the plants that have to be protected. The male insect is attracted to the sex pheromone, touches the source and picks up some of the glue together with a (sub)lethal dose of the insecticide. If such a male copulates with a female later on, there is a good chance that she is poisoned as well. This method is used with success, for

example, against *Pectinophora gossypiella* in cotton fields in Egypt[69] and against *Ephestia kuehniella* in flour mills in Italy[70].

In developing countries, the newer, expensive pesticides are not always available. Because the threshold for damage is much higher than in first-world countries, and the costs of labour are much lower, the application of sex pheromones in pest control programs could be a solution. Sex pheromones are already used in the control of some insect pest species in third-world countries. One established example is the use of the sex pheromone of *Phthorimaea operculella*, which was identified in 1976 by Persoons et al.[43]. The IPO-DLO synthesises this sex pheromone on a commercial scale[71]. This sex pheromone has been applied in Peru, Venezuela and Tunisia for years with great success in mass trapping of *Phthorimaea operculella*[72]. It appears to be cheaper and more effective than the formerly used pesticides.

## 1.5 *Symmetrischema tangolias*

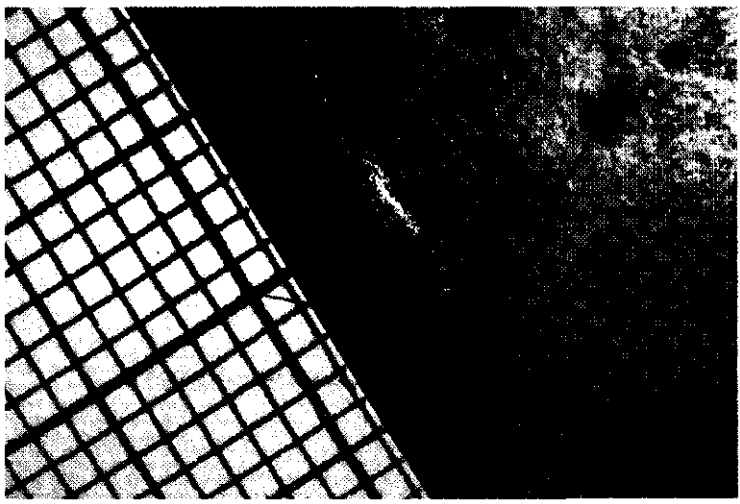


***Fig. 1.3***
**Photographic image of *Symmetrischema tangolias* on the surface of a potato tuber. The millimetre paper at the lower left side of the picture gives an impression of the insect's dimensions.**

### 1.5.1 Nomenclature

The moth *Symmetrischema tangolias* (Gyen) (figure 1.3) was described for the first time as *Phthorimaea plaesiosema* by Turner in 1919[73]. Several synonyms for this moth have been used since: *Phthorimaea melanoplintha* and *Gnorimoschema tuberosella*[74]. The most commonly used name for this moth has been *Symmetrischema plaesiosema* (Turner)[75]. In 1990, Hodges noticed that *Symmetrischema plaesiosema* had already been described as *Symmetrischema*

*tangolias* by Gyen and, therefore, changed the species name from *plaesiosema* to *tangolias*[76]. Until now, the name *Symmetrischema tangolias* is still valid. A specific English name does not exist for this moth but in Peru, it is simply referred to as 'Symmetrischema'. Local farmers in Peru also name this moth: 'la polilla de la papa' (translation: the potato moth), which is confusing because this name is also used for another devastating pest on potatoes, *Phthorimaea operculella*. The latter is closely related to *Symmetrischema tangolias* and occurs in the same regions[77].

### 1.5.2 Biology, occurrence and impact

The potato tuber moth *Symmetrischema tangolias* is a severe pest on potatoes in the field and in storehouses in Peru. In 1952, this moth was described as a potential threat to potato[78], but it was not until 1982 that it became a major pest[79]. The biology of this species has been examined in detail[75]. The total life-cycle is strongly temperature dependent and varies between 40 and 75 days. The pupae of this species are easily separated into males and females by the external characteristics which are shown in figure 1.4. The adults can be sexed by their reproductive organs.

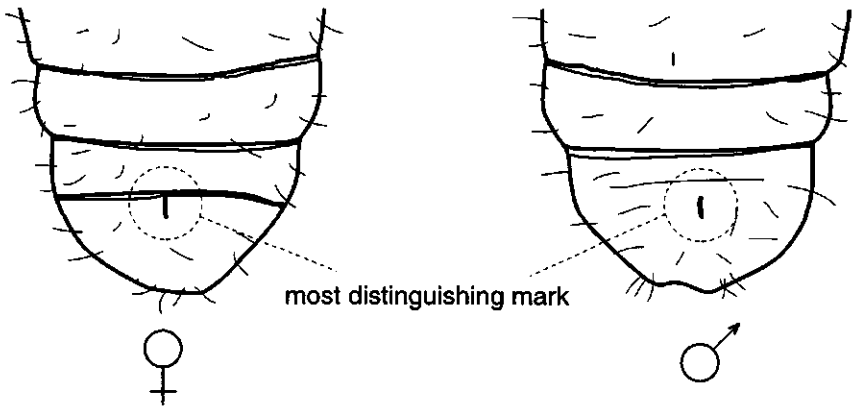



*Fig. 1.4* **External characteristics to distinguish between male and female pupae of *Symmetrischema tangolias*. The last two segments of the male pupae are grown together.**

The main distribution areas for *Symmetrischema tangolias* are the higher regions of the Peruvian Andes[63] and, although this species has been reported in Australia, it seems that it was introduced there rather than being an endemic species[63,80]. In 1993, *Symmetrischema tangolias* appeared in Bolivia for the first time[81]. In the field the larvae bore into the stems

of potato plants, which causes the plants to break and die. In storehouses larvae mine into potato tubers making them unsuitable for human consumption. Nevertheless, infested tubers are often planted, which causes further spread of the pest. In Peru, seed potatoes are generally stored in large storehouses of co-operatives where they are sometimes literally covered with pesticides. Amounts of 1.3 g malathion per kg potatoes have been observed. In the Peruvian Andes, small farmers keep the potatoes indoors or in small open storehouses. These potatoes are not treated with pesticides and are therefore an ideal food for *Symmetrischema tangolias*. Crop losses can reach up to 100%[81]. Today *Symmetrischema tangolias* is considered to be an even greater pest than *Phthorimaea operculella* in Peru[82].

## 1.6 *Scrobipalpuloides absoluta*

### 1.6.1 Nomenclature

The moth *Scrobipalpuloides absoluta* (Meyrick) (figure 1.5) was described by Meyrick in 1917 for the first time as *Phthorimaea absoluta*[83]. Povolný named this species *Scrobipalpula absoluta*[74]. In his paper, he remarked that *Scrobipalpula absoluta* is frequently confused with 'the tomato pinworm' *Keiferia lycopersicella* (Walsingham), which is closely related and sometimes occurs in the same regions as *Scrobipalpula absoluta*[74]. Clarke transferred this species to the genus *Gnorimoschema*[84], however, in 1975 Povolnýchanged the genus name back to *Scrobipalpula*[85]. The present name for this species was established in 1987 by Povolný[86] as *Scrobipalpuloides absoluta*. Povolný indicated that this species differed too much from the genus *Scrobipalpula* and therefore, he placed this species in the genus *Scrobipalpuloides*. The commonly used English name for *Scrobipalpuloides absoluta* is 'tomato leafminer' and the Spanish name for this species is: 'Oruga minadora de hoja y tallo' (translation: leaf and stem mining caterpillar).

### 1.6.2 Biology, occurrence and impact

The tomato leafminer, *Scrobipalpuloides absoluta*, is presently considered the most devastating pest of tomatoes in Peru, Chile, Brazil, Argentina, Bolivia, Venezuela and Colombia[87-97]. It prefers the lower, warmer regions, although the holotype of this species has been collected in Huancayo, 3500 metres above sea level[86]. The biology and occurrence has been studied in many countries[89-97]. The total life cycle is strongly

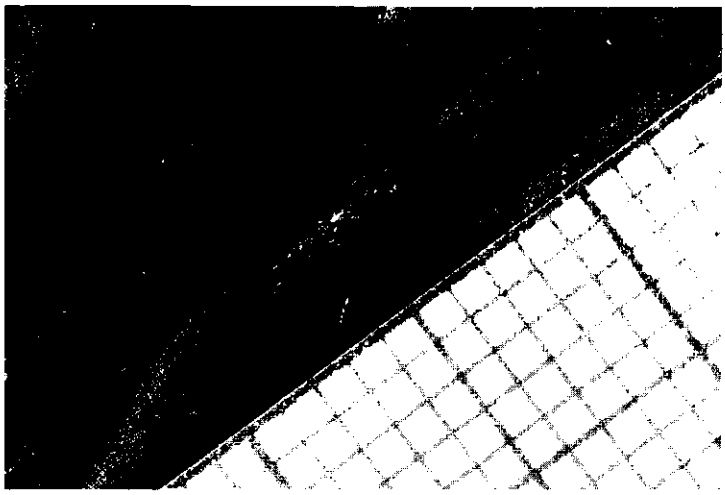


*Fig. 1.5*
**Photographic image of *Scrobipalpuloides absoluta* on the leaf of a tomato plant. The millimetre paper at the lower right side of the picture gives an impression of the insect's dimensions.**

temperature dependent and varies from 20 to 35 days. Adults and pupae of *Scrobipalpuloides absoluta* have the same external characteristics as *Symmetrischema tangolias*, and based on this, they can be separated into males and females. Although it seems that *Scrobipalpuloides absoluta* prefers the tomato plant as its host, it can also develop on several other members in the Solanaceae family, like potato and tobacco[95]. The moth's larvae mine leaves and fruits of tomato plants causing considerable damage. Larvae living inside leaves or fruits are difficult to reach with pesticides. Nevertheless, it seems that this is still the only way to control this pest[98]. *Scrobipalpuloides absoluta* is resistant to organophosphate pesticides in Bolivia and it was established that applying the synthetic pyrethroid, fenvalerate every two weeks, is the most effective way of controlling *Scrobipalpuloides absoluta*[87,88]. In spite of this, farmers in Paraguay often apply pesticides twice every three days[81]. Tomato farming is a large-scale industry in South America. Farms of 150 hectares are common in Peru and in Chile, the co-operatives can reach up to 10,000 hectares[99]. The majority of the tomato crop is processed and exported[99]. This export is essential for these South American countries to obtain foreign currency.

## 1.7 Motivation and scope for this thesis

The outline for the research described in this thesis was formulated when the Centro Internacional de la Papa, Lima, Peru (CIP), together with the Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands decided to write a joint project proposal on the isolation, identification and the application of the sex pheromones of *Symmetrischema*

*tangolias* and *Scrobipalpuloides absoluta*. The Department of Organic Chemistry of the Wageningen Agricultural University (OC-WAU), The Netherlands was willing to act as the third partner in this research project. The project was financially supported by the Netherlands' Minister for Development Co-operation (DGIS).

Moths like *Symmetrischema tangolias* and *Scrobipalpuloides absoluta* have sex pheromones, which might be useful as an alternative way to control these insect pests. The aim of the present study was to isolate, identify and synthesise these sex pheromones and to determine whether the synthetic sex pheromones can be implemented into an integrated pest management (IPM) program with regard to these two pest species. A further aim was to study analytical pathways for the identification of sex pheromones and related compounds.

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# Chapter 2

## Mass spectrometry of dimethyl disulphide derivatives as a tool for the determination of double bond positions in lepidopteran sex pheromones and related compounds*

### 2.1 Introduction

Mass spectrometry is a widely applied technique for the analysis of (volatile) organic molecules. The quality of information obtained, in combination with its sensitivity makes this technique particularly useful for the analysis of volatile straight chain lepidopteran sex pheromone compounds. By linking the mass spectrometer to a gas chromatograph a complex sex pheromone extract can be examined without the need for prior isolation of the individual components. Not only the molecular mass of a sex pheromone component, but information about its functional group and number of double bonds is obtained as well. In cases of methyl-branched, or epoxidized sex pheromones, the position of the methyl group or epoxide can be determined through mass spectrometry (MS) alone[1,2].

Although attempts have been made[4,5], the determination of double bond positions in linear (poly-)unsaturated sex pheromone components and related compounds, without prior derivatisation of the double bonds, is difficult by MS examination alone[3]. The difficulty arises because after eliminating functional groups in the mass spectrometer, the radical sites in the olefins that are formed, migrate freely through the molecule (accompanied by hydrogen rearrangement)[3,6]. Only in cases where molecules possesses two conjugated double bonds, can the positions be deduced from the MS fragmentation of the non-derivatised molecule[7-9]. In these situations the ω-end of the molecule provides two characteristic fragments as illustrated in figure 2.1. This approach can be extrapolated to determine the double bond positions in molecules with three[10] and possibly more conjugated double bonds.

* Parts of this chapter have been published: Griepink, F.C., van Beek, T.A., Visser, J.H., Posthumus, M.A., Voerman, S. and de Groot, Ae. 1996. *Tetrahedron Lett.*, 37, 411-414.

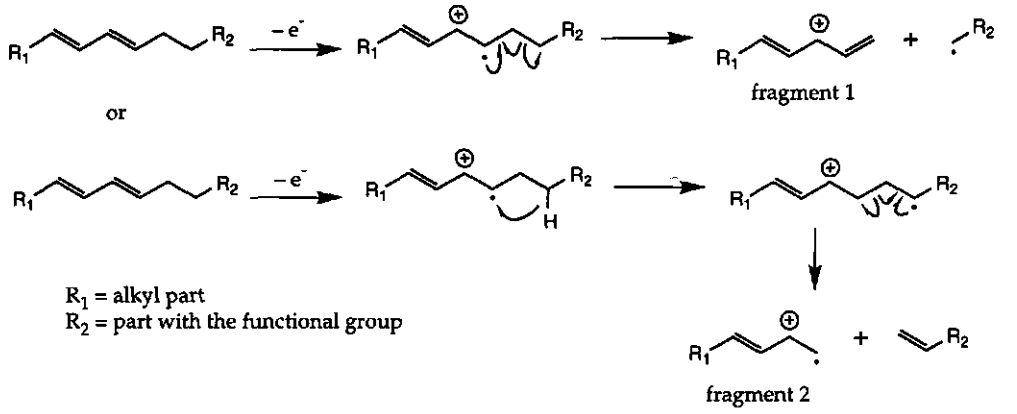


*Fig. 2.1* **Characteristic mass spectrometric fragments that occur for conjugated straight chain molecules.**

A number of procedures have been described for the indirect determination of double bond positions in straight chain unsaturated molecules. Probably the oldest one is to treat the unsaturated molecule with ozone and analyse the obtained aldehyde fragments by GC and MS[11] (figure 2.2).

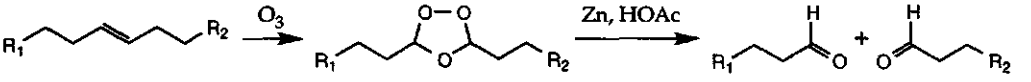


*Fig. 2.2* **Ozonolysis of double bonds prior to analysis.**

Other procedures involve the addition of certain molecules to the double bond(s), to produce a derivative that exhibits a specific mass fragmentation pattern from which the original position(s) of the double bond(s) can be deduced. As mentioned before, the position of an epoxide can be determined directly by MS. A double bond which has reacted with, for example, *m*-chloroperbenzoic acid (*m*-CPBA), and is converted to its corresponding epoxide will fragment next to the epoxide and in this way reveal the

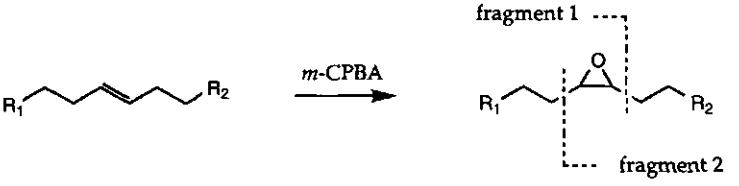


*Fig 2.3* **The conversion of an unsaturated straight chain compound to its epoxide, plus the expected fragments that will form in the mass spectrometer.**

position of the original double bond (figure 2.3)[2]. The epoxides can also be hydrated and converted into their trimethylsilyl ethers before analysis (figure 2.4)[12].

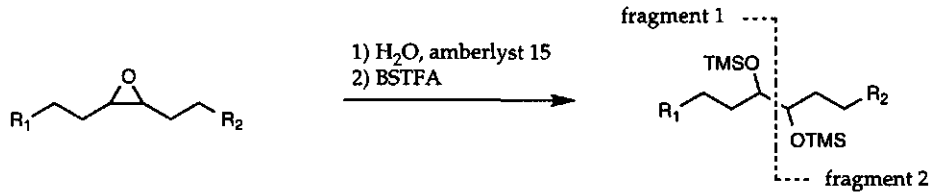


*Fig 2.4* **The hydration of an epoxide and reaction with bis(trimethylsilyl)trifluoroacetamide (BSTFA), plus the expected fragments that will form in the mass spectrometer.**

Methoxylation of the double bonds followed by MS analysis is another established method (figure 2.5)[3].

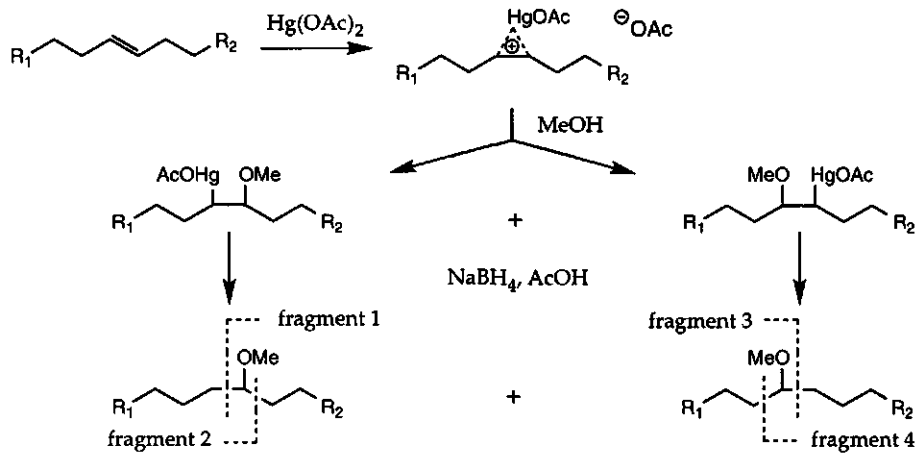


*Fig 2.5* **The reaction of an unsaturated linear compound with mercuric acetate, methanol and sodium borohydride to yield two methoxylated products which will fragment in the mass spectrometer next to the methoxy groups.**

The major drawback of the above mentioned derivatisation procedures is that they require micrograms of starting material. Therefore, they are less suitable for the analysis of insect sex pheromones where often only nanogram quantities are available. A more sensitive approach is to derive the unsaturated molecule with gaseous nitric oxide (NO) inside the mass spectrometer itself, however, this method is restricted to straight chain molecules with a triple or quadruple (cis) homo-conjugated system (figure 2.6)[13]. The position of a double bond close to the aliphatic end of the molecule can be determined

with this approach, however, only the position of that double bond is then determined[14].

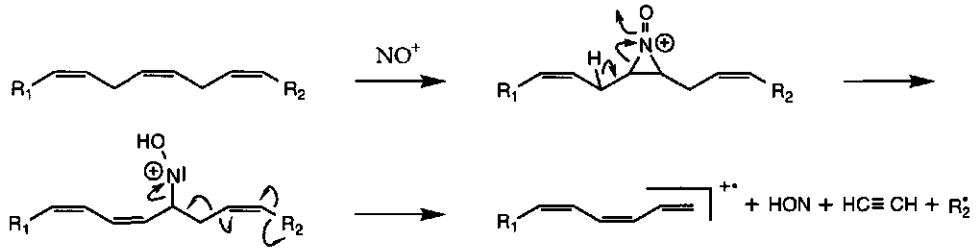


*Fig 2.6* **The reaction of a homo-conjugated triene with nitric oxide (NO) inside the mass spectrometer.**

The fragment which arises from the chemical ionization (CI) with nitric oxide is also detected (at low relative intensities) in the normal electron impact (EI) mass spectrum. This indicates how easy the double bonds in the initially formed radical migrate along the chain (to form a conjugated system which subsequently fragments in a similar way as shown in figure 2.1)[3,6]. The relative intensity of this particular fragment in the EI mass spectrum increases when the ionization energy is reduced.

A sensitive and more broadly applicable procedure is the derivatisation of double bonds with dimethyl disulphide (DMDS). This procedure has been described mainly for molecules with just one or two double bonds[15-17]. In one case it has been described as a tool for the determination of the double bond positions in an alkatriene[18]. However, more than one microgram of compound was used for the derivatisation reaction and analysis. Moreover, no functional group was present in the original molecule and the double bonds were separated by more than three methylene groups which facilitates the analysis considerably[19].

## 2.2 Methods and materials

*Reaction conditions*

Approximately 1 mg (4 µmol) of acetate in 140 µl of freshly distilled DMDS and a crystal of iodine (±5 mg, or ±0.5 mg when mentioning low iodine concentrations) in a 4 ml vial was sealed and heated for 16 hr at 60°C. The reaction was quenched with a few drops of saturated aqueous $Na_2S_2O_3$ (until the red colour of the iodine faded). The organic layer was collected and filtered and dried simultaneously by passing it through a Pasteur pipette filled with dry $Na_2SO_4$.

*Mass spectrometry*
Most of the mass spectrometry was performed on a Finnigan MAT 95 mass spectrometer (70 eV), coupled to a Varian GC equipped with a split/splitless injection system. The injection volumes varied between 1-2 µl (splitless). The column was a J&W 25 m DB-5 fused silica column, 0.25 mm id and 0.25 µm film thickness. Conditions were: Carrier gas helium; column temperature 250 or 260°C. The mass spectra of figures 2.33 through 2.35 were recorded on a Hewlett Packard 5970 quadropole mass selective detector (MSD). Chromatographic conditions were the same as described for the Varian GC only a Hewlett Packard GC was used instead.

## 2.3 The analysis of DMDS derivatised double bonds

A double bond which has reacted with DMDS preferably breaks at the former double bond position in the mass spectrometer. From the obtained fragments, the position of the original double bond can be deduced. The DMDS derivatives are prepared by heating the unsaturated compound with DMDS and iodine. The structure of the DMDS derivatives depends on the number of double bonds present in the molecule, the concentration of DMDS and iodine, and probably also on the heating time and temperature.

### 2.3.1 Mono-unsaturated molecules

The mechanism for the addition of DMDS to a double bond is illustrated in figure 2.7. Iodine initially reacts with DMDS to form methylthio-iodide which subsequently reacts which the double bond. The obtained sulphonium-iodide intermediate reacts with a second molecule of DMDS. A molecule of methylthio-iodide is regenerated in this step, therefore, the iodine acts in this case as a catalyst[16]. The addition of DMDS to the sulphonium-iodide intermediate is assumed to be anti (figure 2.7). Therefore, the addition of DMDS to (Z)-double bonds leads to the threo product whereas the addition to (E)-double bond leads to the erythro product.
The initial attack of the methylthio-iodide to the double bond can take place from the upper or lower side of the molecule thus two enantiomers are formed. The structure of the sulphonium-iodide intermediate highly favours the attack of a DMDS molecule from the opposite side of the sulphonium group, therefore no diastereomer formation is observed. The presence of a single product peak in the gas chromatogram confirms this mechanism.

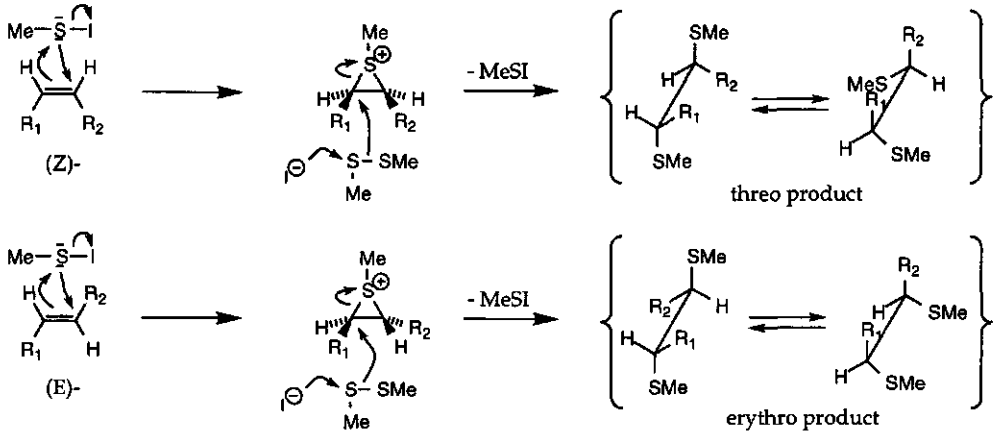


*Fig. 2.7* **Proposed reaction mechanism for the formation of DMDS derivatives of mono-unsaturated straight chain molecules.**

As an example, the reaction of (E)-3-tetradecenyl acetate (**1**) with DMDS is illustrated in figure 2.8.

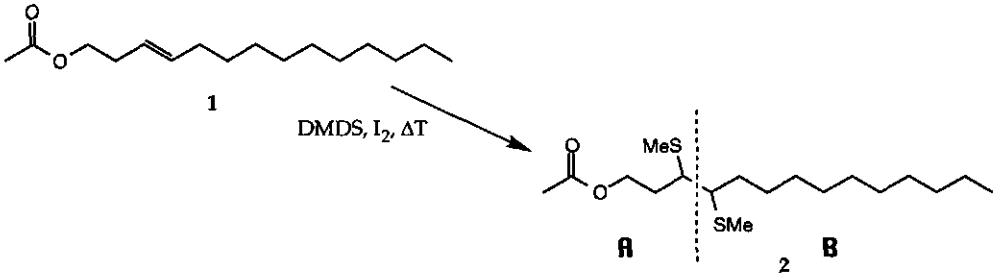


*Fig. 2.8* **The reaction of (E)-3-tetradecenyl acetate (1) with DMDS gives 3,4-bis(methylthio)tetradecyl acetate (2). The dashed line represents the position where the molecule is expected to break in the mass spectrometer yielding fragments A and B.**

The DMDS derivatives are rather stable, which is also expressed by a usually clearly visible molecular ion ($M^{+\cdot}$). In the mass spectrometer, the DMDS derivative will lose one and sometimes more methylthio groups (SMe) leading to fragments $M^{+\cdot} - 47$ (or $M^{+\cdot} - 48$ for HSMe) and fragment $M^{+\cdot} - 95$ (= 47 + 48). In case that two methylthio groups leave initially the total leaving mass is always 95 amu (atomic mass units).
The mass spectrum of 3,4-bis(methylthio)tetradecyl acetate (**2**) is shown in figure 2.9. The relevant fragments are mentioned in table 2.1.

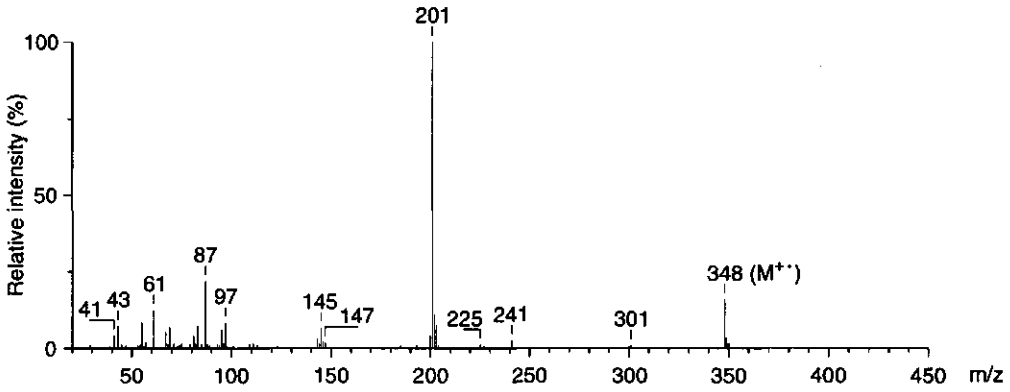


*Fig. 2.9* **Mass spectrum of 3,4-bis(methylthio)tetradecyl acetate (2).**

*Table 2.1* **Relevant mass spectrometric fragments of 3,4-bis(methylthio)tetradecyl acetate (2).**

| *m/z* | composition | source | *m/z* | composition | source |
|---|---|---|---|---|---|
| 348 | $C_{18}H_{36}O_2S_2$ | $M^{+\cdot}$ | 147 | $C_6H_{11}O_2S$ | **A**$^+$ |
| 301 | $C_{17}H_{33}O_2S$ | $M^{+\cdot}$ – SMe | 87 | $C_4H_7S$ | **A**$^+$ – acetate |
| 241 | $C_{15}H_{29}S$ | $M^{+\cdot}$ – SMe – acetate | 201 | $C_{12}H_{25}S$ | **B**$^+$ |

The peak with the highest intensity at *m/z* 201 represents fragment **B** of the DMDS derivative (figure 2.8). Fragments **A** and **A** – SMe have low intensities, but fragment **A** – acetate is clearly visible. The intensity of fragment $M^{+\cdot} - 95$ is very small in this case (<0.1%) and consequently filtered out of the mass spectrum of figure 2.9.

### 2.3.2 Double-unsaturated molecules

Double-unsaturated molecules may react in different ways with DMDS. The distance between the two double bonds determines the final product. When the two double bonds are separated by more than three methylene groups, the molecule simply reacts twice with DMDS to form exclusively an open di-adduct. When the two double bonds are separated by less than three methylene groups, a cyclic thio-ether is formed exclusively. In the particular case when the double bonds are separated by exactly three methylene groups, both types of DMDS reaction product (open or closed) can be present. As an example (E,Z)-3,8-tetradecadienyl acetate (**3**) is taken. This molecule has exactly three methylene groups between the two double bonds and the DMDS reaction product

consists partly of the open DMDS di-adduct 3,4,8,9-tetrakis-(methylthio)tetradecyl acetate (**4**) and partly of the cyclic thio-ether 2-(methylthio-hexane-1-yl)-6-(3-methylthio-ethylpropanoate-3-yl)-tetrahydrothiopyran (**5**) (figure 2.10).

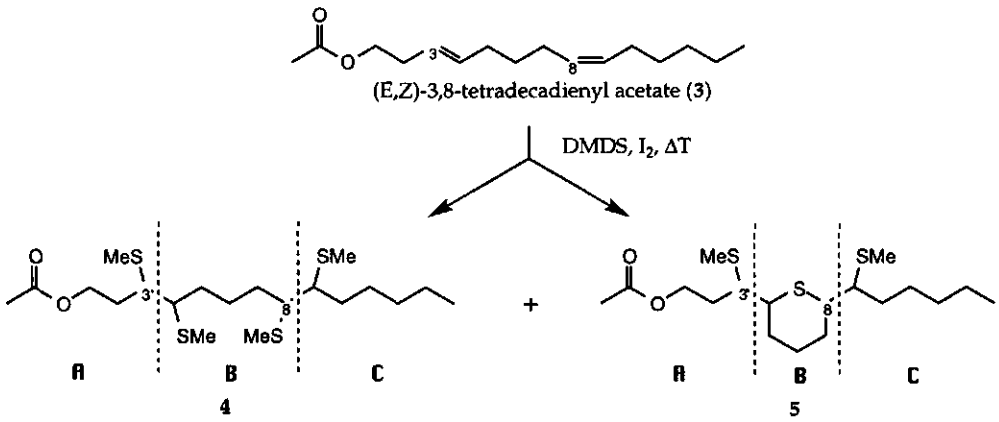


*Fig 2.10* **Exactly three methylene groups between the two double bonds in (E,Z)-3,8-tetradecadienyl acetate (3) results in the two types of DMDS derivatives: 4 (open) and 5 (closed).**

The mass spectrum of the open DMDS di-adduct **4** is shown in figure 2.11. The relevant fragments are mentioned in table 2.2. The principle of ring-closure is discussed further in this chapter.

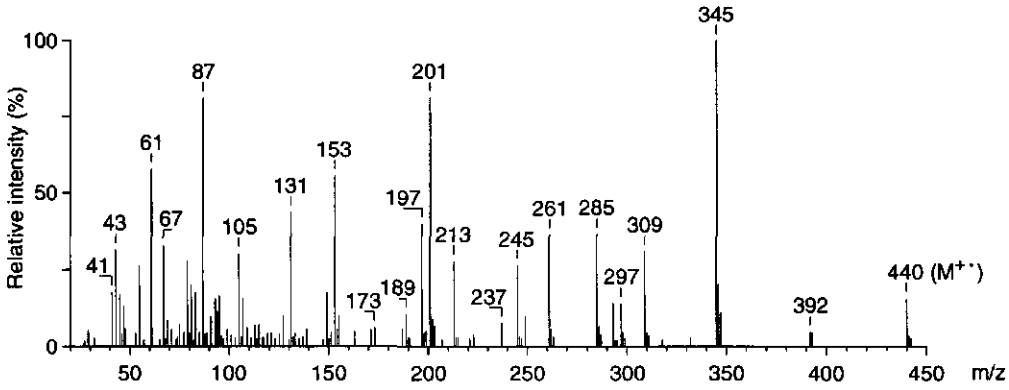


*Fig. 2.11* **Mass spectrum of the open DMDS di-adduct 3,4,8,9-tetrakis(methylthio)tetradecyl acetate (4) from (E,Z)-3,8-tetradecadienyl acetate (3).**

The mass spectrometric fragmentation pattern of **4** can be interpreted in a straightforward manner. It appears that the loss of a methylthio group from an already-formed mass

spectrometric fragment results in the loss of 48 amu. The loss of two methylthio groups, therefore, results in the loss of 96 amu and not of 95 amu, as is observed when the two methylthio groups are lost initially by the molecular ion.

*Table 2.2* **Mass spectrometric fragments of the open DMDS di-adduct 3,4,8,9-tetrakis(methylthio)-tetradecyl acetate (4).**

| *m/z* | composition | source | *m/z* | composition | source |
|---|---|---|---|---|---|
| 440 | $C_{20}H_{40}O_2S_4$ | $M^{+\cdot}$ | 201 | $C_{10}H_{17}S_2$ | **AB**$^+$ – HSMe – acetate |
| 392 | $C_{19}H_{36}O_2S_3$ | $M^{+\cdot}$ – HSMe | 213 | $C_{11}H_{17}O_2S$ | **AB**$^+$ – 2× HSMe |
| 345 | $C_{18}H_{33}O_2S_2$ | $M^{+\cdot}$ – 2× SMe | 153 | $C_9H_{13}S$ | **AB**$^+$ – 2× HSMe – acetate |
| 285 | $C_{16}H_{29}S_2$ | $M^{+\cdot}$ – 2× SMe – acetate | 293 | $C_{14}H_{29}S_3$ | **BC**$^+$ |
| 309 | $C_{13}H_{25}O_2S_3$ | **AB**$^+$ | 245 | $C_{13}H_{25}S_2$ | **BC**$^+$ – HSMe |
| 261 | $C_{12}H_{21}O_2S_2$ | **AB**$^+$ – HSMe | 197 | $C_{12}H_{21}S$ | **BC**$^+$ – 2× HSMe |
| 249 | $C_{11}H_{21}S_3$ | **AB**$^+$ – acetate | 87 | $C_4H_7S$ | **A**$^+$ – acetate |

When the double bonds in the original molecule are separated by less than four methylene groups, the formation of the DMDS derivative develops in a different way. The proposed reaction mechanism is illustrated in figure 2.12. One of the methylthio groups of the DMDS derivative of the first double bond attacks one of the carbons of the sulphonium ion that is formed as an intermediate from the second double bond. In this way, a cyclic thio-ether is formed. The iodine in this reaction is not only the catalyst but is also consumed as methyl iodide during the reaction and thus considered as a reactant[16].

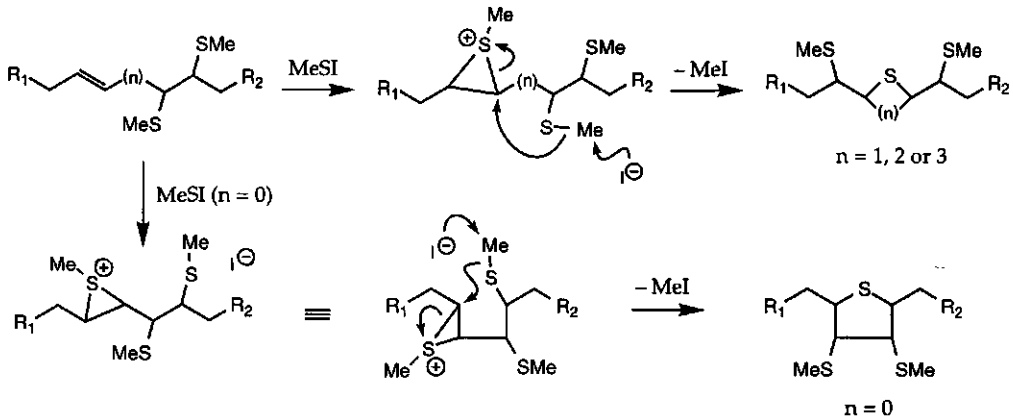


*Fig 2.12* **Proposed mechanism for the formation of DMDS derivatives from molecules with two double bonds which are separated by three or less methylene groups. For details see text.**

In case n = 0, thus when the double bonds in the original molecule are conjugated, the most remote methylthio group that is attached to the first double bond attacks the most remote carbon atom of the sulphonium ion that is formed from the second double bond. In this way a tetrahydrothiophene is obtained with the two methylthio groups attached to the ring (figure 2.12). The mass spectrum of the resulting bis(methylthio)tetrahydrothiophene (n = 0) derivative is recognisable by two intense peaks: $M^{+\cdot} - 95$ and $M^{+\cdot}$ – (95 + functional group) due to the easy loss of the two methylthio groups under formation of a stable thiophene and the subsequent loss of the functional group[16]. During the reaction of DMDS with poly-unsaturated compounds, diastereomers are formed, this in contrast to the reaction of DMDS with mono-unsaturated compounds (§ 2.3.1).
The derivatisation of (E,Z)-3,7-tetradecadienyl acetate (**6**) with DMDS is taken as an example of the formation of a cyclic thio-ether with the two methylthio groups outside the tetrahydrothiophene ring (figure 2.13).

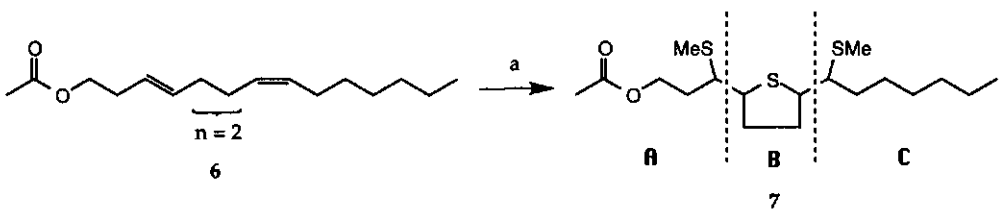


*Fig. 2.13* **Expected product 2-(1-methylthio-heptan-1-yl)-5-(3-methylthio-ethylpropanoate-3-yl)-tetrahydrothiophene (7) from the DMDS derivatisation of (E,Z)-3,7-tetradecadienyl acetate (6). Reagents: a) DMDS, $I_2$, $\Delta T$.**

The mass spectrum of compound 7 is shown in figure 2.14.

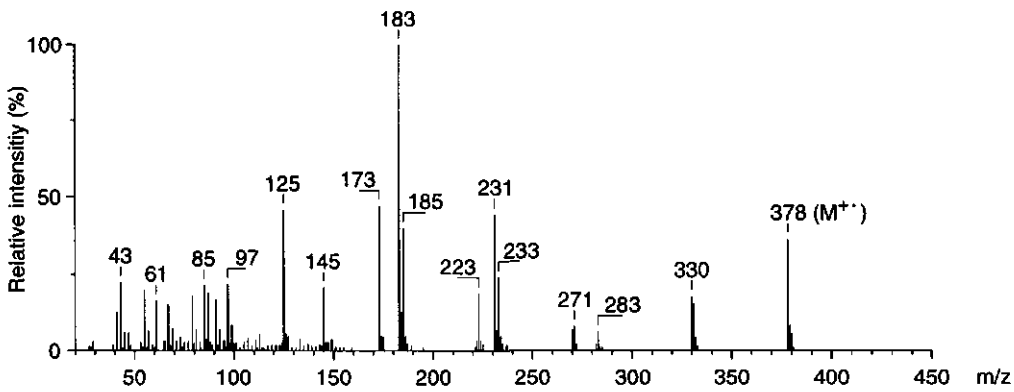


*Fig. 2.14* **Mass spectrum of the DMDS derivative product 7.**

The relevant fragments are mentioned in table 2.3.

*Table 2.3* **Major mass spectrometric fragments of the DMDS derivative of (E,Z)-3,7-tetradecadienyl acetate (6), compound 7.**

| *m/z* | composition | source | *m/z* | composition | source |
|---|---|---|---|---|---|
| 378 | $C_{18}H_{34}O_2S_3$ | $M^{+\cdot}$ | 185 | $C_9H_{13}O_2S$ | **AB**$^+$ – HSMe |
| 330 | $C_{17}H_{31}O_2S_2$ | $M^{+\cdot}$ – HSMe | 173 | $C_8H_{13}S_2$ | **AB**$^+$ – acetate |
| 283 | $C_{16}H_{27}O_2S$ | $M^{+\cdot}$ – 2× SMe | 125 | $C_7H_9S$ | **AB**$^+$ – HSMe – acetate |
| 271 | $C_{15}H_{27}S_2$ | $M^{+\cdot}$ – SMe – acetate | 231 | $C_{12}H_{23}S_2$ | **BC**$^+$ |
| 223 | $C_{14}H_{23}S$ | $M^{+\cdot}$ – 2× SMe – acetate | 183 | $C_{11}H_{19}S$ | **BC**$^+$ – HSMe |
| 233 | $C_{10}H_{17}O_2S_2$ | **AB**$^+$ | 145 | $C_8H_{17}S$ | **C**$^+$ |

Again, the molecular ion ($M^{+\cdot}$) *m/z* 378 is clearly visible. Also the loss of the methylthio and acetate groups from the $M^{+\cdot}$ is observed. Fragment **BC** appears to lose its methylthio group very easily and, in this way, forms the fragment with the highest intensity *m/z* 183. Fragments **A**, **A** – acetate and **A** – SMe are not very intense. The same was observed for the related fragments of DMDS derivative **2** (figure 2.9).

The reaction products depend on the reaction conditions. If the concentration of iodine is low, mainly the derivatives are formed as described in figures 2.10, 2.12 and 2.13. Symmetrical cyclic thio-ethers are then formed exclusively[20]. These cyclic thio-ethers always bridge the two nearest possible carbon atoms. If the iodine concentration is increased it appears that other cyclic thio-ethers are formed as well. The reaction of (Z,Z)-9,12-tetradecadienyl acetate (**8**) with DMDS in the presence of a high iodine concentration is taken as an example. The proposed reaction mechanism that leads to two symmetrical and the two non-symmetrical cyclic thio-ethers is shown in figure 2.15. Routes I and III lead to the formation of two symmetrical cyclic thio-ether tetrahydrothiopyran **9** and thietane **11**, respectively. The routes II and IV give rise to the two non-symmetrical cyclic thio-ethers, the tetrahydrothiophenes **10** and **12**.

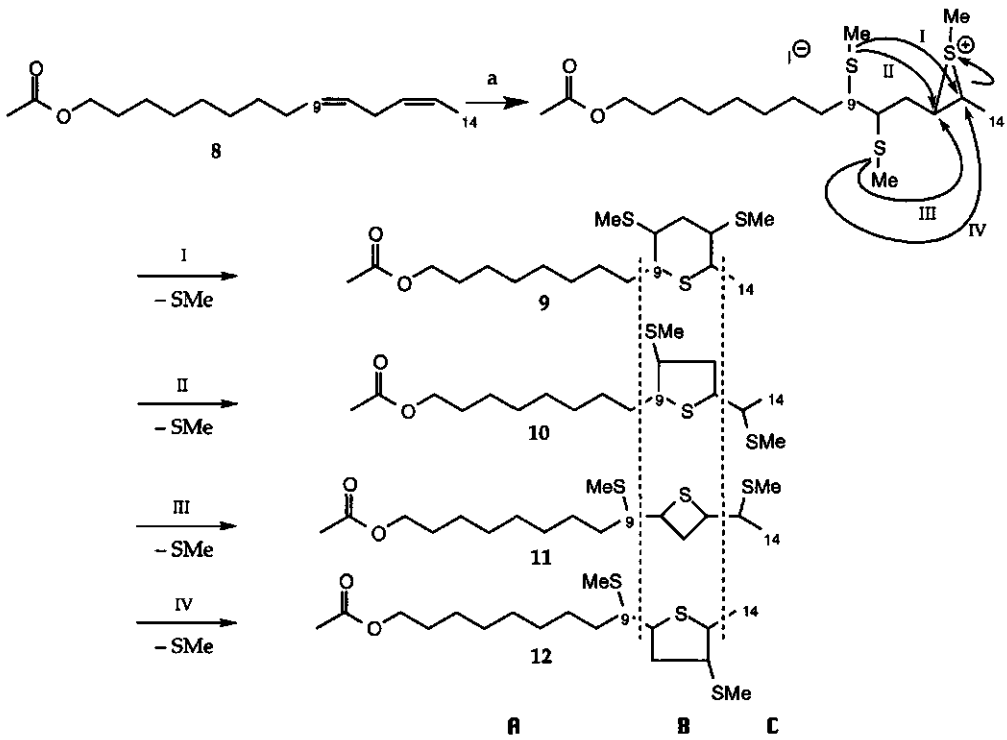


*Fig. 2.15* **Proposed mechanism for the formation of the four possible DMDS derivatives (9 - 12) from (Z,Z)-9,12-tetradecadienyl acetate (8). Reagents: a) DMDS, $I_2$, $\Delta T$.**

The gas chromatogram of the reaction product mixture is shown in figure 2.16.

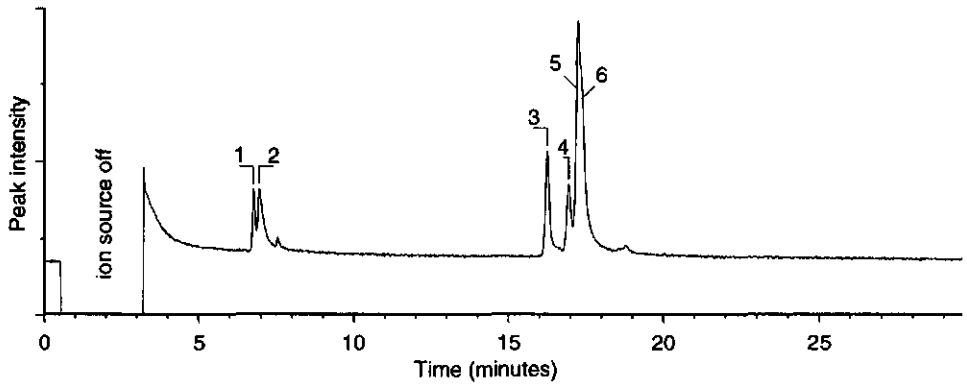


*Fig. 2.16* **Gas chromatogram of the reaction mixture of (Z,Z)-9,12-tetradecadienyl acetate (8) with DMDS at high iodine concentrations. Detector: mass spectrometer.**

Peak numbers 1 and 2 represent derivatives that have reacted in a different way with DMDS, which are discussed later in this chapter. Peak numbers 3 through 6 represent the 'normal' cyclic thio-ethers. The mass spectra of these compounds are shown in figures 2.17 through 2.20.

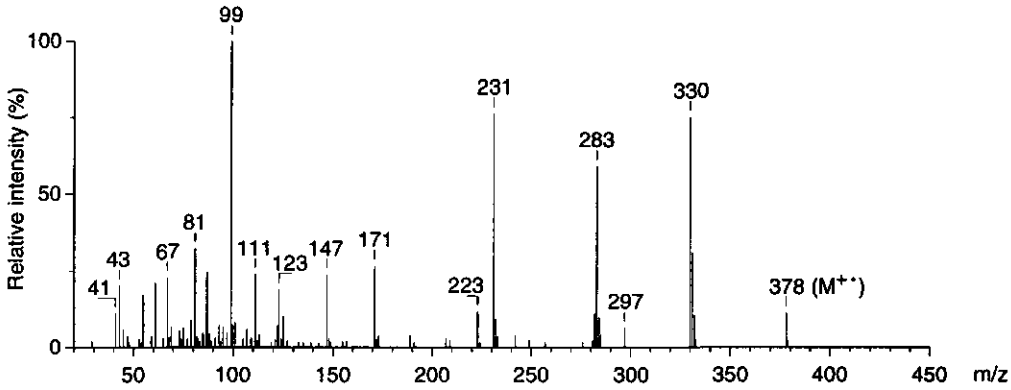


*Fig 2.17* **Mass spectrum of peak number 3 from the gas chromatogram of figure 2.16.**

The mass spectrum of peak number 3 shows an intense peak *m/z* 231 which indicates a fragment **A** as can be expected from structures **11** or **12**. The presence of the fragments **A** – acetate (*m/z* 171), **A** – acetate – SMe (*m/z* 123), **BC** (*m/z* 147) and **BC** – SMe (*m/z* 99) supports this hypothesis. For structure **11**, fragments **AB** (*m/z* 255) and **AB** – acetate (*m/z* 195) are expected (see also figure 2.21). Since the mass spectrum of figure 2.17 lacks these fragments, it is concluded that peak number 3 has structure **12**.

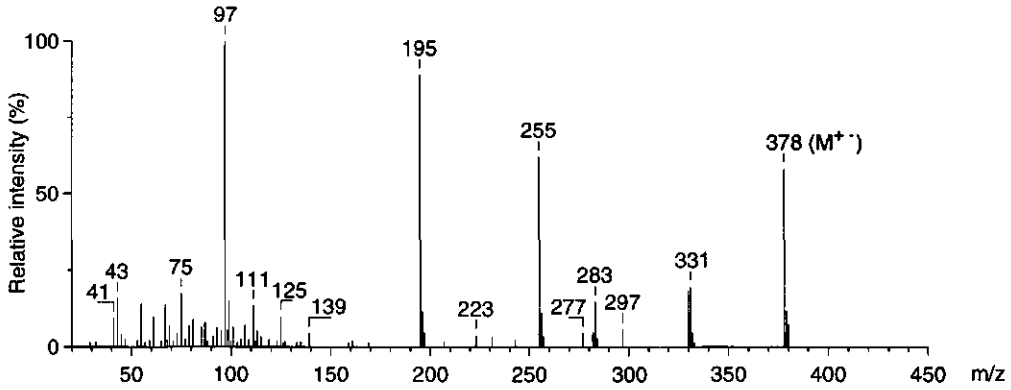


*Fig 2.18* **Mass spectrum of peak number 4 from the gas chromatogram of figure 2.16.**

The mass spectrum of peak number 4 is dominated by the $M^{+\cdot}$ (*m/z* 378) and peaks at

*m/z* 255 and *m/z* 195. The latter two fragments result from the loss of HSMe and HSMe plus acetate from fragment **AB** (*m/z* 303) possibly from structures **10** or **11**. If peak number 4 had structure **11**, fragments *m/z* 231, *m/z* 171 or *m/z* 123 representing fragments **A**, **A** – acetate or **A** – acetate – HSMe of structure **11** respectively, should be present (see figure 2.21). Because these fragments are not significantly present, it is concluded that peak 4 has structure **10**.

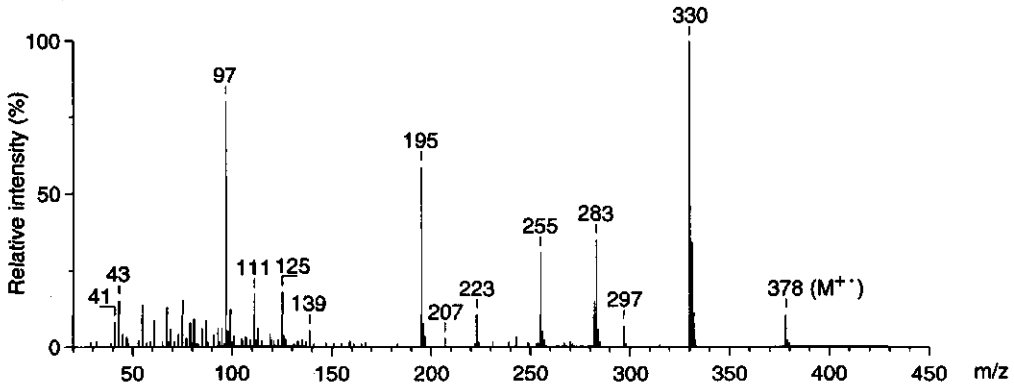


*Fig 2.19* **Mass spectrum of peak number 5 from the gas chromatogram of figure 2.16.**

The mass spectrum of peak number 5 shows similarities with that of peak number 4 (figure 2.18). It is assumed therefore, that peak number 5 is a diastereomer of peak number 4 and, thus, has structure **10**.

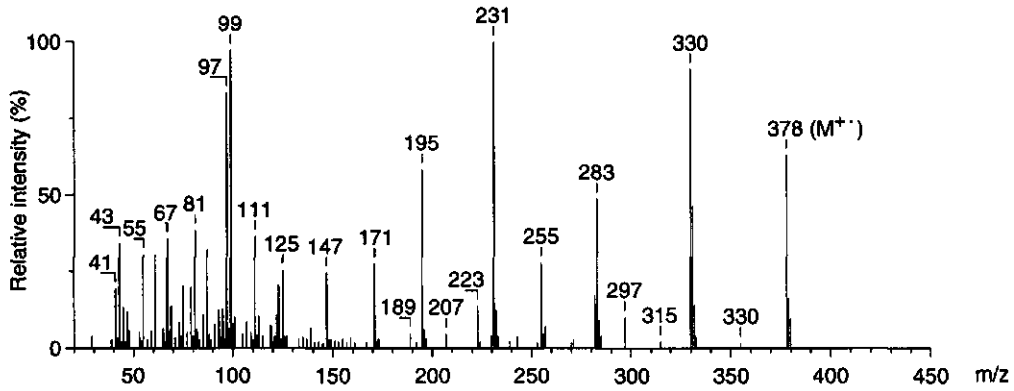


*Fig 2.20* **Mass spectrum of peak number 6 from the gas chromatogram of figure 2.16.**

The mass spectrum of peak number 6 possesses all the characteristics of structure **11**. The fragmentation pattern is shown in figure 2.21.

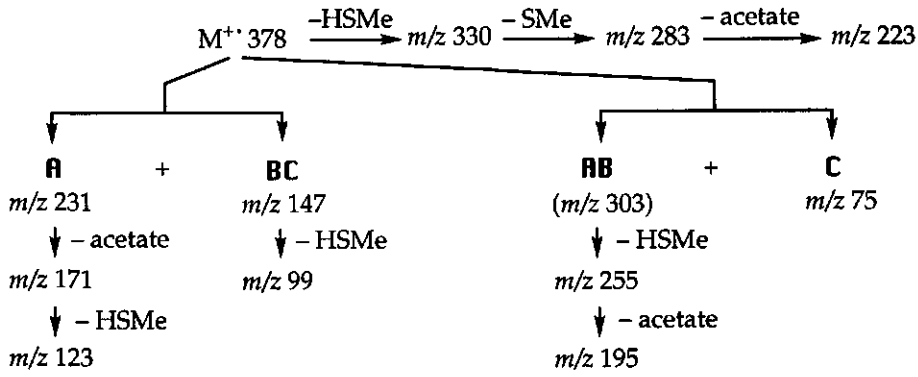


*Fig 2.21* **Fragmentation pattern for DMDS derivative 11. Fragments between brackets were not detected.**

For the symmetrical tetrahydrothiopyran (**9**) a mass spectrum would be expected dominated by peaks $M^{+\cdot} - 95$ (*m/z* 283) and $M^{+\cdot} - 95$ – acetate (*m/z* 223)[17]. Because no spectrum with these characteristics could be found, it is assumed that this DMDS derivative was not present. The formation of the six membered sulphur containing ring is probably less favourable under the reaction conditions used.

In the DMDS derivatisation reaction mixture of poly-unsaturated compounds, incompletely derivatised structures can be detected as well. The latter are again, strongly dependent on the reaction conditions. For example, in case of the derivatisation of (Z,Z)-3,8-tetradecadienyl acetate (**13**) with DMDS, derivatives **14** and **15** (figure 2.22) were identified in the reaction mixture.

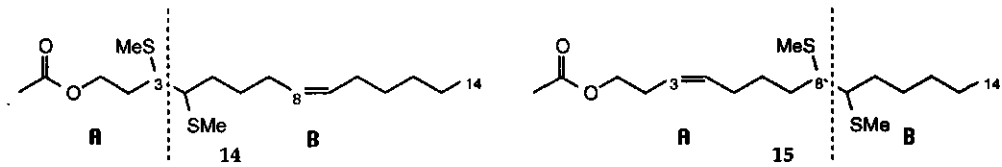


*Fig. 2.22* **Incompletely with DMDS derivatised (Z,Z)-3,8-tetradecadienyl acetate (13) products 3,4-bis-(methylthio)-(Z)-8-tetradecenyl acetate (14) and 8,9-bis(methylthio)-(Z)-3-tetradecenyl acetate (15).**

The mass spectra of compounds **14** and **15** exhibit $M^{+\cdot}$ peaks at *m/z* 346. Because the molecular mass of the original molecule is 252 amu, the additional 94 amu of the DMDS derivative must have been the result of the addition of two methylthio groups without ring-closure. Fragment **A** of **14** (mass spectrum not shown) is similar to that of **2**

(figure 2.9). Fragment **B** of **14** carries two hydrogens less than the corresponding fragment in **2**, therefore, the mass of fragment **B** of **14** is 2 amu lower as well. The mass spectrum of **15** is shown in figure 2.23. The relevant fragments are mentioned in table 2.4.

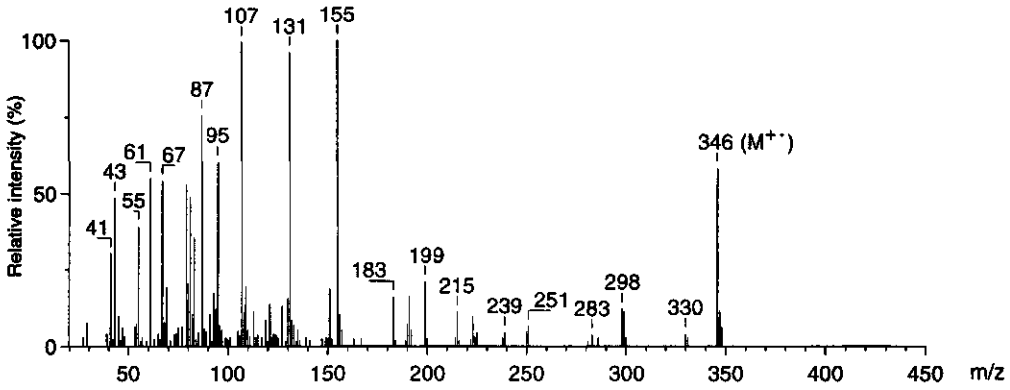


*Fig. 2.23* **Mass spectrum of the incompletely reacted DMDS derivative 15.**

*Table 2.4* **Mass spectrometric fragments of the incompletely reacted DMDS derivative 15.**

| *m/z* | composition | source | *m/z* | composition | source |
|---|---|---|---|---|---|
| 346 | $C_{18}H_{34}O_2S_2$ | $M^{+\cdot}$ | 215 | $C_{11}H_{19}O_2S$ | **A**$^+$ |
| 298 | $C_{17}H_{28}O_2S$ | $M^{+\cdot}$ – HSMe | 155 | $C_9H_{15}S$ | **A**$^+$ – acetate |
| 131 | $C_7H_{15}S$ | **B**$^+$ | 107 | $C_8H_{11}$ | **A**$^+$ – acetate – HSMe |

It was observed that in case of a homo-conjugated system (double bonds separated by one methylene group) another reaction product is formed as well. Because molecular ions ($M^{+\cdot}$) of incompletely DMDS-reacted derivatives originating from homo-conjugated compounds are always well visible[21], the molecular ion peak at *m/z* 316 of peak 2 of figure 2.16 cannot be explained by assuming that the corresponding molecule is incompletely derivatised. It seems that this peak represents an excessively reacted derivative instead. In the product mixture of the derivatisation of (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**) with DMDS, a peak is encountered with similar mass spectrometric characteristics as peak 2 of figure 2.16. The molecular ion ($M^{+\cdot}$) of this compound is detected at a mass of 2 amu lower (*m/z* 314) than that of peak 2 of figure 2.16. The mass spectra of both these DMDS derivatives show several related fragments (figures 2.24 and 2.25 respectively). It is, therefore, assumed that the fragmentation patterns of these two DMDS derivatives follow comparable routes.

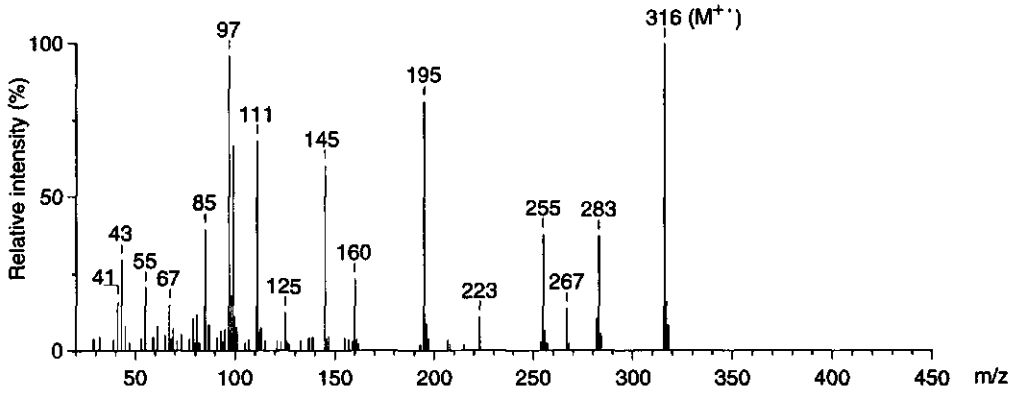


*Fig. 2.24* **Mass spectrum of an with DMDS unually reacted derivative originating from (Z,Z)-9,12-tetradecadienyl acetate (8). (peak 2 from the gas chromatogram of figure 2.16).**

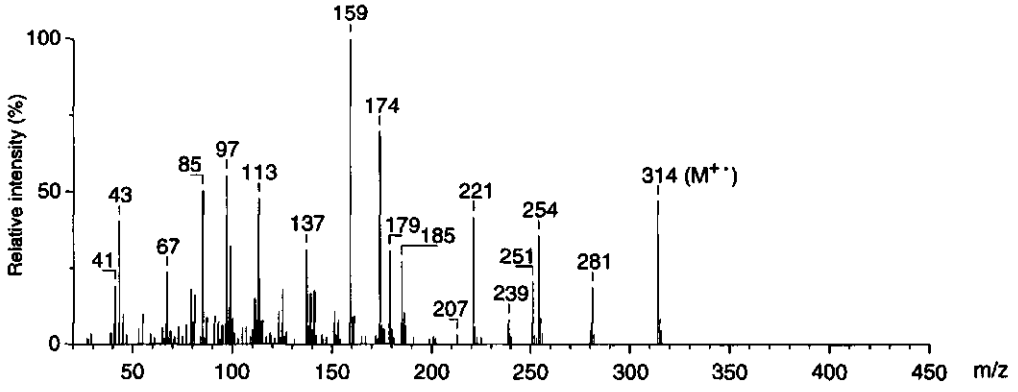


*Fig. 2.25* **Mass spectrum of a with DMDS unusually reacted derivative from (E,Z,Z)-3,8,11-tetradecatrienyl acetate (16).**

It has been considered that these two peaks were incompletely derivatised molecules with undetected molecular ion peaks at m/z of 344 and 346 respectively. The fragments 316 and 314 must then originate from the molecular ion that has lost a fragment of 30 amu. The loss of two consecutive methyl groups is forbidden by mass spectrometric rules and an initial loss of the two methyl groups together as one ethane molecule under the direct formation of the di-thio-ether is rejected as unrealistic. It is therefore proposed that these two products represent di-thio-ethers which have been formed through a second ring-closing reaction as illustrated in figure 2.26.

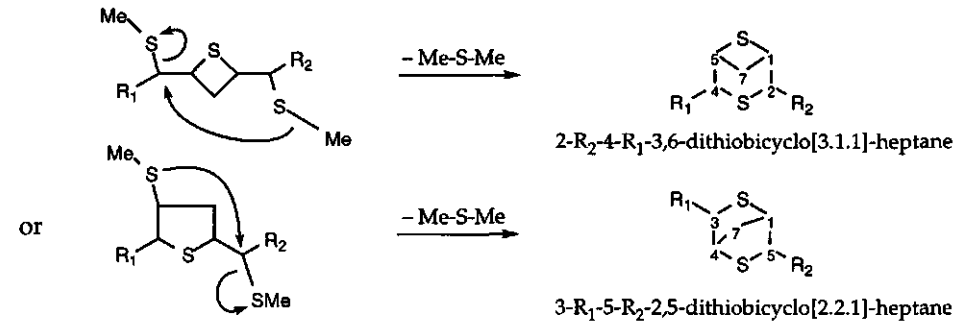


*Fig. 2.26* **Proposed mechanisms for the formation of di-thio-ethers.**

The proposed fragmentation patterns are shown in figures 2.27 and 2.28 respectively.

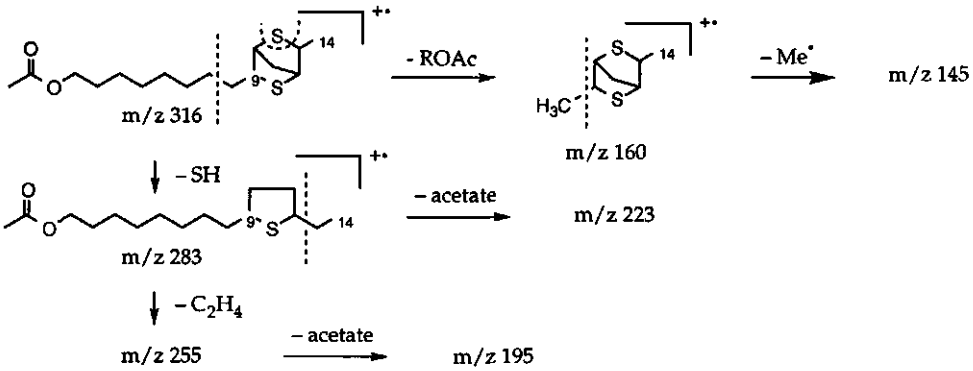


*Fig.* 2.27 **Proposed mass spectrometric fragmentation pattern for the di-thio-ether derivative originating from (Z,Z)-9,12-tetradecadienyl acetate (8).**

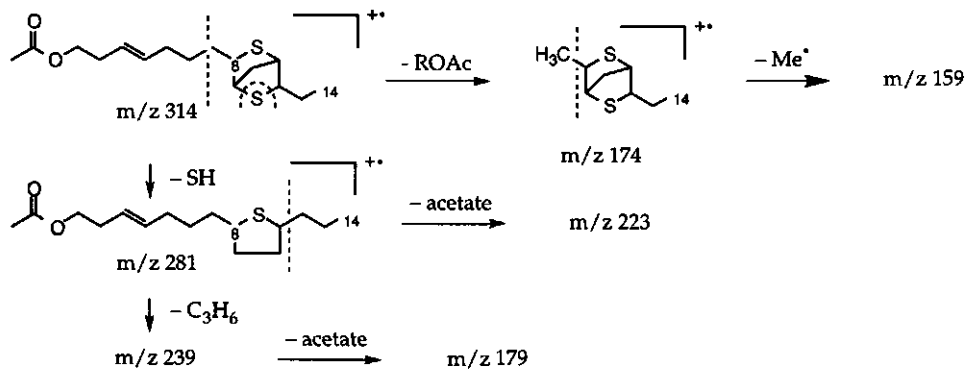


*Fig. 2.28* **Proposed mass spectrometric fragmentation pattern for the excessively with DMDS reacted derivative originating from (E,Z,Z)-3,8,11-tetradecatrienyl acetate (16).**

It is not known whether the di-thio-ethers would have structure bicyclo [3.1.1] or bicyclo [2.2.1]. Because several isomers are observed, probably both isomers are present. The ions at *m/z* 160 and *m/z* 145 of figure 2.24 and at *m/z* 174 and *m/z* 159 of figure 2.25 could be related. Because the latter two fragments are 14 amu higher in mass, these ions originate then from the ω-end of the derivative. The proposed fragmentation routes are not in contradiction with this.

### 2.3.3 Triple-unsaturated molecules

Straight chain molecules with three double bonds react with DMDS in a similar way as the less unsaturated ones do. Also the formation of cyclic thio-ethers follow the same rules as mentioned before. The major difference is that the products are more complicated. Triple-unsaturated compounds have more possibilities for the formation of diastereomers, consequently the gas chromatogram of the reaction mixture is more complex. Still, the main product formed in the presence of a low iodine concentration, is the symmetrical DMDS derivative with one methylthio group at both positions next to the cyclic thio-ethers (preferably thietanes or thiophenes rather than thiopyranes).

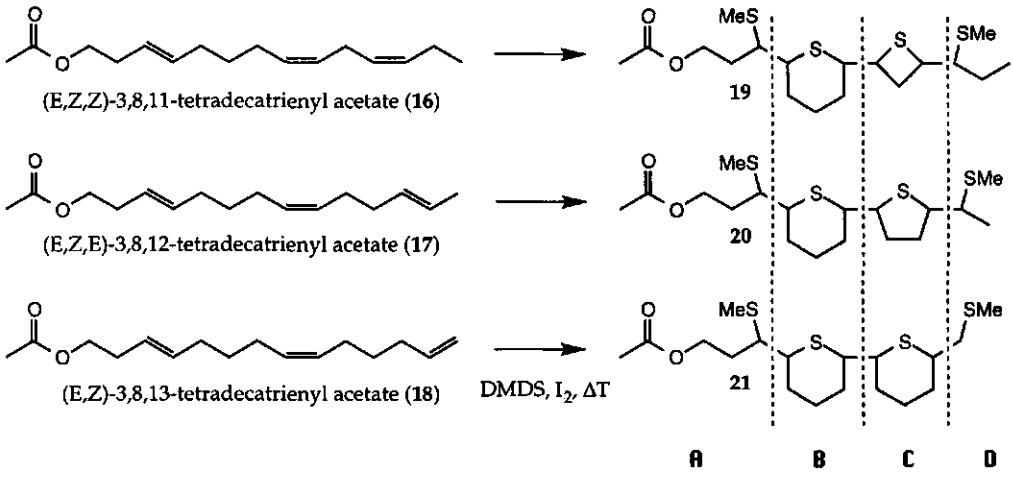


*Fig. 2.29* **Main DMDS derivatives formed from the triple-unsaturated compounds 16, 17 and 18.**

Three structurally related triple-unsaturated acetates: (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**), (E,Z,E)-3,8,12-tetradecatrienyl acetate (**17**) and (E,Z)-3,8,13-tetradecatrienyl acetate (**18**) were synthesised (see chapter 5), derivatised with DMDS and subjected to mass spectrometric analysis in order to see if the obtained derivatives are distinguishable by their MS, and to see if it is possible to locate all double bond positions. The main product formed for each of these compounds is shown in figure 2.29.

The mass spectra of **19**, **20** and **21** are shown in figures 2.30 through 2.32 respectively.

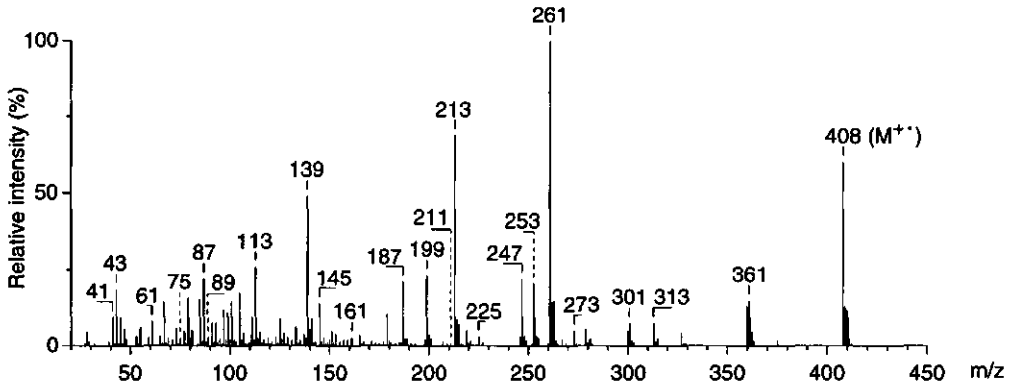


*Fig. 2.30* **Mass spectrum of the DMDS derivative 19 originating from (E,Z,Z)-3,8,11-tetradecatrienyl acetate.**

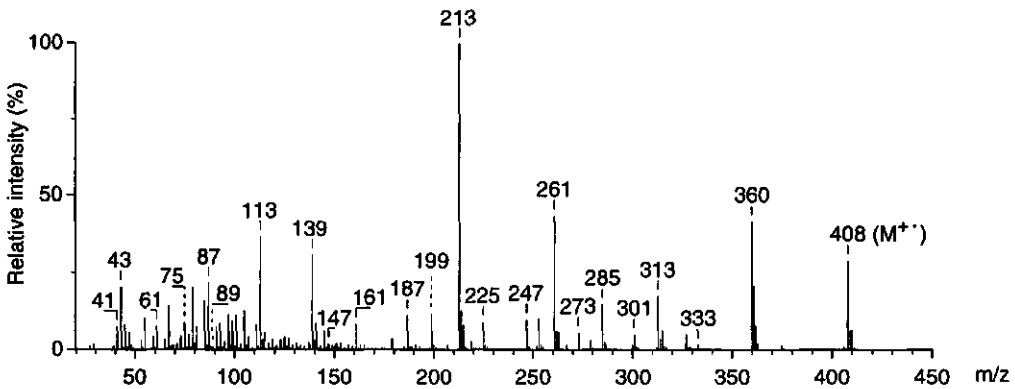


*Fig. 2.31* **Mass spectrum of the DMDS derivative 20 originating from (E,Z,E)-3,8,12-tetradecatrienyl acetate.**

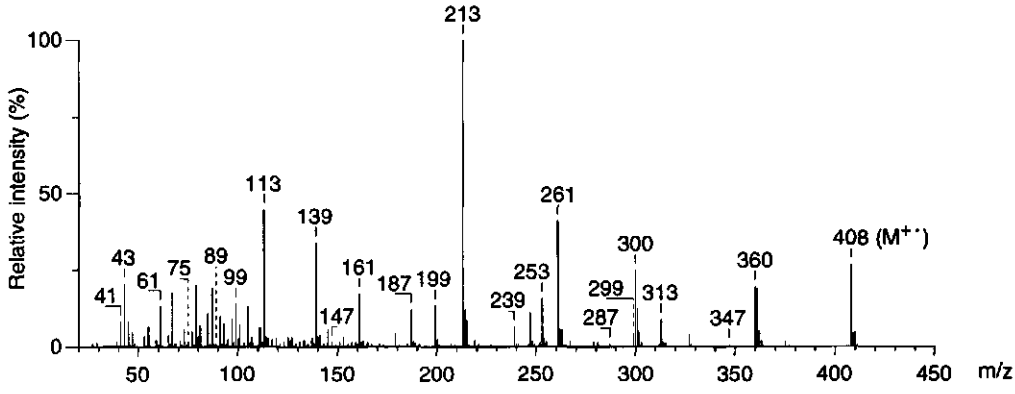


*Fig. 2.32* **Mass spectrum of the DMDS derivative 21 originating from (Z,Z)-3,8,13-tetradecatrienyl acetate.**

All three DMDS derivatives have the same configuration with respect to the first two double bonds. The expected, and for this part of the molecule characteristic, fragments are mentioned in table 2.5. The expected fragments for the distinguishing differences are shown in table 2.6.

*Table 2.5* **Specific mass spectrometric fragments shared by all three DMDS derivatives 19, 20 and 21.**

| *m/z* | composition | source | *m/z* | composition | source |
|---|---|---|---|---|---|
| 408 | $C_{18}H_{32}O_2S_4$ | $M^{+\cdot}$ | 139 | $C_8H_{11}S$ | **AB**$^+$ – HSMe – acetate |
| 360 | $C_{17}H_{28}O_2S_3$ | $M^{+\cdot}$ – HSMe | 147 | $C_6H_{11}O_2S$ | **A**$^+$ |
| 313 | $C_{16}H_{25}O_2S_2$ | $M^{+\cdot}$ – 2× SMe | 99 | $C_5H_7O_2$ | **A**$^+$ – HSMe |
| 301 | $C_{15}H_{25}S_3$ | $M^{+\cdot}$ – acetate – SMe | 87 | $C_4H_7S$ | **A**$^+$ – acetate |
| 253 | $C_{14}H_{21}S_2$ | $M^{+\cdot}$ – acetate – 2× SMe | 261 | $C_{12}H_{21}S_3$ | **BCD**$^+$ |
| 247 | $C_{11}H_{19}O_2S_2$ | **AB**$^+$ | 213 | $C_{11}H_{17}S_2$ | **BCD**$^+$ – HSMe |
| 199 | $C_{10}H_{15}O_2S$ | **AB**$^+$ – HSMe | 161 | $C_7H_{13}S_2$ | **CD**$^+$ |
| 187 | $C_9H_{15}S_2$ | **AB**$^+$ – acetate | 113 | $C_6H_9S$ | **CD**$^+$ – HSMe |

The distinguishing fragments for **20** and **21** are well visible in their mass spectra (table 2.6). However, for **19** the key fragments are not very obvious, or are detected at low intensities. The ratio of the peak intensities of **CD** – HSMe : **CD** is 10 - 15 for compound **19** but only between 2.6 and 4.5 for the other two compounds **20** and **21**. This high intensity ratio (>6.5) of fragments originating from the ω-side of the molecule has also been found for DMDS derivatives originating from (Z,Z)-9,12-tetradecadienyl acetate (**8**), (Z,Z)-3,6-hexadecadienyl acetate (**22**) and (E,Z,Z)-4,7,10-tridecatrienyl acetate (**23**), but not in DMDS

derivatives of, for example, (E,Z)-3,7- and (Z,Z)-3,8-tetradecadienyl acetate (**6** and **13** respectively). The molecules **8**, **22** and **23** have the same homo-conjugated double bond system which is absent in **6** and **13**. It appears that this intensity ratio can be used to discriminate between the DMDS derivatives originating from compounds with and without homo-conjugation in their double bond system.

*Table 2.6* **Distinguishing mass spectrometric fragments which are expected for each of the three DMDS derivatives 19, 20 and 21.**

| *m/z* | composition | source | *m/z* | composition | source |
|---|---|---|---|---|---|
| Distinguishing fragments for **19** | | | 259 | $C_{12}H_{19}S_3$ | **ABC**$^+$ – acetate |
| 319 | $C_{14}H_{23}O_2S_3$ | **ABC**$^+$ | 211 | $C_{11}H_{15}S_2$ | **ABC**$^+$ – acetate – HSMe |
| 271 | $C_{13}H_{19}O_2S_2$ | **ABC**$^+$ – HSMe | 89 | $C_4H_9S$ | **D**$^+$ |
| Distinguishing fragments for **20** | | | 273 | $C_{13}H_{21}S_3$ | **ABC**$^+$ – acetate |
| 333 | $C_{15}H_{25}O_2S_3$ | **ABC**$^+$ | 225 | $C_{12}H_{17}S_2$ | **ABC**$^+$ – acetate – HSMe |
| 285 | $C_{14}H_{21}O_2S_2$ | **ABC**$^+$ – HSMe | 75 | $C_3H_7S$ | **D**$^+$ |
| Distinguishing fragments for **21** | | | 287 | $C_{14}H_{23}S_3$ | **ABC**$^+$ – acetate |
| 347 | $C_{16}H_{27}O_2S_3$ | **ABC**$^+$ | 239 | $C_{13}H_{19}S_2$ | **ABC**$^+$ – acetate – HSMe |
| 299 | $C_{15}H_{23}O_2S_2$ | **ABC**$^+$ – HSMe | 61 | $C_2H_5S$ | **D**$^+$ |

It is striking that the relative intensities of the key fragments can change considerably if the mass spectra of **19**, **20** and **21** are recorded on a quadropole mass spectrometer. This type of mass spectrometer promotes the occurrence in the mass spectrogram of fragments with lower masses (*m/z* <100). The mass spectra of **19**, **20** and **21** recorded on a quadropole

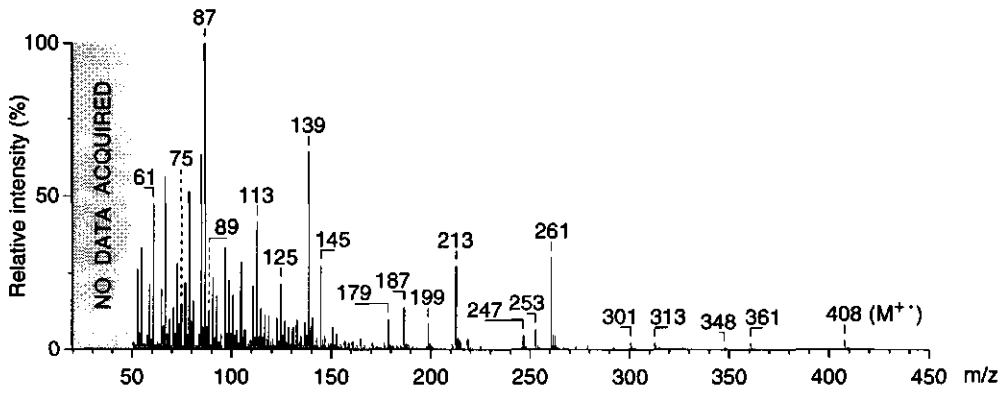


*Fig. 2.33* **Mass spectrum of the DMDS derivative 19 taken on a quadropole mass spectrometer.**

mass spectrometer are shown in figures 2.33 through 2.35.

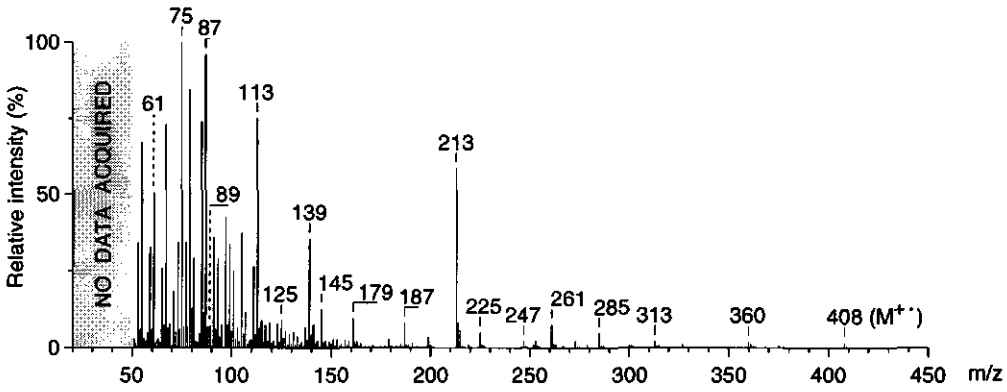


*Fig. 2.34* **Mass spectrum of the DMDS derivative 20 taken on a quadropole mass spectrometer.**

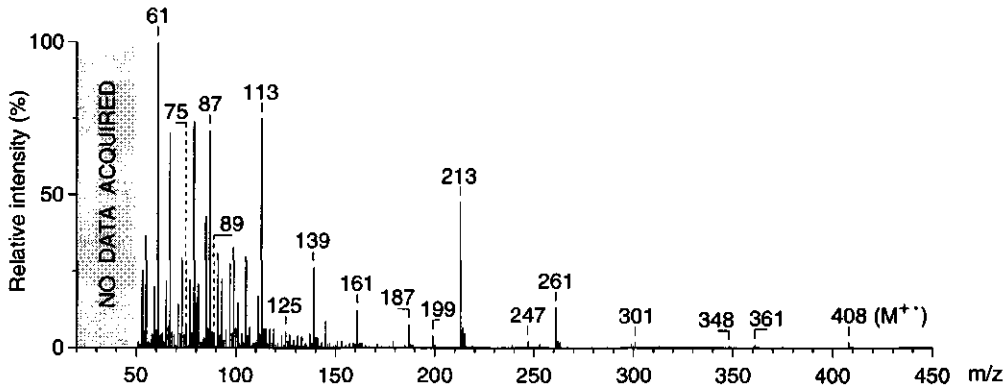


*Fig. 2.35* **Mass spectrum of the DMDS derivative 21 taken on a quadropole mass spectrometer.**

The key fragments **D** of compounds **20** and **21** (*m/z* 75 and *m/z* 61 respectively), have become the 100% intensity peaks. The relative intensity of fragment D (*m/z* 89) of **19**, although not the 100% peak, is significantly higher in comparison to the relative intensities of this fragment in the mass spectra of **20** and **21**. It is taken into account that the relative intensity of the isotope peak of fragment *m/z* 87, due to the presence of sulphur (relative intensity $^{34}S$ = 4.21%)[22], is predominantly responsible for the relative intensities of the fragments *m/z* 89 in case of compounds **20** and **21**.

## 2.3 Conclusions and discussion

The DMDS derivatisation reacted is very useful for the analysis of sex pheromones and related compounds. This analytical approach has proven to be of major importance for the identification of the sex pheromone compounds of *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*, the latter one in particular (chapter 5). The derivatisation reaction can be scaled down to sub-microgram levels and still provide sufficient information for the determination of the double bond positions. An extra benefit of this approach is that the raw biological starting material does not have to be extracted before the derivatisation reaction with DMDS. For the identification of the sex pheromone of *Scrobipalpuloides absoluta*, sex pheromone glands were directly collected in DMDS which was also used for the derivatisation reaction (chapter 5). It appears possible to determine the position of three double bonds in sex pheromone like structures through DMDS derivatisation of the compounds followed by mass spectrometric analysis. To the best of knowledge, this fact and a never before reported type of di-thio-ether which is formed from homo-conjugated sex pheromone compounds. The detection of such structures is, therefore, a strong indication of the presence of a homo-conjugated system in the original molecule.

## 2.4 References and notes


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# Chapter 3

## Isolation, identification and synthesis of the sex pheromone of *Symmetrischema tangolias**

### 3.1 Introduction

Today, the potato tuber moth, *Symmetrischema tangolias* (Gyen) (figure 1.3) is considered the most important pest of potatoes in Peru[1] and is recognised as a pest in neighbouring countries. The larvae of this moth mine the stems of potato plants causing them to break and die. In potato storage facilities, larvae often bore into potato tubers making them unsuitable for human consumption. In contrast to seed-potatoes, which are protected by large amounts of chemicals, consumer-potatoes are unprotected and thus, very vulnerable to this moth. Losses up to 100% are caused by this pest[2].
The use of a sex pheromone in the control of a pest population proved to be very effective with *Phthorimaea operculella* (Zeller)[3] which is closely related to *Symmetrischema tangolias*. Therefore, it is expected that the sex pheromone of *Symmetrischema tangolias* might be useful in the control of this pest as well. For this reason a project was initiated to identify the sex pheromone of *Symmetrischema tangolias*.

### 3.2 Methods and materials

*Insects*
The laboratory culture of *Symmetrischema tangolias* was started from pupae which were collected in a storehouse for potatoes in Cajamarca, Peru, in November 1991. The moths were reared on potato tubers (cv. Bintje) under the following conditions, 22 ± 1°C at day and 17 ± 1°C at night, 65 ± 5% relative humidity, and a 12L : 12D photoperiod. The potatoes were provided with small punched holes in which the females could lay their eggs.

* This chapter is based on the following paper: Griepink, F.C., van Beek, T.A., Visser, J.H., Voerman, S. and de Groot, Ae. 1995. *J. Chem. Ecol.*, 21, 2003-2013.

After four days the tubers, loaded with eggs, were placed into transparent boxes (29 (l) × 16 (w) × 10 (h) cm) with a 1 cm layer of clean sand. About 40 days later the pupae were collected, washed with a 3% hypochlorite solution and separated into males and females. The moths were fed on a 10% honey solution.

*Pheromone extracts*

Pheromone glands from 3-day-old virgin females were collected at 7-10 hr into the scotophase as female moths were observed to call during this time. The tips of the abdomen, including the intersegmental membrane[4], were used for extract preparation. The extracts were stored under nitrogen at –20°C. Bioassays were carried out by offering a piece of filter paper[5] loaded with 2 FE (female equivalents) of extract to male moths. Bioactive fractions elicited intense "flutter" responses and attempts to copulate with the filter paper. Sex pheromone glands were collected from about 2000 virgin female moths over a period of six weeks, and kept in 10 ml double-distilled and degassed hexane. The extract was concentrated to approximately 1.5 ml under a gentle stream of nitrogen and fractionated on a 6 ml Baker 10 SPE column packed with 500 mg 45 µm, 60Å silicagel. The column was successively eluted with 1.5 ml portions of respectively 0, 2, 5, 10, 20, 50 and 100% of *tert*-butyl methyl ether in hexane. The column was sucked dry between two fractions. Only the 5%-fraction generated a behavioural response.

*Electroantennograms (EAG's)*

Antennae from 3-day-old males were used for EAG recordings. The antenna was cut off at the base. About four segments of the tip were removed to enable electrical contact with the recording electrodes. The tip and basal part were connected to glass electrodes filled with a 0.1 M KCl solution. All tetradecenyl acetate isomers used (>99% purity), were obtained from the IPO-DLO pheromone bank[6]. Test cartridges were prepared by applying 100 µl of a 5 ppm solution of the acetate in hexane (= 0.5 µg active ingredient) on a piece of filter paper[5]. After evaporation of the solvent the paper was put into a Pasteur pipette. A Pasteur pipette filled with a filter paper containing 25 µl of a 1% (v/v) of (Z)-3-hexenyl-acetate (Carl Roth, Karlsruhe, Germany) in paraffin oil (Merck, Uvasol) served as reference stimulus. Silver wires connected the electrodes to the recording instruments: Grass HIP16A input probe, Grass P16D DC-amplifier (rise time: 30 ms), Philips PM3302 oscilloscope, Krenz TRC 4010 transient-recorder and Estate PC AT386. For details on the recording and calculation of EAG data see[7].

*Coupled gas chromatography-electroantennographic detection (GC-EAD)*

All the GC-EAD measurements were carried out at Syntech Laboratories, Hilversum, The Netherlands. The GC was a Chrompack 9000 gas chromatograph equipped with a

split/splitless injection system. Injections were done in splitless mode only. The column was a J&W 12 m DB-5 (5% phenyl methyl polysiloxane), 0.20 mm id and 0.33 µm film thickness. The sample was equally split between a flame ionisation detector (FID) and the EAG detector. Conditions were: carrier gas, helium; inlet pressure, 150 kPa; temperature programming, 100°C (0 min hold) to 220°C (0 min hold) at 10°C/min; injector and detector temperature, 250°C. The EAG recorder, software, IDAC (Intelligent Data Acquisition Controller) interface board for the AT486 PC and other peripheral equipment were manufactured by Syntech Laboratories.

*Dual column GC*

Gas chromatographic analyses were performed on a Hewlett Packard (HP) 5890A gas chromatograph equipped with a split/splitless injection system, a 1 : 1 inlet splitter and two fused silica columns each equipped with an FID. The two columns were a J&W 60 m DB-1 (100% methyl polysiloxane), and a J&W 60 m DB-WAX (polyethylene glycol), both having 0.25 mm id and 0.25 µm film thickness. Conditions were: carrier gas, hydrogen; inlet pressure, 20 psi; linear velocity, 35 cm/s; temperature programming, 50°C (0 min hold) to 238°C (8 min hold) at 4°C/min; injector temperature 220°C; detector temperature 260°C. The real retention times instead of the adjusted retention times were used for retention index (RI) calculations[8]. The RI's were calculated by comparing the retention times of the components of interest with those of the $C_7$ - $C_{23}$ alkanes. For the GC spiking experiments, a reference compound was added to the gland extract in such an amount so the peak of interest would rise about 10 - 50%. Both the extract and the extract combined with the reference compound were examined by GC. Compounds were considered identified when on both columns the peaks of interest would rise by the same amount due to the added compound.

*Preparative GC*

Preparative GC was performed on an HP 5890 series II gas chromatograph with an HP 7673 automatic sampler. The temperature programmable injector (controller model 504) and trap unit (controller model 580) were obtained from Gerstel, Mülheim an der Ruhr, Germany. The column was a 60 m J&W DB-WAX, 0.53 mm id and 1.0 µm film thickness. Conditions were: carrier gas, hydrogen; inlet pressure, 150 kPa; temperature programming, 150°C (1 min hold) to 230°C (1 min hold) at 5°C/min; injector temperature programming, 50°C (1 min hold) to 240°C (1 min hold) at 15°C/s; detector temperature 260°C.

*Dimethyl disulphide (DMDS) derivatisation*

Approximately 10 µl of freshly distilled DMDS and 5 µl of 5% iodine in diethyl ether were added to 20 female equivalents of extract from which the solvent had been evaporated by a gentle stream of nitrogen. The mixture was heated for 16 hr at 60°C in a small airtight flask. The reaction was quenched by the addition of a drop of saturated aqueous $Na_2S_2O_3$ solution. The organic phase was extracted three times with 100 µl hexane and concentrated to approximately 10 µl by evaporating the solvent at 40°C in a nitrogen atmosphere[9,10].

*Coupled gas chromatography mass spectrometry (GC-MS)*

GC-MS was performed on a Finnigan MAT 95 mass spectrometer (70 eV), coupled to a Varian GC equipped with a split/splitless injection system. Injections were done in splitless mode only (1-2 µl). The column was a J&W 25 m DB-5 fused silica column, 0.25 mm id and 0.25 µm film thickness. Conditions were: carrier gas, helium; temperature programming, 50°C (2 min hold) to 100°C (0 min hold) at 25°C/min and then to 280°C (8 min hold) at 4°C/min; injector and transferline temperature, 250°C.

*NMR*

NMR spectra were recorded on a Bruker AC-E 200 at 200 MHz in $CDCl_3$ (100.0% D, Janssen Chimica). Chemical shifts are reported in parts per million (δ) relative to TMS. The chemical shift of $CHCl_3$ (δ = 7.24) was used as internal reference.

*Synthesis*

All the used chemicals were of pro analyse quality. 3-Decyn-1-ol (**24**) was purchased from ABCR Chemie, Karlsruhe, Germany. The final synthetic products were purified on a 200 cm × 0.8 cm Lewatit SP 1080/$Ag^+$ column[11] to obtain an isomeric purity of more than 99%. Products were stored under nitrogen at –20°C with 0.1% 2,6-di-*tert*-butyl-4-methyl-phenol (BHT) as antioxidant[12].

*Wind tunnel bioassays*

Details about the wind tunnel bioassays are given in chapter 6.

## 3.3 Results and discussion

An extract was made from the sex pheromone glands of *S. tangolias* and purified to obtain a single active fraction. A 2-female equivalent of this extract, when exposed to male moths, elicited intense 'flutter' responses. The EAG responses to this active fraction and

the total extract were not significantly different. From GC-EAD measurements of this fraction it was concluded that EAG-active peaks eluted only during a short period of the entire GC run. The limited resolution of the GC-EAD equipment made it impossible to discriminate between the four peaks in the gas chromatogram of the active fraction on DB-1 (figure 3.1).

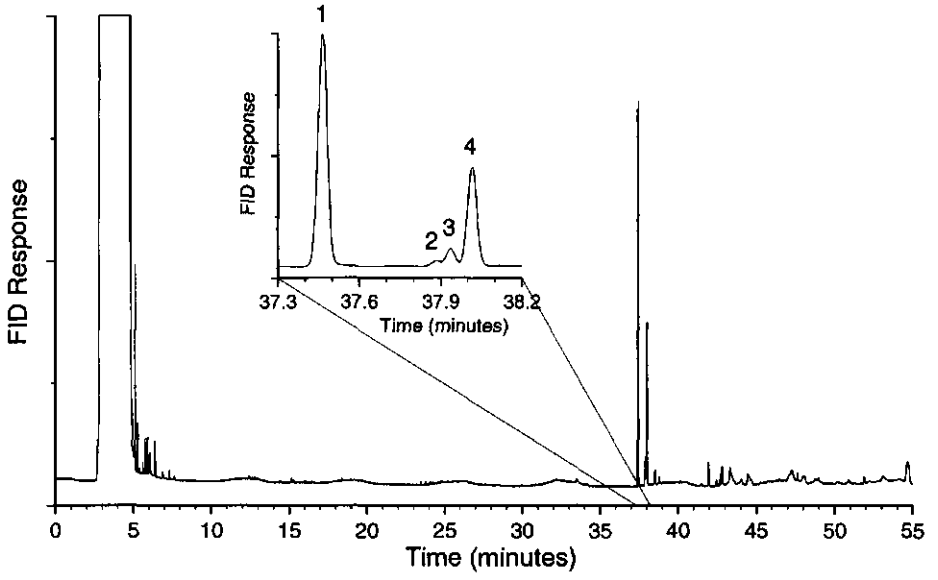



*Fig. 3.1* **Gas chromatogram of the biologically active fraction (splitless 2 µl, on the 60 m DB-1 column). The inset displays the time interval where EAG activity was observed. For an explanation of the peak numbers 1-4 see text.**

By comparison of the calculated RI's on both the DB-1 and DB-WAX columns with those calculated for reference compounds from the IPO pheromone bank[6], it became obvious that peaks 2, 3 and 4 were tetradecenyl acetate isomers. The mass spectra of these peaks were in agreement with this hypothesis. Peak 1 could be tentatively identified as a non-conjugated tetradecadienyl acetate isomer because of its typical extra difference in RI on the DB-1 and DB-WAX columns. The mass spectrum of peak 1 (figure 3.2) supported this hypothesis.

Peaks 2, 3 and 4 were identified, respectively, as (Z)-5-tetradecenyl acetate (**25**), (Z)-7-tetradecenyl acetate (**26**) and (E)-3-tetradecenyl acetate (**1**) by comparing the MS and the RI's with those obtained from reference compounds.

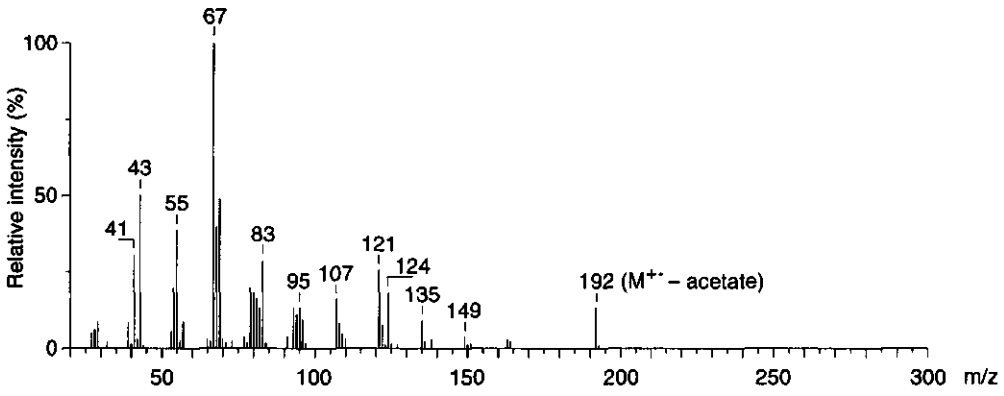



*Fig. 3.2* **Mass spectrum of peak 1 (compound 6). The molecular ion ($M^{+\cdot}$) could not be detected.**

GC spiking experiments with **1**, **25** and **26** provided additional evidence. The GC chromatograms of the spiking experiment of the extract with additional (Z)-7-tetradecenyl acetate is shown in figure 3.3 as illustration.

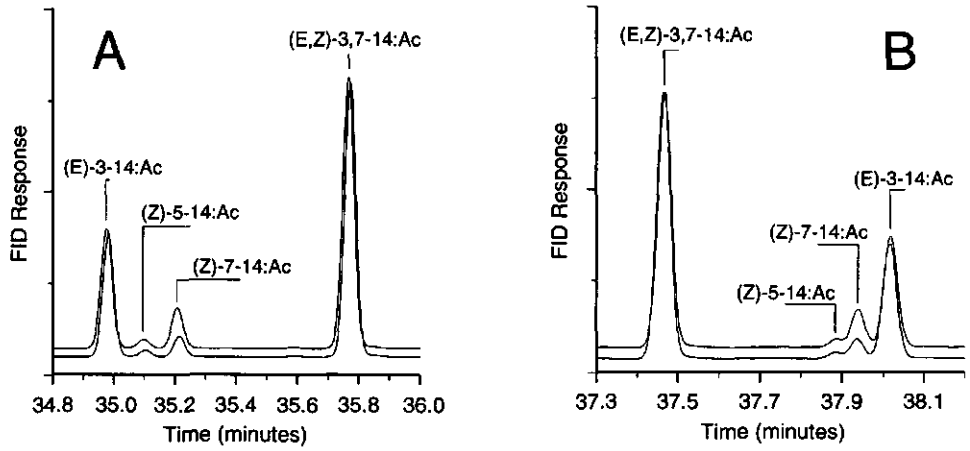



*Fig. 3.3* **GC spiking chromatograms of the sex pheromone extract with and without additional (Z)-7-tetradecenyl acetate (26); (A) on the DB-WAX column, (B) on the DB-1 column.**

About 10 micrograms of the two main peaks, 1 and 4, were isolated with preparative GC, which proved sufficient for NMR analysis. The NMR spectrum of peak 4 was identical to that of synthetic (E)-3-tetradecenyl acetate.

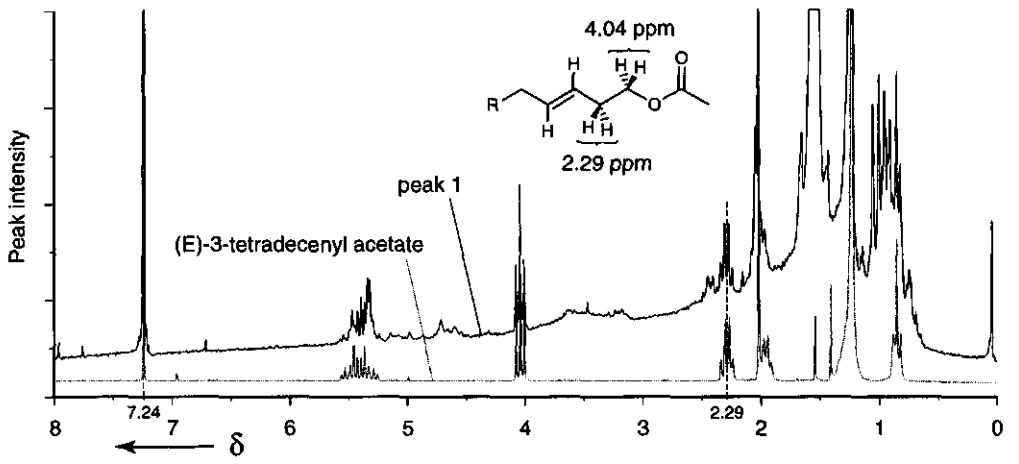



*Fig. 3.4* **NMR spectra of synthetic (E)-3-tetradecenyl acetate (1) and peak 1 of figure 3.1 (compound 6).**

The NMR spectrum of peak 1 showed the same signal at 2.29 ppm as the NMR spectrum of synthetic (E)-3-tetradecenyl acetate, which is characteristic for a double bond at the 3-position (figure 3.4). Comparison of the olefinic part of the NMR spectrum of peak 1 with those of synthetic (Z)-3- and (E)-3-tetradecenyl acetate suggested that the double bond at the 3-position of peak 1 had the (E)-configuration. The extract was derivatised with DMDS to get information about the position of the second double bond. The mass spectrum of the DMDS derivative of peak 1 is shown in figure 3.5. The fragmentation pattern suggested peak 1 to be a 3,7-tetradecadienyl acetate isomer (see also chapter 2).

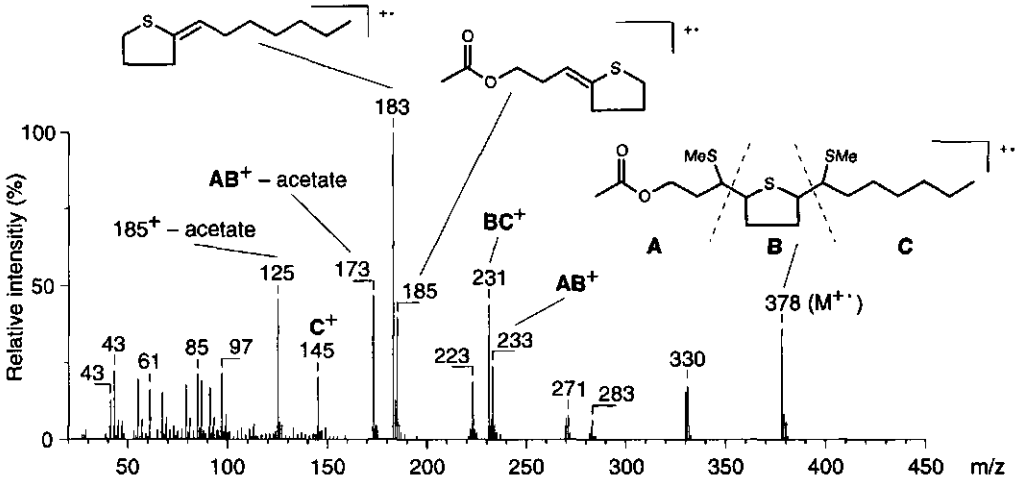



*Fig. 3.5* **Mass spectrum of the DMDS derivative of peak 1. Molecular ion ($M^{+\cdot}$) at m/z 378.**

To determine the E/Z-configuration of the double bonds, all tetradecenyl acetate isomers were tested with EAG (figure 3.6).

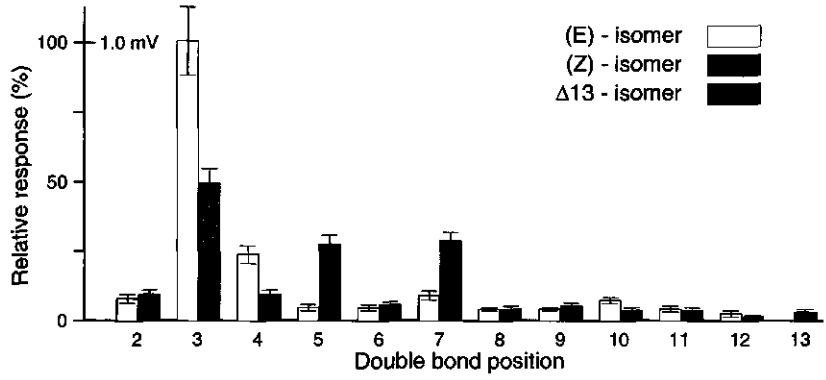
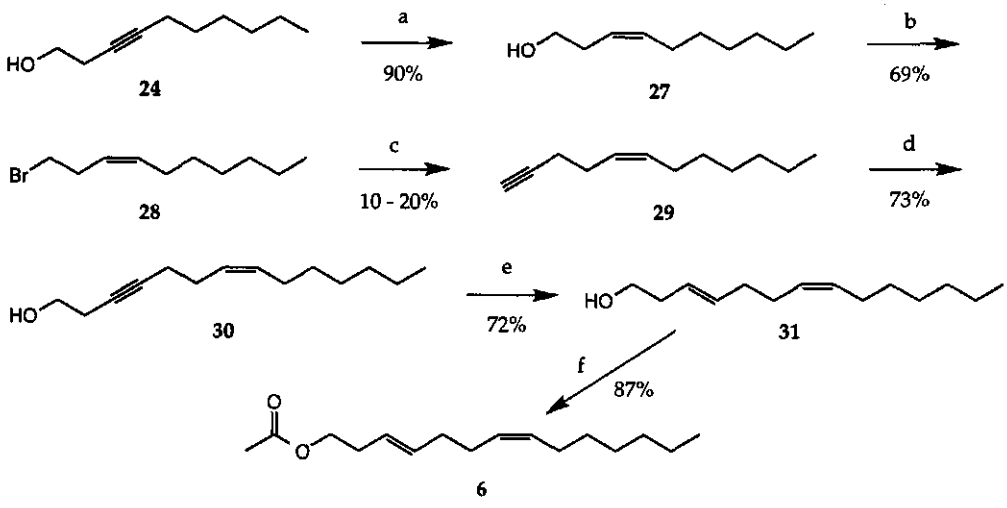



*Fig. 3.6* **Electroantennogram responses of all tetradecenyl acetate isomers. Bars indicate 95% confidence intervals. N = 15.**

The difference in response between the (E)-7- and the (Z)-7-isomer suggested the (E,Z)-3,7-configuration for peak 1. This was confirmed through synthesis. The molecule (E,Z)-3,7-tetradecadienyl acetate (**6**) was initially synthesised according to scheme 3.1 in an isomeric purity of more than 99% (GC).




*Scheme 3.1* **Reagents: a) P-2 Ni, $H_2$, ethanol; b) $CBr_4$, $Ph_3P$, THF; c) lithium acetylide-ethylene-diamine complex, THF, HMPA; d) n-BuLi, ethylene-oxide, THF, HMPA; e) $LiAlH_4$, THF, diglyme; f) $Ac_2O$, HOAc.**

For this route, 3-decyn-1-ol (**24**) was hydrogenated to the (Z)-alkene **27** in the presence of a nickel catalyst[13]. The alcohol group in **27** was subsequently converted into the bromide **28**[14]. The carbon chain of **28** was elongated with lithium acetylide[15] to **29** and next with ethylene-oxide[16] to give **30**. Alkenynol **30** was reduced with lithium aluminium hydride ($LiAlH_4$)[17] to the corresponding (E)-isomer **31** and acetylated to obtain the sex pheromone compound **5** in a overall yield of 5 - 10%. Both MS and [$^1$H]NMR[18] spectra of synthetic **6** were identical to those obtained for peak 1.

The low overall yield of this synthetic route was mainly the result of a side reaction in step c) where the 1-bromo-(Z)-3-decene (**28**) under the used basic reaction conditions predominantly (>75%) gives elimination of HBr under formation of the diene **33** rather than to couple with the lithium acetylide (figure 3.7).

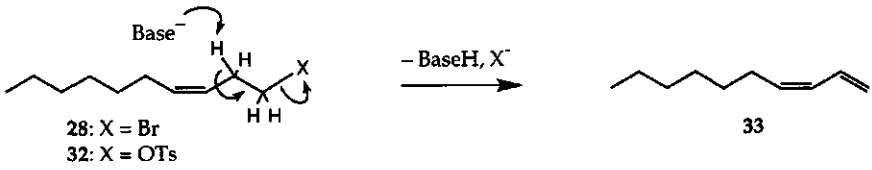
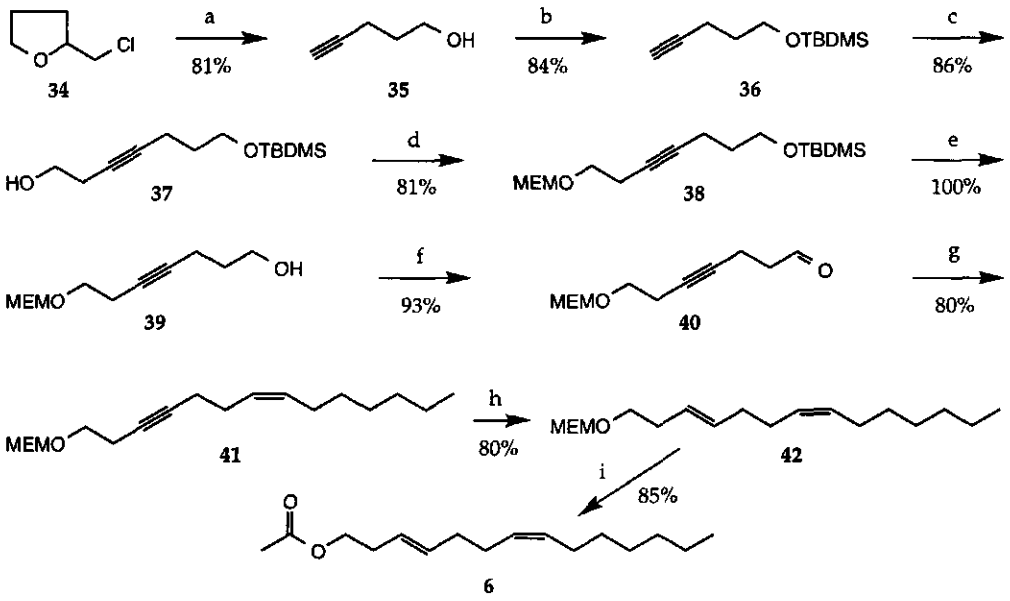



*Fig. 3.7* **Proposed mechanism for the side reaction step c) of scheme 3.1.**

This reaction has been carried out with the tosylate **32** instead of the bromide **28** to force the reaction in favour of substitution over elimination[19]. The main product remained the diene **33** and the yield of the desired adduct **29** was even lower.

More efficient routes for the synthesis of **6** have been investigated and a second route is shown in scheme 3.2. For this route the commercially available tetrahydrofurfuryl chloride (**34**) was converted into pentynol **35**[20]. Compound **35** was protected with *tert*-butyldimethylsilyl chloride (TBDMSCl)[21] and elongated with ethylene oxide[15] to obtain compound **37**. The free hydroxyl function of **37** was protected with β-methoxyethoxy-methyl chloride (MEMCl)[22] and next the TBDMS group could be selectively removed with tetrabutylammonium fluoride (TBAF)[21] to obtain the half protected alkyn diol **39**. The free alcohol function of **39** was oxidised in high yield[23] to the aldehyde **40**. A Wittig reaction[24] of heptyl-triphenylphosporane with aldehyde **40** gave compound **41**. A ten fold excess of the heptyl-triphenylphosphonium iodide with potassium hexamethyl-disilazide as base had to be used to obtain a good yield. The (E)-reduction[17] of the triple bond of **41** resulted in compound **42** which was deprotected and acetylated in one step[25] to the sex pheromone compound **6**. The overall yield was 24% based on **34**. The final product was not purified on the Lewatit SP 1080/$Ag^+$ column. The overall yield of a desired 99+% pure product will therefore be lower.




*Scheme 3.2* **Reagents: a) $NaNH_2$, $NH_3$; b) TBDMSCl, DMF, imidazole; c) n-BuLi, ethylene oxide, THF, HMPA; d) MEMCl, $CH_2Cl_2$, diisopropylamine; e) TBAF, THF; f) $Ag_2CO_3$, celite, benzene; g) Wittig, KHMDS, heptyl-triphenylphosphine iodide, THF; h) $LiAlH_4$, THF, diglyme; i) $FeCl_3$, $Ac_2O$.**

This second route seems to be advantageous over the first one, however, the use of expensive reagents like TBDMSCl and $Ag_2CO_3$ makes this route less economical.

The ratio of the four identified acetates in the gland extract was: (E,Z)-3,7- : (E)-3- : (Z)-7- : (Z)-5 - = 63 : 31 : 5 : 1. Different mixtures of identified components were tested in the wind tunnel. The best catch results were obtained with a 2 : 1 mixture containing two components, namely (E,Z)-3,7-tetradecadienyl acetate (6) and (E)-3-tetradecenyl acetate (1) (see chapter 6). This mixture was tested in potato fields and storage facilities in Peru. Catches up to 120 and 450 individuals per night per trap for field and storehouse experiments, respectively, were achieved with the synthetic *S. tangolias* sex pheromone.

The field catches emphasised the great attractiveness of the synthetic pheromone blend since no *S. tangolias* could be detected by us in an extensive search of the potato field and the surrounding areas. The catches in the storehouses dropped considerably after the first couple of days. Replacement of the traps by new ones did not improve the catches. This means that traps indoors are able to diminish pest populations quite rapidly and may be efficient for pest control in storehouses through mass trapping of the pest insects.

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# Chapter 4

## Determination of the sex pheromone gland content of *Symmetrischema tangolias* by means of direct gland introduction into a two-dimensional gas chromatograph*

### 4.1 Introduction

When calling, female moths release a sex pheromone to attract males of the same species. The released amounts are species related and depend further on the age of the insect as well as the time of the day[1]. The first day(s) after emergence from the pupae, the female does not always produce sex pheromone. The amount of sex pheromone in the sex pheromone gland (hereafter called gland) and its variation during a 24-hr period is also species related. The gland contents vary from individual to individual throughout the day. Again, this depends on the species, and even within a single species it appears that rearing temperature, inbreed, as well as the techniques used strongly affect the outcome of such studies[2,3].

In some species the sex pheromone is only present in the glands during the calling period, suggesting that the production is regulated and at the same time, limited to that period[4]. For other species the sex pheromone amount in the glands is rather stable during the day (when the moth is not active) and lower during the calling period[5]. This suggests that the sex pheromone production is continuous and probably only controlled by the sex pheromone titre in the gland[5].

The sex pheromone gland contents can be examined following several approaches. The most established one is to extract the glands and analyse the extract by means of GC (together with an internal standard)[1,5]. This labour-intensive method has the disadvantage that the analysis of low concentrated samples or samples that contain unstable compounds, cannot be achieved in a reliable way. The extraction step can be omitted by introducing the glands directly into the GC. This approach has been reported before[6,7],

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however only by using GC's with standard (continuously heated) injectors. These injectors have the drawback that they heat up (or cool down) very slow. Therefore, the authors had to seal the samples in glass tubes and had to use special solid phase GC injectors in order to prevent the premature release of volatile compounds[6,7]. By using a temperature programmable GC injector which can be cooled during the sample preparation, and flash heated during injection, the samples can be introduced directly into the equipment without the need for prior sealing.

This approach is described here as an application to determine the sex pheromone amounts in the glands of *Symmetrischema tangolias* during the 24-hrs dark-light cycle. The two-dimensional GC (2D-GC) system, which was used for this research, made it possible to work with impure and complex samples (like whole insect glands). The first GC serves as the clean-up step for the actual analysis which is conducted on the second GC. The sex pheromone of *S. tangolias* has been identified as a 2 : 1 mixture of (E,Z)-3,7-tetradecadienyl acetate (**6**) and (E)-3-tetradecenyl acetate (**1**) (see chapter 3). In addition, two related minor compounds were identified in the gland extract, namely (Z)-7-tetradecenyl acetate (**26**) and (Z)-5-tetradecenyl acetate (**25**). The peaks in the gas chromatogram for the latter two minor compounds were not resolved under the GC conditions used here. Therefore, the combined amounts of (Z)-7- and (Z)-5-tetradecenyl acetate (**26** + **25**) in the glands are presented in this chapter.

## 4.2 Methods and materials

*Insects*

The procedure for rearing *Symmetrischema tangolias* is described in § 3.2. The moths for the present experiments were of the 22$^{nd}$ - 26$^{th}$ laboratory generation (>2.5 years in rearing). Counting from the day that they emerged from the pupae, females were three days old when used.

*Sample preparation*

Pheromone glands, including the intersegmental membranes, were cut off from the abdomen and placed in special glass GC liners for the temperature programmable injector. A schematic drawing of the liners used is shown in figure 4.1. Each glass liner (Gerstel, Mülheim an der Ruhr, Germany) was filled with a small amount of silylated glass wool at the ferrule side and one gland per liner was put into the glass wool from that side. Liners with glands were sealed with aluminium foil and stored at –20°C until use. Liners were reusable immediately after analysis due to the high desorption temperatures.

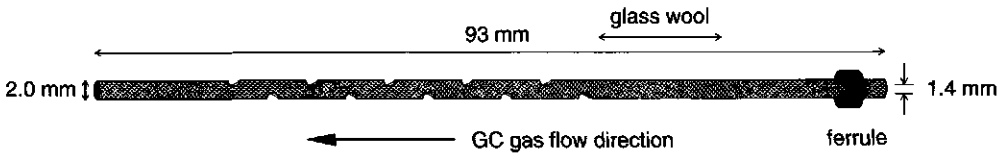



*Fig. 4.1* **Schematic drawing of the glass GC liners used.**

*Two Dimensional Gas Chromatography (2D-GC)*

2D-GC was performed on two, interconnected HP 5890 series II gas chromatographs. The column of the first GC was a 25 m J&W DB-5, 0.20 mm id and 0.33 µm film thickness. The column of the second GC was a 40 m J&W DB-WAX, 0.187 mm id and 0.3 µm film thickness. The two columns were connected by a dual column switching system (DCS). Each GC was equipped with a flame ionisation detector (FID). The cooled injection system (CIS) with a model 504 programmable temperature vaporiser (PTV), model 580 fraction collector and other peripheral equipment including the DCS were obtained from Gerstel, Mülheim an der Ruhr, Germany. A schematic drawing of the GC equipment used is shown in figure 4.2.

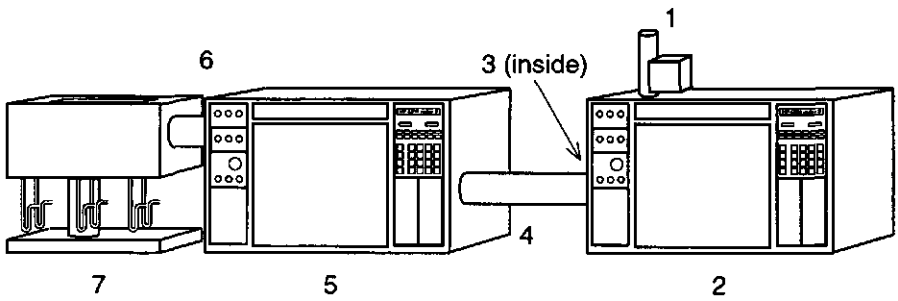



*Fig. 4.2* **Schematic drawing of the two-dimensional GC equipment. 1) temperature programmable injector, 2) first GC, 3) dual column switching system (inside), 4) transfer line between the two GC's, 5) second GC, 6) transfer line between the second GC and the fraction collector, 7) fraction collector.**

Individual glass liners with pheromone glands were placed inside the injector. The volatile part of the sample was evaporated by flash heating the programmable injector to 350°C at 10°C/s. By keeping the temperature of the first GC at 40°C, the sample was focused on the initial part of the first column. The transfer line, which connected the two GC's, was kept at –20°C with cold nitrogen gas, from 0.5 minute before the DCS valve opened, until 0.5 minute after the DCS valve closed. In this way, the peaks of interest,

which were cut from the first column by the DCS valve, were (re)focused in the transfer line. This was not strictly necessary but enhanced the quality of separation on the second column. The focused peaks in the transfer line were subsequently injected into the column of the second GC by flash heating the transfer line to 220°C at 20°C/s. About 2 - 5% of the gas flow of the first column was sent to an FID to monitor the run of the first GC. The second column was connected to an FID or to the fraction collector. In the latter case only 2 - 5% of the gas flow of the second column was sent to an FID. The fraction collector could be used to collect up to six peaks (or time windows) from the second column. All the equipment, except for the second GC, was controlled by a computer. The pneumatic scheme is shown in figure 4.3.

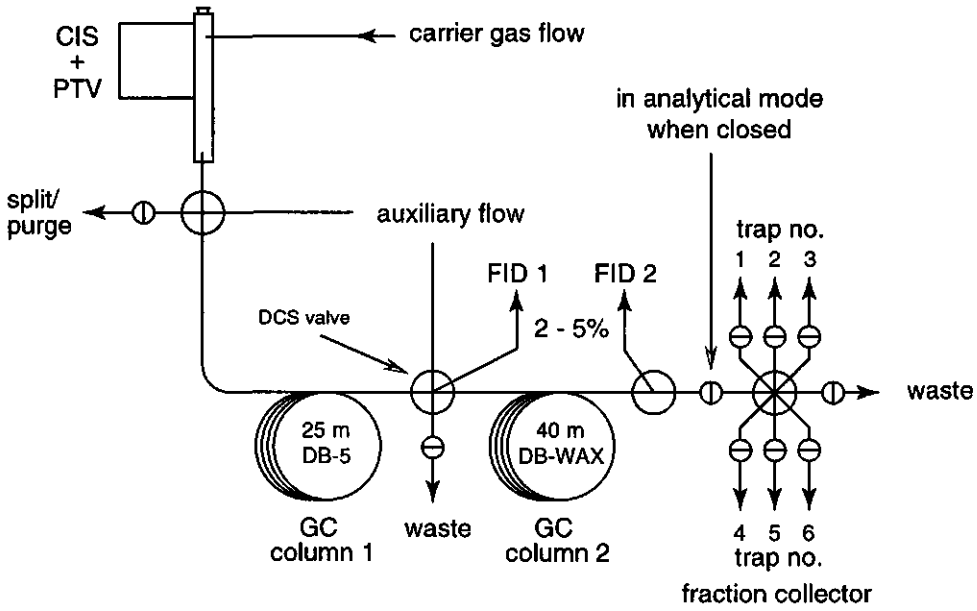



*Fig 4.3* **Pneumatic scheme of the two-dimensional GC. All the carrier gas flow is sent to FID 2 when the equipment is set into analytical mode. CIS: cooled injection system. PTV: programmable temperature vaporiser.**

Further conditions were: Carrier gas, hydrogen; inlet pressure, 250 kPa; detector temperatures, 260°C. The temperature program of the GC ovens and programmable injector plus the timed events of the splitter and DCS valve are visualised in figure 4.4. The DCS valve opened at t = 30 minutes and closed again at t = 32 minutes. The sample was injected into the second column by flash heating the transfer line at t = 33 minutes.

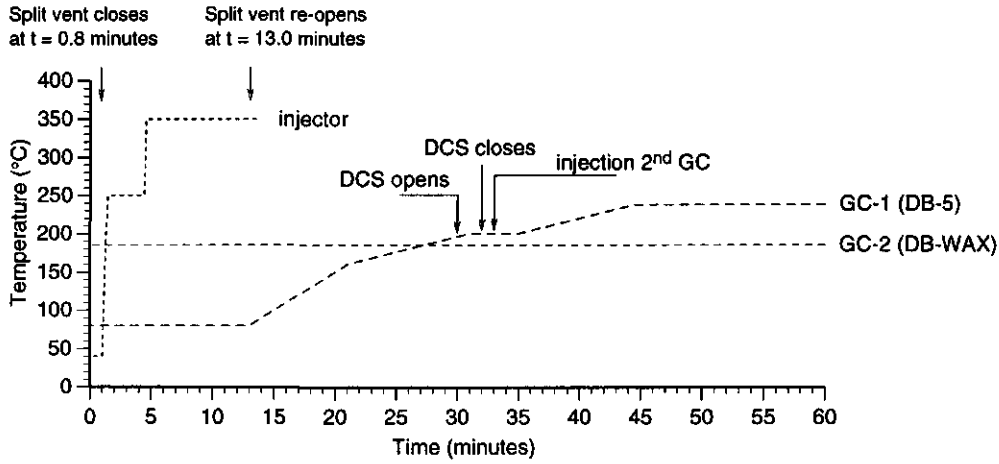



*Fig. 4.4* **Temperature programs of the two GC's and the injector plus the timed events of the splitter and DCS valve.**

*Gland sex pheromone amount measurement calibration*

(E)-8-tetradecenyl acetate in amounts of 0.1 to 1000 ng of was injected into the 2D-GC system under similar conditions as the test samples. The areas obtained (means of three replicates) were regressed against concentration that provided a straight line with an $r^2 = 0.9997$ (coefficient of determination, Hewlett Packard 32S calculator) what was used for external standardisation. The quantitative calculations were done by comparing the observed integrated peak areas of each pheromone component peak with this external standard. Sex pheromone compounds and external standard had the same relative FID response.

## 4.3 Results and discussion

It was found that, when the injector was heated for less than 13 minutes or set at a lower temperature than 350°C, sex pheromone components stayed behind in the liner (gland). This was tested by desorbing the same liners a second time. The shape of the injector temperature program was the result of a trial and error process to determine the optimal conditions. Perhaps that the first temperature plateau at 250°C in the injector temperature program (figure 4.4) was not essential, however, this was not further tested. The overall temperature program of the first GC was chosen in such a way that the peaks of interest eluted within a reasonable time. The conditions of the second GC provided a base-line

separation of the two main compounds (E,Z)-3,7-tetradecadienyl acetate and (E)-3-tetradecenyl acetate and the mixture (Z)-7- plus (Z)-5-tetradecenyl acetate. A typical set of gas chromatograms from the 2D-GC is shown in figures 4.5 and 4.6. When the peaks of the second GC, eluting from 43.5 to 45.0 minutes (figure 4.6), were collected and offered to conspecific males, they induced a full behavioural response. Males exhibited an intense wing fluttering and they attempted to copulate with the glass trap.

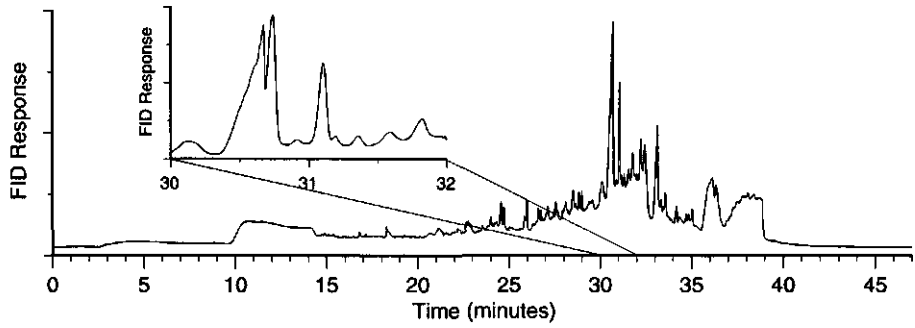



*Fig. 4.5* **Gas chromatogram of one sex pheromone gland of *Symmetrischema tangolias* on the 2D-GC, FID response of the first GC (DB-5). The inset displays the part of the chromatogram which was cut by the DCS valve and sent to the second column.**

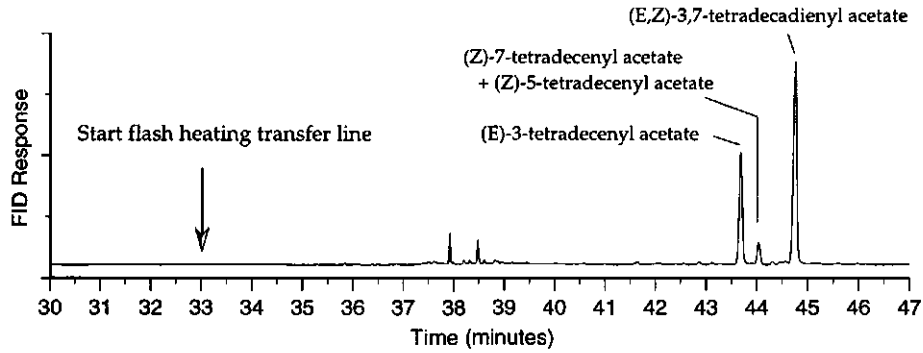



*Fig 4.6* **Gas chromatogram of one sex pheromone gland of *Symmetrischema tangolias* on the 2D-GC, FID response of the second GC (DB-WAX).**

When the temperature of the second GC was set at 165°C, the (Z)-7- and (Z)-5-tetradecenyl acetate peaks could be resolved as well. Where measured, the contribution of (Z)-5-tetradecenyl acetate did not exceed 0.5% of the total amount of sex pheromone. The identity of the peaks was confirmed through spiking experiments.

The quantitative detection limit of the two-dimensional GC system for these experiments was experimentally determined at 0.5 ng per sex pheromone component. When reference

samples were injected, amounts smaller than 0.1 ng could be detected well, however, with intact glands there was always some noise from unidentified co-eluting impurities.
For the gland content examination, samples were taken every two hours during the 24-hrs dark-light cycle, however, due to the limited number of replicates, the 95% confidence intervals turned out to be relatively large. Therefore, the measured amounts were pooled to compare data in four time periods. The amounts of sex pheromone in the glands during these time periods are shown in figure 4.7.

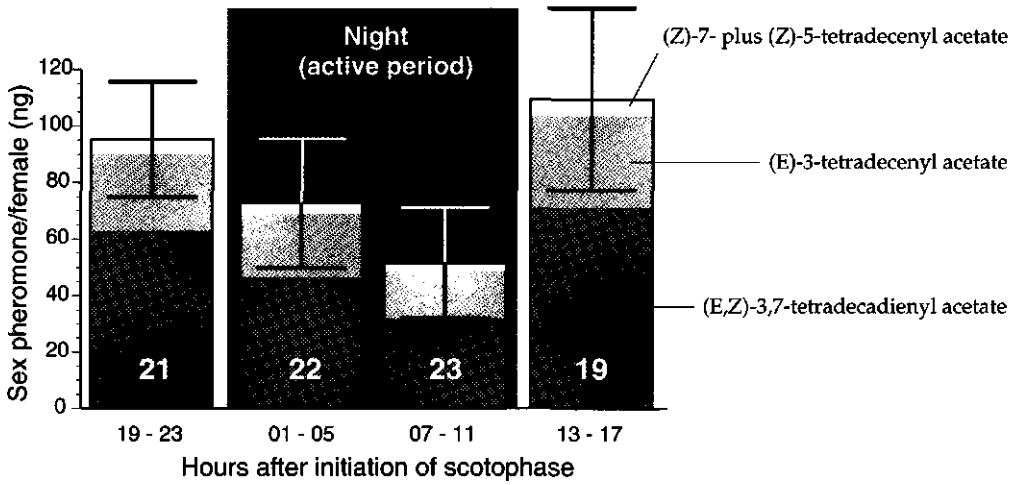



*Fig. 4.7* **Amount and composition of the sex pheromone gland contents of *Symmetrischema tangolias* during the 24-hrs dark-light cycle. Bars indicate 95% confidence intervals for the total amounts. White numbers in the columns represent the number of replicates.**

The measured sex pheromone amounts appeared to be highly variable from individual to individual and varied from 3.8 to 350.0 ng/♀. From figure 4.7 it is concluded that the sex pheromone amount is significantly lower during the calling period 7 - 11 hr after initiation of the scotophase (dark period), when compared to the two periods (13 - 17 and 19 - 23 hr) in the photophase (light period). When the total night and day periods were compared, it appeared that they were significantly different (F-test, $P < 0.001$). The estimated means for night and day are 62.2 and 101.5, respectively, with a standard error for the difference between the means: sed = 11.3[8,9]. At the moment that the lights went on in the rearing chambers (at 12 hr), it was observed that calling activity stopped immediately. The results obtained suggest that the production of sex pheromones does not stop immediately whereas release of sex pheromone does. During the photophase, the sex pheromone accumulates in the gland. It is therefore concluded that *S. tangolias*

produces sex pheromone continuously probably only regulated by the gland sex pheromone titre.

Other lepidopteran species have been reported to have higher sex pheromone amounts in their glands when compared to *Symmetrischema tangolias*, for example: *Adoxophyes orana*, 182 ± 15 ng/♀ in the calling period[10]; *Cryptophlebia leucotreta*, 412 ± 186 ng/♀ in the calling period[3]; *Trichoplusia ni*, 740 ng/♀ in the photoperiod and 420 ng/♀ in the scotophase, (estimated from a reported graph)[5]. During the entire period of measurements the number of females in the (well ventilated) rearing chambers varied. A higher density of females may have affected their amounts of sex pheromone[4,11]. It was not tested whether the density of females in the rearing chambers and the amount of sex pheromone correlated.

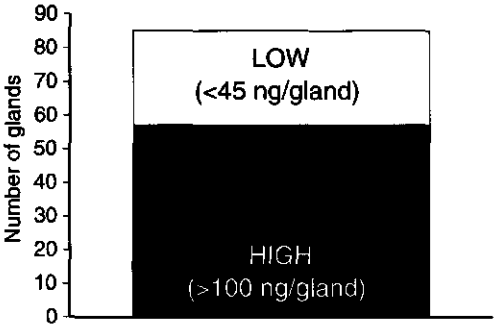
From figure 4.7, it seems that the sex pheromone composition remains stable during the entire 24-hrs dark-light cycle. In order to check this more precisely, the total amounts of sex pheromone compounds, per time period, were set to 100% and the relative amounts of individual components were calculated. The results are shown in table 4.1.

*Table 4.1* **Average relative amounts of pheromone compounds found in the sex pheromone glands of *Symmetrischema tangolias* during four periods of the light-dark cycle.**

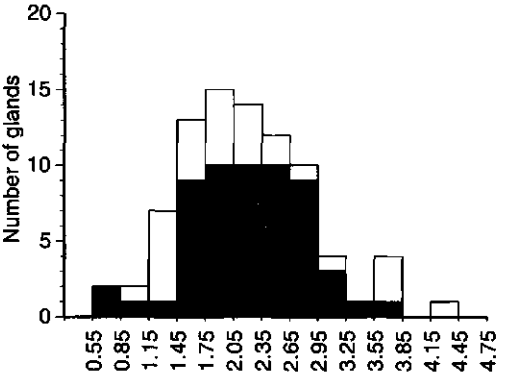
| Hours after initiation of scotophase | Compound | Composition per ♀ (%) mean ♀ | ± | 95% CI |
|---|---|---|---|---|
| 01 - 05 | (E,Z)-3,7-tetradecadienyl acetate | 63.9% | ± | 3.4% |
| | (E)-3-tetradecenyl acetate | 30.6% | ± | 3.0% |
| | (Z)-7- + (Z)-5-tetradecenyl acetate | 5.5% | ± | 0.7% |
| 07 - 11 | (E,Z)-3,7-tetradecadienyl acetate | 59.9% | ± | 3.9% |
| | (E)-3-tetradecenyl acetate | 32.9% | ± | 3.5% |
| | (Z)-7- + (Z)-5-tetradecenyl acetate | 7.2% | ± | 1.4% |
| 13 - 17 | (E,Z)-3,7-tetradecadienyl acetate | 64.6% | ± | 3.1% |
| | (E)-3-tetradecenyl acetate | 29.8% | ± | 2.7% |
| | (Z)-7- + (Z)-5-tetradecenyl acetate | 5.6% | ± | 1.0% |
| 19 - 23 | (E,Z)-3,7-tetradecadienyl acetate | 64.6% | ± | 2.0% |
| | (E)-3-tetradecenyl acetate | 29.9% | ± | 2.1% |
| | (Z)-7- + (Z)-5-tetradecenyl acetate | 5.5% | ± | 0.4% |
| total period | (E,Z)-3,7-tetradecadienyl acetate | 63.1% | ± | 1.6% |
| | (E)-3-tetradecenyl acetate | 30.9% | ± | 1.4% |
| | (Z)-7- + (Z)-5-tetradecenyl acetate | 6.0% | ± | 0.5% |

All confidence intervals of the mean relative amounts of individual compounds overlap. It is, therefore, concluded that the ratio of the different components in the pheromone blend does not fluctuate during the 24-hrs dark-light cycle.

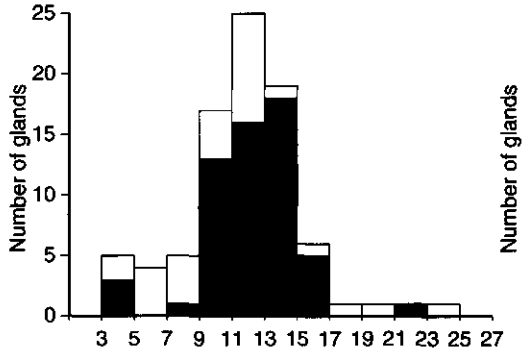
In addition, the distribution of ratios of the pheromone components in the individual glands was examined. The glands (N = 85) were divided into three groups, namely low, medium and high pheromone titre. All three groups contained approximately the same number of measurements (figure 4.8 A). The ratios of the sex pheromone components, plotted in histograms, are shown in figure 4.8.



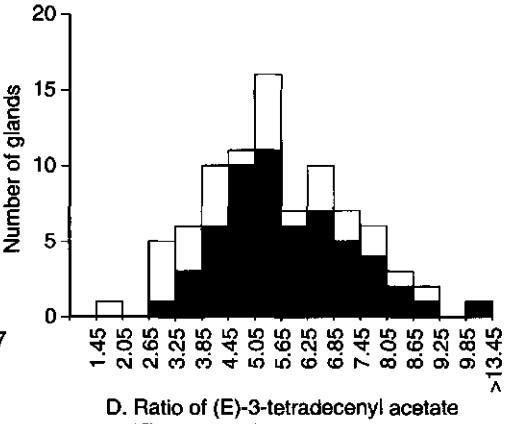

(A) The sex pheromone amount measurements, divided in three equal groups: low, medium and high.




A. Ratio of (E,Z)-3,7-tetradecadienyl acetate to (E)-3-tetradecenyl acetate.




C. Ratio of (E,Z)-3,7-tetradecadienyl acetate to (Z)-7- plus (Z)-5-tetradecenyl acetate.




D. Ratio of (E)-3-tetradecenyl acetate to (Z)-7- plus (Z)-5-tetradecenyl acetate.

*Fig. 4.8* **The ratios of the different sex pheromone constituents of *Symmetrischema tangolias* presented in histograms, subdivided into low, medium and high individual measurements.**

The distribution is more or less similar for all three combinations. All ratios show large variation. The three titres, i.e. low, medium and high, are randomly distributed in this respect. It appears, therefore, that the variation in ratios is not related to the absolute sex pheromone quantities in the glands.

From this research it is concluded that two-dimensional GC, combined with the programmable injector described , is an excellent tool to unravel pheromone titres and the composition of individual glands. The amount of sex pheromone in the glands of *Symmetrischema tangolias* is at its lowest level during the calling period. The commonly accepted assumption that the calling period is the best time for the collection of glands for analyses does not hold for *Symmetrischema tangolias*.

## 4.4 References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# Chapter 5

## Isolation, identification and synthesis of the sex pheromone of *Scrobipalpuloides absoluta**

### 5.1 Introduction

*Scrobipalpuloides absoluta* (Meyrick) (figure 1.5) is a severe pest on tomatoes in large parts of South America, notably Peru, Chile, Brazil, Argentina, Bolivia, Venezuela and Colombia[1]. The unbridled use of pesticides against this species has led to resistance and forced farmers to find other ways to control this pest. The use of sex pheromones, as part of an integrated pest management (IPM) program, has proven to be efficient in the control of the related species *Phthorimaea operculella* (Zeller) by mass trapping[2]. For this insect the use of sex pheromones appears to be even more effective and cheaper than the use of pesticides[1]. It is expected that if a sex pheromone of *Scrobipalpuloides absoluta*, was available, it would also be effective in the control of this species. For this reason a research project was started to elucidate the structures of the sex pheromone components of *Scrobipalpuloides absoluta*.

### 5.2 Methods and materials

The recording of *electroantennograms (EAG's)*, *synthesis*, the use of *coupled gas chromatography-electroantennographic detection (GC-EAD)*, the *dual column GC* and the *two-dimensional GC (2D-GC)* have been described already in chapters 3 and 4. For the technical details about these techniques, see chapters 3.2 and 4.2.

*Insects*

A laboratory culture of *Scrobipalpuloides absoluta* was started from pupae, collected in greenhouses of the International Potato Center (CIP) in Lima, Peru, in July 1992.

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*S. absoluta* was reared in cages on tomato plants (cv. Money Maker) under climatological rearing conditions resembling the natural environment of this species as much as possible: 26 ± 1°C at day and 22 ± 1°C at night, 65 ± 5% relative humidity and a 15.5L : 8.5D photoperiod. The larvae pupated in soil underneath the tomato plants. After approximately 35 days, the first adults appeared in the cages. From that moment on the newly-emerged moths were collected twice a day and separated into males and females before any copulation could take place. Moths were fed on a 10% honey solution.

*Pheromone extracts*

Three to four-day-old virgin females were collected at the end of the scotophase because females were observed to call at this age and in this time period. Slightly more than the tips of the abdomens were cut off[3] and kept in double-distilled and degassed hexane. All extracts were stored under nitrogen at –20°C until required for use. Bioassays were carried out by offering a piece of filter paper[4] loaded with 2 FE (female equivalents) of extract to conspecific males. Bioactive fractions elicited intense 'flutter' responses and attempts to copulate with the filter paper.

*Dimethyl disulphide (DMDS) derivatisation*

Approximately 50 µl of freshly distilled DMDS and 5 µl of a 5% iodine solution in diethyl ether were added to 10 sex pheromone glands in a 1 ml vial (Chrompack, Bergen op Zoom, The Netherlands) under nitrogen. The vial was sealed and heated for 16 hr at 60°C in an oven. The reaction was quenched by the addition of aqueous saturated $Na_2S_2O_3$ solution. Usually, one drop was sufficient which could be observed by the fading of the dark red colour of the iodine. A spatula tip of NaCl (for drying) and 200 µl distilled hexane was added. The organic phase was separated with a syringe and concentrated to approximately 10 µl by evaporating the solvent at 40°C in a nitrogen atmosphere[5,6].

*Coupled gas chromatography-mass spectrometry (GC-MS)*

GC-MS was performed on a Finnigan MAT 95 mass spectrometer (at 70 eV unless stated otherwise), coupled to a Varian GC equipped with a split/splitless injection system. Injections were done in splitless mode only (1-2 µl). The column was a J&W 25 m DB-5 fused silica column, 0.25 mm id and 0.25 µm film thickness. Conditions were: carrier gas, helium; temperature programming, 50°C (2 min hold) to 100°C (0 min hold) at 25°C/min and then to 280°C (8 min hold) at 4°C/min; injector and transfer line temperature, 250°C.

*Field tests and statistical analysis.*

Red rubber septa (Phase Separations, Waddinxveen, The Netherlands) were loaded with 1 mg of synthetic sex pheromone mixture in 200 µl $CH_2Cl_2$. All the synthetic compounds

contained approximately 0.1% of 2,6-di-*tert*-butyl-4-methylphenol (BHT) as antioxidant[7]. The field tests were performed in a tomato field in the centre of a 150 ha tomato farm in Ica, Peru. A completely randomised block design with four blocks was used. The treatments were six traps (a control and five experimental ones). During the experimental period the numbers of captured moths were counted on a daily base. Since in the first period (six days) traps were used that were different from those used in the remaining part of the experimental period (20 days), cumulative counts of the first week and those of the remaining period were analysed separately.
On the logarithmically transformed total counts for the two separate periods ANOVA was performed using the model:

$$^{10}\log(Y_{ij}) = \mu + \mathbf{block}_i + \mathbf{trap}_j + e_{ij}$$

where $\mu$ is the general mean, $block_i$ = effect of the $i^{th}$ block (i = 1...4), $trap_j$ = effect of the $j^{th}$ trap (j = 1...6). The error terms $e_{ij}$ are assumed to be independently normally distributed with mean 0 and variance $\sigma^2$. Block and trap effects were assessed using the F-ratios resulting in F-tests. Pairwise differences between treatment-means on the logarithm-scale were tested using a t-test. Differences between treatments were considered to be non-significant at $P \geq 0.05$. Analyses were performed using the Genstat statistical program[8,9].

*NMR.*
NMR spectra were recorded on a Bruker DPX-400 at 400 MHz in $CDCl_3$ (99.6% D, Janssen Chimica). Chemical shifts are reported in parts per million ($\delta$) relative to TMS. The chemical shift of $CHCl_3$ ($\delta$ = 7.24) was used as internal reference .

## 5.3 Results and discussion

### 5.3.1 Identification and synthesis

A hexane extract was prepared from 50 sex pheromone glands of virgin females. This extract elicited a clear-cut behavioural response when offered immediately after its preparation to conspecific males. The attractiveness was lost after one week, although the extract had been stored at –20°C under $N_2$. This suggested that one or more of the sex pheromone components were unstable. Therefore, it was postulated that the sex pheromone contained aldehydes or compounds with several conjugated double bonds.

GC-EAD recordings of a fresh gland extract (20 FE) revealed that there was only a small time window where EAG active peaks eluted (figure 5.1).

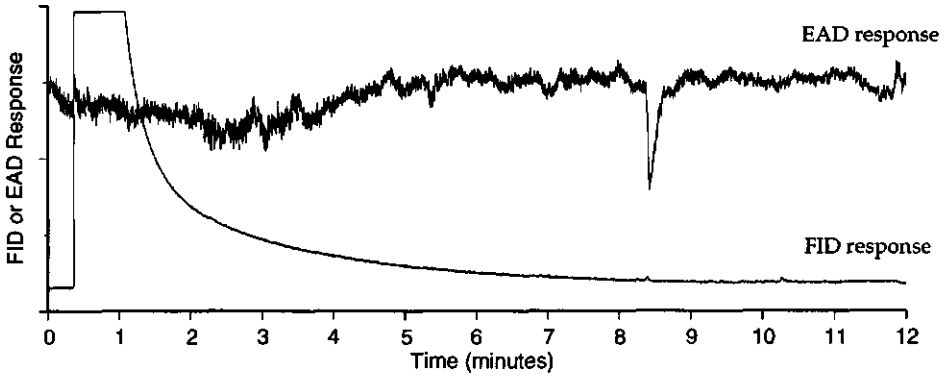



*Fig. 5.1* **GC-EAD of a sex pheromone gland extract of *Scrobipalpuloides absoluta*.**

The resolution of the used GC-EAD equipment was insufficient to determine whether just one, or more compounds were responsible for the EAD activity. Examination of a fresh gland extract (20 FE) with GC-MS under similar conditions as the GC-EAD revealed two possible sex pheromone candidate peaks in a ratio of 92 : 8.

The mass spectrum of the major sex pheromone peak is shown in figure 5.2.

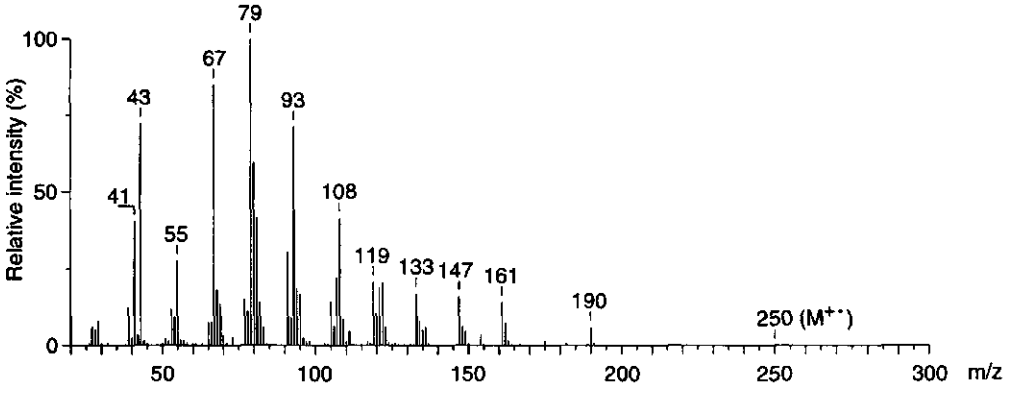



*Fig. 5.2* **Mass spectrum of the major sex pheromone peak found in the sex pheromone gland extract of *Scrobipalpuloides absoluta*. The molecular ion ($M^{+\cdot}$) was detected at *m/z* 250.**

The molecular ion ($M^{+\cdot}$) at $m/z$ 250 and the further fragmentation pattern, which is characteristic for a straight carbon chain, suggested the major sex pheromone component to be a tetradecatrienyl acetate. The mass spectrum of the minor sex pheromone peak is shown in figure 5.3.

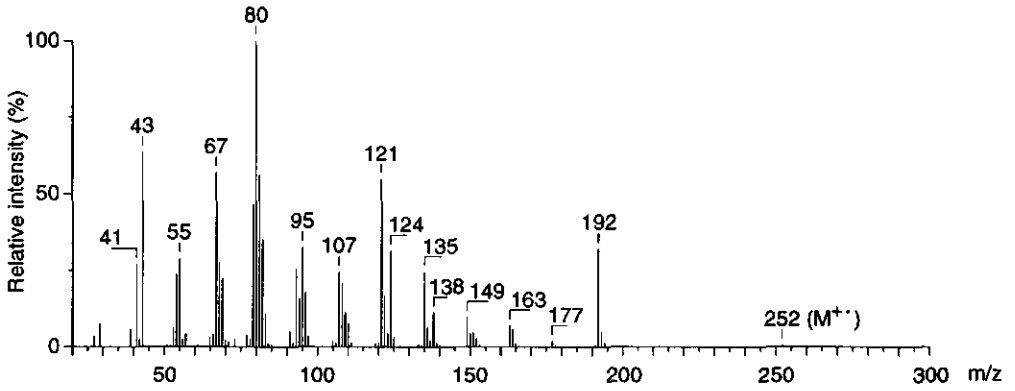



*Fig. 5.3* **Mass spectrum of the minor sex pheromone peak found in the sex pheromone gland extract of *Scrobipalpuloides absoluta*. The molecular ion ($M^{+\cdot}$) was detected at *m/z* 252.**

The mass spectrum of this peak revealed the same characteristics as that of the major sex pheromone component. The molecular mass of $m/z$ 252 suggested that the minor sex pheromone component was a tetradecadienyl acetate.

The examination of the sex pheromone gland extract by dual column GC gave no clear information about its constituents on the DB-WAX column as none of the peaks dominated. On DB-1 however, the two sex pheromone peaks could be designated. The calculated retention indices (RI's) of 1755.5 and 1753.0 for the major and the minor sex pheromone components, respectively, were in agreement with the assumption that both of these compounds were linear acetates with a carbon chain length of fourteen. Without the matching RI's on the polar DB-WAX column, it was impossible to determine whether or not these compounds had a conjugated double bond system. By means of two-dimensional preparative GC, it was possible to collect approximately 100 ng of the pure major sex pheromone component by means of direct introduction of four sex pheromone glands (without prior extraction) into the equipment (see for details chapter 4). The two gas chromatograms of the two-dimensional GC are shown in figure 5.4.

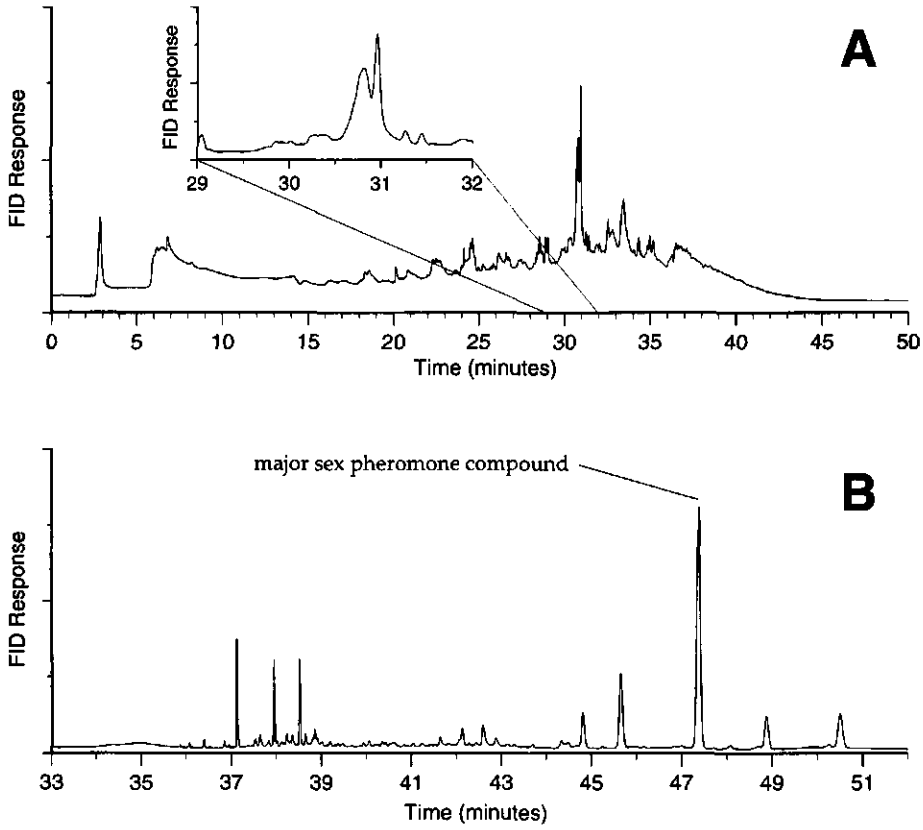



*Fig. 5.4* **Gas chromatogram of four sex pheromone glands of *Scrobipalpuloides absoluta* which were introduced, via a temperature programmable injector, into a two-dimensional GC. A) FID response of the first GC (DB-5). The inset shows the part that was sent to the second GC. B) FID response of the second GC (DB-WAX) (in analytical mode).**

The different peaks in the range from 43 to 51 minutes were collected individually but only the major peak (figure 5.4 B) elicited a behavioural response in conspecific males. The glass trap with this compound elicited an intense 'flutter' responses, which, however, stopped after about 15 seconds. No attempts to copulate with the trap were observed. This glass trap was rinsed with 10 µl hexane, which was concentrated to 1 µl, and injected splitless on the dual column GC. In this way, the RI on the polar DB-WAX column was established (RI: 2213.7). The RI for this compound was expected to have a value of more than 2300 in case it had two or more of the double bonds conjugated. With a RI of 2213.7, it was concluded that this compound could not possess conjugated double bonds.

The unusually large fragment at *m/z* 108 in the mass spectrum of the major sex pheromone component (figure 5.2) is known to occur in triple-unsaturated homo-conjugated linear compounds (figure 5.5)[10].

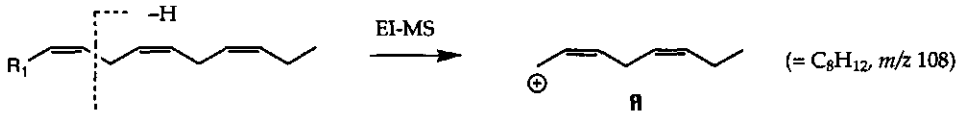



*Fig. 5.5* **Triple-unsaturated homo-conjugated linear compounds show a particular fragment with electron impact mass spectrometry (EI MS).**

In case the major sex pheromone compound had such a homo-conjugated system the peak at *m/z* 108 was expected to increase when the mass spectrum was taken at a lower ionisation voltage, analogous to reported mass spectra from compounds with a homo-conjugated double bond system[10]. Figure 5.6 shows the 18 eV mass spectrum of the major sex pheromone component.

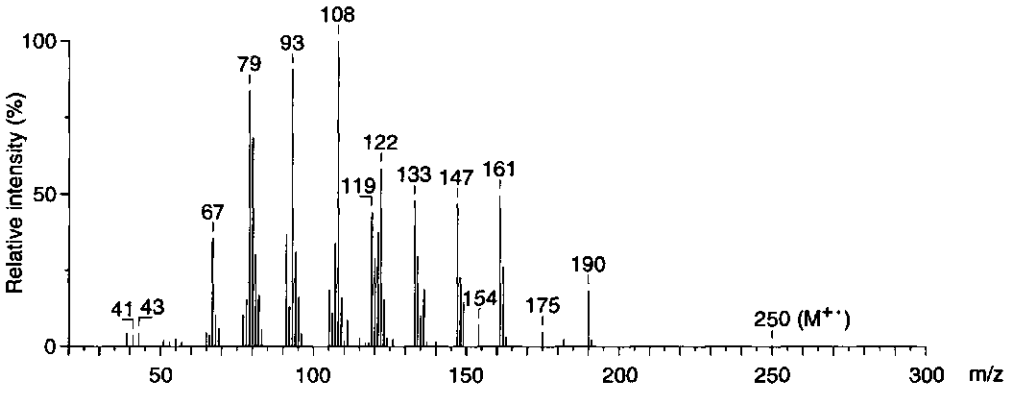
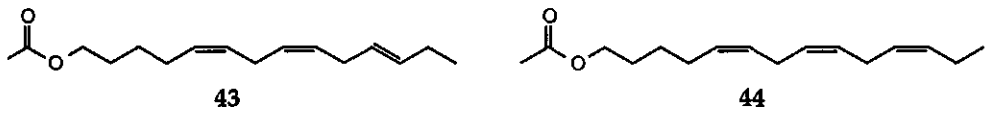



*Fig. 5.6* **Low ionisation voltage (18 eV) mass spectrum of the major sex pheromone component of *Scrobipalpuloides absoluta*.**

Because of the intensity increase of the peak at *m/z* 108 in the 18 eV mass spectrum, it was postulated that the major sex pheromone component was a 5,8,11-tetradecatrienyl acetate isomer. Two of the isomers, namely (Z,Z,E)-5,8,11-tetradecatrienyl acetate (**43**) and (Z,Z,Z)-5,8,11-tetradecatrienyl acetate (**44**) (figure 5.7), were synthesised in small quantities by similar techniques as described further in this chapter.



43 44

*Fig. 5.7* **The two synthesised triple homo-conjugated acetates (Z,Z,E)-5,8,11-tetradecatrienyl acetate (43) and (Z,Z,Z)-5,8,11-tetradecatrienyl acetate (44), which were possible candidates for the major sex pheromone compound.**

A small amount of a mixture of **43** and **44** was added to four sex pheromone glands of *S. absoluta* and injected into the two-dimensional GC. The gas chromatogram (figure 5.8) revealed that **43** and **44** were not identical with the major sex pheromone component.

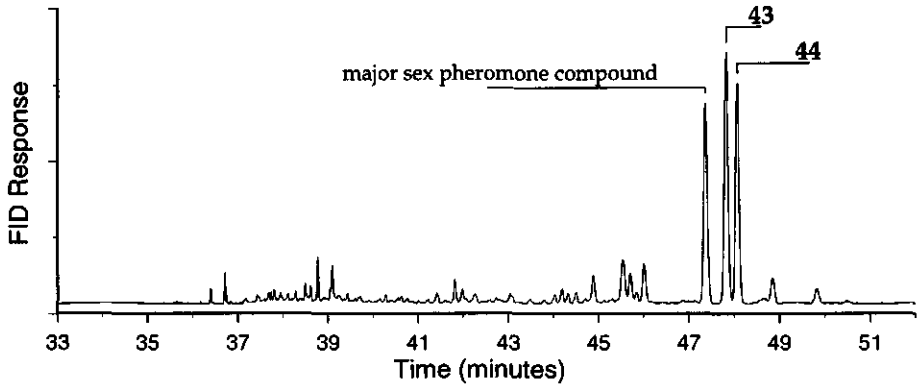



*Fig. 5.8* **A gas chromatogram of four sex pheromone glands of *Scrobipalpuloides absoluta* spiked with a mixture of (Z,Z,E)-5,8,11-tetradecatrienyl acetate (43) and (Z,Z,Z)-5,8,11-tetradecatrienyl acetate (44). The figure displays the gas chromatogram of the second GC (DB-WAX) of the two-dimensional GC.**

From a large number of mono- and poly-unsaturated acetates and alcohols, which were available at IPO-DLO[11] it was established that straight chain molecules with (E) double bond(s) eluted faster on the polar DB-WAX column than the corresponding molecules with (Z) double bond(s). The RI's for non-available, poly-unsaturated straight chain compounds could be predicted (to a certain level) through extrapolation of the obtained RI values of available mono- and poly-unsaturated compounds. The all-trans isomer of 5,8,11-tetradecatrienyl acetate would, of course, elute first of all possible 5,8,11-triple-unsaturated compounds and it could be predicted that even the all-trans isomer of 5,8,11-tetradecatrienyl acetate would elute after the major sex pheromone compound. In other words, the major sex pheromone compound could not have the 5,8,11-tetradecatrienyl acetate configuration.

By means of electroantennogram (EAG) recordings of the related mono-unsaturated compounds, it is sometimes possible to gain information about the positions and stereochemistry of the double bonds in poly-unsaturated sex pheromone compounds. For that reason, all the mono-unsaturated tetradecenyl acetates were tested by EAG recordings. The result is shown in figure 5.9.

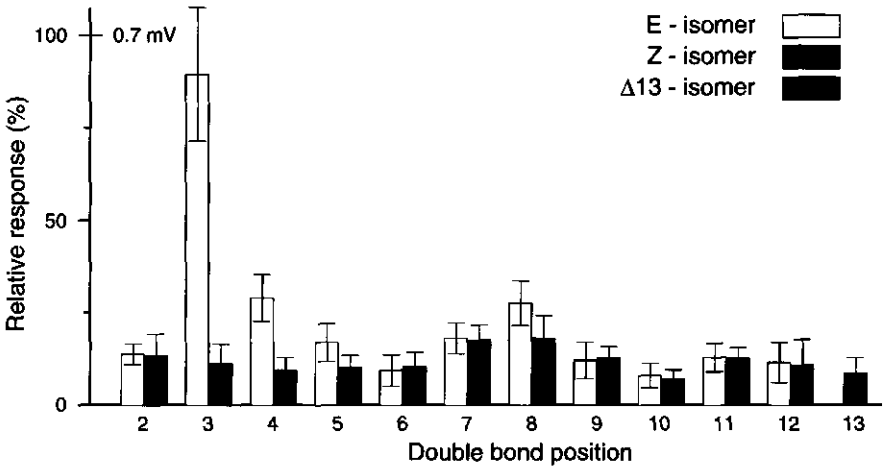



*Fig. 5.9* **Electroantennogram responses of male *Scrobipalpuloides absoluta* to all tetradecenyl acetate isomers. Bars indicate 95% confidence intervals. N = 15.**

In contrast to the clear-cut conclusions from the EAG recordings of *Symmetrischema tangolias* (chapter 3), for *Scrobipalpuloides absoluta* only the (E)-3-isomer gave a significantly larger response than all the other isomers. This suggested that at least one of the sex pheromone components possessed a double bond at the 3-position. The large difference in response between the (E)- and the (Z)-isomer indicated that the stereochemistry of this double bond was most likely to be (E).

It was decided to convert the sex pheromone compounds with dimethyl disulphide (DMDS) into the corresponding DMDS derivatives. Subsequent MS analysis should provide the essential information for deducing the original double bond positions. Because it was observed that the sex pheromone extract lost its attractiveness when stored for one week, 20 sex pheromone glands were excised from females and treated directly with DMDS without prior extraction. DMDS derivatives of the double-unsaturated (minor) and of the triple-unsaturated (major) sex pheromone compounds were detected in the reaction mixture. The gas chromatogram of the DMDS reaction mixture is shown in figure 5.10.

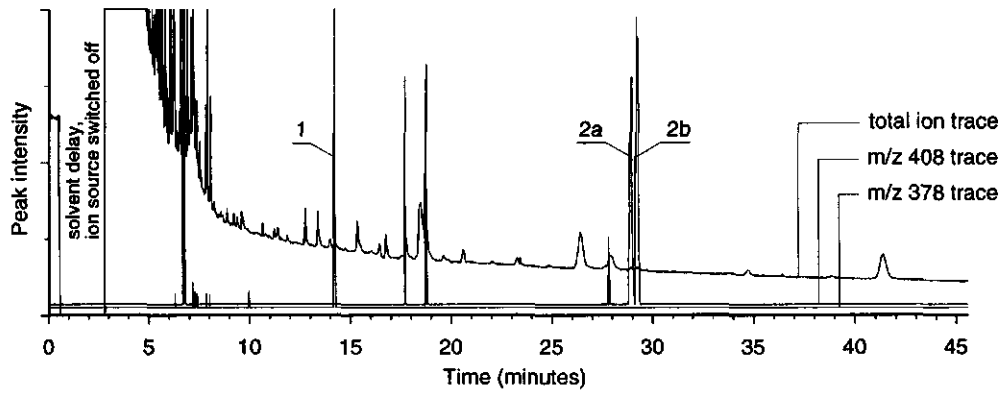



***Fig. 5.10*** **Gas chromatogram of the reaction mixture of 20 *Scrobipalpuloides absoluta* sex pheromone glands which were treated with DMDS. Peak 1: DMDS derivative of the minor component, peak 2a,b: DMDS derivatives of the major component.**

The DMDS derivative peaks were relatively small but were unambiguously identified in the matrix by selective ion monitoring of the expected molecular ion fragments; *m/z* 378 for the minor, and *m/z* 408 for the major sex pheromone component (see also chapter 2). The DMDS derivative of the major sex pheromone component showed two peaks (peaks 2a,b figure 5.10) with virtually identical mass spectra. These peaks were thought to be diastereoisomers.

The mass spectrum of the DMDS derivative of the minor pheromone component is shown in figure 5.11.

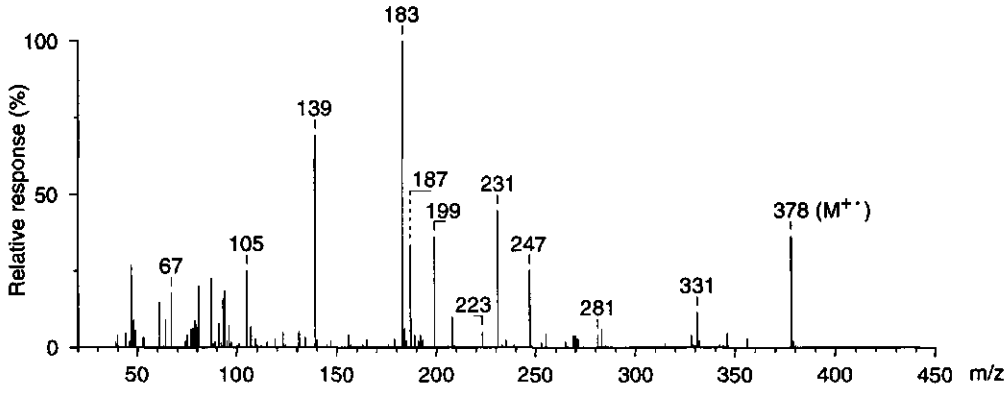
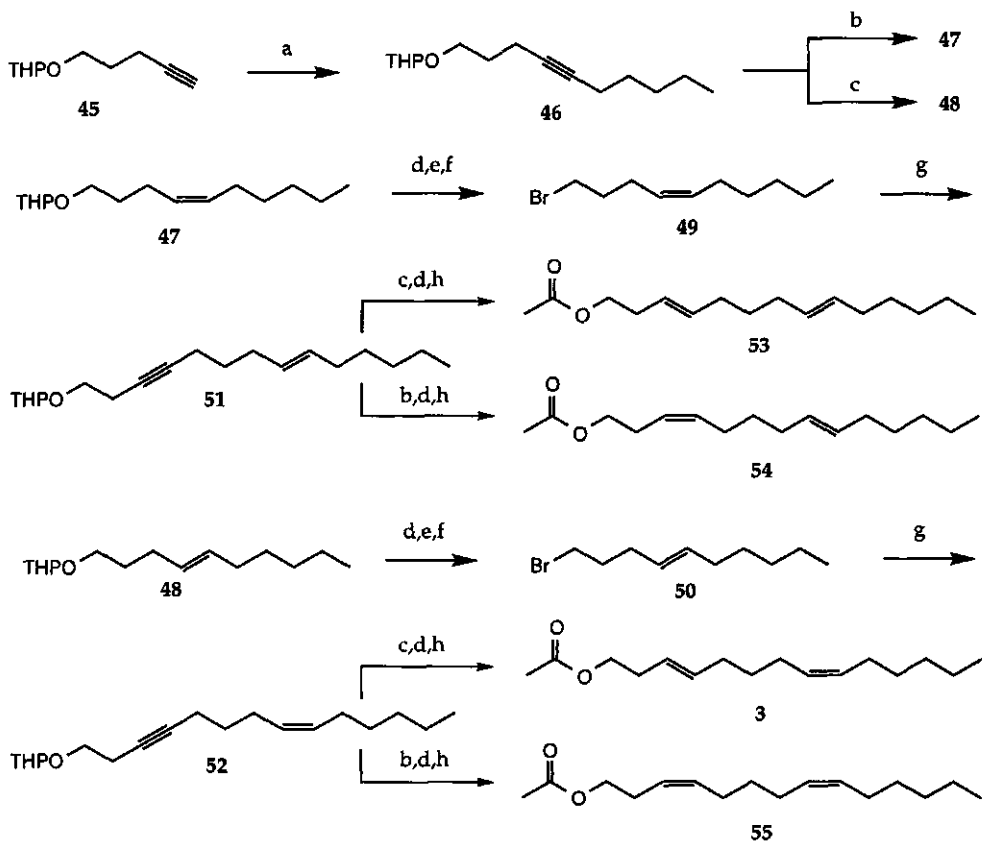



***Fig. 5.11*** **Mass spectrum of the DMDS derivative of the double-unsaturated minor sex pheromone component of *Scrobipalpuloides absoluta*. The molecular ion ($M^{+\cdot}$) was detected at *m/z* 378.**

The mass spectrum of the DMDS derivative of the minor pheromone component gave a fragmentation pattern which corresponded to a 3,8-tetradecadienyl acetate as the original molecule (see also chapter 2). To untangle the E/Z-configuration of the double bonds, all four isomers of 3,8-tetradecadienyl acetate were synthesised in a stereoselective procedure as visualised in scheme 5.1.




*Scheme 5.1* **Reagents: a) n-BuLi, bromopentane, THF, HMPA; b) P-2 Ni, $H_2$, EtOH; c) $LiAlH_4$, THF, diglyme, 140°C; d) PTSA, $H_2O$, MeOH; e) *p*-TsCl, KOH, ether, 0°C; f) LiBr, DMSO, 80°C; g) 3-butyn-1-ol-THP-ether, n-BuLi, THF, HMPA; h) $Ac_2O$, DMAP.**

Compound **45** was prepared from **35** (scheme 3.2) and dihydropyran (not visualised). The lithium salt of the THP protected 4-pentyn-1-ol (**45**) was alkylated with bromopentane to give the protected alkynol **46** in 84% yield[12]. After reduction of the triple bond with lithium aluminium hydride ($LiAlH_4$)[13] or catalytically with $H_2$ and nickel[14], the molecules were deprotected with *p*-toluenesulfonic acid (PTSA) in a 10% $H_2O$ in MeOH solution, tosylated, and converted into the corresponding bromoalkenes **49** and **50** in 52% and 79%

yield respectively[15], based upon **46**. These bromoalkenes reacted with the lithium salt of THP protected 3-butyn-1-ol to give the protected compounds **51** and **52** in more than 80% yield[12], based upon **49** and **50**. The stereoselective reduction and deprotection of **51** and **52**, followed by acetylation, gave all the stereoisomers **3** and **53 - 55**[16-19].

The RI's of **3** (DB-1 and DB-WAX) matched accurately those obtained for the minor sex pheromone component. Moreover, electroantennography measurements showed that **3** induced a significantly larger response than the other three isomers **53 - 55** when exposed to the antennae of male *S. absoluta*. (figure 5.12). Therefore, the minor sex pheromone component was identified as (E,Z)-3,8-tetradecadienyl acetate.

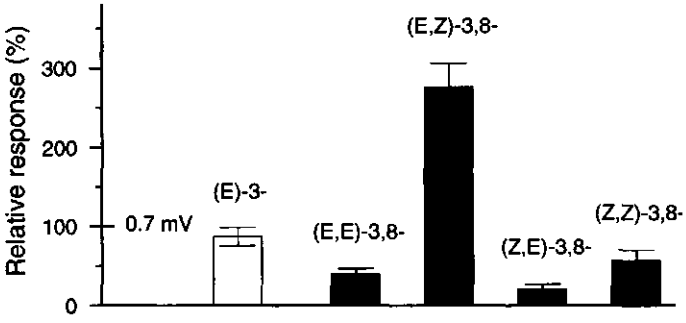



*Fig. 5.12*
**EAG responses of male *Scrobipalpuloides absoluta* to all 3,8-tetradecadienyl acetate isomers compared to (E)-3-tetradecenyl acetate. Bars indicate 95% confidence intervals. N = 12 - 14.**

The mass spectrum of one of the two DMDS derivatives of the major sex pheromone component is shown in figure 5.13.

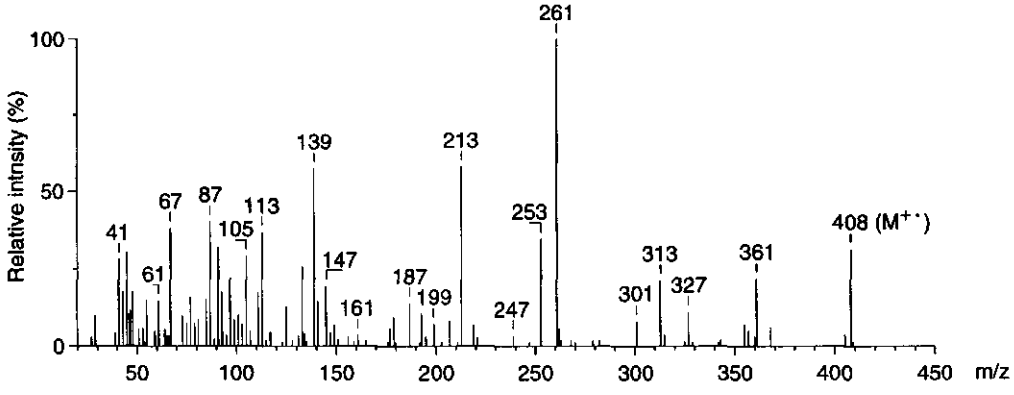
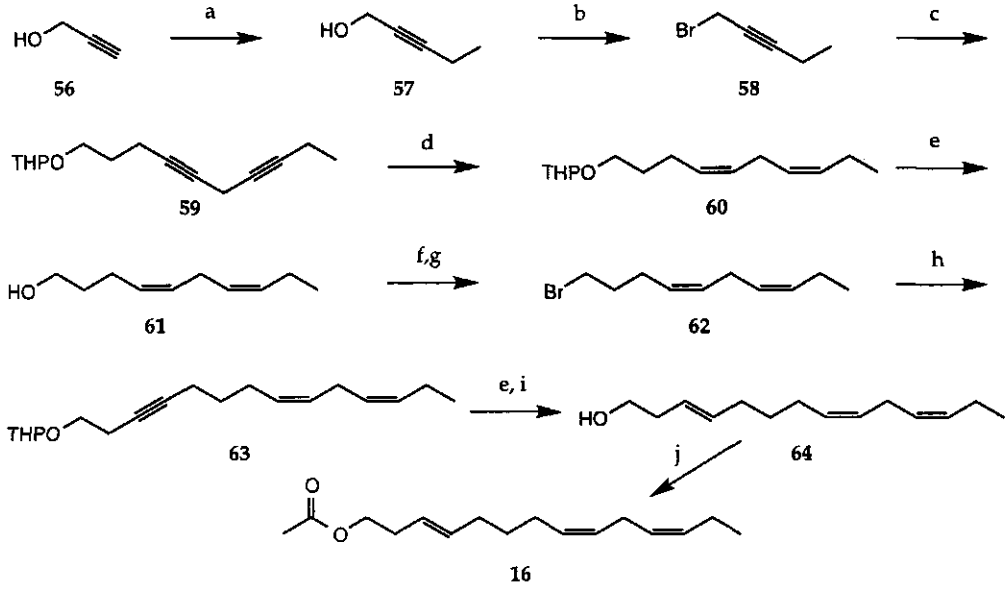



*Fig. 5.13* **Mass spectrum of the DMDS derivative of the triple-unsaturated major sex pheromone component of *Scrobipalpuloides absoluta*. The molecular ion ($M^{+\cdot}$) was detected at *m/z* 408.**

From the MS fragmentation pattern of the DMDS derivative of the major triple-unsaturated component, in first instance only the double bonds at positions 3 and 8 could be determined. The stereochemistry of the double bonds at the 3- and 8-positions was expected to be similar to that of the minor sex pheromone component, considering the large difference in EAG response of **3** when compared to EAG's of the other three isomers **53** - **55** (figure 5.12). RI calculations indicated that the stereochemistry of the third double bond was most likely (Z)-11-, (E)-12- or at the Δ13-position. It was decided to synthesise all these isomers.

The first triple-unsaturated candidate, (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**), was synthesised in a stereoselective way as visualised in scheme 5.2.

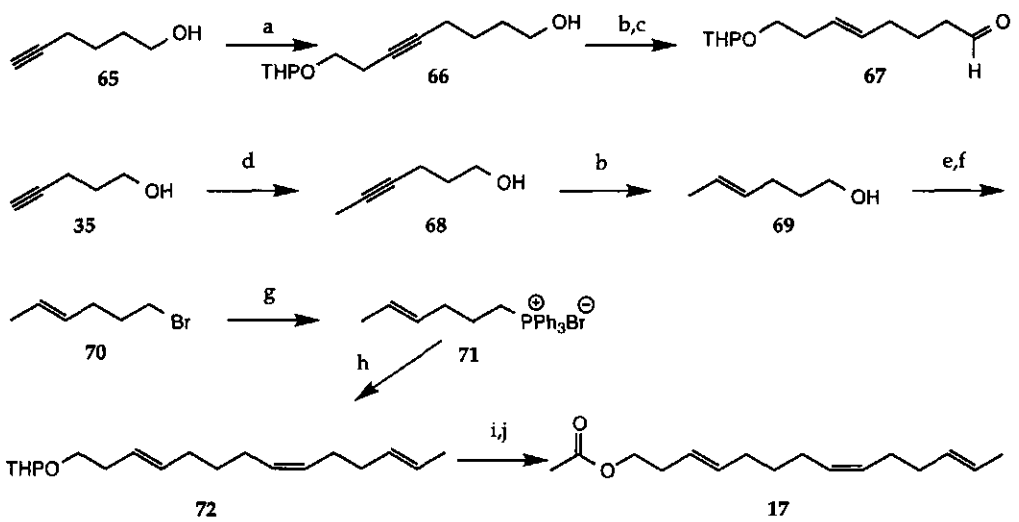



*Scheme 5.2* **Reagents: a) $LiNH_2$, $NH_3$, EtBr; b) $PBr_3$, ether; c) 4-pentyn-1-ol-THP-ether (45), EtMgBr, CuI, THF; d) P-2 Ni, $H_2$, EtOH; e) PTSA, $H_2O$, MeOH; f) *p*-TsCl, KOH, ether, 0°C; g) LiBr, DMSO, 80°C; h) 3-butyn-1-ol-THP-ether, n-BuLi, THF, HMPA; i) $LiAlH_4$, THF, reflux; j) $Ac_2O$, DMAP.**

1-Bromo-2-pentyn (**58**) was prepared in two steps from propargyl alcohol (**56**) in a 56% yield based upon **56**[15]. The Grignard derivative of the THP protected 4-pentyn-1-ol (**45**) was alkylated with bromide (**58**) to give the THP protected diynol **59**[15]. This diynol was catalytically hydrogenated[14] with $H_2$ and deprotected with PTSA to give (Z,Z)-4,7-decadienol **61** in 68% yield based upon **45**. Bromide **62** was obtained in a 58% yield from the dienol **61** as described before. This bromide was subsequently coupled with the

lithium salt of 3-butyn-1-ol-THP-ether to give compound **63** in 82% yield. Deprotection and reduction of **63** afforded the trienol **64** which was subsequently acetylated to give the sex pheromone compound **16** in 19% overall yield based upon **45**[20].

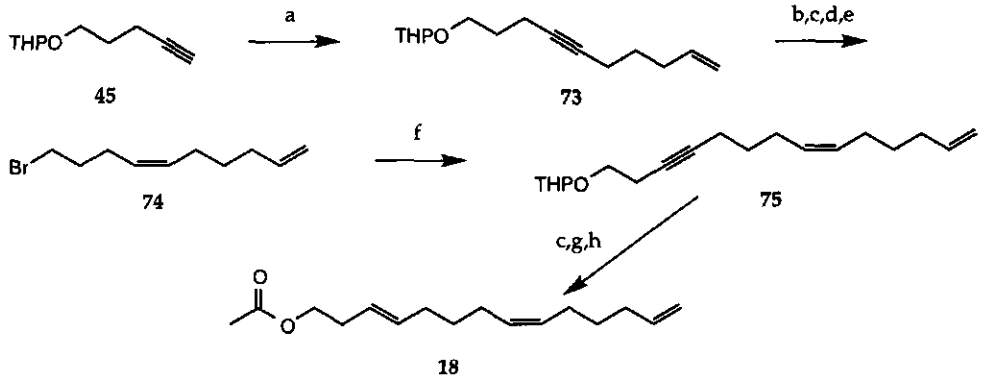
The second triple-unsaturated candidate, (E,Z,E)-3,8,12-tetradecatrienyl acetate (**17**), was synthesised in a stereoselective way as visualised in scheme 5.3.




*Scheme* 5.3 **Reagents: a) $LiNH_2$, $NH_3$, bromoethanol-THP-ether, THF; b) $LiAlH_4$, THF; c) PFC, $CH_2Cl_2$; d) $LiNH_2$, $NH_3$, $CH_3I$; e) *p*-TsCl, KOH, ether; f) LiBr, DMSO; g) $PPh_3$, MeCN; h) t-BuOK, THF, 67, HMPA; i) PTSA, $H_2O$, MeOH; j) $Ac_2O$, DMAP.**

The di-lithium salt of commercially available 5-hexyn-1-ol was coupled in liquid ammonia with the THP-ether of bromoethanol to compound **66**. The semi-protected diol was subsequently trans reduced by lithium aluminium hydride and oxidised to the aldehyde **67** with pyridinium fluorochromate (PFC) in an overall yield of 24% based upon the 5-hexyn-1-ol. 4-Pentyn-1-ol (**35**) was coupled with methyl iodide, trans-reduced and converted into bromide **70** in approximately 40% yield based upon **35**. Compound **70** was converted into its phosphonium salt and submitted to a Wittig reaction with aldehyde **67** to give compound **72** in a low yield. Deprotection of the alcohol and acetylation gave compound **17**[21].

The third triple-unsaturated candidate, (E,Z)-3,8,13-tetradecatrienyl acetate (**18**), was synthesised in a stereoselective way as visualised in scheme 5.4.




*Scheme 5.4* **Reagents: a) n-BuLi, 1-bromo-4-pentene, THF, HMPA; b) P-2 Ni, $H_2$, EtOH; c) PTSA, $H_2O$, MeOH; d) *p*-TsCl, KOH, ether, 0°C; e) LiBr, DMSO, 80°C; f) 4-lithium, 3-butyn-1-ol THP ether, THF, HMPA; g) $LiALH_4$, THF; h) $AC_2O$, DMAP.**

The synthesis of compound **18**[22] was comparable to that of **3** (scheme 5.1). Instead of bromopentane, the commercially available 1-bromo-4-pentene was used in step a) of scheme 5.4. None of the presented synthetic routes were optimised.

The RI's (on DB-1 and DB-WAX) and MS of **16**, and the DMDS derivative of **16**, were identical to those obtained for the major sex pheromone component. A 92 : 8 blend of synthetic (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**) and synthetic (E,Z)-3,8-tetradecadienyl acetate (**3**) elicited an intense 'flutter' response and attempts to copulate with the source when offered to *S. absoluta* males.

### 5.3.2 Field tests

To determine the attractiveness of the identified compounds in the natural environment of *S. absoluta*, field experiments were conducted in Peru. The following mixtures of the (synthetic) sex pheromone components were tested:

| | | | | | |
|---|---|---|---|---|---|
| Control: | solvent only | | | | |
| SA 1: | (E,Z,Z)-3,8,11-14:Ac (**16**) : (E,Z)-3,8-14:Ac (**3**) = | 100 | : | 0 | |
| SA 2: | (E,Z,Z)-3,8,11-14:Ac (**16**) : (E,Z)-3,8-14:Ac (**3**) = | 95 | : | 5 | |
| SA 3: | (E,Z,Z)-3,8,11-14:Ac (**16**) : (E,Z)-3,8-14:Ac (**3**) = | 92 | : | 8 | (= gland ratio) |
| SA 4: | (E,Z,Z)-3,8,11-14:Ac (**16**) : (E,Z)-3,8-14:Ac (**3**) = | 80 | : | 20 | |
| SA 5: | (E,Z,Z)-3,8,11-14:Ac (**16**) : (E,Z)-3,8-14:Ac (**3**) = | 0 | : | 100 | |

The population density of *S. absoluta* was very high in the test area, which was located in the coastal desert, approximately 20 kilometres south of Ica in Peru. Already while installing the traps with the sex pheromone dispensers, males from the surroundings flew in. Nevertheless, trap catches remained low in the first period (day 1 - 6). This was probably caused by the small container traps (Ø 10 cm) which were used initially, or perhaps because the traps were positioned above the vegetation level. Also the amount of 1 mg sex pheromone mixture per dispenser could be too high for the males to locate them. It was observed that male *S. absoluta* approached the traps rapidly to a distance of about 5 cm, and then flew sideways. In the second period (day 7 - 26), the small container traps were replaced by larger open bowls (Ø 25 cm) which were placed directly onto the soil. Now the numbers of captured moths increased more than tenfold. Table 5.1 shows the total of four repetitions (four blocks) and average catch per trap-type per day.

*Table 5.1* **Total numbers of *Scrobipalpuloides absoluta* males caught in Ica, Peru in September 1995. Period 1: days 1 - 6. Period 2: days 7 - 26. Traps for which the means on the logarithmic scale do not differ statistically at a 2-sided t-test at level of significance $\alpha = 0.05$ have the same letter. For further explanation see text.**

| | | control | SA 5 | SA 3 | SA 4 | SA 1 | SA 2 |
|---|---|---|---|---|---|---|---|
| *period 1* | totals | $48^{a}$ | $68^{a}$ | $357^{b}$ | $388^{b}$ | $384^{b}$ | $574^{b}$ |
| average per trap per day | | 2.0 | 2.8 | 14.9 | 16.2 | 16.0 | 23.9 |
| *period 2* | totals | $2359^{c}$ | $5134^{d}$ | $18235^{e}$ | $18511^{e}$ | $19835^{e}$ | $21034^{e}$ |
| average per trap per day | | 29.5 | 64.2 | 227.9 | 231.4 | 247.9 | 262.9 |

Block effects were not significant in both periods. From table 5.1 it is concluded that in period 1 the control and the SA 5 trap caught significantly less moths than the other traps. In period 2 the control trap caught fewer than all the other traps and SA 5 caught significantly fewer moths than SA 1 through SA 4.

The numbers of captured moths in one of the control traps rose dramatically after changing trap types. Perhaps that the control dispenser was contaminated with sex pheromone at the moment dispensers were changed. In a second set of field tests, we experienced that the numbers of trapped moths decreased after some days because of depletion of the local population. This effect was observed in a field tests near the International Potato Center (CIP) in Lima, Peru, where the population density of *S. absoluta* was low. Replacement of the pheromone dispensers, by new ones did not improve trap catches. However, the trap catches in Ica did not decrease in time. The high population density of *S. absoluta*, and also the small size of the test area (20 × 30 meter) in

comparison to the surrounding untreated fields (ca. 150 ha.) probably caused that moths flew in continuously.

## 5.4 Discussion

The sex pheromone of *Scrobipalpuloides absoluta* consists of two compounds, which have the same stereochemistry at the (E)-3- and (Z)-8-double bond positions. This phenomenon has been found as well in the sex pheromone for *Phthorimaea operculella*, which is closely related to *Scrobipalpuloides absoluta*. The sex pheromone of *Phthorimaea operculella* consists of (E,Z)-4,7-tridecadienyl acetate (**76**) and (E,Z,Z)-4,7,10-tridecatrienyl acetate (**23**)[23] (figure 5.14).

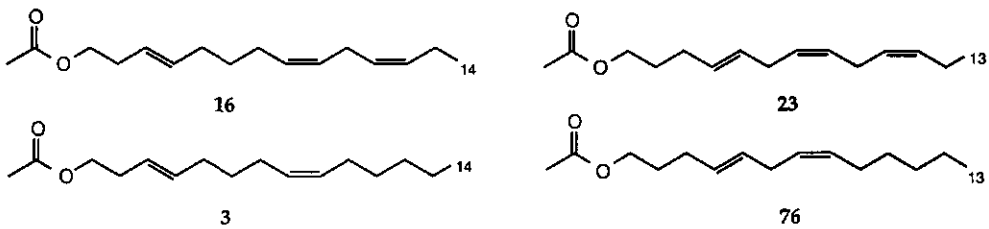



***Fig. 5.14*** **Similarities between the sex pheromones of *Scrobipalpuloides absoluta* (16,3) and *Phthorimaea operculella* (23,76).**

From the experience with the DMDS derivatisations of triple-unsaturated linear compounds, it appeared, afterwards, possible to locate all three double bond positions in the molecule (see also chapter 2). The DMDS method can be helpful for future analysis of sex pheromones.

An extract of sex pheromone glands lost its activity when stored for longer than one week. However, the identified sex pheromone compounds are not particular unstable. Perhaps that other chemical factors in the extract catalysed the decomposition of the pheromone components.

A random reduction procedure has been reported recently by Attygalle *et al.* for the identification of the major component of the *S. absoluta* sex pheromone[24]. With that procedure, the poly-unsaturated sex pheromone compounds of the gland extract are reduced to mono-unsaturated components. Although the authors noticed the existence of a second compound in the sex pheromone, they were unable to resolve its structure. With their method, the two sex pheromone components of *S. absoluta* provide only a single set

of mono-unsaturated compounds. For that reason, it was not possible for them to identify both components.

The synthetic sex pheromone of *S. absoluta* is very effective in attracting and trapping males of this species. Therefore, it can be applied for monitoring purposes. The large numbers of captured moths indicate that the synthetic sex pheromone may also be suitable for mass trapping of these pest insects. It is surprising that the addition of the minor sex pheromone compound did not increase the trap catches in the field. In the laboratory, it appeared that this minor component is essential to maintain a lasting behavioural response. Perhaps that lower amounts of sex pheromone in the dispensers are more discriminative. This experiment, and large scale field tests will be carried out in South America in the near future in order to determine and perhaps even improve the efficiency of the synthetic sex pheromone for the control of *Scrobipalpuloides absoluta*.

## 5.4 References and notes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16. NMR data for (E,E)-3,8-tetradecadienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 0.85 (t, 3H, *J* = 7.0 Hz; H-14), 1.26 (br m, 6H; H-11 to H-13), 1.37 (tt, 2H, *J* = 7.4, 7.5 Hz; H-6), 1.96 (br m, 6H; H-5, H-7, H-10), 2.01 (s, 3H; $O_2CMe$), 2.28 (dt, 2H, *J* = 6.9, 6.9 Hz; H-2), 4.03 (t, 2H, *J* = 6.9 Hz; H-1), 5.35 (m, 3H; H-4, H-8 and H-9), 5.48 (dt, 1H, *J* = 15.3, 6.9 Hz; H-3). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 14.0 (q), 20.9 (q), 22.5 (t), 29.2 (t), 29.3 (t), 31.4 (t), 31.9 (t)(double peak?), 32.0 (t), 32.5 (t), 64.1 (t), 125.2 (d), 129.8 (d), 130.8 (d), 133.3 (d), 171.0 (s).
17. NMR data for (E,Z)-3,8-tetradecadienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 0.86 (t, 3H, *J* = 6.9 Hz; H-14), 1.27 (br m, 6H; H-11 to H-13), 1.37 (tt, 2H, *J* = 7.4, 7.5 Hz; H-6), 1.98 (br m, 6H; H-5, H-7, H-10), 2.00 (s, 3H; $O_2CMe$), 2.28 (dt, 2H, *J* = 6.9, 6.9 Hz; H-2), 4.03 (t, 2H, *J* = 6.9 Hz; H-1), 5.32 (m, 3H; H-4, H-8 and H-9), 5.44 (dt, 1H, *J* = 15.3, 6.9 Hz; H-3). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 13.9 (q), 20.9 (q), 22.5 (t), 26.6 (t), 27.1 (t), 29.4 (t)(double peak?), 31.5 (t), 31.9 (t), 32.1 (t), 64.0 (t), 125.2 (d), 129.3 (d), 130.2 (d), 133.2 (d), 171.0 (s).
18. NMR data for (Z,E)-3,8-tetradecadienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 0.86 (t, 3H, *J* = 6.9 Hz; H-14), 1.27 (br m, 6H; H-11 to H-13), 1.39 (tt, 2H, *J* = 7.3, 7.4 Hz; H-6), 1.98 (br m, 6H; H-5, H-7, H-10), 2.02 (s, 3H; $O_2CMe$), 2.34 (dt, 2H, *J* = 7.2, 7.0 Hz; H-2), 4.03 (t, 2H, *J* = 7.0 Hz; H-1), 5.36 (br m, 3H; H-4, H-8 and H-9), 5.48 (dt, 1H, *J* = 10.9, 7.2). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 14.0 (q), 20.9 (q), 22.5 (t), 26.7 (t), 26.8 (t), 29.3 (t), 29.5 (t), 31.4 (t), 32.1 (t), 32.6 (t), 64.0 (t), 124.5 (d), 129.8 (d), 130.9 (d), 132.7 (d), 171.1 (s).

19. NMR data for (Z,Z)-3,8-tetradecadienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 0.86 (t, 3H, *J* = 6.9 Hz; H-14), 1.29 (br m, 6H; H-11 to H-13), 1.38 (tt, 2H, *J* = 7.5, 7.5 Hz; H-6), 2.01 (br m, 6H; H-5, H-7, H-10), 2.01 (s, 3H; $O_2CMe$), 2.34 (dtd, 2H, *J* = 7.2, 7.0, 0.9 Hz; H-2), 4.03 (t, 2H, *J* = 7.0 Hz; H-1), 5.33 (br m, 3H; H-4, H-8 and H-9), 5.48 (dtt, 1H, *J* = 10.9, 7.2, 1.6 Hz; H-3). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 14.0 (q), 20.9 (q), 22.5 (t), 26.8 (t)(two peaks), 26.9 (t), 27.2 (t), 29.4 (t), 29.6 (t), 31.5 (t), 63.9 (t), 124.5 (d), 129.2 (d), 130.4 (d), 132.6 (d), 171.0 (s).

20. NMR data for (E,Z,Z)-3,8,11-tetradecatrienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 0.95 (t, 3H, *J* = 7.5 Hz; H-14), 1.40 (tt, 2H, *J* = 7.4, 7.5 Hz; H-6), 2.02 (s, 3H; $O_2CMe$), 2.03 (br m, 6H; H-5, H-7 and H-13), 2.29 (dtd, 2H, *J* = 6.8, 6.9, 1.0 Hz; H-2), 2.74 (dd, 2H, *J* = 6.0, 6.4 Hz; H-10), 4.04 (t, 2H, *J* = 6.9 Hz; H-1), 5.33 (br m, 5H; H-4, H-8, H-9, H-11 and H-12), 5.49 (dtt, 1H, *J* = 15.2, 6.8, 1.2 Hz; H-3). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 14.3 (q), 20.5 (t), 21.0 (q), 25.9 (t), 27.0 (t), 29.7 (t), 32.4 (t), 32.5 (t), 64.5 (t), 125.8 (d), 127.7 (d), 128.7 (d), 130.1 (d), 132.2 (d), 133.6 (d), 171.5 (s).

21. NMR data for (E,Z,E)-3,8,12-tetradecatrienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 1.37 (tt, 2H, *J* = 7.4, 7.5 Hz; H-6), 1.60 (br d, 3H, *J* = 4.7 Hz; H-14), 2.00 (s, 3H; $O_2CMe$), 2.00 (br m, 8H; H-5, H-7, H-10 and H-11), 2.27 (dtd, 2H, *J* = 6.8, 6.9, 1.0 Hz; H-2), 4.03 (t, 2H, *J* = 6.9 Hz; H-1), 5.35 (m, 5H; H-4, H-8, H-9, H-12 and H-13), 5.47 (dtt, 1H, *J* = 15.2, 6.8, 1.2 Hz; H-3). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 17.8 (q), 20.9 (q), 26.6 (t), 27.3 (t), 29.3 (t), 31.9 (t), 32.1 (t), 32.6 (t), 64.0 (t), 125.0 (d), 125.3 (d), 129.4 (d), 129.6 (d), 130.9 (d), 133.2 (d), 170.9 (s).

22. NMR data for (E,Z)-3,8,13-tetradecatrienyl acetate: [$^1$H]NMR ($CDCl_3$, 400 MHz); δ 1.35 (tt, 2H, *J* = 7.2, 7.5 Hz; H-11 or H-6), 1.39 (tt, 2H, *J* = 7.3, 7.4 Hz; H-6 or H-11), 1.98 (s, 3H; $O_2CMe$), 1.98 (br m, 8H; H-5, H-7, H-10 and H-12), 2.26 (dtd, 2H, *J* = 6.8, 6.9, 1.0 Hz; H-2), 4.01 (t, 2H, *J* = 6.9 Hz; H-1), 4.89 (ddt, 1H, *J* = 10.1, 2.0, 1.2 Hz; $H_\alpha$-14), 4.95 (ddt, 1H, *J* = 17.1, 2.0, 1.8 Hz; $H_\beta$-14), 5.31 (m, 3H; H-4, H-8 and H-9), 5.46 (dtt, 1H, *J* = 15.2, 6.8, 1.2 Hz; H-3), 5,75 (ddt, 1H, *J* = 17.1, 10.1, 6.8 Hz; H-13). [$^{13}$C]NMR ($CDCl_3$, 100 MHz); δ 20.8 (q), 26.5 (t), 26.5 (t), 28.8 (t), 29.3 (t), 31.6 (t), 32.0 (t), 33.2 (t), 63.9 (t), 114.3 (d), 125.2 (d), 129.6 (d)(two peaks), 133.1 (d), 138.6 (d), 170.8 (s).

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# Chapter 6

## Wind tunnel bioassays of the *Symmetrischema tangolias* sex pheromone*

### 6.1 Introduction

The question of how an insect responds to chemical compounds isolated and identified from the sex pheromone glands, is only answered through behavioural bioassays. The most simple bioassay is through direct offering of a test sample to conspecific males. If they recognise the odour blend of a sex pheromone, they beat their wings ('fluttering') and attempt to locate the source. This activity shows that the sex pheromone gland extract possesses biological activity and thus contains the sex pheromone components. This method, however, should be regarded only as first step in assessing the complex behavioural responses involved. More detailed information about the quality of a particular sample is obtained through field experiments or wind tunnel bioassays in the laboratory. Many variables affect the behaviour of insects orienting to an odour source: the chemical composition and concentration of the blend, visual surroundings, temperature, relative humidity, wind speed and turbulence, and even the composition of the odour plume[1-6]. These factors are not completely under control in field experiments. However, in a well designed wind tunnel, these variables can be controlled and reproduced.

The potato tuber moth, *Symmetrischema tangolias* (Gyen), is an important pest on potatoes at higher elevations in South America. Four compounds, identified in extracts from sex pheromone glands of female *S. tangolias*, could be part of the sex pheromone (see chapter 3). These compounds included two major compounds, (E,Z)-3,7-tetradecadienyl acetate (63%) and (E)-3-tetradecenyl acetate (31%), and two minor compounds (Z)-7-tetradecenyl acetate (5%) and (Z)-5-tetradecenyl acetate (1%). In wind tunnel bioassays, the behavioural relevance of these compounds in the pheromone blend can be assessed.

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A new wind tunnel for studies of insect behaviour have been built at IPO-DLO. This wind tunnel was designed as a return-flow tunnel in order to regulate the temperature and humidity of the air through feedback control. Special precautions were taken to prevent odour contamination of the wind tunnel by (a) a continuous partial refreshment of the air, (b) two activated charcoal filter units present in the upstream as well as the downstream air flow, and (c) the materials used for the construction of the tunnel, like glass for the flight section and stainless steel for the other compartments, which were sealed with silicone packing, silicone tubing, and aluminium tape. This chapter gives a description of this wind tunnel and presents the first results which were obtained with *S. tangolias*.

## 6.2 Methods and materials

*Insects*

For rearing conditions see chapter 3.2. All bioassays were conducted with 3-day-old males from the $12^{th}$ - $15^{th}$ generation (>1.8 years in rearing).

*Wind tunnel*

The tunnel consists of several parts that follow in succession in the direction of the air-flow (see figure 6.1): (F) a Fischbach compact fan type SD9-070/D50-4 and stepless speed controller FDR 200/3, with, at a distance of 21 cm from the ventilator outlet, a perforated metal plate (40 × 55 cm, holes 10 mm, 50% open area) that forces the flow to use the complete cross-section of the tunnel, (G) an activated charcoal filter consisting of 28 zigzag placed Norithene Norit filter plates type C233008, (H) two Camfil Airopac 90 air filters placed parallel, (also like A) sharp bends with corner vanes, (K) the plant section, (L) four stainless steel damping screens (Dinxperlo, mesh size 40, 0.14 mm wire diameter, 61% open area) placed at 10 cm intervals, (P) the flight section measuring 3.0 (l) × 1.3 (w) × 0.8 (h) m, with streamlined corners, (Q) two damping screens (as item L), (A) sharp bends with corner vanes, (B) an activated charcoal filter (as item G), and (C, D and E) a bypass flow circuit to the air-conditioning unit with outlet and inlet isolated by two perforated metal plates (see air-conditioning and ventilation). In order to house the filter units, the dimensions of the lower parts of the wind tunnel are 122 (w)× 61 (h) cm, whereas the diameter of the flight section including the upwind and downwind regions with damping screens, measures 130 cm wide and 80 cm high. At each side of the flight section, three sash windows counterbalanced with constant-force springs (Elmekanic type BBSR20) make this section very accessible.

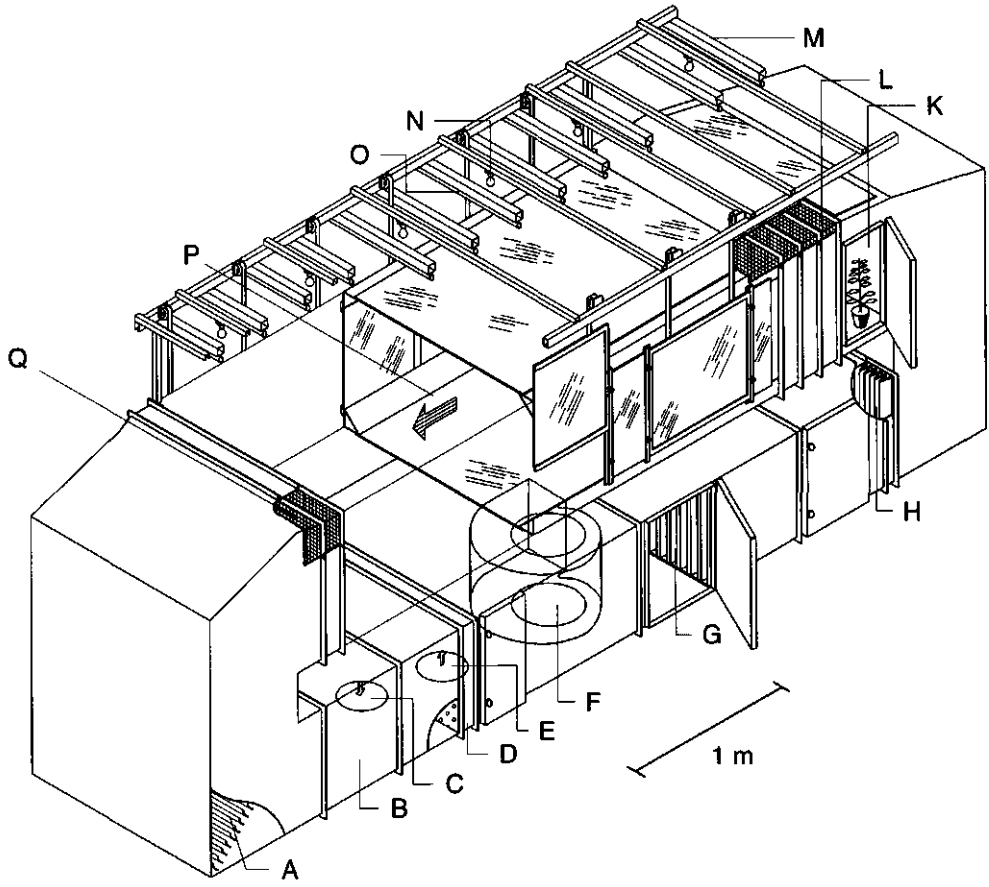


*Fig. 6.1* **Schematic drawing of the wind tunnel. A: corner vanes; B: activated charcoal filter; C: outlet for air returning to air-conditioning unit; D: two perforated metal plates; E: inlet for air coming from air-conditioning unit; F: centrifugal ventilator; G: activated charcoal filter; H: air filter; K: plant section; L: four damping screens; M: metal support for the lamps; N: incandescent lamp; O: fluorescent lamp; P: flight section, arrow indicates wind direction; Q: two damping screens.**

*Illumination*

A metal frame (figure 6.1, M) above the flight section supported the illumination unit, which contains 10 fluorescent lamps, 21 to 34 cm apart, and 10 incandescent lamps spaced 60 cm apart, and 2 fluorescent and 2 incandescent lamps above the plant section. The fluorescent lamps are Philips high-frequency type TLD 50W/84HF and are connected to one dimmer (Philips LPS 100). The light-intensity of the incandescent lamps can be controlled by a second dimmer. Spacing of the lamps was optimised to obtain a uniform light-intensity distribution in the flight section. Figure 6.2 illustrates the light-intensity

distribution measured with 10 TL lamps and 10 incandescent lamps of 60W each set at highest intensity. Lux measurements were done with a Li-Cor photometer Li-189 equipped with a Li-210 SA photometric sensor.

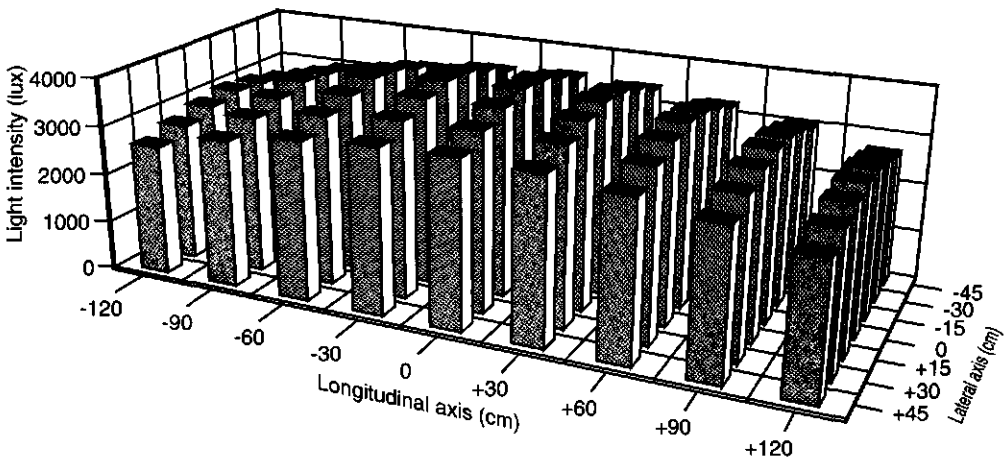


*Fig. 6.2* **Light-intensity distribution on the floor of the flight section at maximal setting of incandescent and fluorescent lamps above the flight section. The light-intensity in the middle of the flight section, at x,y co-ordinates (0,0), is 3960 lux at the bottom and 5100 lux at 40 cm height. Lateral axis scale is drawn at the upwind side of the flight section.**

In all experiments with *S. tangolias*, to simulate night conditions, 10 red incandescent lamps of 20W each were set at the lowest intensity and all fluorescent lamps were switched off. Light-intensity measured 0.7 lux in the middle of the flight section at floor level.

*Airspeed distribution*

The distribution of airspeeds in the flight section was measured with a Lambrecht 642 thermo-anemometer and turned out to be rather uniform. The uniform air velocity profile results from the damping screens (figure 6.1 L and Q) installed at the upwind and downwind sides of the flight section. Figure 6.3 shows the vertical distribution of wind speed deviations measured at 50 cm/s through the central axis of the wind tunnel in two positions: (a) in the plant section 5 cm in front of the first damping screen (figure 6.1 K), and (b) 30 cm downwind from the fourth damping screen in the flight section. It became obvious that the tunnel bends created an uneven air velocity distribution, i.e. speeds were higher at the outside than the inside, which is corrected by the four damping screens before entering the flight section. At 100% setting of the centrifugal ventilator the airspeed is 2.6 m/s in the flight section.

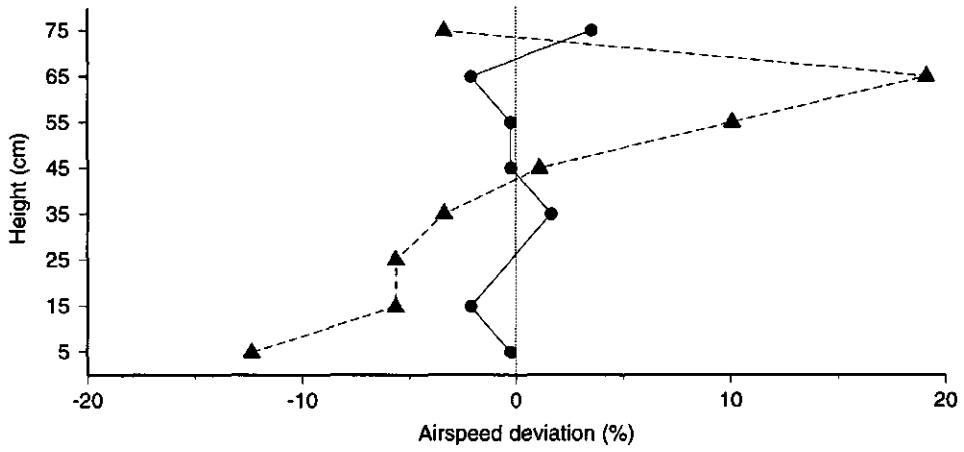


*Fig. 6.3* **The vertical distribution of deviations in airspeed before, in the plant section (broken line), and after the four damping screens, in the flight section (solid line), measured at the central axis of the wind tunnel at an average speed of 50 cm/s.**

*Air-conditioning and ventilation*

The temperature and humidity of air flowing through the wind tunnel can be controlled in the range of 17 to 30°C (± 0.5°C) and 40 to 70% relative humidity (RH) (± 4% RH). The upper limit of the humidity range is 70% RH which prevents capillary condensation of water in the pores of the activated charcoal filters that can affect the efficiency of air purification. Air-conditioning is obtained in a bypass flow circuit which is connected with the tunnel through an inlet tube close to the tunnel ventilator, and an outlet further upstream. The diameter of both inlet and outlet tubes measure 25 cm. In order to prevent the flow short-cutting, the inlet and outlet are isolated by two metal plates with staggered perforations (15 mm diameter, 44% open area). The plates are spaced 10 cm apart. The sensors for temperature QAM 21 and humidity QFM 63, both Landis & Gyr, are located at the downwind side in the flight section. The air in the wind tunnel is refreshed by forced ventilation of the air-conditioning unit with outside air. The rate of refreshment was measured by controlled injection of $CO_2$ in the flight section unto a stable equilibrium, when the rate of injection is the rate of ventilation, and, after injection had stopped, the subsequent decrease in $CO_2$ concentration. Measurements were done with a Fuji 3300 $CO_2$ infrared gas analyser. At a set flow through the air-conditioning unit of 1400 $m^3/h$ and measured in a wide range of airspeeds in the wind tunnel (0.5 - 2.0 m/s), the ventilation was 400 $m^3/h$. When injection of $CO_2$ had stopped, the time to reach half of the concentration measured at equilibrium was 2 minutes, and complete recovery to normal $CO_2$ levels (320-340 ppm), i.e. complete refreshment, was obtained in 15 minutes.

*Wind tunnel bioassays*

Two delta traps (IPO-DLO), with black painted interiors[7], were placed 30 cm apart at the upwind side of the wind tunnel at 10 cm distance of the four damping screens (figure 6.4). The bottom of each trap had a sticky surface and moths that touched the surface could not escape. Test samples were pipetted onto slips of filter paper[8] which were hung horizontally inside the traps. Filter papers were treated with 100 µl of a 1 ppm solution (100 ng active ingredients) of a (mixture of) acetate(s) in bi-distilled hexane. Hexane was allowed to evaporate before the filter papers were brought into the wind tunnel. Wind tunnel conditions were: temperature 17 ± 0.5°C; 65 ± 4% RH; 12 cm/s wind speed. The temperature and relative humidity settings in the wind tunnel corresponded with those in the rearing rooms. The bottom of the wind tunnel had a symmetrical pattern of grey circles (10 cm diameter) each separated by 30 cm on a white background.

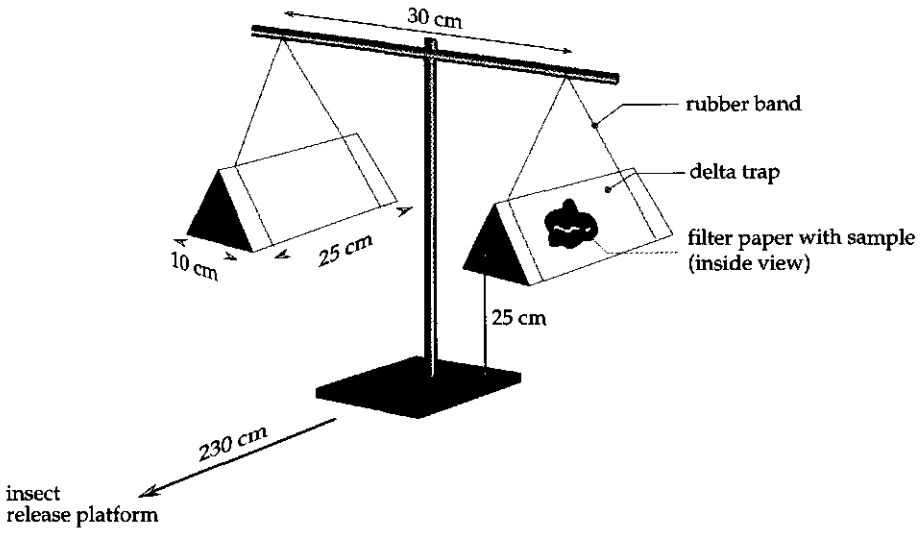


*Fig. 6.4* **Schematic drawing of the positions of the traps in the wind tunnel as it was used in the bioassays with *Symmetrischema tangolias*.**

A transparent release box (29 (l) × 16 (w) × 10 (h) cm) with the males was transferred from the rearing room to the wind tunnel a few hours into scotophase, protected against light and temperature fluctuations. The release box was placed on a platform at the downwind side of the wind tunnel. The upper edge of the box was set at the same height (25 cm) as the filter paper samples in the traps. Moths were given 15 minutes to acclimatise. Tests were started by carefully pulling the lid from the release box. Moths in the wind tunnel were then left undisturbed for six hours. The positions of the test samples were alternated

were then left undisturbed for six hours. The positions of the test samples were alternated (left/right) every second day. All captured males were counted and the numbers were subjected to Wilcoxon-matched-pairs-signed-rank test[9].

## 6.3 Results and discussion

A wind speed of 12 cm/s seems low but this was necessary because at higher speeds very few moths flew. With the low light-intensity it was difficult to follow individual moths, however, good results were obtained by releasing a group of moths and counting trap catches after six hours. The interior of the traps were painted black to reduce visibility of captured insects that may form an extra stimulus[4].

(E,Z)-3,7-Tetradecadienyl acetate (**6**) and (E)-3-tetradecenyl acetate (**1**) were each tested against a control (hexane). It was found that (E)-3-tetradecenyl acetate alone was not attractive, whereas (E,Z)-3,7-tetradecadienyl acetate alone was significantly attractive (P = 0.01) (36% captured against 3% for the control, 6 replicates, total number of tested moths = 185).

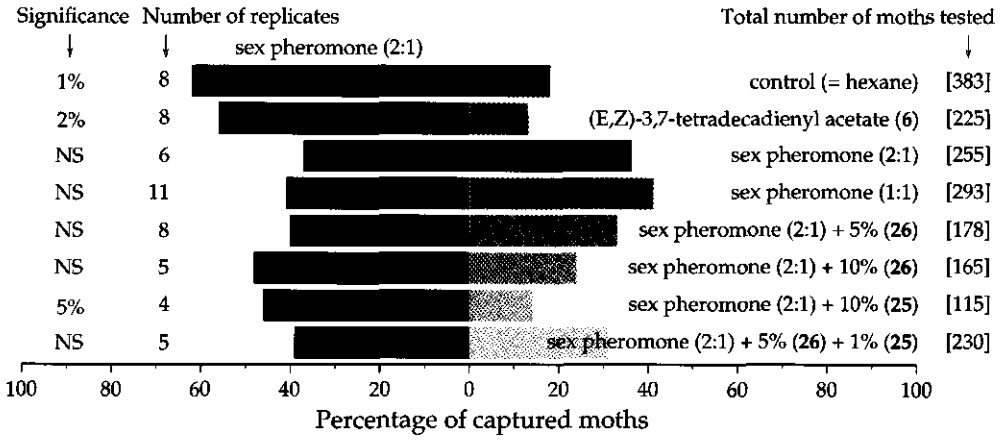


*Fig. 6.5* **Trap catches of male *Symmetrischema tangolias* with various blends of gland components carried out in a wind tunnel. 'Sex pheromone' represents a mixture of (E,Z)-3,7-tetradecadienyl acetate (6) and (E)-3-tetradecenyl acetate (1). (Z)-7-tetradecenyl acetate (26); (Z)-5-tetradecenyl acetate (25). Significance calculated according to Wilcoxon-matched-pairs-signed-rank test, 2-tailed. NS means P > 0.1.**

A 2 : 1 mixture of (E,Z)-3,7-tetradecadienyl acetate and (E)-3-tetradecenyl acetate, respectively, (sex pheromone 2 : 1) appeared also highly attractive (figure 6.5). However, when the latter two tests were compared, it appeared that the sex pheromone (2 : 1) caught a higher percentage of the males than (E,Z)-3,7-tetradecadienyl acetate alone (62% against 36%). A mixture of the unattractive (E)-3-tetradecenyl acetate plus the attractive (E,Z)-3,7-tetradecadienyl acetate resulted in a blend which is significantly more attractive than its individual constituents.

Several mixtures were tested against the 2 : 1 sex pheromone blend to determine whether the addition of the minor sex pheromone gland constituents (Z)-7-tetradecenyl acetate (**26**) and (Z)-5-tetradecenyl acetate (**25**) improves the attractiveness of the pheromone blend (figure 6.5).

No difference in attractiveness was found between a 2 : 1 and a 1 : 1 sex pheromone blend. The addition of the minor gland component **26** to the sex pheromone mixture, at a dose of 5 or 10%, did not change the attractiveness. However, the addition of 10% **25** to the (2 : 1) sex pheromone blend significantly decreased attractiveness. The addition of 1% (Z)-5-tetradecenyl acetate (**25**) plus 5% (Z)-7-tetradecenyl acetate (**26**) to the reference mixture did not affect its attractiveness. The latter mixture resembles the ratio extracted from the sex pheromone glands. Therefore, the function of the (Z)-5- and (Z)-7-tetradecenyl acetates remains unclear. It may be that the trap catches in the wind tunnel do not reveal subtle behavioural differences. Only the 2 : 1 sex pheromone was tested in Peru and showed to be highly attractive to male *S. tangolias* in both field and storage facilities.

It is concluded that male *S. tangolias* are able to distinguish between different pheromone mixtures in the wind tunnel. EAG recordings (chapter 3) revealed that male *S. tangolias* were able to perceive the minor gland constituent (Z)-5-tetradecenyl acetate. From the present wind tunnel tests it is concluded that they also respond to this compound but only in higher doses than normally occur in the gland extracts, and then as repellent. The method of pheromone application to the males (as a continuous plume) works very well for this species. In some (individual) cases 100% of the released males were recaptured and the average trap catches for attractive mixtures was more than 80% (total of the two traps).

The wind tunnel bioassays have led to a highly attractive pheromone mixture for male *S. tangolias* which can be used in an integrated pest management program for this species.

## 6.4 References and notes


1. McDonough, L.M., Davis, H.G., Chapman, P.S. and Smithhisler, C.L. 1993. Response of male codling moths (*Cydia pomonella*) to components of conspecific female sex pheromone glands in flight tunnel tests. *J. Chem. Ecol.*, 19, 1737-1748.
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6. Mafra-Neto, A. and Cardé, R.T. 1994. Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. *Nature*, 369, 142-144.
7. The delta traps were obtained from the IPO-DLO, Wageningen, The Netherlands.
8. 6 x 0.8 cm, Schleicher & Schuell 589[2].
9. Siegel, S. 1956. *Nonparametric statistics for the Behavioral Sciences.* John Wiley, New York.

# Chapter 7

## General discussion

The moths, *Symmetrischema tangolias* and *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae), are severe pests of potato and tomato in South America. This thesis describes the isolation, identification and synthesis of the sex pheromones of these two species. In chapter one, an overview was presented of all sex pheromones and attractants which are currently known in the Gelechiidae. The sex pheromones identified for *Symmetrischema tangolias* and *Scrobipalpuloides absoluta* (figure 7.1) appear to be structurally related to already identified sex pheromones and attractants.

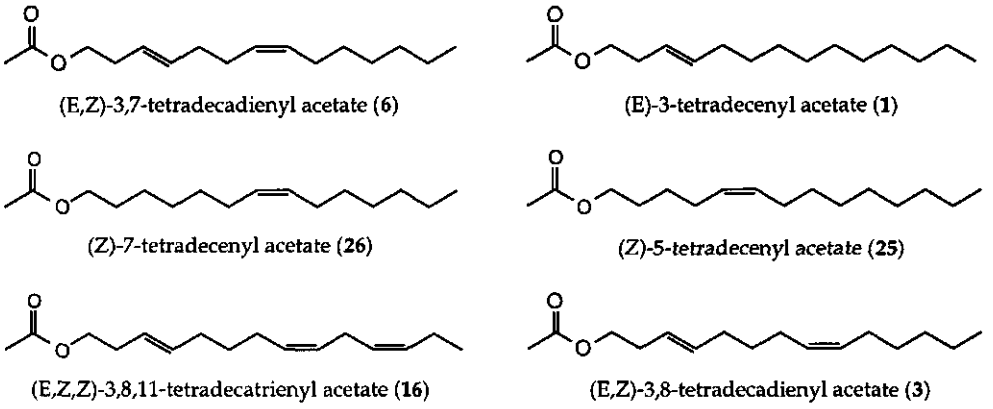



***Fig. 7.1*** **Identified sex pheromone components for *Symmetrischema tangolias* (6,1) plus the two minor sex pheromone gland constituents (26,25) and the identified sex pheromone components for *Scrobipalpuloides absoluta* (16,3).**

The mono-unsaturated compounds **1**, **25** and **26** have already been described as constituents of sex pheromones and attractants in a number of lepidopteran species (figure 7.2)[1]. Considering the number of publications about sex pheromones and related compounds, the occurrence of the mono-unsaturated sex pheromone compound **1** is relatively scarce compared to the two other mono-unsaturated sex pheromone gland constituents **25** and **26**. The double unsaturated sex pheromone compounds **6** and **3** (for *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*, respectively), plus the triple-unsaturated sex pheromone compound **16** (for *Scrobipalpuloides absoluta*) have never been

reported for another insect sex pheromone or attractant. The two double bonds in (E,Z)-3,7-tetradecadienyl acetate (**6**) are separated by two methylene moieties, which is unique in this group of tetradecadienyl acetates (figure 7.2). However, this phenomenon of double bonds separated by two methylene groups has been found in other lepidopteran sex pheromones. The sex pheromone of *Pectinophora gossypiella* (gossyplure), for example, consists of a 1 : 1 mixture of (Z,E)- and (Z,Z)-7,11-hexadecadienyl acetate.

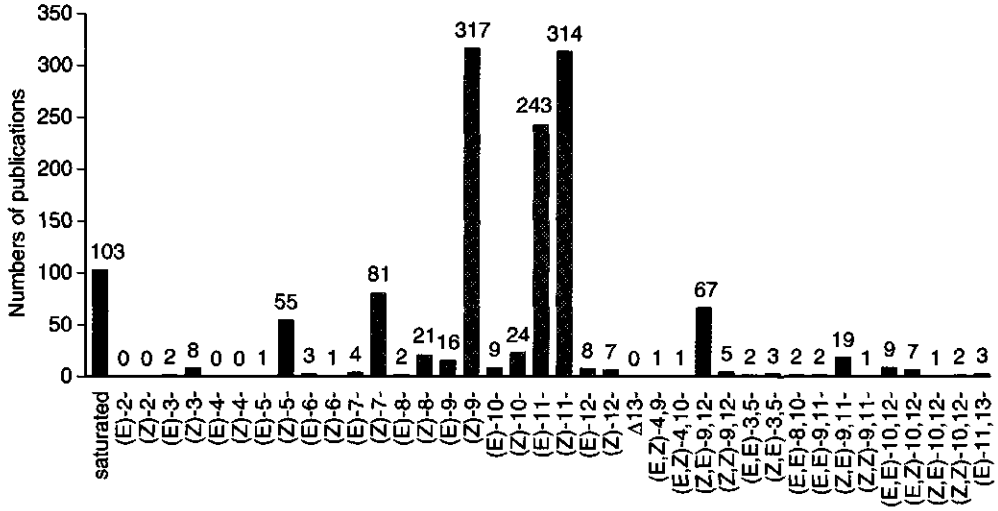
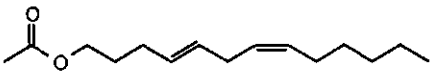
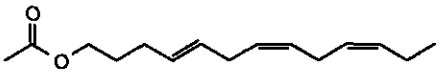



*Fig. 7.2* **The numbers of publications about lepidopteran species having a linear tetradecyl chain with an acetate as the functional group in their sex pheromone or attractant[1].**

For the identification of the *Scrobipalpuloides absoluta* sex pheromone the EAG measurements with mono-unsaturated test compounds only pointed towards the double bond at the (E)-3-position (chapter 5), this in contrast to *Symmetrischema tangolias* were EAG measurements provided a clear indication about the double bond configurations (chapter 3). The two other double bonds at the 8- and 11-positions, of the triple unsaturated sex pheromone compound of *Scrobipalpuloides absoluta*, could not be identified following this approach. The responses to both (E)-11- and (Z)-11-tetradecenyl acetate were low. Considering the 8-position, it appears that the relative response of the (E)-8-isomer was even higher than that of the corresponding (Z)-8-isomer. Thus it seems that when the double bonds in a molecule are homo-conjugated, the EAG recordings do not give higher responses for the related mono-unsaturated compounds. EAG measurements with *Phthorimaea operculella* (which also possesses homo-conjugated double bonds in its sex pheromone compounds, figure 7.3) gave similar poor results[2].



76 (PTM1)



23 (PTM2)

*Fig. 7.3* **Sex pheromone components of *Phthorimaea operculella*, a species closely related to *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*.**

It is not known whether these EAG results for homo-conjugated sex pheromone compounds can be considered a general rule.

## 7.1 Three-dimensional structures of sex pheromone and consequences for their activity

The actual recognition of a molecule, and thus the EAG response, is primarily the result of the binding of the molecule with a receptor protein in the dendritic membrane of a sensory neurone. The three-dimensional (3D) conformation of the molecule and the way it approaches and binds with the receptor protein are essential[3]. Figure 5.12 shows that (E,E)-3,8- and (Z,Z)-3,8-tetradecadienyl acetate (**53** and **55** respectively) elicit lower EAG responses from male *Scrobipalpuloides absoluta* than the mono-unsaturated (E)-3-tetradecenyl acetate (**1**), however, they both show higher EAG responses than (Z,E)-3,8-tetradecadienyl acetate (**54**). When compared to the actual sex pheromone compound (E,Z)-3,8-tetradecadienyl acetate (**3**), compounds **53** and **55** differ in stereochemistry only at one of the double bond positions whereas compound **54** differs at both positions. It seems, therefore, that the presence of an additional double bond at the right position, but with the wrong stereochemistry reduces the actual fit of the molecule with the receptor protein. Because the EAG responses of compounds **53** and **55** are in the same range, it appears to make no difference which of the double bond positions (3 or 8) has the wrong stereochemistry (compared to the actual sex pheromone compound **3**). This would be in disagreement with the opinion that first the functional group, and subsequently the rest of the molecule in consecutive order of double bonds binds with the receptor protein[3].
The structural formulas of the identified sex pheromone molecules do not give a very realistic view of their actual shape (figure 7.1). For a better understanding of the actual shape of these sex pheromone compounds, the 3D structures were assessed by means of MOPAC calculations[4,5] and illustrated in figure 7.4. It is stressed that the linear carbon chains of these types of structures are not rigid but always maintain a large degree of rotational freedom.

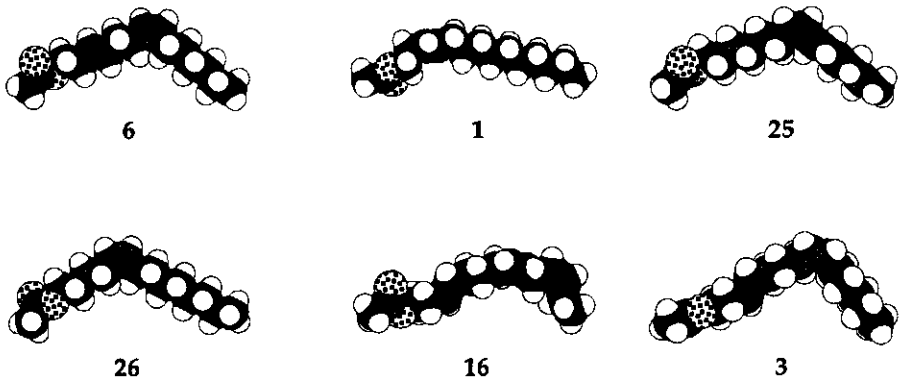



*Fig. 7.4* **3D representation assessed by means of MOPAC[4,5] calculations of the identified sex pheromone components of *Symmetrischema tangolias* (6,1) plus the two minor sex pheromone gland constituents (25,26) and the identified sex pheromone components of *Scrobipalpuloides absoluta* (16,3). Grey, carbon; speckled, oxygen; white, hydrogen.**

It is observed that the three-dimensional shapes of these chemically closely related molecules show substantial differences. The deformation of the carbon chain in (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**) may explain that the mono-unsaturated (Z)-8- and (Z)-11-tetradecenyl acetate were unable to elicit distinct EAG responses (chapter 5).
That the recognition and the resulting behavioural response is not always determined by the 3D-structure of the sex pheromone compounds involved, is illustrated in *Chilo suppressalis*, for which (Z)-11-hexadecenal (**77**) acts as a strong attractant. This moth does not respond at all to the structurally closely related (Z)-9-tetradecenyl formate (**78**)[6]. Figure 7.5 indicates that both molecules possess almost identical 3D structures. In this case, the functional group seems more important than the overall 3D-structure of the molecule.

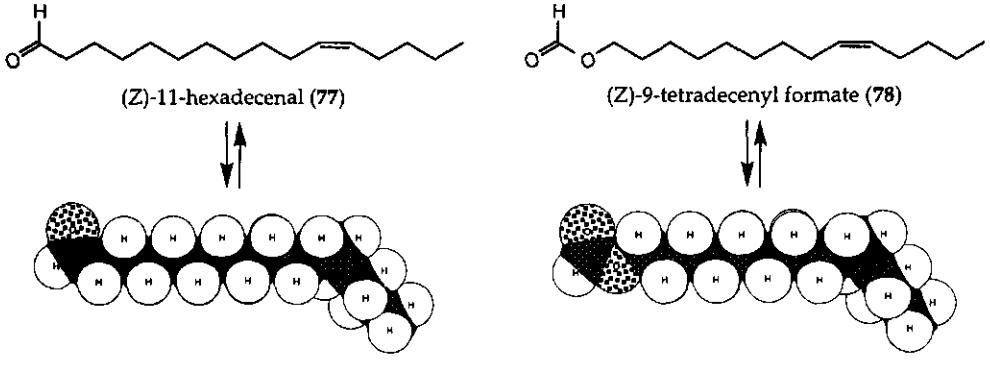
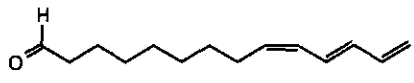
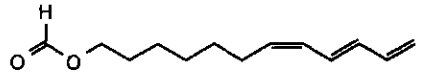



*Fig. 7.5* **The sex pheromone compound (Z)-11-hexadecenal (77) of *Chilo suppressalis* and the total inactive but 3D structurally related molecule (Z)-9-tetradecenyl formate (78).**

However, for another species, *Ectomyelois ceratoniae*, it has been found that the structurally related (Z,E)-7,9,11-dodecatrienyl formate (**80**) is as effective to formulate attractive mixtures as the natural sex pheromone compound (Z,E)-9,11,13-tetradecatrienal (**79**)[7] (figure 7.6).



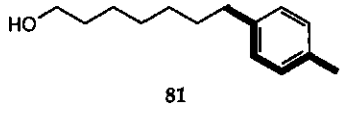
(Z,E)-9,11,13-tetradecatrienal (**79**)



(Z,E)-7,9,11-dodecatrienyl formate (**80**)

*Fig. 7.6* **The sex pheromone mimic (Z,E)-7,9,11-dodecatrienyl formate (80) is as effective as the natural sex pheromone compound (Z,E)-9,11,13-tetradecatrienal (79) for *Ectomyelois ceratoniae*.**

This result is important for the practical use of the sex pheromone (or attractant) because the formate is much more stable than the corresponding aldehyde. Not only the functional group, but also the total sex pheromone structure, with respect to the 3D-conformation, can be altered with retention of part of the attractiveness. This is shown with *Cydia pomonella* which is attracted to 7-(*p*-tolyl)-heptanol (**81**)[8]. This compound is structurally related to the sex pheromone (E,E)-8,10-dodecadienol (**82**) of this moth (figure 7.7).



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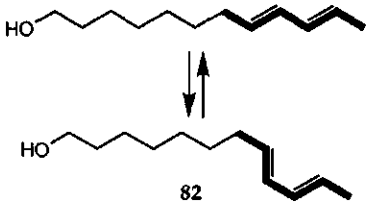

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*Fig. 7.7* **Sex pheromone of *Cydia pomonella* (82) and its structurally related attractant (81).**

## 7.2 Sex pheromones and the probability of resistance

It would be interesting to examine whether these sex pheromone mimics are attractive to the whole population or just a part of it. When only a, to the mimic more sensitive, part of the population is caught, a selection pressure exists which may eventually create a population that is less sensitive to this mimic. A similar story may be valid to some degree, for the (synthetic) sex pheromones blends. In chapter 4 it was reported that the natural ratio of the main sex pheromone compounds in the pheromone glands of

*Symmetrischema tangolias* is not strict, but shows a lot of variation. Although the relation between the sex pheromone gland contents and the air-emitted sex pheromone is not known, the same ratio 2 : 1 of the two main sex pheromone components found, on average, in the sex pheromone glands appears to be highly attractive to *Symmetrischema tangolias* males. On the other hand, a ratio of 1 : 1 of the same components does not attract fewer males. The same is true for *Scrobipalpuloides absoluta* since, in field traps, no significant differences in attractiveness were observed between different ratios of the sex pheromone components (chapter 5). For that reason it is not expected that the use of (synthetic) sex pheromones in the control of these two species will induce a shift in the natural population so that the (synthetic) sex pheromone will eventually lose its attractiveness. The closely related species *Phthorimaea operculella* has been successfully controlled for over ten years through application of its synthesised sex pheromone without a change in attractiveness.

## 7.3 Biosynthesis

The biosynthesis of lepidopteran sex pheromones has been examined in several species. It appears that evolutionarily higher moth species possess a unique Δ-11 desaturase enzyme in addition to the ubiquitous Δ-9 desaturase enzyme[9]. In more primitive species a Δ-10 desaturase enzyme is supposed to play a role in sex pheromone biosynthesis[10]. The starting materials for the biosynthesis of sex pheromones commonly are esterified fatty acids like those shown in figure 7.8.

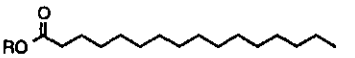
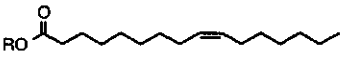
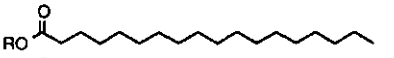
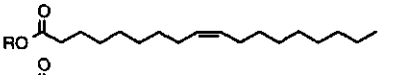
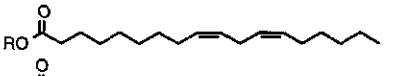
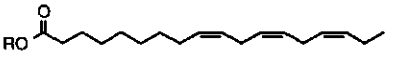
| | | |
|---|---|---|
| palmitate | hexadecanoic acid R ester (83) |  |
| palmitoleate | (Z)-9-hexadecenoic acid R ester (84) |  |
| stearate | octadecanoic acid R ester (85) |  |
| oleate | (Z)-9-octadecenoic acid R ester (86) |  |
| linoleate | (Z,Z)-9,12-octadecadienoic acid R ester (87) |  |
| linolenate | (Z,Z,Z)-9,12,15-octadecatrienoic acid R ester (88) |  |

*Fig. 7.8* **Common starting materials for the biosynthesis of moth sex pheromones.**

By means of the unique Δ-11 desaturase combined with more common fatty-acid biochemistry, the existence of a wide variety of lepidopteran sex pheromones can be explained. With this knowledge, the biosynthetic routes for the sex pheromone

components of *Symmetrischema tangolias* (**1**, **6**, **25** and **26**) and *Scrobipalpuloides absoluta* (**3** and **16**) are proposed. The minor sex pheromone compound **26** can be biosynthesised by two different routes, namely via stearate as the starting material, selective oxidation by the Δ-11 desaturase enzyme, and subsequent β-oxidation, etc. (not illustrated), or via palmitoleate as the starting material, and subsequent β-oxidation etc. (figure 7.9). Other closely related species, like *Phthorimaea operculella* and *Keiferia lycopersicella*, are not reported to use the Δ-11 desaturase enzyme[9]. It is therefore assumed that *Symmetrischema tangolias* does not use this special enzyme either and, thus, biosynthesises its sex pheromone compound **26** as indicated in figure 7.9.

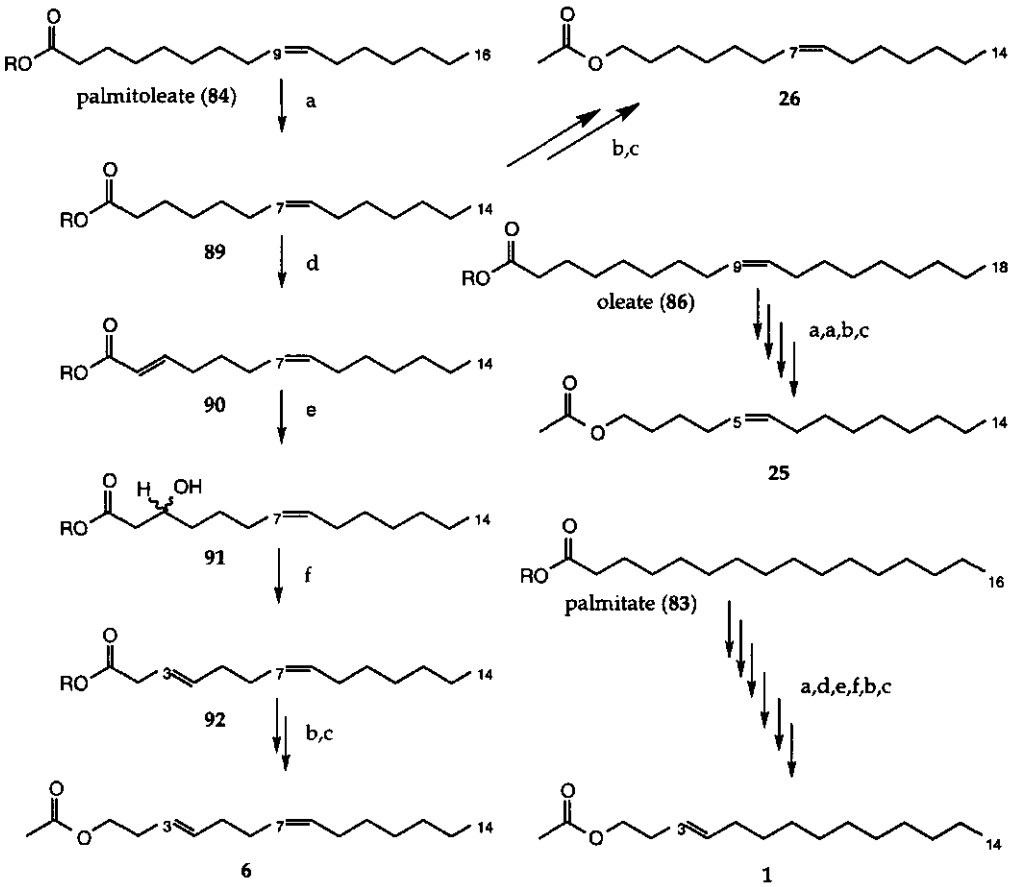



*Fig. 7.9* **Proposed steps in the biosynthesis of the sex pheromone components of *Symmetrischema tangolias*. (a) β–oxidation, (b) ester reduction, (c) acetylation, (d) oxidation, (e) hydration, (f) selective dehydration.**

The chain shortening of palmitoleate via a β-oxidation step, followed by subsequent ester reduction and acetylation gives compound **26**. For compound **6**, palmitoleate is shortened to the tetradecenoate ester (**89**) and subsequently oxidised to enoyl ester **90**. The double bond at the 2-position is hydrated to alcohol **91** and subsequently dehydrated to form the (E)-3-bond in **92**. The ester is reduced and acetylated to give compound **6**. The other major compound **1** is formed by the same steps as **6**, only starting with palmitate instead of palmitoleate. Compound **25** is formed through 2 consecutive β-oxidation steps and subsequent ester reduction and acetylation starting from oleate.

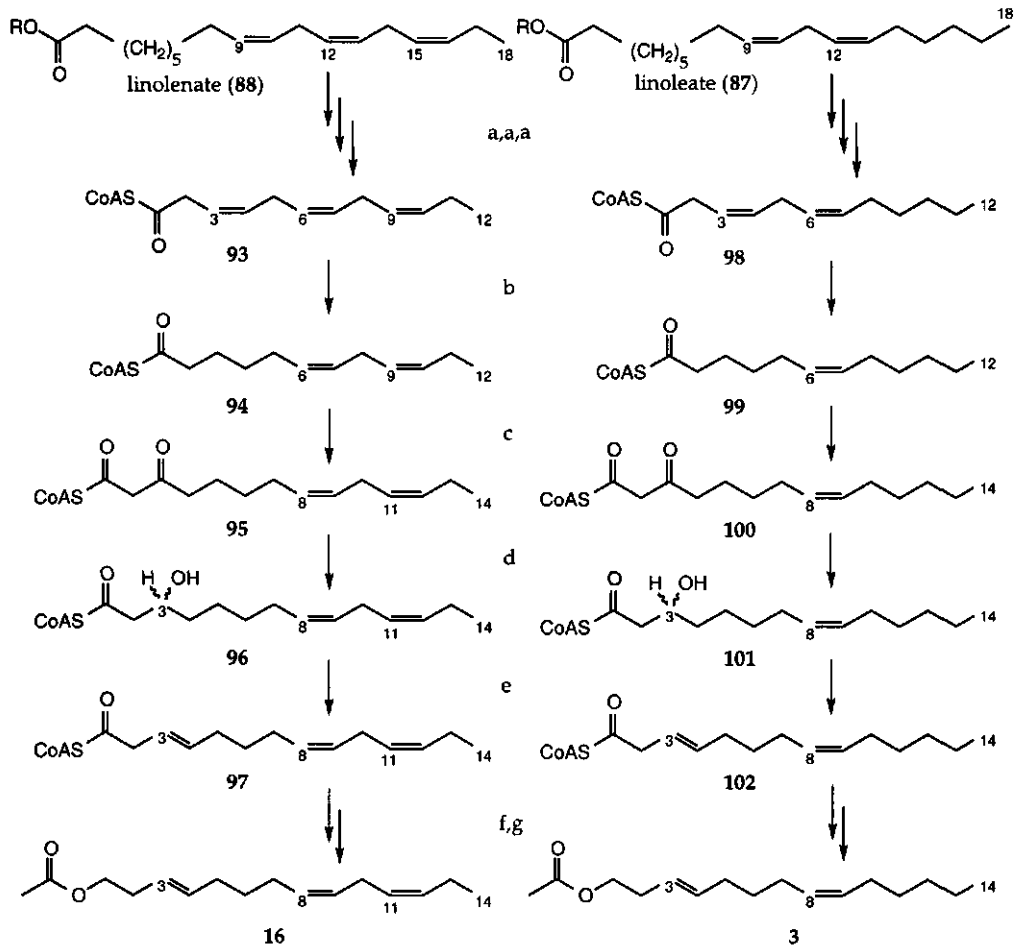



*Fig. 7.10* **Proposed steps in the biosynthesis of the sex pheromone components of *Scrobipalpuloides absoluta*. (a) β-oxidation, (b) β-double bond reduction, (c) Acetyl CoA, (d) keto reduction, e) selective dehydration, (f) ester reduction, (g) acetylation.**

Both sex pheromone compounds of *Scrobipalpuloides absoluta* are formed via the same proposed biosynthetic route (figure 7.10). The sole difference is the starting material. Considering the biosynthesis, and with the choice between (E,Z,Z)-3,8,11-, (E,Z,E)-3,8,12- or (E,Z)-3,8,13-tetradecatrienyl acetate, in retrospect, it can be concluded that the (E,Z,Z)-3,8,11-isomer was indeed the most likely candidate for the triple unsaturated sex pheromone compound of *Scrobipalpuloides absoluta* (chapter 5).

## 7.4 The link between laboratory results and practical application

The environmental conditions in the wind tunnel bioassays for the *Symmetrischema tangolias* pheromone were, of course, different from the natural conditions. Factors like light, airspeed distribution, temperature and humidity were kept constant and, although *S. tangolias* flew well in the wind tunnel tests, other species may not do so. The creation of some turbulence or the addition of host plants is then advisable. Wind tunnel results can not be extrapolated directly to field conditions. Before the sex pheromone compound (E,Z)-3,7-tetradecadienyl acetate (**6**) for *S. tangolias* had been synthesised, bioassays were performed with a mixture of two (available) mono-unsaturated tetradecenyl acetates, namely (E)-3-tetradecenyl acetate (**1**) and (Z)-7-tetradecenyl acetate (**25**). *S. tangolias* males responded to this mimic sex pheromone under laboratory conditions. In the Peruvian fields, however, it appeared that this mimic, although very attractive to a yet unidentified species, was not attractive at all to *S. tangolias*[12].

Also qualitative conclusions from field results must be drawn with care. The best catching mixture for a particular species at one location is, at another location, not necessarily the most attractive mixture. However, the ratio of the two constituents found in the sex pheromone glands of *Scrobipalpuloides absoluta* (the rearing started with individuals collected from Peru) was similar as reported for specimens originating from Brazil[13].

An attractive mixture alone is not enough to ensure high trap catches. The trap design and positioning are important factors in trapping efficiency. If the traps are small or placed in a wrong position, it is possible that an attractive sex pheromone mixture does not capture many insects. This was observed with field tests for *Scrobipalpuloides absoluta* in Peru (chapter 5). Male *Scrobipalpuloides absoluta* flew in from the surroundings at the moment that the sex pheromone dispensers were exposed, but due to the choice of trap, the numbers of caught males were low. The application of a larger trap type which was placed, at the same time, at a lower position, increased the number of caught males more than tenfold.

The solvent in which the sex pheromones are dissolved before filling the dispensers is also an important factor. The IPO-DLO uses dichloromethane because this solvent

promotes the adsorption of the sex pheromone into the rubber of the dispensers. Also the release concentration-curve is favourable in comparison with, for example, hexane as solvent[14]. So far, the life time of the dispensers with the sex pheromone for the two moth species examined is not precisely known. Dispensers with the sex pheromone for *Phthorimaea operculella*, last for about three months[15]. This sex pheromone consists of a mixture of (E,Z)-4,7-tridecadienyl acetate (**76**) and (E,Z,Z)-4,7,10-tridecatrienyl acetate (**23**) (figure 7.3). Especially the triple-unsaturated compound (**23**) is expected to be less stable than the triple-unsaturated (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**) of the *Scrobipalpuloides absoluta* sex pheromone because of its double homo-conjugation. It is, therefore, expected that the dispensers with *Scrobipalpuloides absoluta* sex pheromone will last for at least three months. The double-unsaturated (E,Z)-3,7-tetradecadienyl acetate (**6**) of the *Symmetrischema tangolias* sex pheromone appears to be very stable under field conditions[15]. In Peru, farmers claimed that the dispensers were still attractive to males after two years, although a comparative test against new dispensers was not carried out.

## 7.5 Some notes on the commercial synthesis of pheromone

The highest possible yield is mostly the primary objective in chemical synthesis research. In chapter 3 two routes were presented for the synthesis of (E,Z)-3,7-tetradecadienyl acetate. The oxidation step of alcohol **39** to aldehyde **40** (scheme 3.2) is performed in high yield using the very expensive silver carbonate on celite. The high yield is not essentially the most important lead to follow in commercial synthetic applications. To demonstrate that the cost of a reaction step depends on several factors, the (estimated) price for the mentioned oxidation step is calculated for five different reaction procedures (figure 7.11). The following formula is used for the calculations:

$$\text{product price (kDfl / mol)} = \frac{\text{SM amount} \times (\text{SM price} + \text{reaction price}) + \text{infrastructural costs}}{\text{reaction yield} \times \text{SM amount}}$$

| | |
|---|---|
| kDfl: | Dutch guilders × 1000 |
| SM: | starting material (mol) |
| SM price: | price of starting materials (kDfl / mol) |
| reaction price: | total price of all necessary reagents (kDfl / mol SM) |
| infrastructural costs: | Total of estimated infrastructure and labour costs per day × the number of days necessary (≈ 2 kDfl / day) |

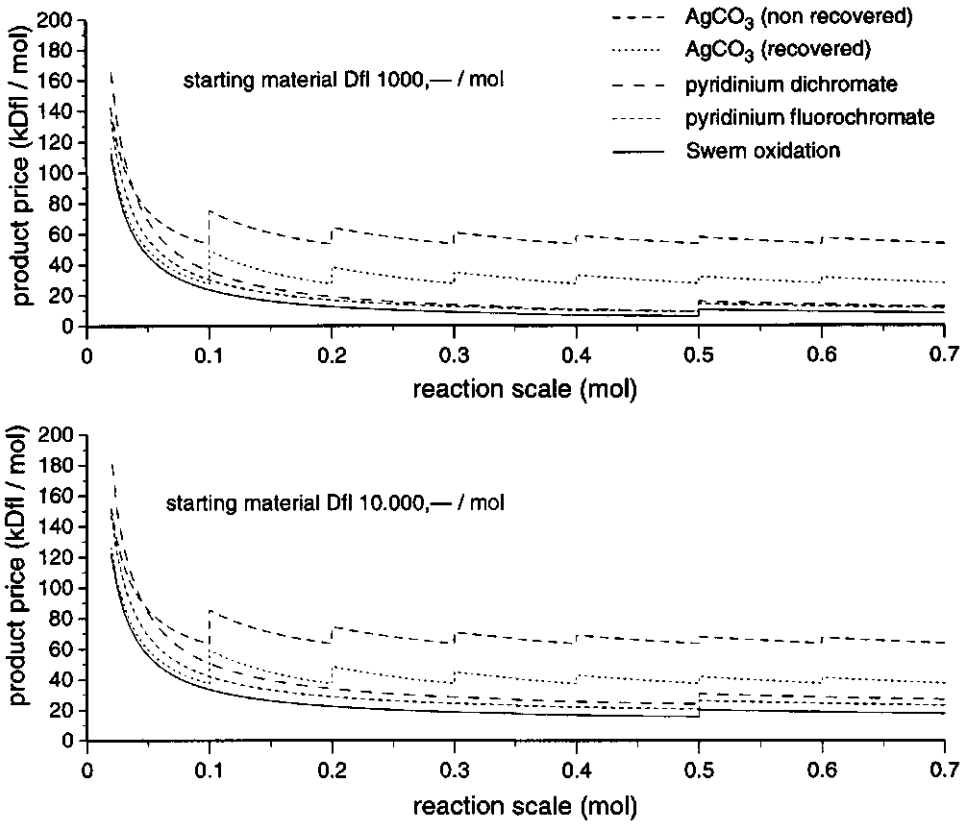



*Fig. 7.11* **Calculation of production costs for a single oxidation step by using different methods. For further explanation see text.**

The upper graph of figure 7.11 assumes a price of Dfl 1000,–/mol for the starting material and the lower graph a price of Dfl 10.000,–/mol (which is more realistic at that stage in the synthetic route). The values for the other used variables are based on our experience except for the Swern oxidation which is based on literature values[11]. The reaction with silver carbonate is calculated, in addition, with the assumption that the reagent is recovered (90%) after the reaction. The leaps in the graphs are caused by set reaction scale limits for the silver carbonate oxidation of 0.1 mol per day and for the other oxidations of 0.5 mol per day.

From the graphs, it is concluded that for the large-scale commercial synthesis of sex pheromones, the oxidation with silver carbonate is too expensive. The Swern oxidation seems to be the cheapest solution for this reaction step but it must be noted that a protective group as the tetrahydropyranyl ether is probably not stable under the acidic reaction conditions. The higher yields obtained with pyridinium fluorochromate (PFC)

compensate for the higher costs of this reagent when compared to pyridinium dichromate (PDC). It is therefore concluded that PFC would have been the best choice for this reaction step. Figure 7.11 shows that the choice of reaction conditions depends, not solely on the price of the reagents, but also on the price of the starting material and this should, therefore, be estimated for every reaction step.

## 7.6 References and notes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# Chapter 8

## Summary

Sex pheromones are substances which are used by insects to attract a partner with the intention to mate. Pheromones are essential for the species survival because without them the partner cannot be located. When the chemical structures are known, the sex pheromones could be applied for pest control of that species. Sex pheromones are produced by the insect itself and they are attractive in very low concentrations. The development of resistance against sex pheromones, in contrast to pesticides, is considered unlikely. Owing to the species specificity and the low amounts necessary, the natural environment of the pest is less afflicted by the use of sex pheromones in comparison to conventional pesticides.

This thesis describes the isolation, identification and possible applications of the sex pheromones of two South-American moths, *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*. The research was carried out as a co-operative project between the Research Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands, the Department of Organic Chemistry of the Wageningen Agricultural University (OC-WAU) and the International Potato Center, Lima, Peru (CIP). The project was financially supported by the Netherlands' Minister for Development Co-operation.

The moth *Symmetrischema tangolias* (Gyen), synonym: *Symmetrischema plaesiosema* (Turner), occurs mainly in the higher regions of the Andes in Peru and Bolivia where it is a severe pest of potatoes. The larva lives in the fields in the stems of potato plants and in storage places in the tubers. The harvested potatoes are stored in open facilities which are easy accessible to the insects. The potato growing is essential for the local food provision in the above-mentioned areas.
The sex pheromone of *Symmetrischema tangolias* has been identified (chapter 3) as a 2 : 1 mixture of (E,Z)-3,7-tetradecadienyl acetate (**6**) and (E)-3-tetradecenyl acetate (**1**). In the sex pheromone glands, two additional minor, to the sex pheromone related, compounds have been identified, namely (Z)-5-tetradecenyl acetate (**25**) and (Z)-7-tetradecenyl acetate (**26**) (figure 8.1). The ratio of these compounds in the sex pheromone gland is: 63 : 31 : 5 : 1 for **6** : **1** : **26** and **25** respectively.

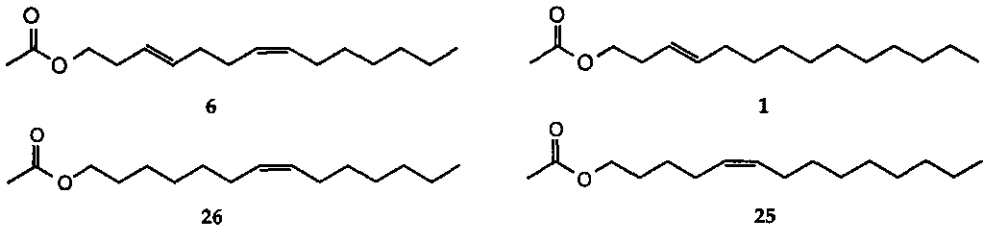


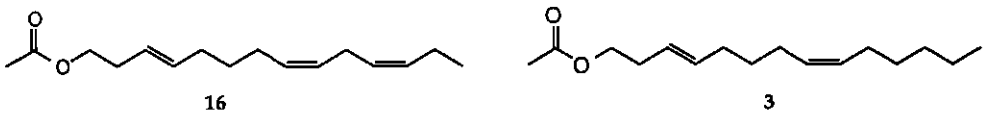

*Fig. 8.1* **Identified sex pheromone components of *Symmetrischema tangolias* (6,1) plus the two minor sex pheromone gland constituents 26 and 25.**

In a new wind tunnel at IPO-DLO, several mixtures of the identified and synthesised sex pheromone components have been tested in different ratios (chapter 6). A mixture of **6** and **1** appears to be more attractive than the two components separately. Mixtures of **6** and **1** in ratios of 1 : 1 and 2 : 1 are equally attractive. The addition of small amounts of minor components **25** and **26** does not affect the attractiveness of the sex pheromone blend. The function of these two minor components therefore remains unclear.
The amounts and ratios of the four gland constituents were measured during the 24-hrs dark-light cycle (chapter 4). A new approach was followed which involved the direct introduction of sex pheromone glands into the gas chromatograph (GC) by using a special temperature programmable GC-injector. With this method it was possible to examine glands without prior processing which provides a clear advantage over earlier described methods. It turns out that the total amount of sex pheromone in the glands varied strongly from individual to individual (3.8 - 350 ng/♀). The total sex pheromone amounts were significantly lower in the scotophase than in the photophase (62.2 and 101.5 ng/♀ respectively). The ratios of the various gland constituents showed a symmetrical distribution which did not fluctuate during the 24-hrs dark-light cycle.
The synthetic sex pheromone was highly attractive to male *Symmetrischema tangolias* in field tests conducted in potato fields and in storage facilities in Peru. The local farmers were exited about the results and wish to apply the sex pheromone as soon as it becomes available. Although the synthesis of the mono-unsaturated compound **1** is easy, large scale synthesis of the double-unsaturated compound **6** (which has been never reported before) is problematic.

The moth *Scrobipalpuloides absoluta* (Meyrick), synonym: *Scrobipalpula absoluta* (Meyrick), lives in low altitude areas of South America. The larva is a leafminer of tomatoes and has developed into a devastating pest in tomato cultivation, especially in Brazil, Peru and Chile. In contrast to small-scale potato crops in higher parts of the Andes, the tomato

growing is a large-scale and professional business. The harvested tomatoes are largely processed and exported. Therefore, tomato growing is of national economic interest for the involved countries.

The sex pheromone of *Scrobipalpuloides absoluta* has been identified (chapter 5) as a 9 : 1 mixture of (E,Z,Z)-3,8,11-tetradecatrienyl acetate (**16**) and (E,Z)-3,8-tetradecadienyl acetate (**3**) respectively (figure 8.2).



16 3

*Fig. 8.2* **Identified sex pheromone components of *Scrobipalpuloides absoluta* (16,3).**

The ratio of these two identified compounds in the sex pheromone gland is 92 : 8 (for **16** : **3** respectively). On a Peruvian tomato farm, several mixtures of the identified compound were tested in ratios between 100 : 0 and 80 : 20, for **16** : **3** respectively. All tested mixtures turned out to be highly attractive to *Scrobipalpuloides absoluta* males, however, none of them appeared superior. Both the identified sex pheromone components **16** and **3** are unique to this species. The synthesis of the minor compound **3** can be increased easily, however, large-scale synthesis of **16** may provide more problems due to its three double bonds.

Various techniques were used to identify the sex pheromones of *Symmetrischema tangolias* and *Scrobipalpuloides absoluta*. By means of GC, the chain lengths, functional groups and number of double bonds could be established. The active components were found in the gas chromatogram by means of electroantennography (EAG-detector). This detection technique is based on the application of the insect's antenna (olfactory organ) as a detector. From the gas chromatographical research it turned out that the sex pheromone candidates for both species belonged to the same group of linear unsaturated compounds all with a chain length of 14 carbons. The mono-unsaturated components **1**, **25** and **26** could be identified with related reference compounds which were all available at IPO-DLO. Of the double-unsaturated compound **6** of the *Symmetrischema tangolias* sex pheromone sufficient amounts could be isolated by means of preparative GC to unambiguously determine the (E)-3-double bond with NMR. Through EAG-recordings of all mono-unsaturated related compounds the (Z)-7-double bond was postulated as the other double bond in molecule **6**. The synthesis of **6** confirmed the postulated structure. By means of derivatisation of sex pheromone compounds with dimethyl disulphide (DMDS) subsequently followed by analysis of the derivatives obtained with mass

spectrometry, the double bond positions in the mono- and double-unsaturated compounds could be determined directly. For compound **3** the two double bonds could be located at positions 3 and 8 with the DMDS method. All four possible (E/Z) isomers were synthesised, tested with EAG-recordings and compared to the analytical obtained data on the minor sex pheromone component of *Scrobipalpuloides absoluta*. Through this the identity of **3** could be confirmed. With the DMDS derivatisation technique two of the three double bonds in **16** could be unambiguously located at positions 3 and 8, similar to the minor component **3**. The obtained EAG results suggested the same E/Z configuration for these two double bond positions in **16**. GC-analysis of available mono- and poly-unsaturated related compounds left three possibilities for the structure of **16**. The identity of **16** was determined through synthesis of all three possibilities and comparison of the analytical data with the data obtained for the sex pheromone compound.
A closer investigation of the DMDS derivatives of these latter three triple-unsaturated compounds revealed the possibility for the direct determination of all three double bond positions solely through interpretation of mass spectra. This has not been reported before for these types of molecules. For this reason, and because of its importance for this kind of research, a separate chapter (chapter 2) was dedicated to it.

The discussion contains a proposal for the biosynthetic formation of the identified sex pheromones. Also the price control in commercial synthesis of sex pheromones is mentioned and illustrated through an example. Both pheromones of *Symmetrischema tangolias* and *Scrobipalpuloides absoluta* contain a synthetically problematic compound. Considering the need for these sex pheromones it is recommended to continue the research for more efficient synthetic routes.

# Capítulo 9

## Resumen

Las feromonas sexuales son sustancias utilizadas por los insectos para atraer a la pareja con la intención de parearse. Estas sustancias son esenciales para la supervivencia de estas especies ya que sin ellas la pareja no podría ser localizada. Al conocerse la estructura química de las feromonas sexuales, éstas podrían ser utilizadas para el control de plaga de estas especies. Las feromonas sexuales son producidas por los propios insectos y pueden atraer a concentraciones muy bajas. Al contrario que el uso de pesticidas, el desarrollo de resistencia frente a las feromonas sexuales es improbable. Debido a que las feromonas sexuales son específicas para cada especie y la cantidad necesaria es baja, su uso afectaría menos al ambiente natural de la plaga en comparación con los pesticidas.

Esta tesis describe el aislamiento, identificación y posible aplicación de las feromonas sexuales procedentes de dos polillas de Sudamerica: *Symmetrischema tangolias* y *Scrobipalpuloides absoluta*. Esta investigación se ha llevado a cabo dentro de un proyecto de colaboración entre el Instituto de Investigación para la Protección de Plantas, Wageningen, Holanda (IPO-DLO), el departamento de química orgánica de la Universidad de Agricultura de Wageningen (OC-WAU), Holanda y el Centro Internacional de la Papa (CIP), Lima, Perú. El proyecto fue financiado por el Ministerio Holandés para el Desarrollo (DGIS).

La polilla *Symmetrischema tangolias* (Gyen), sinónimo: *Symmetrischema plaesiosema* (Turner), se encuentra principalmente en las regiones altas de Los Andes en Perú y Bolivia, siendo esta la plaga de la papa en esta región. La larva vive en el campo en las partes firmes de la planta de la papa y en los lugares de almacenamiento de los tubérculos. La papa cosechada es almacenada al aire libre, siendo de fácil acceso para los insectos. El cultivo de papa es esencial como provisión de alimentos para la región. Al estar disponibles las feromonas sexuales podrían ser utilizadas en el control de la plaga.
La feromona sexual de *Symmetrischema tangolias* ha sido identificada (capítulo 3) como una mezcla 2 : 1 de acetato de (E,Z)-3,7-tetradecadienilo (**6**) y acetato de (E)-3-tetradecenilo (**1**). En las glándulas que contienen las feromonas sexuales se han identificado dos compuestos adicionales minoritarios relacionados con ellas. Estos han

sido identificadas como: acetato de (Z)-7-tetradecenilo (**25**) y acetato de (Z)-5-tetradecenilo (**26**) (figura 8.1). Tales compuestos se encuentran en la glándulas que contienen las feromonas sexuales en una proporción de 62 : 31 : 6 : 1 para **6 : 1 : 25** y **26** respectivamente.

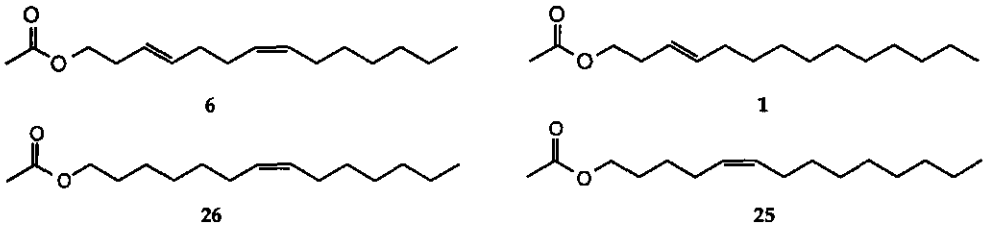



*Fig. 8.1* **Feromonas sexuales identificadas a partir de *Symmetrischema tangolias* (6,1) y los componentes minoritarios 26 y 25 presentes en las glándulas.**

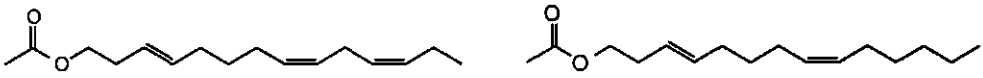
Varias mezclas de los componentes identificados a partir de la feromona sexual han sido evaluadas en un nuevo tunel de viento del IPO-DLO a distintas proporciones (capitulo 6). La mezcla de **6** a **1** parece ser la más atractiva en comparación con cada uno de los componentes por separado. Mezclas de **6** a **1** a razón de 1 : 1 y 2 : 1 son igualmente atractivas. Añadiendo un poco de los componentes minoritarios **25** y **26**, no se altera el efecto atractivo de la mezcla. Por lo tanto, aún no se ha clarificado la función de estos componentes minoritarios.

Las cantidades y proporciones de estos cuatro compuestos de la glándula han sido medidos durante las 24-hr de fotoperíodo (capítulo 4). Se ha seguido una nueva aproximación que consiste en introducir directamente las glándulas que contienen feromonas sexuales dentro del cromatógrafo de gas (GC) utilizando un GC-inyector programador de temperatura. Con este método es posible examinar las glándulas sin procesamiento previo, lo cual tiene ventajas sobre el procesamiento descrito en los métodos. Se observó que la cantidad total de feromona sexual en las glándulas varía ampliamente de individuo a individuo (3.8-350 ng/♀). Las cantidades totales son significativamente menores en la escoto-fase, en comparación con la fotofase de las 24 hr fotoperíodo (62.2 and 101.5 ng/♀ respectivamente). La proporción a la que se encuentran los distintos componentes de las glándulas muestra una distribución simétrica y además es constante durante el fotoperíodo. Se concluye por lo tanto que *Symmetrischema tangolias* produce feromonas sexuales continuamente las cuales son sólo reguladas por el título de la glándula.

En base a estudios llevados a cabo en los campos de papa y en lugares de almacenamiento en en Perú, se concluye que las feromonas sexuales sintéticas atraen con gran fuerza a los machos de *Symmetrischema tangolias*. La población local estaba muy entusiasmada con los resultados y deseosa de utilizar las feromonas sexuales tan pronto como estuvieran

disponibles. A pesar de que la síntesis del compuesto **1** mono-insaturado es sencilla, la síntesis a gran escala del compuesto **6** doble-insaturado (no publicada hasta el momento) es más difícil.

La polilla *Scrobipalpiloides absoluta* (Meyrick), sinónimo *Scrobipalpula absoluta* (Meyrick), habita en las zonas bajas de Sudamerica. La larva invade las hojas de tomate y se ha convertido en una plaga devastadora para el cultivo de tomates, especialmente en Brazil, Perú y Chile. A diferencia del cultivo a pequeña escala de papas en las regiones altas de los Andes, el tomate se cultiva a gran escala y representa un importante negocio. Los tomates cosechados son, en su mayoría, procesados y exportados. Por lo tanto, el cultivo de tomate es de interés económico para los paises involucrados.
La feromona sexual de *Scrobipalpuloides absoluta* ha sido identificada (capítulo 5) como una mezcla 9 : 1 de acetato de (E,Z,Z)-3,8,11-tetradecatrienilo (**16**) y acetato de (E,Z)-3,8-tetradecadienilo (3) respectivamente (figura 8.2).



**16** **3**

***Fig. 8.2*** **Componentes de la feromona sexual de *Scrobipalpuloides absoluta*.**

La proporción a la que se encuentran los compuestos identificados de la glándula que contiene feromonas sexuales es de 92 : 8 ( para **16 : 3** respectivamente). En una hacienda de tomate Peruana, varias mezclas del compuesto identificado han sido evaluadas en proporciones que varían entre 100 : 0 y 80 : 20, para **16 : 3** respectivamente. Todas las mezclas evaluadas resultaron ser muy atractivas para los machos de *Scrobipalpuloides absoluta*, sin embargo, ninguna de ellas resultó ser superior. Ambos compuestos identificados **16 y 3** son únicos para estas especies. La síntesis del compuesto minoritario **3** puede ser fácilmente aumentada a gran escala, sin embargo, la síntesis del compuesto **16** puede traer problemas debido a sus tres dobles enlaces homo-conjugados.

Se han aplicado varias técnicas han sido aplicadas para la identificación de las feromonas sexuales de *Symmetrischema tangolias* y *Scrobipalpuloides absoluta.* Por medio del GC, la longitud de las cadena, los grupos funcionales y el número de dobles enlaces, pueden ser determinados en las moléculas de las feromonas sexuales candidatas. Estas fueron identificadas en el cromatógrafo de gas utilizando un detector antena-gráfica (EAG-detector). Esta técnica está basada en el uso de la antena de los insectos (órganos olfatorios) como detector. Los estudios realizados con el cromatógrafo de gas indican que las feromonas sexuales candidatas para ambas especies pertenecen al mismo grupo de

compuestos lineales insaturados, todos con una longitud de 14 carbonos. Los compuestos mono-insaturados **1,25** y **26** pueden ser identificados utilizando un compuesto de referencia relacionado que se encuentra en IPO-DLO. El compuesto **6** de la feromona sexual de *Symmetrischema tangolias*, que es doble insaturado, puede aislarse utilizando el GC preparativo para determinar sin ambigüedades el (E)-3-doble enlace utilizando NMR (resonancia magnética nuclear). En base al registro del EAG de todos los compuestos relacionados mono-insaturados se ha postulado, el (Z)-7-doble enlace como el otro doble enlace en la molécula **6**. La síntesis de **6** confirmó dicha estructur. La derivación de compuestos de las feromonas sexuales utilizando disulfidos dimetílicos (DMDS) seguida del análisis de los derivados obtenidos por espectometría de masa, permite la determinación directa de los dobles enlaces en los compuestos mono y doble insaturados. Con respecto a la identidad de **6**, este método proporciona solo evidencia adicional, sin embargo, para el compuesto **3**, los dos dobles enlaces pueden ser localizados en las posiciones **3** y **8**. Los cuatro posibles isómeros (E/Z) fueron sintetizados, analizados con los registros EAG y comparados con los datos analíticos obtenidos para el componente minoritario de las feromonas sexuales de *Scrobipalpuloides absoluta*. De esta manera, la identidad de **3** pudo ser confirmada. Con la técnica de derivación DMDS dos de los tres dobles enlaces en **16** han podido ser localizados sin ambigüedades en las posiciones **3** y **8**, al igual que el componente minoritario **3**. Los resultados previos de EAG sugieren la misma configuración (E,Z) para estas dos posiciones dobles en **16**. Los análisis por GC de los compuestos mono y doble-insaturados relacionados proporcionan tres posibilidades para la estructura **16.** La identificación de **16** fue confirmada al sintentizar las tres posiblidades y comparar los datos analíticos obtenidos con los datos del compuesto de las feromonas sexuales.

Una investigación más detallada de los derivados DMDS de estos compuestos triple-insaturados reveló la posibilidad de determinar de manera directa, la posición de los tres dobles enlaces exclusivamente a través de la interpretación de la espectrometría de masa. Esto no ha sido publicado antes para este tipo de moléculas. Por esta razón, y debido a su importacia para este tipo de investigación, se le ha dedicado un capítulo aparte (capítulo 2).

La discusión contiene una propuesta para la biosíntesis de las estructuras de las feromonas sexuales identificadas. Así mismo, se menciona y se ilustra a través de un ejemplo el precio para el control comercial de la síntesis de feromonas sexuales. Tanto las feromonas de *Symmetrischema tangolias* como las de *Scrobipalpuloides absoluta* contienen un compuesto cuya síntesis es problemática. Debido a la potencialidad del mercado para estas feromonas sexuales, se recomienda continuar la investigación sobre rutas de síntesis más eficientes.

# Hoofdstuk 10

## Samenvatting

Seksferomonen zijn vluchtige verbindingen die door insecten worden verspreid om daarmee een partner voor de paring te lokken. Ze zijn essentieel voor het voortbestaan van de soort, omdat een partner zonder de hulp van seksferomonen niet gevonden kan worden. Wanneer de chemische structuur van de seksferomonen voor een bepaalde soort bekend is, kunnen deze natuur-identieke stoffen na synthese gebruikt worden bij de bestrijding van die soort. Seksferomonen worden door het insect zelf geproduceerd, zijn specifiek voor de soort en zeer attractief in bijzonder lage concentraties. Het ontwikkelen van resistentie tegen seksferomonen wordt, in tegenstelling tot veel pesticiden, onwaarschijnlijk geacht. Vanwege de soortspecificiteit en lage dosering wordt bij het gebruik van seksferomonen als bestrijdingsmiddel de natuurlijke omgeving van het insect veel minder belast dan bij het spuiten met conventionele pesticiden.

Dit proefschrift beschrijft de isolatie, identificatie en mogelijke toepassingen van de seksferomonen van twee uit Zuid-Amerika afkomstige motten: *Symmetrischema tangolias* en *Scrobipalpuloides absoluta*. Het project is uitgevoerd in een samenwerkingsverband van het Instituut voor Planteziektenkundig Onderzoek (IPO-DLO), Wageningen, Nederland, de vakgroep Organische Chemie van de Landbouwuniversteit Wageningen, Nederland (OC-LUW) en het International Potato Center (CIP), Lima, Peru. Het onderzoek is mogelijk geworden door de financiële steun van het Directoraat-Generaal Internationale Samenwerking (DGIS).

De mot *Symmetrischema tangolias* (Gyen), synoniem: *Symmetrischema plaesiosema* (Turner), leeft voornamelijk in de Andes van Peru en Bolivia, waar dit insect een ernstige plaag vormt voor de lokale aardappelteelt. De larve leeft in het veld in de hardere delen van de plant en in opslagplaatsen in de aardappelen zelf. Gerooide aardappelen worden bewaard in opslagplaatsen die voor insecten gemakkelijk toegankelijk zijn. De aardappelteelt in die gebieden is van essentieel belang voor de voedselvoorziening.
Het seksferomoon van *Symmetrischema tangolias* is geïdentificeerd (hoofdstuk 3) als een 2 : 1 mengsel van (E,Z)-3,7-tetradecadienylacetaat (**6**) en (E)-3-tetradecenylacetaat (**1**). In de klieren die het seksferomoon aanmaken zijn naast deze twee verbindingen nog twee

andere nauw aan elkaar verwante verbindingen aangetroffen, namelijk (Z)-7-tetradecenylacetaat (**25**) en (Z)-5-tetradecenylacetaat (**26**) (figuur 10.1). De onderlinge verhouding van deze vier verbindingen in de seksferomoonklier is **6** : **1** : **26** : **25** = 63 : 31 : 5 : 1.

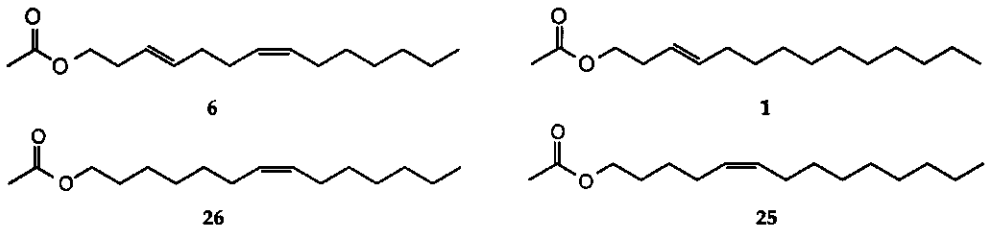



*Fig. 10.1* **De beide geïdentificeerde seksferomooncomponenten van *Symmetrischema tangolias* (6,1) plus twee in de seksferomoonklier aangetroffen verwante verbindingen 25 en 26.**

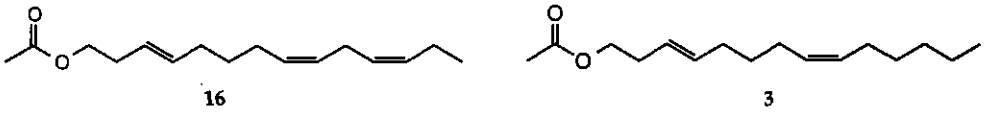
In een nieuwe windtunnel van het IPO-DLO zijn diverse mengsels van synthetisch **6**, **1**, **25** en **26** in verschillende verhoudingen tegen elkaar getest (hoofdstuk 6). Het bleek dat een mengsel van **6** en **1** attractiever is dan de beide verbindingen apart. Mengsels van **6** en **1** in de verhoudingen 1 : 1 en 2 : 1 zijn even attractief. Het toevoegen van een beetje **25** of **26** aan het seksferomoon heeft geen invloed op de attractiviteit ervan. De biologische significantie van deze componenten **25** en **26** is daarmee niet duidelijk.

De hoeveelheden en de onderlinge verhouding in de klieren gedurende het etmaal zijn onderzocht voor de verbindingen **6**, **1** en **25** plus **26** (hoofstuk 4). Hiervoor is gebruikgemaakt van tweedimensionale gaschromatografie waarbij intacte seksferomoon-klieren konden worden geïnjecteerd met behulp van een speciale temperatuur programmeer-bare injector. De gebruikte methode maakt het mogelijk om seksferomoon-klieren te analyseren zonder enige voorbewerking wat een belangrijk voordeel oplevert in vergelijking met de tot nu toe beschreven methoden. Uit dit onderzoek is gebleken dat de hoeveelheid seksferomoon in de klieren van *Symmetrischema tangolias* sterk varieert van individu tot individu (3,8 - 350,0 ng/♀). De hoeveelheden per klier zijn significant lager gedurende de actieve periode in vergelijking met de inactieve periode (gemiddeld 62,2 en 101,5 ng/♀ respectievelijk). De onderlinge verhouding van de verschillende componenten vertoont een symmetrische spreiding en is verder constant gedurende de gehele dag.

Het synthetisch seksferomoon is zeer attractief voor *Symmetrischema tangolias* mannetjes zoals is gebleken uit veldtesten die zijn uitgevoerd in aardappelvelden en opslagplaatsen in Peru. De lokale bevolking is zeer enthousiast en wil dit seksferomoon zo snel mogelijk gaan gebruiken. Alhoewel de mono-onverzadigde component **1** eenvoudig is te synthetiseren, vormt de grootschalige synthese van de (nog niet eerder in de literatuur

beschreven) dubbel-onverzadigde component (**6**) tot nu toe een probleem.

De mot *Scrobipalpuloides absoluta* Meyrick, synoniem: *Scrobipalpula absoluta* (Meyrick), leeft in de lager gelegen gebieden in Zuid-Amerika. De larve mineert tomatenplanten en veroorzaakt daarmee een enorme schade, met name in Brazilië, Peru en Chili. In tegenstelling tot de kleinschalige aardappelteelt boven in de Andes is de tomatenteelt zeer grootschalig en professioneel van opzet. De tomaten worden na verwerking grotendeels geëxporteerd en bescherming van de tomatenteelt is dus van economisch belang voor de betreffende landen.
Het seksferomoon voor *Scrobipalpuloides absoluta* is geïdentificeerd (hoofdstuk 5) als een 9 : 1 mengsel van (E,Z,Z)-3,8,11-tetradecatrienylacetaat (**16**) en (E,Z)-3,8-tetradecadienylacetaat (**3**) (figuur 10.2).



**16** **3**

*Fig. 10.2* **Geïdentificeerde seksferomooncomponenten van *Scrobipalpuloides absoluta*.**

Deze twee verbindingen zijn in een verhouding van 92 : 8 (voor **16** : **3** respectievelijk) in de seksferomoonklieren aangetoond. In een tomatenkwekerij in Peru zijn diverse onderlinge verhoudingen van deze twee componenten getest in verhoudingen van 100 : 0 tot 80 : 20 (voor **16** : **3** respectievelijk). Alle geteste mengsels bleken uitermate attractief. Geen van de geteste mengsels is echter significant attractiever dan de andere (hoofdstuk 5). Beide verbindingen **3** en **16** zijn uniek voor *Scrobipalpuloides absoluta* en nog niet eerder beschreven voor andere insecten. De synthese van component **3** is eenvoudig op te schalen maar die van component **16** kan mogelijk problemen opleveren vanwege de drie dubbele banden in het molecuul.

Voor de identificaties van beide seksferomonen zijn diverse technieken gebruikt. Met behulp van gaschromatografie (GC) konden ketenlengte, functionele groep en het aantal dubbele banden worden vastgesteld van kandidaat-seksferomoonverbindingen. Deze verbindingen werden gevonden in het gaschromatogram met behulp van een electroantennografie-detector (EAG-detector). Bij deze detectietechniek functioneert de antenne (het reukorgaan) van het insect als de eigenlijke detector. Uit het gaschromatografisch onderzoek bleek dat de seksferomoonkandidaten voor beide mottensoorten tot dezelfde groep van onverzadigde acetaten met een ketenlengte van 14 koolstofatomen behoorden. De mono-onverzadigde verbindingen **1**, **25** en **26** konden

worden geïdentificeerd met behulp van referentieverbindingen die voor deze groep van verbindingen allemaal op het IPO-DLO aanwezig waren. Van de dubbel-onverzadigde seksferomooncomponent (**6**) van *Symmetrischema tangolias* kon er zoveel worden geïsoleerd, dat met behulp van NMR de (E)-3-dubbele band ervan kon worden vastgesteld. Door vervolgens alle aan deze groep verwante verbindingen te testen met EAG kon de (Z)-7-dubbele band worden gepostuleerd als tweede dubbele band in molecuul **6**. De synthese van **6** bevestigde deze gepostuleerde structuur. Door de seksferomooncomponenten te derivatiseren met dimethyldisulfide (DMDS) en de derivaten vervolgens met massaspectrometrie te analyseren, konden dubbelebandposities ook rechtstreeks worden bepaald in de enkel- en dubbel-onverzadigde componenten. Voor **6** gaf dit in een extra bevestiging van de structuur. Voor component **3** konden hiermee de dubbele banden op de 3- en 8-positie gelokaliseerd worden. Door alle vier de mogelijke E/Z-isomeren te synthetiseren en deze onderling te vergelijken met EAG en met de verzamelde analytische gegevens van de seksferomoonkandidaat werd de structuur van **3** bevestigd. Met deze derivatiserings-techniek konden ook twee van de drie dubbelebandposities in **16** ondubbelzinnig worden bepaald. Deze bevonden zich, evenals bij component **3**, op de posities 3 en 8. De eerder verkregen EAG-resultaten van **3** plus de drie isomeren van **3** suggereerden dezelfde E/Z-stereochemie voor deze twee dubbele banden in **16**. GC-analyse van de retentie-indices van beschikbare enkel- en meervoudig-onverzadigde verwante verbindingen lieten drie mogelijkheden over voor de uiteindelijke structuur van component **16**. De identiteit van component **16** werd bevestigd door deze drie drievoudig-onverzadigde verbindingen te synthetiseren en de analytische gegevens te vergelijken met die van de seksferomooncomponent.
Bij nader onderzoek van de DMDS-derivaten van deze drie laatstgenoemde drievoudig-onverzadigde verbindingen bleek dat alle drie de dubbelebandposities ook door een interpretatie van de massaspectra van het DMDS-derivaat van **16** konden worden bepaald. Dit was nog niet eerder beschreven voor dit soort verbindingen. Vanwege deze resultaten én het gebleken belang van deze techniek voor dit soort onderzoek is er een apart hoofstuk (hoofdstuk 2) aan gewijd.

De discussie bevat een voorstel voor de biosynthetische vorming van de geïdentificeerde seksferomooncomponenten. Ook wordt er ingegaan op het belang van kostenbeheersing in de commerciële synthese van seksferomonen. De seksferomonen van zowel *Symmetrischema tangolias* als *Scrobipalpuloides absoluta* bevatten een lastig te synthetiseren component. Vanwege de mogelijke afzetmarkt voor beide geïdentificeerde verbindingen verdient het aanbeveling om het onderzoek hiernaar voort te zetten.

## Curriculum Vitae

Frans Christiaan Griepink werd geboren op 27 oktober 1964 te 's-Gravenhage. In 1983 behaalde hij het diploma Atheneum-B aan het Revius Lyceum te Doorn. Na aanvankelijk een jaar werktuigbouw te hebben gestudeerd aan de Technische Universiteit te Delft startte hij in 1984 met de studie Moleculaire Wetenschappen aan de Landbouwuniversiteit te Wageningen (LUW). Van september 1987 t/m september 1988 werd de studie voor ruim 12 maanden onderbroken ter vervulling van de militaire dienstplicht (ambulancechauffeur bij de luchtdoelartillerie), waarna de studie werd voortgezet. Afstudeervakken werden gedaan bij de vakgroepen Organische Chemie (dr. T.A. van Beek en prof. dr. Ae. de Groot) en Entomologie (dr. J.J.A. van Loon en prof. dr. L.M. Schoonhoven). In het kader van een Erasmus-uitwisseling werd stage gelopen bij de afdeling Chemie van de Royal Veterinary and Agricultural University te Kopenhagen, Denemarken (K. Jørgensen MSc en prof. dr. L.H. Skipsted). Het doctoraal examen werd behaald in augustus 1991. In de periode oktober 1991 - oktober 1995 was hij werkzaam als assistent in opleiding (AIO)[1]. Het werk hiervoor werd uitgevoerd aan de vakgroep Organische Chemie van de LUW, onder leiding van dr. T.A. van Beek en prof. dr. Ae. de Groot, en aan het Instituut voor Plantenziektenkundig Onderzoek van de Dienst Landbouwkundig Onderzoek (IPO-DLO) onder leiding van dr. J.H. Visser en drs. S. Voerman. Het project werd gefinancierd door het Directoraat-Generaal Internationale Samenwerking (DGIS).

Per 1 juni 1996 is hij werkzaam bij de afdeling Ecologie en Biologische Bestrijding van Insecten van het IPO-DLO.

[1]Het tijdens deze periode uitgevoerde onderzoek staat beschreven in dit proefschrift.