Doctoral Thesis

Olfactory cues in host plant location of the oriental fruit moth, Cydia molesta

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Olfactory cues in host plant location of the oriental fruit moth, 

_Cydia molesta_

A dissertation submitted to the

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Doctor of Natural Science

presented by

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2003
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Summary

The oriental fruit moth, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae) is an economically important pest of stone fruit orchards with peach being its primary host. Apple orchards commonly provide a post peach-harvest resource, and apples are recently increasing in importance as a late season host. Considerable levels of *C. molesta* damage to apple orchards have been widely observed in recent years, and the area of infestation is rapidly expanding. Interventions against *C. molesta* are typically based on male catches in pheromone traps, but it was yet unknown whether the presence of males indicates the simultaneous presence of females. The aim of this study was to devise a monitoring strategy for females using plant derived volatiles.

The first part of the study emphasised the need for a suitable experimental set-up for the observation of the olfactory behaviour of female *C. molesta* exposed to host plant odours in a bioassay. Three different olfactometer designs were evaluated, (1) a linear and (2) a Y-tube olfactometer, to observe the behaviour of females as individuals, and (3) a dual-choice arena, to observe the behaviour of females as groups. Controlled room temperature, relative humidity, light intensity, airflow, age of the moths and odour source were kept constant during experiments. The odour source tested was a peach top shoot excised immediately before experiments commenced. Peach shoot volatiles attracted female *C. molesta* in all the three olfactometers used. The dual choice arena out-rated the other two set-ups for screening potentially attractive odour sources: (1) the dual choice arena was the only olfactometer that allowed for a differentiation of the response of mated and virgin females. (2) Movements of groups of insects tested followed a diurnal cycle which mimicked natural conditions. (3) Manipulation on individuals was reduced and flight was not precluded.
The second part of the study investigated a possible preference for peach or apple foliage volatiles, and focused on the identification of peach shoot volatiles as a source of possible attractants for female *C. molesta*. The olfactory behaviour of mated females exposed to excised green shoot odours of either peach or apple tree was observed using a dual choice arena. Peach and apple shoots volatiles were both attractive to mated female *C. molesta*. No preference was observed between the top shoot odours from peach and apple. Twenty-two compounds were identified in headspace volatiles of peach shoots using gas chromatography-mass spectrometry. Green leaf volatiles accounted for more than 50% of the total emitted volatiles. A bioassay-assisted fractionation using different sorbent polymers indicated an attractant effect of compounds with a chain length of 6-8 carbon atoms. The major compounds of this fraction were tested either singly or in combinations for behavioural response of females. Significant bioactivity was found for a three-component mixture composed of (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol and benzaldehyde in a 4:1:1 ratio. This synthetic mixture elicited a similar attractant effect as the full natural blend from peach shoots as well as the bioactive fraction.

The last part of the study also investigated a possible preference of *C. molesta* for peach or apple, this time focusing on the attractiveness of fruit volatiles of both host plants. The olfactory behaviour of mated females exposed to fruit odours of both peach and apple was observed using a dual choice arena. Volatiles emitted from immature and mature peach and apple fruit were attractive to mated female oriental fruit moth. Female did not discriminate between odours emitted by the two major host plants. The same odour sources were behaviourally ineffective for virgin females. A major component of apple fruit volatiles, butyl hexanoate, attracted female *C. molesta*. Data were particularly promising for mated females.

In conclusion this study resulted in a new bioassay method suitable for testing female *C. molesta* response to large numbers of odour sources. It further detected two
different attractant for females, a three component blend derived from peach shoot volatiles, and a single compound from apple fruit volatiles. Further evaluations of these natural resources from host plants of *C. molesta* are recommended under field conditions.
1b Riassunto

_Cydia molesta_ (Busck) (Lepidoptera: Tortricidae) è un fitofago di rilevante impatto economico per le colture drupacee, in particolare pesco. Recentemente _C. molesta_ sta assumendo rilevanza economica anche per le pomacee dove, in particolare nelle regioni di attiguità colturale, il fitofago può spostarsi a fine raccolta del pesco, per trovarvi un’importante riserva di cibo. L’area di infestazione su melo è di fatto in rapida espansione e, negli ultimi anni, danni anche ingenti sono stati osservati in diversi continenti. Attualmente, la strategia di lotta integrata per il controllo del tortricide è perlopiù basata sulla soglia di catture dei maschi, che non assicura tuttavia sulla contemporanea presenza delle femmine. Lo scopo di questo dottorato è di studiare lo sviluppo di un sistema di monitoraggio di _C. molesta_ alternativo, o complementare, a quello attuale basato, appunto sulla cattura delle femmine mediante kairomoni.

La prima parte dello studio ha enfatizzato la necessità di disporre di un biosaggio ottimale con cui osservare il comportamento olfattivo di femmine di _C. molesta_, in risposta ai semiochimici rilasciati da piante ospiti allo stato volatile. A questo scopo, si è voluto comparare l’efficacia di tre olfattometri a corto raggio d’azione, diversamente configurati. I primi due, un olfattometro lineare e un olfattometro Y, consentivano di osservare il comportamento di singoli individui. Il terzo, un’arena a duplice possibilità di scelta, consentiva di osservare il comportamento di gruppi di individui. I parametri di temperatura, umidità relativa, intensità luminosa, flussi di aria, età degli insetti e fonte odorosa rimanevano costanti durante gli esperimenti. La fonte di emissione dei semiochimici era rappresentata da un getto verde di pesco, reciso immediatamente prima degli esperimenti. I getti apicali sono risultati significativamente attrattivi indipendentemente dal tipo di olfattometro impiegato. Dei tre strumenti confrontati, l’arena a duplice possibilità di scelta è apparsa più appropriata per saggio semiochimici potenzialmente attrattivi: (1) in questo
olfattometro è stato possibile osservare una differenza tra la risposta di femmine vergini e femmine accoppiate, (2) il naturale ritmo circadiano degli insetti durante le osservazioni viene riprodotto, (3) le manipolazioni a danno degli individui sono ridotte, mentre le possibilità di volo non sono precluse.

La seconda parte dello studio è stata incentrata sul confronto tra la risposta di *C. molestia* a semiochimici di pesco e melo nell'intento di riscontrare una eventuale preferenza per l'una o l'altra pianta ospite. Tutti i biosaggi sono stati condotti nell'arena a duplice scelta osservando il comportamento olfattivo di gruppi di femmine accoppiate in risposta a getti di pesco e melo utilizzati come fonti di semiochimici. I getti di entrambe le piante sono risultati ugualmente attrattivi per femmine di *C. molestia*, le quali non hanno di fatto mostrato significativa preferenza per pesco o melo messi a confronto. Mediante analisi gas-cromatografica a spettrometria di massa dei campioni contenenti semiochimici volatili raccolti dalla cosiddetta "head-space", sono stati identificati 22 composti. La frazione maggiore consiste dei cosiddetti "green leaf volatiles", presenti per oltre il 50% del totale. Si è adottato un nuovo metodo di frazionamento della miscela naturale di volatili basato sull'utilizzo di filtri selettivi per numero di atomi di carbonio dei composti. Questo metodo ha consentito di suddividere la miscela naturale in 5 frazioni di volatili. Successivamente, un biosaggio che testava le frazioni così ottenute, ha indicato bioattività per una frazione contenente composti volatili da *n*-C6 a *n*-C8. I composti maggiori di questa frazione, singolarmente o in differenti combinazioni, sono stati testati su gruppi di femmine per verificarne l'attrattività. Una combinazione composta da (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol, e benzaldeide nel rapporto relativo di 4:1:1, è risultata significativamente attrattiva per femmine accoppiate di *C. molestia*. Questa miscela, paragonata alla miscela naturale di volatili e alla frazione bioattiva, ha mostrato la stessa efficacia in termini di attrattività.
Nella terza parte dello studio si è completato il confronto tra la risposta di *C. molest*a a semiochimici di pesco e melo nell'intento di riscontrare una eventuale preferenza per una delle due piante ospiti. I biosaggi sono stati condotti nell'arena a duplice scelta osservando il comportamento olfattivo di gruppi di femmine accoppiate in risposta a frutti maturi di pesco e melo. Frutti-noce e frutti maturi di entrambe le piante ospiti sono risultati ugualmente attrattivi per gruppi di femmine accoppiate, le quali non hanno di fatto mostrato significativa preferenza per pesco o melo messi a confronto. Contrariamente, le stesse fonti di semiochimici sono risultate non attrattive nei confronti di femmine vergini. Nella parte finale dello studio si è saggiato su femmine di *C. molest*a l'attrattività di butyl hexanoate, uno dei maggiori composti volatili dei futtti maturi di melo. Al termine di una serie di biosaggi, butyl hexanoate é risultato un attrattivo per femmine di *C. molest*a. I risultati sono stati particolarmente incoraggianti per le femmine accoppiate.

In conclusione, questo studio ha condotto allo sviluppo di un nuovo metodo di biosaggio idoneo a saggiare la risposta di femmine di *C. molest*a ad un largo numero di fonti di semiochimici. Inoltre ha cosentito di individuare due differnti attrattivi, una miscela di tre componenti volatili rilasciati dai getti di pesco, e un singolo componente volatile rilasciato dai frutti di melo. Ulteriori valutazioni di questi composti attrattivi prodotti dalle piante ospiti di *C. molest*a sono auspicabili in condizioni di campo.
Seite Leer / Blank leaf
2 General introduction

Cydia molesta (Busck) (Lepidoptera: Tortricidae) is a member of the sub-family Olethreutinae, commonly known as the Oriental Fruit Moth or, less often, the Oriental Peach Moth or the Peach Tip Moth. Various other generic names have been used for this moth, including Laspeyresia, Grapholitha and Grapholita. According to the current classification (Schoonhoven et al., 1998), C. molesta can be considered an oligophagous pest since it attacks a number of plant species grouped in the family of Rosaceae, and only one shrub in the family of Myrtaceae (Table 1). The main host plants are peach followed by plum, quince and cherry, among stone fruit orchards, and apple and pear among pome fruit orchards. The centre of origin of this species is thought to be Northwest China, where the main natural hosts are likely Prunus spp. (Rothshild and Vickers, 1991). This pest, which causes economic damage at relatively low population densities, is a multivoltine species. The number of generations per season ranges from 2 up to 7, depending on temperature. The life cycle is basically synchronised with that of its host plants, so to guarantee availability of food sources for the larvae (Ivancich Gambaro, 1987).

On peaches, eggs are laid adjacent to young shoots (on the under side of leaves) or on the smooth surface of new twigs. Ovipositing females prefer smooth surface to pubescent substrates. Therefore, females rarely lay eggs on peach fruits, while they lay eggs on apple fruits and on the smooth upper surface of leaves (Peterson and Haeussler, 1930). C. molesta larvae cause damage to their host by tunnelling and feeding in the shoots and/or fruits.

Mating is facilitated by calling behaviours in which both sexes are involved. On the long range, males detect the calling virgin females using the pheromone released by them. The pheromone blend consists of 4 identified compounds (Cardé et al., 1979; Han et al. 2001). The female calling period seems to be regulated by a circadian rhythm and it extends from about 3 hours before to 1 hour after sunset.
Table 1. Host plants and distribution of *Cydia molesta* (after Rothschild and Vickers 1991)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>Distribution</th>
<th>Infesting shoots</th>
<th>Infesting fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MYRTACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eugenia myrianthus</em></td>
<td>Argentine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>ROSACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cotoneaster spp.</em></td>
<td>Europe, Africa, Asia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Crataegus spp.</em></td>
<td>Eurasia</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td><em>Cydonia vulgaris</em></td>
<td>Eur., Asia N. Am, Australasia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Eriobotrya japonica</em></td>
<td>Asia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Malus silvestris</em></td>
<td>Europe, N. America, Australasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Mespilus germanica</em></td>
<td>Eurasia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Photinia spp.</em></td>
<td>North America</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus amygdalus</em></td>
<td>Eurasia, N. America, Australasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus armeniaca</em></td>
<td>Europe, N. America</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus cerasus</em></td>
<td>Eur., Asia N. Am, Australasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus domestica</em></td>
<td>Eur., Asia N. Am, Australasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus ilicifolia</em></td>
<td>North America</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><em>Prunus japonica</em></td>
<td>Asia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus spp.</em></td>
<td>North America, Australasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus laurocerasus</em></td>
<td>Eurasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus persica</em></td>
<td>Eur., Asia, N.S. Amer., Austral.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus persica var. nectar.</em></td>
<td>N. Amer., Europe, Australasia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pyrus communis</em></td>
<td>Eur., Asia, N.S. Amer., Austral.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Pyrus japonica</em></td>
<td>Asia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Pyrus pyrifolia</em></td>
<td>Asia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Rosa spp.</em></td>
<td>Eur., Asia, N. Amer., Africa</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(Rothschild and Vickers, 1991). On the short range, males display characteristic courtship behaviour, which is rather unusual among Lepidoptera (Lofstedt et al. 1990; Baker et al. 1981; Birch and Haynes 1982). A few centimetres from the female, the male turns away and repeatedly extrudes and retracts its abdominal hair pencils, propelling volatile chemicals over the female. The compounds released are ethyl trans-cinnamate, \((R)\)-(\(-\))-mellein, methyl jasmonate and methyl 2-epijasmonate and are possibly sequestered from the host plants during larval development (Baker et al. 1981). Females begin to lay eggs after a preoviposition period of 1-10 days, depending on temperature. Timing of oviposition covers approximately the same
daily period as mating, but at temperatures exceeding 21°C, females may begin to oviposit well before the usual period.

The oriental fruit moth has recently gained economic importance as increasing infestation of pome-fruits, in particular apples, are documented from different continents. The presence of *C. molesta* on pome-fruits was reported since the early 1960s and the 1970s (Roehrich, 1961; Bovey, 1979). During recent years, heavy infestation from peach to apple orchards was observed from July onwards in the northeastern region of Emilia-Romagna in Italy (reviewed by Pollini and Bariselli 1993; Dorn et al., 2001). In the northern Italy region of Trentino fruits were attacked from the end of May with damage levels of 3.5% at the beginning of June (Bradlwarter et al., 1999). Infestation on apples was reported in Brazil from 1982 (Reis et al., 1988; Hickel and Ducroquet, 1998). In northern China, the first and the second generations infested the new shoots of peaches and apples, the second and third generations infested peach, and the third to fifth generations infested apples and pears (Zhao et al., 1989). The presence of *C. molesta* on apples was documented in the Russian regions of Krasnodar and Crimea (Popovich, 1982; Sokolova and Trikoz, 1985), and recently in North America (e.g. Rashid et al., 2001).

As a consequence of this new scenario, the current integrated management of *C. molesta* may result inadequate, especially in the areas where stone and pome orchards are associated. Integrated pest management (IPM) was defined by the FAO in 1968 as a pest management system in the context of the associated environment and the population dynamics of the pest species. It utilizes all suitable techniques and methods, which are compatible and possible, to maintain the pest population at levels below those causing economically unacceptable damage or loss. The monitoring of the oriental fruit moth is based on male captures by means of pheromone traps (e.g. Cravedi and Molinari, 1995). IPM growers spray their crops with selective Insect Growth Regulators (IGR) once the economic threshold has been reached. However, the correlation between dispersal of male *C. molesta* attracted to a pheromone trap and the level of infestation has not yet been investigated.
A most recent study shows that the crepuscular flight pattern of the experimental moths in relation to photointensity is similar to that reported from field studies, and females have a higher flight capacity than males when tested on a computer-linked flight mill (Hughes and Dorn, 2002). This new study supports an extended release-recapture study carried out in the field with thousands of baited traps (Yetter and Steiner, 1932; Steiner and Yetter, 1933) but yet without statistical data analysis. After the flight, female are still capable of laying a sizable number of eggs (Hughes and Dorn, 2002). Thus female immigrants may pose a threat to apple orchards in summer and fall, and they cannot be monitored with pheromone traps. Using host-plant odour to monitor female C. molesta on a short-range would overcome the above-mentioned problem. As for many lepidopteran female insects, the primary sensory modality involved in host-plant attraction is considered to be chemical (reviewed by Honda, 1995; Hern and Dorn, 2002).

Accordingly, the purpose of this study was to investigate host plant volatiles with the aim to identify a single compound or a mixture of compounds that attract female C. molesta, as a base for a monitoring tool with efficacy on females. The study addressed the following research questions: (1) what is the composition of volatiles from the main host plant of C. molesta? (2) Does C. molesta prefer apple as compared to peach plant volatiles? (3) Do the main host plants release volatiles which are of potential use for monitoring females in orchards?
3 Bioassay approaches to observing behavioural responses of adult female *Cydia molesta* to host plant odour

3.1 Abstract

Three different olfactometers were evaluated in order to develop a bioassay procedure testing for the olfactory responses of *Cydia molesta*. Females were tested individually using a linear and a Y-tube olfactometer, and in groups using a dual-choice arena. Room temperature, relative humidity, light intensity, airflow, age of the moths and odour source were kept constant during experiments. The odour source tested was a green shoot of peach excised 10-min before experiments started. *C. molesta* females showed a significant response to this plant odour in all olfactometers. A number of qualitative aspects were found to be in favour of the dual choice arena as a tool for screening potentially attractive odour sources. It allowed for a differentiation of the response of mated and virgin females. Experimental conditions allowed the circadian rhythm of insects to be mimicked. Manipulation of individuals is reduced and flight is not precluded.

3.2 Introduction

Semiochemicals released by the plant are considered the primary modality for host plant finding of female lepidopteran insects (Honda, 1995). Pre-alighting discrimination of a potential host plant implies that the relevant characteristics are perceived at a distance. As such, these cues are expected to influence orientation and choice by the females. To investigate the chemical basis of pre-alighting discrimination olfactometers that deliver odours to test insects in moving air have been developed and used with success (Eigenbrode and Bernays, 1997). Many different designs have been elaborated (e.g. Finch, 1986). In tortricid moths, for example, a Y-tube olfactometer method has been developed to study the dose-
response of virgin and mated *Cydia pomonella* L. to \(\alpha\)-farnesene (Hern and Dorn, 1999). The host-plant discrimination of adult *Cydia (Grapholita) molesta* (Busck) (Lepidoptera: Tortricidae) females remains to be evaluated (Dorn et al., 2001). This herbivore is typically associated with peach (*Prunus persica*) as its primary host, where it infests shoots as well as fruits (Rothshild and Vickers, 1991). Nevertheless it has recently gained importance as a key pest of pome fruits in some areas of Italy and South America (Reis et al., 1988, Civolani et al., 1998). While petri dish studies showed an attraction of the neonate *C. molesta* larvae to peach twigs and fruit skin (Bouzouane et al., 1987), the olfactory response of adult females to host plant odours is largely unknown as only a preliminary study is available (Natale et al., 1999). Wind tunnel studies, though well established for males (e.g. Rumbo and Vickers, 1997) yielded only low responses in females of *Cydia* species. In *C. pomonella* the percentage of individuals attracted to host odours from branches with and without fruits counted only 18 and 4\%, respectively (Bengtsson et al., 2001) and attraction was similarly low in *C. molesta* (D. Natale et al., unpublished). Our preliminary study on this species indicated that olfactometers might represent an appropriate tool to observe the females' short-range olfactory response (Natale et al., 1999).

The purpose of the present study was to observe the response of female *C. molesta* to host-plant volatiles, specifically to peach shoot volatiles. We evaluated two classical olfactometer set-ups, in which observations are carried out on individuals, and a newly developed binary choice set-up, in which observations are carried out on groups of insects. The set-ups tested were on one hand (a) a linear tube olfactometer and (b) a Y-tube olfactometer, and on the other hand (c) a dual-choice arena. Experiments were conducted to elucidate (1) the proportion of responding females versus females making no choice, and (2) the preference of females with respect to the odour source.
3.3 Materials and Methods

Plants. Three-year-old peach trees (*Prunus persica* cv. Red Heaven) were purchased from a commercial station in Switzerland (Hauenstein, Rafz). Plants were maintained in an environmental growth chamber (Conviron, Inc.) at 24°C (day) and 16°C (night), L16 : D8, 50-70% r.h. For the bioassays a green (not lignified) shoot with 6 ± 1 leaves was used as an odour source. The green shoot was excised from a peach plant 10 min before the experiments commenced.

Insects. *Cydia molesta* pupae were purchased from a commercial station in Italy (BT, Todi-Perugia). Moths had been bred in a culture for approximately 66 generations. Larvae were reared on an artificial diet based on corn semolina, wheat germ and brewers yeast, as described by Ivaldi-Sender (1974). On arrival, pupae were placed in a climatic chamber (Conviron, Inc.) at 25°C, L16 : D8, 60-70% r.h., inside a plastic cylinder (10 x 25 cm), and supplied with honey solution. Adults were never exposed to host plant chemicals before the bioassays and were considered "naive". Only female *C. molesta* were tested. Before mating, pupae of both sexes were confined pair wise in a plastic cylinder. For the bioassay, virgin or mated females, aged from 3 to 5 days, were selected at random from the cages 3 hours before the onset of scotophase. Prior to each set of bioassays, adults were allowed to acclimatise for one hour in the bioassay room. Individual moths were used only once.

Bioassay. The olfactory response of female *C. molesta* to odour emitted by green peach shoots was tested in three separate devices. Light intensity, room temperature and relative humidity were kept constant for all set-ups. For all devices the airflow was filtered through an activated-charcoal filter, regulated by a float flow meter and moistened through a glass chamber containing water. The airflow was pumped into the odour chambers connected to the olfactometers at a rate of 700 ± 10 ml/min at the entrance. Devices were placed on a table at a vertical distance of 130cm from 7
parallel tubes of cool white light "Lux line plus 36W" (Sylvania®) providing a uniform light intensity of 2400 lux. Bioassays were conducted at 24 ± 1°C and 55 - 65% r.h. All bioassays were carried out over a number of days. In order to avoid positional bias, the odour chambers were rotated for every replicate. Olfactometers were cleaned every trial session by using liquid laboratory glassware cleaning solution (Sigmaclean®), acetone (purity > 90%), hexane (purity > 90%) and heat treatment (250°C, 8 ± 2 hours).

Linear tube olfactometer. The purpose of using this device was to investigate the directional movement of individual females in a stream of odour. The parameter "activity" was defined as the capability of females to displace upwind in the tube. The linear tube olfactometer was adapted from that of Pimbert (1987) with slight modifications. The test chamber consisted of a glass tube 30 cm long and 2.5 in cm diameter, with a score line drawn at 18 cm from the entrance. The odour source, a peach shoot, was placed in a separate odour chamber connected to the linear tube by Teflon tubing. The odour chamber was a dilated glass cylinder consisting of two parts connected with ground glass joint (12 cm in long, 7 cm diameter) and with double tubulation at the two ends. During the bioassay individual female C. molesta kept in a plastic cylinder (7.5 cm long, 3 cm diameter, with one end covered by a mesh and the opposite end open) were released at the opening of the linear tube and observed for a maximum of 5 min. A pilot study had shown that the number of responding females remains nearly constant when exposure to the odour is prolonged over this period.

As a control, female C. molesta were exposed to clean air in a parallel linear tube olfactometer. Positive response was recorded when a moth crossed the score line within 5 min of release and stayed ahead of the score line for at least 1 min. No-response was recorded when the moth did not cross the score line within 5 min from release. Fifty females were tested per treatment. The shoot was changed every trial session (i.e. 10-15 individuals tested).
Y-tube olfactometer. The purpose of using this device was to investigate a preference of individual females exposed to a dual choice of odours. For this set-up we recorded the parameter "preference" defined as the capability of females to choose between one of two odour sources. The test chamber consisted of a Y-shaped glass tube, with each arm 23 cm long and 2.5 cm in diameter, as described previously (Bertschy et al., 1997). As an odour chamber, we used a dilated glass cylinder (35 cm long, 6.5 cm diameter) consisting of two parts with a ground glass joint. The odour chamber contained a peach shoot, while the control chamber remained empty. A score line was drawn on the two arms of the olfactometer at 10 cm from the joint. For the bioassay, individual C. molesta females were released at the open end of the common arm of the Y-tube. A choice was recorded when the moth crossed the score line within 5 min from release and stayed in the portion of the arm behind the score line for at least 1 min. A no-choice was recorded when the moth remained in the common arm for more than 5 min. Fifty replicates were carried out per treatment. The shoot was changed every trial session (i.e. 10-15 individuals tested).

Dual choice arena (Figure 3.1). The purpose of using this device was to investigate "preference", as defined above, of groups of females. The test chamber consisted of a dilated glass bottle (10 l volume; 41 cm long; 22 cm diameter) with no bottom and covered at the two ends with fine nylon mesh. The mesh at the large end was pierced with two 2 cm diameter holes, 18 cm apart. Odour sources were placed in 300 ml flasks with top tubulation, which were connected to the holes in the mesh. A pilot study using chemicals in up to 10 small capillaries, showed a positive olfactory response of females. The odour chamber was entered statistically more frequently as compared to blank, indicating the turbulences caused by inserting material into the odour chamber do not prevent the response of the moths (Natale et al. unpublished). Based on these preliminary results, peach shoots were included in the odour chamber. For the bioassay, 30 female C. molesta were released into the arena simultaneously. "Preference" was recorded as the percentage of females choosing
one of the two odour sources by counting the number of individuals in either of the two odour chambers. To evaluate the time course of the number of females making a choice, observations were made at hourly intervals for the complete duration of the trial session, using red light for counting during scotophase. Three replicates of 30 moths each were implemented. The bioassay, starting 3 hours before the onset of scotophase, was performed for 14 hours, ending 3 hours after the onset of photophase. The shoot was changed every trial session (i.e. group of moths tested).

Figure 3.1. A schematic representation of the dual-choice arena set-up. Thirty female C. molesta were placed into the arena. A preference for volatile chemicals from the green peach shoot was recorded as the number of moths collected in either odour chamber within a fixed time.
**Statistical analysis.** Due to differences in the design of the three devices, data were analysed separately. All statistical models included the effects of the odour source, mating status of females and the day. Results of bioassays carried out in the linear tube olfactometer and the Y-tube olfactometer were analysed with Logistic Regression (Trexler and Travis, 1993), using the LOGIT module of SYSTAT 8. Data obtained with the dual choice arena were analysed using a Generalised Linear Model with a normal distribution and identity link (Crawley, 1993). Model simplification was carried out as described by Trexler and Travis (1993) by deleting a single term from a hierarchy of models and assessing the change in the log likelihood ratio (G² test statistic). Data obtained with the Y-tube olfactometer and dual choice arena were also analysed using a Chi-square test (Sokal and Rohlf, 1995) to test for differences between odours.

### 3.4 Results and Discussion

Previous investigations of the olfactory behaviour of *C. molest*a were limited to wind tunnel trials dealing with male response to sex pheromones (e.g. Willis and Baker, 1994, Valeur and Lofstedt, 1996, Rumbo and Vickers, 1997), except for our own pilot trial (Natale et al., 1999). All attempts to study the behaviour of female *C. molest*a in the wind tunnel failed (D. Natale et al., unpublished), while a positive response to plant odours was obtained in the Y-tube olfactometer in the mentioned pilot study. It showed that female *C. molest*a were attracted to host plant volatiles in the linear and the Y-tube olfactometers as well in the dual choice arena. All the results were similarly straightforward to interpret, as there was no interaction between the factors. We argue that the dual choice arena is the trial design which provides a maximal benefit.

In the linear tube olfactometer, both mated and virgin *C. molest*a females were significantly more active when exposed to the plant odour as compared to the blank. Females were attracted to peach shoot odours (Figure 3.2). In fact when the factor
plant odour was removed from a model that included the three independent factors (plant odour plus mating status plus day), the decrease in G was significant (DF = 1; G = 117.4; P < 0.001). When the factor plant odour was removed from two other models, which included plant odour plus day and plant odour plus mating status, the decrease in G was again significant (DF = 1; G = 117.3; P < 0.001 and DF = 1; G = 118.9; P < 0.001). In addition, mating status and day did not influence the females’ activity, as the decrease in G was not significant for these two factors. No interaction was significant. The observation of 50 moths required a total of four days. Each observation day of required a working time of approximately 1.5 hours, amounting to total working time of approximately 6 hours for completion of an experiment.

**Female C. molesta**

<table>
<thead>
<tr>
<th></th>
<th>Positive response</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant odour</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>Blank</td>
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<td>40</td>
</tr>
<tr>
<td><strong>Virgins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant odour</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Blank</td>
<td>14</td>
<td>36</td>
</tr>
</tbody>
</table>

Figure 3.2. Activity of mated and virgin female *C. molesta* in two parallel linear tube olfactometers reported as positive response. Females were exposed either to peach shoot odour or to blank (clean air). Numbers within bars refer to the number of moths crossing the score line. N = 50. Differences between odours are significant, G = 117.4, P > 0.001; logistic regression.
In the Y-tube olfactometer, both mated and virgin *C. molest* females showed a significant preference for the plant odour as compared to the blank (Figure 3.3a). In fact, when the factor day was removed from the model, which included the two independent factors day and mating status, the decrease in G was significant (DF = 1; G = 14.6; P < 0.001). The two-way interaction was not significant. In addition, the number of mated females choosing plant odour was 24 vs. 9 choosing blank ($\chi^2 = 6.8$, P < 0.01), and the number of virgin females choosing plant odour was 15 vs. 0 choosing blank ($\chi^2 = 15$, P < 0.001). The observation of 50 moths required a total of four days. Each observation day required working time of approximately 1.5 hours, amounting to total working time of approximately 6 hours for completion of an experiment. In the dual choice arena, both mated and virgin *C. molest* females showed a significant preference for the plant odour as compared to the blank (Figure 3.3b). In fact, when the factor plant odour was removed from a model, which included the three independent factors, plant odour, mating status and day, the F-value was significant (DF = 1, 8; F-value = 37.04; P < 0.001). Also when the factor mating status was removed from a model, which included the three independent factors, the F-value was significant (DF = 1, 8; F-value = 6.2; P < 0.05). This indicates that in this device the behavioural response of mated and virgin female was different. No interaction was significant. In addition, the number of mated females choosing plant odour was 33 vs. 5 choosing blank ($\chi^2 = 20.6$, P < 0.001) and the number of virgin females choosing plant odour was 18 vs. 2 choosing blank ($\chi^2 = 12.8$, P < 0.001). A time course experiment indicated that moths do not move back to the test chamber once in the odour chamber (Table 3.1), which rules out contact chemoreception as a cue for host choice under our experimental conditions. One trial lasted 14 hours. As all moths remain in an odour chamber once they had entered it, the observation could be limited to a final count at the end of the trial session. In this case the working time was reduced to 0.5 hours, resulting in a total working time of approximately 1.5 hours for completion of three replicates.
This study evaluated the suitability of two classical olfactometer set-ups, testing individual insects, versus a newly developed dual choice set-up, testing groups of insects.

**Female C. molesta**

<table>
<thead>
<tr>
<th></th>
<th>□ plant odour</th>
<th>□ blank</th>
<th>P</th>
<th>N. making no choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>mated</td>
<td>24</td>
<td>9</td>
<td>**</td>
<td>17</td>
</tr>
<tr>
<td>virgins</td>
<td>15</td>
<td></td>
<td>***</td>
<td>35</td>
</tr>
</tbody>
</table>

Figure 3.3. Preference of mated and virgin female C. molesta for peach shoot odour in (a) the Y-tube olfactometer and (b) the dual choice arena. (a) Numbers within bars refer to the number of moths crossing the score line. N = 50. * = P < 0.05  ** = P < 0.01  *** = P < 0.001. (b) Numbers refer to moths collected in either odour chamber. N = 3 replicates with 30 moths each. *** = P < 0.001.
Table 3.1. Time-course experiment in the dual choice arena. Observed numbers of female *C. molesta* in the test chamber and the two odour chambers are recorded at intervals of 1h. The last column indicates the backward movement by moths from the odour chambers to the test chamber calculated after hourly updating. Scotophase (21.00 – 6.00) began 3 hours after start of the bioassay, photophase 3 hours before its termination. Data from 3 replicates are given as x/x/x. n.a. = non applicable

<table>
<thead>
<tr>
<th>time (hours)</th>
<th>observed in test chamber</th>
<th>odour chambers control</th>
<th>calculated backward movements from the odour chambers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>test</td>
<td>test</td>
<td>control</td>
</tr>
<tr>
<td>19.00</td>
<td>30/30/30</td>
<td>0/0/0</td>
<td>0/0/0 n.a. n.a. n.a.</td>
</tr>
<tr>
<td>20.00</td>
<td>28/28/28</td>
<td>2/1/2</td>
<td>0/1/0 n.a. n.a. n.a</td>
</tr>
<tr>
<td>21.00</td>
<td>25/27/25</td>
<td>4/2/5</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
<td>22.00</td>
<td>24/25/22</td>
<td>5/4/8</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
<td>23.00</td>
<td>24/25/22</td>
<td>5/4/8</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
<td>24.00</td>
<td>24/25/22</td>
<td>5/4/8</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
<td>1.00</td>
<td>24/25/22</td>
<td>5/4/8</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
<td>2.00</td>
<td>24/25/22</td>
<td>5/4/8</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
<td>3.00</td>
<td>24/25/22</td>
<td>5/4/8</td>
<td>1/1/0 0/0/0</td>
</tr>
<tr>
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<td>24/25/22</td>
<td>5/4/8</td>
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<tr>
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<td>24/25/22</td>
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</tr>
<tr>
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<td>20/21/17</td>
<td>8/6/13</td>
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</tr>
<tr>
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<td>20/19/16</td>
<td>8/8/14</td>
<td>2/3/0 0/0/0</td>
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<td>18/18/16</td>
<td>10/9/14</td>
<td>2/3/0 0/0/0</td>
</tr>
</tbody>
</table>

All three tools proved suitable for the purpose of the study. The binary choice approaches have the advantage of demonstrating the preference of *C. molesta* females for peach odour over clean air, whereas the linear tube does not allow for an analysis of odour choice. The olfactory responses of individually- and group- tested females were relatively similar, even though a direct quantitative comparison of the results is not possible due to the different designs and different durations of the
bioassays. However, a number of qualitative aspects are in favour of the dual choice arena. It allowed for differentiation of response between mated and virgin females. More mated than virgin females responded to plant odour, but preference to odour as compared to clean air was similar. In addition it provides the test insect with a free choice of locomotory activity, including flight. The relatively narrow access holes leading from the large test chamber to the odour sources may have eliminated some of the random wandering that characterised female behaviour in the Y-tube. In a recent paper, a binary choice device oriented vertically was used to test the olfactory preference of individual parasitoid Hymenoptera, and similar differences as compared to a Y-tube olfactometer were suggested (Halvill et al., 2000). Further, periods of maximal choices of females exposed to the odours for several hours (Table 3.1) coincide with the circadian rhythm observed in C. molest a in the laboratory (Hughes and Dorn, 2002) as well as in the field (Roehrich, 1961), with maximal activities just before the onset of scotophase and during the first hours of the photophase. A final benefit of the dual choice arena in testing groups of insects is its working time effectiveness; completion of an experiment requires only one fourth of the time needed for a Y-tube or a linear tube olfactometer experiment.
4 Response of female *Cydia molesta* (Lepidoptera: Tortricidae) to plant derived volatiles

4.1 Abstract

Peach shoot volatiles were attractive to mated female oriental fruit moth, *Cydia molesta* (Busck), in a dual choice arena. No preference was observed between leaf odours from the principle host plant, peach, and the secondary host plant, apple. Twenty-two compounds were identified in headspace volatiles of peach shoots using gas chromatography-mass spectrometry. Green leaf volatiles accounted for more than 50% of the total emitted volatiles. A bioassay-assisted fractionation using different sorbent polymers indicated an attractant effect of compounds with a chain length of 6-8 carbon atoms. The major compounds of this fraction were tested either singly or in combinations for behavioural response of females. Significant bioactivity was found for a three-component mixture of (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol and benzaldehyde in a 4:1:1 ratio. This synthetic mixture elicited a similar attractant effect as the full natural blend from peach shoots as well as the bioactive fraction.

4.2 Introduction

The primary sensory modality involved in host plant finding of female lepidopteran insects is considered to be chemical (reviewed by Honda, 1995; Hern & Dorn, 2002). A possible strategy for monitoring female herbivores could thus rely on chemical stimuli derived from host plant (Dorn *et al.*, 2001). The semiochemicals mediating the host location behaviour of the oriental fruit moth, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae), are unknown (e.g. Natale *et al.*, 1999; Dorn *et al.*, 2001). This species is an important pest of stone fruits, particularly peach, where it infests
the growing shoots at the beginning of the season (Rothshild & Vickers 1991). As the season progresses, it also damages fruits and is also found in apple orchards (Pollini & Bariselli, 1993). This is surprising as this species was considered to be oligophagous, and damage to apples was considered rare in Western Europe until the late 1970s (Bovey, 1979). In recent years, however, considerable levels of *C. molesta* damage to apple orchards have been widely observed in several fruit growing regions including Latin America, Asia and Europe (Popovich, 1982; Reis et al., 1988; Zhao et al., 1989; Hickel & Ducroquet, 1998; Bradlwarter et al., 1999). Monitoring of *C. molesta* predominantly relies on pheromone trapping of male moths (Vickers et al., 1985). However, the flight performance of this species exhibits marked sexual differences, and gravid females can be considered to be the main colonists (Dorn et al., 2001; Hughes & Dorn, 2002). In the field female *C. molesta* are capable of making inter-orchard flights (Yetter & Steiner, 1932; Steiner & Yetter, 1933). This can pose a serious threat to apple cultivation in orchards in the vicinity of peach crops. Development of a semiochemical-based monitoring strategy is thus desirable (Dorn et al., 2001). In addition, host plant odours which are attractive to female *C. molesta* could also be used in an attract and kill deployment. The goal of this study was to identify compounds derived from host plant or mixtures of compounds which are attractive to *C. molesta* females. First, the response of females to peach and apple shoots was characterized. As both odour sources were similarly attractive, it was focused on peach as the main host plant. The headspace volatiles of peach shoots were analyzed using combined gas chromatography-mass spectrometry. Subsequently, they were fractionated using different sorbent polymers which are able to trap compounds based on their volatility range. As bioassays indicated an attractant effect of compounds of a distinct range of carbon atoms, the hypothesis was tested that the major compounds of this chain length, either singly or in combination, would elicit the desired behavioural effect in *C. molesta* females.
4.3 Materials and Methods

Insects and Plants
Pupae of *C. molesta* were purchased from a commercial station in Italy (BioTechnologie B.T., Todi-Perugia). The colony originated from individuals collected in peach orchards in Emilia-Romagna (northern Italy). Moths were bred in culture for approximately 68 generations. Larvae were reared on an artificial diet based on corn semolina, wheat germ and brewers yeast as described by Ivaldi-Sender (1974). On arrival, pupae were placed inside a plastic box (30 x 30 x 30 cm) and supplied with a honey solution. Pupae and emerging adults were maintained at 24 ± 1°C, 60 ± 10% relative humidity, and a photoperiod of 16L: 8D. A previous study indicated that mated females responded better than virgins when exposed to peach shoots in a dual choice arena (Natale *et al.*, in press). For the bioassays, 3- to 5-day-old mated females were chosen without a conscious bias from the cage. Before the test started, adults of the two sexes were singled out based on the slight sexual dimorphism, the females being larger-sized. Additionally, the mated status of the female moths was checked by dissecting under the microscope the bursa copulatrix for presence of a spermatophore at the end of experiments. All tested individuals were females which were successfully mated. Moths were used only once and were not exposed to odour sources before the bioassay.

Three-year-old potted plants of peach and apple (*Prunus persica* L. Batsch cv. Redhaven and *Malus domestica* L. Borkh cv. Golden Delicious) were used for bioassays and collection of volatiles. Plants were maintained outdoors at 20 ± 5°C.

Chemical Analysis
Volatiles from excised shoots of peach were sampled using a dynamic headspace sampling system similar to that described by Boevé *et al.* (1996). A dilated glass cylinder with a glass joint (500-ml) was used as a collection chamber in which airflow was generated using a vacuum pump. Incoming air was filtered with
an activated-charcoal filter (Supelco, Mounting Clip for S-Trap, Buchs SG Switzerland) connected by Teflon tubing. A cylindrical trap (Supelco) filled with 300 mg of Tenax-GR 60/80 was plugged to the chamber by means of a PTFE-lined cap. The sorbent trap was connected to the vacuum using Teflon tubing. The flow rate was set at 100 ml/min by a flow meter connected between the pump and the trap. The flow meter was set and adjusted during the early phase of the collections to ensure that the correct flow was obtained. A moisture-removing filter (Supelco, 400cc 1/4") for the adsorption of condensation, observed to form within the tubing, was connected between the Tenax trap and the flow meter. Headspace collections lasted 3 hours and were performed at 22°C, 60 ± 10% relative humidity.

Identification with Coupled Gas Chromatography-Mass Spectrometry. Samples were analyzed using a Hewlett Packard GC-MS instrument (GC 6890 mass selective 5973) equipped with a HP1, polydimethyl siloxane column with nominal film thickness 1μm, diameter 0.25 mm, and length 30 m. The initial oven temperature was 40°C. The oven was heated up to 220°C at a rate of 8°C/min. A post run of 10 min at 300°C was applied to remove impurities from the column. Analyses were carried out using a thermal-desorption system (Unity, Markes Int. Ltd™, Rhondda Cynon Taff, UK.), in which the desorbed headspace volatiles were transferred to the GC-column without use of a solvent. Volatiles were desorbed from the Tenax trap with helium (99.99%) for 5 min, starting at 50°C and then up to 300°C at approx. 20°C/min, and transferred to the cold trap (-10°C) which was packed with a bed of Tenax GR and Carbopak B of 4 cm and 2 cm in length, respectively. The cold trap was subsequently heated up to 300°C at approx. 60°C/s for 3 min. The desorption flow was kept at 30 ml/min for all analyses. The thermal desorber was operated with a double split, i.e. the split was operational during both the sample tube and the cold trap desorption, and the GC was operated splitless. As the desorption flow was kept at 30 ml/min for all analyses and the split flow was 10 ml/min, the total split flow ratio during the thermal desorption was 7.7:1. This split operation was used to prevent overloading of the GC
column. It enhances the chromatographic separation of the components. A second
Tenax trap recollected part of the sample. The transfer line to the GC was kept at
200°C. The identification of chromatographically separated compounds was carried
out using a NIST98 spectral library, a user created library, and matching GC retention
time and mass spectra with authentic standards. Quantification of volatiles was
based on the response factors of the MS detector to the components, and carried out
using a calibration standard containing 50 µl of each component and 50 µl of internal
standard (hexylbenzene, Fluka, purity > 99.8%) (Raffa & Steffeck 1988). Fifteen
headspace collections of peach shoot volatiles were carried out.

Bioassays

Bioassay Arena. All behavioural tests were performed in a dual choice arena (Natale
et al., 2003). The arena consisted of a test-chamber, where insects were released,
and two odour chambers, where insects were captured. Based on preliminary
observations, the test chamber was a bottomless cylindrical glass bottle (10 l volume;
41 cm long; 22 cm diameter) covered at the two ends with fine nylon mesh. The
mesh at the large-sized end was pierced with two 2 cm diameter holes, 18 cm apart.
The two odour chambers, 300 ml flasks each with a tubing at the top, were
connected to the test chamber at the two holes described above. Air was filtered
through an activated-charcoal filter, regulated by a float flow meter and moistened
through a glass chamber containing water. The airflow was pumped into the odour
chambers connected to the olfactometers at a rate of 700 ± 10 ml/min at the
entrance. The arena was placed on a workbench, 130 cm below 7 Lux line plus 36W
“cool white” (Sylvania®, Mississauga, Ontario Canada) lamps which provided a
uniform light intensity of 2400 lux. Bioassays were conducted at 24 ± 1°C, 60 ± 5%
relative humidity. For each replicate the position of the odour chambers was
exchanged in order to avoid positional bias. The arena was cleaned before every trial
session using a laboratory glassware liquid cleaner (Sigmaclean®, Buchs SG
Switzerland), acetone (purity > 90%), hexane (purity > 90%) and heat treatment
(250°C, approx. 8 hours). The bioassays were carried out over a number of days. They started always 3 hours before the onset of the scotophase. A group of 30 females per replicate was released into the test chamber from the smaller end of the arena and allowed to move upwards and to choose one odour source. After 15 ± 1 hours, moths captured in the two odour chambers were recorded as responders. All other moths were removed and classed as non-responders. A pilot study using a fruit volatile ester in small disposable capillary pipettes, showed a positive olfactory response of females, as the odour chamber was entered statistically more frequently as compared to the blank (D. Natale et al., unpublished).

**Female Response to Plant Volatiles.** Peach and apple shoots were used as odour sources in experiments investigating a possible preference of female *C. molestata* for one of the two host plants. Female *C. molestata* lay eggs on the foliage of top shoots, on the lower leaf surface in peaches and on the upper surface in apples. (Rothshild & Vickers 1991). For the bioassay, a top shoot with 6 ± 1 leaves of both peaches and apples was used. The shoots were excised within 10 min before experiments, at the phenological state defined as full leaf unfolding (Dierschke, 1970). The response of mated females to host plant volatiles was tested with 3 experiments in order to elucidate possible preference for (1) peach shoot volatiles tested versus blank, (2) apple shoot volatiles versus blank, and (3) apple shoot volatiles versus peach shoot volatiles.

**Female Response to Fractions.** Fractions of volatiles emitted by excised peach shoots were used as odour source in experiments investigating the attraction of mated female *C. molestata* for candidate active compounds. For this purpose, a bioassay-assisted fractionation was developed. Fractions were obtained from volatiles of excised peach shoots using selective tube sorbents. Based on previous observations, stainless steel cylinders (1/4" x 30 cm), ca. 10 ml volume, filled with sorbent polymers of differing adsorbing strength were used as tubes. The range
adsorbed by each sorbent depends upon the volatility of compounds and therefore their number of carbons in length (in "Guidelines for sorbent selection", Markes Int. Ltd.). A tube sorbent was plugged between a “plant chamber” and an “odour chamber” of dual choice arena. The plant chamber was a flask with the same size as the odour chamber defined above. The goal was to adsorb all the volatiles in the range of a given sorbent from the headspace of the peach shoot in the “plant chamber”, and only allow the remaining fraction to enter the “odour chamber”. Four fractions of volatiles were tested using the following selective sorbents: (1) Porapak N™, 190 mg/tube, of medium sorbent strength with an approximate compound volatility range of n-C5 to n-C8, thus eluting compounds with more than 8 carbons; (2) Carboxen™, 250 mg/tube, of strong sorbent strength with an approximate compound volatility range of n-C5 to n-C30, eluting compounds with more than 30 carbons; (3) Carbopak F™, 40 mg/tube, of medium / weak sorbent strength with an approximate compound volatility range of n-C9 to n-C30, eluting compounds with 5-8 carbons, and (4) Tenax TA™, 120 mg/tube, of weak sorbent strength with an approximate compound volatility range of n-C7 to n-C30, eluting compounds with 5-6 carbons. A fifth empty tube, eluting all compounds from the headspace of peach shoot, was used as a control. The response of mated females to the fractions of peach shoot volatiles as explained above was tested with 5 experiments in which the effect of each single odour source was compared to a blank.

Female Response to Synthetic Chemicals. As the fraction bioassays indicated the attractant effect of compounds of a range n-C6 to n-C8, major compounds of this range were used either singly or in combination as odour sources in experiments investigating the attraction of mated female C. molestata to artificial chemicals. Chemicals used for the bioassay were (Z)-3-hexen-1-ol (Fluka, purity > 99.5 %), (Z)-3-hexen-1-yl acetate (Avocado, purity > 99 %) and benzaldehyde (Aldrich, purity > 99 %) as potentially attractive compounds, cis-β-ocimene (Robertet, purity > 99 %) and (Z)-3-hexen-1-yl butyrate (Aldrich, purity > 99 %) as compounds representative of the
Table 4.1. Artificial combinations or single constituents used in bioassays with chemicals. Ratios used reflect approximate ratios released from the peach shoots. Dosages were prepared using the appropriate number of 0.5 μl microcapillaries to give the required release rate. Quantities released over the trial period were determined gravimetrically (mean ± standard error).

<table>
<thead>
<tr>
<th>constituents</th>
<th>ratio (μl)</th>
<th>release (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLV (Z)-3-hexen-1-yl acetate : (Z)-3-hexen-1-ol</td>
<td>4 : 1</td>
<td>750 ± 0.32 : 175 ± 0.15</td>
</tr>
<tr>
<td>ALD benzaldehyde</td>
<td>1</td>
<td>161 ± 0.07</td>
</tr>
<tr>
<td>MIX (Z)-3-hexen-1-yl acetate : (Z)-3-hexen-1-ol : benzaldehyde</td>
<td>4 : 1 : 1</td>
<td>750 ± 0.37 : 175 ± 0.11 : 161 ± 0.08</td>
</tr>
<tr>
<td>EST (Z)-3-hexen-1-yl butyrate</td>
<td>1</td>
<td>326 ± 0.27</td>
</tr>
<tr>
<td>TRP cis-β-ocimene</td>
<td>1</td>
<td>221 ± 0.4</td>
</tr>
</tbody>
</table>

Table 4.2. Quantities and percentages of major volatile compounds released by peach shoots as observed in the analysis. Rt = retention time. *green leaf volatile. * compounds quantified with relative response of synthetic standard in comparison to internal standard. n = frequency of detection of the compounds in a total of 15 samples.

<table>
<thead>
<tr>
<th>peaks</th>
<th>compounds</th>
<th>carbons</th>
<th>rt (min)</th>
<th>n</th>
<th>ng ± se</th>
<th>% ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>valeric acid</td>
<td>5</td>
<td>5.90</td>
<td>14</td>
<td>0.46 ± 0.04</td>
<td>0.03 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>(E)-2-hexenal*</td>
<td>6</td>
<td>5.15</td>
<td>12</td>
<td>19.8 ± 0.3b</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>(Z)-3-hexen-1-ol*</td>
<td>6</td>
<td>5.30</td>
<td>13</td>
<td>128.5 ± 4b</td>
<td>6.67 ± 0.47</td>
</tr>
<tr>
<td>4</td>
<td>1-hexanol*</td>
<td>6</td>
<td>5.60</td>
<td>13</td>
<td>1.93 ± 0.15</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>benzaldehyde</td>
<td>7</td>
<td>7.54</td>
<td>15</td>
<td>116.4 ± 3b</td>
<td>8.08 ± 2.57</td>
</tr>
<tr>
<td>6</td>
<td>benzonitrile</td>
<td>7</td>
<td>8.09</td>
<td>10</td>
<td>1.29 ± 0.24</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>(Z)-3-hexen-1-yl acetate*</td>
<td>8</td>
<td>8.74</td>
<td>15</td>
<td>613 ± 8</td>
<td>40.62 ± 1.25</td>
</tr>
<tr>
<td>8</td>
<td>methyl benzoate</td>
<td>8</td>
<td>10.53</td>
<td>15</td>
<td>0.71 ± 0.10</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>9</td>
<td>benzylnitrile</td>
<td>8</td>
<td>11.44</td>
<td>11</td>
<td>0.79 ± 0.34</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>methyl salicylate</td>
<td>8</td>
<td>12.55</td>
<td>15</td>
<td>2.00 ± 0.50</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>11</td>
<td>nonatriene</td>
<td>9</td>
<td>11.02</td>
<td>13</td>
<td>1.21 ± 2.13</td>
<td>0.8 ± 0.64</td>
</tr>
<tr>
<td>12</td>
<td>cis-β-ocimene</td>
<td>10</td>
<td>9.57</td>
<td>15</td>
<td>3.61 ± 1.50b</td>
<td>1.96 ± 0.12</td>
</tr>
<tr>
<td>13</td>
<td>3-carene</td>
<td>10</td>
<td>10.20</td>
<td>10</td>
<td>0.16 ± 0.03</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>14</td>
<td>(Z)-3-hexen-1-yl butyrate*</td>
<td>10</td>
<td>12.42</td>
<td>15</td>
<td>9.93 ± 1.13b</td>
<td>2.25 ± 0.70</td>
</tr>
<tr>
<td>15</td>
<td>dodecane</td>
<td>12</td>
<td>12.69</td>
<td>13</td>
<td>1.12 ± 0.14</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>16</td>
<td>(Z)-3-hexen-1-yl hexanoate</td>
<td>12</td>
<td>16.03</td>
<td>15</td>
<td>0.54 ± 0.32</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>17</td>
<td>(Z)-3-hexen-3-yl benzoate</td>
<td>13</td>
<td>19.16</td>
<td>15</td>
<td>1.48 ± 0.50</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>18</td>
<td>tetradecane</td>
<td>14</td>
<td>16.36</td>
<td>15</td>
<td>0.78 ± 0.10</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>19</td>
<td>trans-caryophyllene</td>
<td>15</td>
<td>16.78</td>
<td>15</td>
<td>1.14 ± 0.13</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>20</td>
<td>(E,E)-α-farnesene</td>
<td>15</td>
<td>17.26</td>
<td>14</td>
<td>0.19 ± 0.11</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>21</td>
<td>β-farnesene</td>
<td>15</td>
<td>17.36</td>
<td>15</td>
<td>1.08 ± 0.28</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>22</td>
<td>pentadecane</td>
<td>15</td>
<td>18.02</td>
<td>15</td>
<td>1.02 ± 0.64</td>
<td>0.20 ± 0.03</td>
</tr>
</tbody>
</table>
behaviourally inactive fractions. Based on preliminary observations, pure chemicals were released from disposable 0.5 µl capillary pipettes “end to end” (Hirschmann®, Eberstadt, Germany), baited and immediately placed in the odour chamber. Combinations of chemicals were based on the ratios quantified in the natural blend (see Table 4.1). Quantities released over the trial period of 14 ± 1 hours were determined gravimetrically using a microbalance set with a readability of 1µg (Mettler-Toledo model MT5, San Juan, Puerto Rico). This microbalance has the electronic control unit separated from the mechanical components of the balances minimizing any effects on weight. An automatic vibration damper and a built-in calibration feature eliminate effects of unstable ambient conditions. The response of mated females to chemicals was tested with four experiments in which the activity of compounds, singly or in combination, was compared to a blank.

Data analysis
Numbers of captured moths from all bioassays were analyzed with Generalized Linear Model using a Poisson distribution and a log link (Crawley, 1993).

4.4 Results

Chemical Analysis. Twenty-two compounds were found in the headspace of excised peach shoots (Table 4.2). Compounds ranged from n-C6 to n-C16. The major classes of compounds were terpenoids and esters, which accounted for more than 27%, hydrocarbons for more than 18%, while aldehydes and alcohols accounted for 9% of the total emitted volatiles. From a functional point of view, the green leaf volatiles, (E)-2-hexenal, (Z)-3-hexen-1-ol, 1-hexanol, (Z)-3-hexen-1-yl acetate and (Z)-3-hexenyl butyrate, were the major group accounting for more than 50% of total emitted volatiles. Major compounds identified were an acetate, (Z)-3-hexen-1-yl acetate, an aromatic aldehyde, benzaldehyde, and an alcohol, (Z)-3-hexen-1-ol with a short-chain (n-C6 to n-C8), as well as a monoterpene, cis-β-ocimene, and an ester,
(Z)-3-hexen-1-yl butyrate with a medium-chain (n-C10). (Z)-3-hexen-1-yl acetate, benzaldehyde, cis-β-ocimene, (Z)-3-hexen-1-yl butyrate, and β-farnesene were consistently detected in all fifteen headspace samples analyzed.

**Female Response to Plant Volatiles.** Volatiles from peach and apple shoots attracted mated female *C. molesta* in the dual choice arena (F-value = 11.77; DF = 3, 8; P < 0.001; F-value = 25.28; DF = 3, 8; P < 0.001 respectively) (Figure 4.1). There was no significant preference of female moths for peach shoot volatiles versus apple shoot volatiles (F-value = 1.07; DF = 1, 10; P = 0.33) (Figure 4.1). Movements of female *C. molesta*, from the test chamber to the two odour chambers of the dual choice arena, occurred only in the few hours before the onset of scotophase and after the onset of photophase (Figure 4.2).

![Graph](image)

**Figure 4.1.** Olfactory preference of mated female *Cydia molesta* exposed in a dual choice arena to excised peach shoot vs. blank; excised apple shoot vs. blank; excised peach shoot vs. excised apple shoot. N = 3, 30 moths per replicate. (*** = P < 0.001; Generalized Linear Model).
Female Response to Fractions. There was a significant difference between the 4 fractions of volatiles tested (F-value = 4.28; DF = 9, 29; P<0.05) (Figure 4.3). The two fractions eluted with Tenax TA™ and the Carbopak F™, expected to contain the short-chain compounds n-C5 to n-C6 and n-C5 to n-C8, respectively, attracted female C. molesta in the dual choice arena (F-value = 4.28; DF = 9, 29; P<0.01; F-value = 4.28; DF = 9, 29; P<0.01). The fraction eluted with the empty tube, containing all the peach shoot volatiles and used as a control, also attracted female moths (F-value = 4.28; DF = 9, 29; P<0.01). In contrast, the two fractions eluted with Porapak N™ and Carboxen™, expected to contain medium- and long-chain compounds with more than n-C8, and more than n-C30, respectively, did not attract female C. molesta (F-value = 4.28; DF = 9, 29; P<0.01; F-value = 4.28; DF = 9, 29; P = 0.165). The short-chain compounds identified from peach shoot volatiles (Table 4.2) with (1) n-C5 to n-C6: comprise (E)-2-hexenal, (Z)-3-hexen-1-ol, 1-hexanol, and valeric acid and (2) n-C5 to n-C8: the same four compounds plus benzaldehyde, benzonitrile, (Z)-3-hexen-1-yl acetate, methyl benzoate, benzyl nitrite and methyl salicilate.

![Graph showing cumulative captures of females](image)

**Figure 4.2.** Time course of cumulative captures of mated female Cydia molesta (dual choice arena bioassay) in the odour chamber containing a peach shoot as compared to a blank.
The subsequent bioassays were straightforward, the approach based on the simple assumption that major components of these fractions might be bioactive as has previously been shown for a major constituent of apple fruit volatiles, \((E,E)-\alpha\)-farnesene (Hern & Dorn, 1999). For comparison, bioassays were carried out with the two major constituents of the long-chain carbons \((n-C10)\) identified from peach shoot volatiles (Table 4.1), \(cis-\beta\)-ocimene and \((Z)\)-3-hexen-1-yl butyrate which were assumed to be behaviourally ineffective.

![Image](image_url)

**Figure 4.3.** Bioactivity of fractions of compounds with a different number of carbons as compared to a blank. Attraction of mated female *Cydia molesta* in a dual choice arena to a fraction of compounds with more than 8 carbons; fraction of compounds with more than 30 carbons; fraction of compounds of 5-8 carbons; fraction of compounds of 5-6 carbons, total emitted peach shoot volatiles. \(N = 3; 30\) moths per replicate. (** = \(P < 0.01\); Generalized Linear Model).
Female Response to Synthetic Chemicals. In the search for bioactive single constituents of peach shoot volatiles, the prevailing short-chain compounds in the natural blend (Table 4.1) were tested as synthetic chemicals, singly or in combination, and compared to the two prevailing longer-chain compounds, (Z)-3-hexen-1-yl butyrate and cis-ß-ocimene. There was a significant difference between the five combinations or single constituents tested (F-value = 2.43; DF = 9, 49; P < 0.05) (Figure 4.4). Neither of the two long-chain compounds attracted female moths (EST F-value = 0.54; DF = 1, 9; P = 0.486; TRP F-value = 0.67; DF = 1, 9; P = 0.439). In contrast, a mixture of the two major green leaf volatiles, (Z)-3-hexen-1-yl acetate and (Z)-3-hexen-1-ol, plus benzaldehyde attracted female C. molesta (MIX F-value = 22.75; DF = 1, 9; P < 0.01). However, a mixture of only these two major green leaf volatiles, or the aldehyde tested singly, did not attract female moths (GLV F-value = 0.46; DF = 1, 9; P = 0.517; ALD F-value = 0.22; DF = 1, 9; P = 0.649).

Figure 4.4. Bioactivity of single or combinations of compounds as compared to a blank. Attraction of mated female C. molesta in a dual choice arena to (Z)-3-hexen-1-yl acetate plus (Z)-3-hexen-1-ol (GLV), benzaldehyde (ALD), (Z)-3-hexen-1-yl acetate plus (Z)-3-hexen-1-ol plus benzaldehyde (MIX), (Z)-3-hexen-1-yl butyrate (EST), cis-ß-ocimene (TRP). N = 5; 30 moths per replicate. (** = P < 0.01; Generalized Linear Model).
4.5 Discussion

Olfaction appears to be involved in host habitat location behaviour of mated female *C. molesta*. Female moths, known to oviposit on shoots or foliage of peach and apple trees (Rothshild & Vickers 1991), were attracted to volatiles from shoots of both host plants. No discrimination between the volatiles from peach and apple foliage was found in this oligophagous herbivore. The significance of olfactory stimuli in lepidopteran species is assumed to reflect their level of host specialization. Highly specialized species are expected to be dependent on olfaction, while this sensory modality is considered to be of low importance in highly polyphagous species (Ramaswamy, 1988). The host range of *C. molesta* is confined to plant species in the family Rosaceae, mostly in the genera *Prunus* and *Pyrus*, and to one shrub from the family Myrtaceae, reflecting an intermediate level of specialization.

Analysis of the volatile blend emitted by the main host plant, peach *P. persicae*, revealed the presence of 22 compounds. A previous investigation (Horvat & Chapman, 1990) on leaf volatiles found only two compounds in sizable amounts which is possibly due to methodological differences (see below). In the current study the major constituents were (Z)-3-hexen-1-yl acetate and (Z)-3-hexen-1-ol, accounting for 41 and 7% of the total quantity of volatiles in the shoot headspace, respectively. Both compounds have also been reported from volatile collection of apple foliage (Bengtsson *et al.*, 2001). They are categorised as green leaf volatiles and consist of a number of compounds of saturated or mono-unsaturated aldehydes, alcohols and acetates, which occur in all plants, but in very varying proportions depending on species (Hansson *et al.*, 1999). Antennal receptors of different lepidopteran species were stimulated in response to green leaf volatiles which included (Z)-3-hexen-1-yl acetate (reviewed by Visser, 1986; Bengtsson *et al.*, 2001). This class of compounds, possibly in combination with further constituents of plant odours, is assumed to be involved in herbivore orientation to its host plant (van Tol & Visser, 2002).
An attractive effect on *C. molesta* females was found for these two major green leaf volatiles in combination with benzaldehyde. This aromatic aldehyde has also been detected during certain periods of the season in volatile blends from an apple tree (Bengtsson *et al.*, 2001; A. Vallat & S. Dorn, unpublished). This is, to our knowledge, the first time that host-plant-derived attractants for female *C. molesta* are reported. The ratio of these three compounds tested for the behavioural response of the moth reflects their ratio in the natural blend. Two major compounds from a non-bioactive fraction did not elicit any response in *C. molesta* females. A combination of a minor constituent of the green leaf volatiles, (E)-2-hexenal plus (Z)-3-hexen-1-ol, was behaviourally inactive as well (data not shown). This does not exclude that further combinations may exhibit an attractant effect on the moths. In the apple maggot fruit fly, *Rhagoletis pomonella* (Walsh), a seven-component mix was first reported to be attractive to sexually mature adults (Fein *et al.*, 1982), while a later study identified an even higher effect for a five-component blend (Zhang *et al.*, 1999). Further studies will be needed to analyze to what degree the bioactivity found for the mixture characterised above is sex-specific in *C. molesta*.

New methods were used for the fractionation of peach volatiles and for the bioassay. In a previous study, peach leaves were frozen, ground to a fine powder and then solvent extracted (Horvat & Chapman, 1990). The current study could benefit from the technology of direct thermal desorption of headspace volatiles from intact shoots. This yielded a larger number of quantifiable compounds without the risk of including artefacts caused by oxidation in leaf homogenates. The method of fractionation based on sorbent polymers that trap different compounds within a given volatility range proved to be appropriate for the purpose of this study. An artificial mixture prepared to mimic components of the bioactive fraction was behaviourally effective. This indicates the usefulness of this procedure. It is related to the so-called subtractive combination method defined as “subtracting fractions from the whole blend of compounds for bioassays” (Byers, 1992). However, the current study started with headspace volatiles instead of crude extracts and subtracted all but one fraction
instead of subtracting a single fraction from the total blend. The bioassay was carried out in a dual choice arena under light conditions simulating a diurnal cycle. Major movements of the moths into the odour chambers were recorded before the onset and after the termination of the period without light. This coincides with a previous laboratory study reporting flight activity in *C. molesta* during dusk and dawn, and minimal movement during dark (Hughes & Dorn, 2002). As this photoperiodicity is identical to that observed for this species in the field (Dustan, 1964; Roerich, 1961; Rothschild & Minks, 1974), it is concluded that the bioassay used offers favourable conditions for the assessment of adult behaviour.

In addition to olfaction, vision might also be an important sensory modality for host habitat location as it is in other lepidopteran species (Ramaswamy, 1988). Further work should evaluate the three-compound mixture for trapping *C. molesta* females on a larger scale, paying attention also to the design of the trap. Such investigations might lead to an effective tool for attracting female moths, and to a better understanding of their host plant selection process.
5 Apple and peach fruit volatiles and the apple constituent butyl hexanoate attract female oriental fruit moth, *Cydia molest*a, in the laboratory

5.1 Abstract

Volatiles emitted from immature and mature peach and apple fruits were all attractive to mated female oriental fruit moth, *Cydia molest*a (Busck), in a dual choice arena. Females did not discriminate between odours emitted by these two major host plants. The same natural blends were behaviourally ineffective for virgin females. A major component of apple fruit volatiles, butyl hexanoate, also attracted female *C. molest*a. Mated females were attracted to 2 medium dosages, while virgin females responded positively to the lowest of the 5 dosages tested. The time course of the captures of the moths shows a diurnal activity cycle known from the field. The possible implications of a semiochemical which attracts females are discussed in the context of previous findings that gravid females may immigrate from peaches into apple orchards particularly in the later phase of the season.

5.2 Introduction

In search of compounds which attract female moths, host plants may offer a choice of bioactive natural resources, as the primary sensory modality involved in host plant finding of moths is considered chemical (reviewed by Honda, 1995; Hern and Dorn, 2002). In the oriental fruit moth, *Cydia molest*a (Busck) (Lepidoptera: Tortricidae), such chemicals might be identified in the headspace of fruits or other plant parts from the family Rosaceae. Except for one shrub belonging to the family Myrtaceae, *Eugenia myrianthus* L., stone and pome fruit trees are the only host plants of this oligophagous herbivore.
Originating from northwest China (Roehrich, 1961) *C. molesta* has become widely distributed throughout the stone fruit growing areas of the world, including other parts of Asia, Europe, South and North America, North Africa, the Middle East, New Zealand and Australia (Rothschild and Vickers, 1991). Its principle host plant is considered to be peach where it causes damage even at low population densities, initially on shoots, shifting with progressing season to fruit (Rothschild and Vickers, 1991). From July onwards, heavy infestations proceeded recently from peach to apple orchards in the northern region of Emilia-Romagna in Italy (reviewed by Pollini and Bariselli 1993; Dorn et al., 2001). Infestation on apples was further reported from Brazil (Reis et al., 1988; Hickel and Ducroquet, 1998), from the Russian regions of Krasnodar and Crimea (Popovich, 1982; Sokolova and Trikoz, 1985), and most recently from North America (e.g. Rashid et al., 2001). In northern China, it is the third to fifth generations which infest pome fruits such as apples (Zhao et al., 1989). Mid- to late-season damage in peach occurs mainly, and in apple exclusively, to the growing fruit by the larva which tunnels towards the centre (Rothschild and Vickers, 1991).

In *C. molesta*, a proportion of the population, particularly gravid females, have the capacity to make inter-orchard flights and infest non-contiguous orchards (Hughes and Dorn, 2002). Thus, compounds which attract females are required to complement the well-established pheromone-based techniques to monitor this pest. The objectives were (1) to establish, for the first time, the response of *C. molesta* to volatiles from peach and apple fruits, and (2) to exploit possible detected differences to find female attractants. As there was attraction but no significant differences between the response to the various natural blends investigated, we started by testing single constituents of volatiles from apple fruit which are relatively well known (e.g. Fein et al., 1982). Here, we report on the effect of a major constituent from apple fruit, butyl hexanoate (Fein et al., 1982; Hern and Dorn, in print), on female *C. molesta*. The study relied on a bioassay with a dual choice arena that was elaborated recently (Natale et al., in print).
5.3 Materials and Methods

*Insects and Plants.*

*C. molesta* sexed pupae were purchased from a commercial station in Italy (BT, Todi-Perugia). The indigenous colony originated from moths collected in peach orchards in Emilia Romagna (northern Italy). Moths had been bred in culture for approximately 70 generations. Larvae were reared on an artificial diet based on corn semolina, wheat germ and brewers yeast as described by Ivaldi-Sender (1974). On arrival, pupae were placed in plastic cages (30 x 30 x 30 cm) and maintained at 15°C, 16L : 8D photoperiod and 60 ± 10% relative humidity. A honey solution was added as food supply for emerged adults (placed into the cages). Mated females were obtained by placing pupae of both sexes into the same cage, and by leaving the emerged moths together for a minimum of 3 days. According to Hughes and Dorn (2002), more than 50% of the females are already fertilised one day after emergence. As female are larger than male moths (Hughes and Dorn, 2002) only the larger moths were used for the trials. To verify that inseminated females had been tested, the moths were dissected after completion of the trial, and the bursa copulatrix was examined under the microscope for the presence of a spermatophore. All tested individuals were females that had successfully mated. Virgin females were obtained by placing female pupae into separate cages. For the bioassays, 3- to 5-day-old females were chosen at random from the cage. Moths were used only once and were not exposed to plant-related odour sources before the bioassay.

Three-year-old peach and apple plants (*Prunus persica* cv Redhaven and *Malus domestica* cv Golden Delicious) were used for the bioassays. Potted plants were maintained outdoor at 20 ± 7°C and irrigated when needed.

*Bioassay*

*Bioassay Arena.* All behavioural tests were carried out in a dual choice arena (Natale et al., in print). The arena consisted of a test-chamber, where insects were released,
and two connected odour chambers, where insects were captured. The test chamber was a bottomless dilated glass bottle (10 l volume; 41 cm long; 22 cm diameter) covered at the two ends with fine nylon mesh. The mesh at the larger end of the bottle was pierced with two 2 cm diameter holes, 18 cm apart. The two odour chambers, 500 ml flasks with top tubulation, were connected to the test chamber at the two holes in the mesh as described above. Airflow was filtered through an activated-charcoal filter, regulated by a float flow meter and moistened through a glass chamber containing water. The airflow was pumped into the odour chambers connected to the test chamber of the arena at a rate of 700 ± 10 ml/m at the entrance. The arena was placed on a workbench, 130 cm below artificial cool white light from 7 parallel tubes "Lux line plus 36W" (Sylvania®) which provided a uniform light intensity of 2400 lux. Bioassays were conducted at 24 ± 1°C, 60 ± 5% relative humidity. For each replicate, the position of the odour chambers was exchanged in order to avoid a positional bias. The arena was cleaned after each trial session using successively liquid laboratory glassware cleaning solution (Sigmaclean®), acetone (purity > 90%), hexane (purity > 90%) and heat treatment (250°C, 8 ± 2 hours). All bioassays started always 3 hours before the onset of the scotophase. A group of 30 females per replicate was released into the test chamber from the smaller end of the arena and allowed to move upwards and to choose one odour source. After 15 ± 1 hours, moths captured in the two odour chambers were recorded as responders. All other moths were removed and classified as non-responders. The bioassays were carried out over a number of days and the order in which the treatments were tested was randomised.

**Female Response to Fruit Volatiles.** A peach or an apple fruit was inserted into an odour chamber as odour source in experiments investigating the preference of female *C. molesta* for one of the two host plants. Fruits were picked from potted trees within 10 min before experiments at the phenological stage defined as "bearing green fruit" and "bearing ripe fruit" (Dierschke, 1970) and used only once. The peach and
apple fruits in the "bearing green fruit" stage of weighted 45 ± 0.5 gr. and 70 ± 0.8 gr., respectively. Bearing ripe fruits of peach and apple weighted 135 ± 1.6 gr., and 150 ± 1.5 gr. respectively. The size and the ripeness of fruits chosen for the experiments were similar over a period of several days, allowing for proper bioassays. The behaviour of mated and virgin females was observed in 8 experiments in order to elucidate whether the 4 natural odour sources described above would elicit a positive response in the bioassay, as compared to a blank.

**Female Response to a Synthetic Chemical.** Butyl hexanoate (Aldrich, purity > 98 %) was used as the odour source in experiments investigating the attraction of female *C. molesta* for a specific fruit constituent. Based on preliminary observations indicating attraction for low dosages, the pure chemical was released from different numbers of disposable capillary pipettes “end to end” (Hirschmann®) of 0.5 μl, baited and placed in the odour chamber. The behaviour of mated and virgin females exposed to 5 dosages of butyl hexanoate (0.5, 1, 2, 3, and 5 μl), was observed in 10 experiments in order to elucidate whether this synthetic cue would elicit a positive response in the bioassay, as compared to a blank. The number of pipettes was 1, 2, 4, 6, and 10 respectively for the 5 dosages used. Release rates over the trial period of 15 ± 1 hours were determined gravimetrically for the 5 dosages as 0.32 ± 0.021 mg, 0.5 ± 0.027 mg, 0.8 ± 0.033 mg, 1.36 ± 0.26 mg, and 3.42 ± 0.67 mg.

**Data analysis.**

For all bioassays, data were analysed with a Generalized Linear Model using a Poisson distribution and a log link (Crawley, 1993) based on 5 replicates with 30 moths each.
Figure 5.1. Mean (± SE) number of responding mated (A) and virgin (B) female C. molesta (dual choice arena bioassay) to volatiles from immature peach fruit vs blank; mature peach fruit vs blank; immature apple fruit vs blank; mature apple fruit vs blank. N = 3 replicates with 30 moths each. (*** = P < 0.001; * = P < 0.05 for comparison between each treatment and control based on parameter estimates from Generalised Linear Model for test effect. SE for second blank in Fig. 1B is 0).
5.4 Results

Female Response to Fruit Volatiles. Mated female *C. molesta* were attracted to volatiles from immature and mature peach and apple fruits in the dual choice arena (F-value 41.34; DF=1, 23; P < 0.001) (Figure 5.1). There was no significant difference between the four treatments tested (F-value 0.17; DF=3, 23; P=0.91). Virgin female *C. molesta* were not attracted to volatiles from immature and mature peach and apple fruits (F-value 0.03; DF=1, 23; P=0.87) (Figure 5.1). There was no significant difference between the four treatments tested (F-value 2.46; DF=3, 23; P=0.092). The number of mated and virgin moths that made a choice was relatively uniform throughout the trial (Figure 5.1). The time course of response in both mated and virgin females to the natural blends from peach shows that movement of *C. molesta* occurred in the few hours before the onset of scotophase and in the first hour of photophase (Figures 5.2).

![Graph showing the time course of cumulative captures of mated and virgin female Cydia molesta (dual choice arena bioassay) in the odour chamber containing mature peach fruit as compared to a blank.](image)

**Figure 5.2.** Time course of cumulative captures of mated and virgin female *Cydia molesta* (dual choice arena bioassay) in the odour chamber containing mature peach fruit as compared to a blank.
Figure 5.3. Mean (± SE) number of responding mated (A) and virgin (B) female *C. molesta* (dual choice arena bioassay) to different doses of butyl hexanoate (dark bars) vs blank. N = 5 for each dose of butyl hexanoate tested, 30 moths per trial. (* = P < 0.05 for comparison between each treatment and control based on parameter estimates from Generalised Linear Model for test effect).
Female Response to a Synthetic Chemical. Mated female *C. molest*a were attracted to butyl hexanoate at 2 dosages tested in the dual choice arena (0.5 mg F-value 6.79, DF=1, 9, *P*<0.05; 0.8 mg F-value 6.37, DF=1, 9, *P*<0.05) (Figure 5.3). These moths moved into the odour chamber baited with this single compound already at the onset of the trial, that is during the period preceding scotophase (Figure 5.4). Outside the indicated range the chemical was behaviourally ineffective (0.32 mg F-value 0.78, DF=1, 9, *P*=0.40; 1.36 mg F-value 0.07, DF=1, 9, *P*=0.80; 3.42 mg F-value 1.54; DF=1, 9, *P*=0.25). There was no significant dose dependent effect (F-value 0.32; DF=4, 49; *P*=0.86), nor was the interaction significant between the number of moths captured in each treatment and the dose (F-value 1.21; DF=9, 49; *P*=0.31).

**Figure 5.4.** Time course of cumulative captures of mated female *C. molest*a (dual choice arena bioassay) in the odour chamber containing synthetic butyl hexanoate (0.8 mg) as compared to a blank.
Virgin female *C. molestata* were attracted to butyl hexanoate at the lowest dosage tested (F-value 28.44; DF=1, 9, P<0.001) (Figure 5.3). The doses of 0.5 mg and 0.8 mg which attracted mated females were behaviourally ineffective (F-value 1.42, DF=1, 9, P=0.26 and F-value 0.06; DF=1, 9, P=0.81), as were the two highest doses (1.35 mg F-value 0.09, DF=1, 9, P=0.76; 3.42 mg F-value 0.18; DF=1, 9, P=0.68). There was no significant dose dependant effect (F-value 1.42; DF=4, 49; P=0.24), nor was the interaction significant between the dose and the number of moths captured (F-value 1.99; DF=9, 49; P=0.06). The number of mated and virgin moths that made a choice was relatively uniform throughout the trial, except for virgin moths at the lowest dosage tested (Figure 5.3).

Discussion

Both peach and apple fruits tested were attractive to mated female *C. molestata*. Fruit odours from both host plants elicited similar attraction. Further, both immature and mature fruits of apple and peach were attractive. Females did not discriminate between volatiles from shoots of peach or apple trees either (D. Natale et al. unpublished). Mechanisms underlying the host shift from peach to pome fruit trees, observed from July onwards (Pollini and Bariselli, 1993; Zhao et al., 1989), could comprise various factors, including changing biotic and abiotic conditions.

Significant attraction to freshly picked healthy fruit was not found in the codling moth, *C. pomonella* L. (Lepidoptera: Tortricidae). In this *Cydia* species, mated females were not attracted to volatiles from apples picked in August compared to a blank in a dual choice olfactometer bioassay (Hern and Dorn, 2002). Bioactivity of the headspace volatile from fruits was limited to mated female *C. molestata* as virgin females did not show any significant preference for fruit odours when given a choice with a blank. The response to host plant volatiles, and more generally the interaction with the host plant, may differ according to the mating status of female moths. In previous works reviewing the evolution of host preference and oviposition behaviour in Lepidoptera,
it was assumed that ovipositing females choose one or more host plant species in an attempt to maximize larval survival and growth (e.g. Papaj and Rausher, 1987; Thompson and Pellym, 1991). Therefore, "non-ovipositing" virgin females, are assumed to be less attracted to host plants than mated females. Our results confirm this common hypothesis. Similarly, mated female *C. pomonella* responded more strongly than virgins to *(E,E)-α- farnesene* (Hern and Dorn, 1999).

Activity of female moths exposed to peach and apple fruits in the bioassay was observed before the onset of scotophase and in the first hours of photophase. A similar diurnal cycle was observed in a previous laboratory study on flight activity in *C. molesta* during dusk, dark and dawn (Hughes and Dorn, 2002), and it is identical to that observed in the field (Dustan, 1964; Roerich, 1961; Rothschild and Minks, 1974).

The responsiveness of female *C. molesta*, that is the percentage of females making a choice, ranged from 35 to 49% in mated and from 10 to 39% in virgin females. This is in line with studies on other *Cydia* species and their response to plant-derived semiochemicals. Responses of *C. pomonella* in a Y-tube olfactometer to fruit odours or constituents ranged from 26 to 64% (Hern and Dorn, 1999; 2001; 2002). As the dual choice arena used in the current study may be considered a small wind tunnel, the level of responsiveness observed in *C. molesta* compares well to the 6 - 38% mated *C. pomonella* observed making a choice for infested apples volatiles (Reed and Landolt, 2002), or to 18% making a choice for volatiles from branches with green apples and leaves (Bengtsson et al., 2001) in wind tunnels.

A selected single constituent from apple fruit volatiles, butyl hexanoate, was clearly attractive for female *C. molesta*. This compound accounts for more than 10% of the total headspace volatiles emitted from ripe apples (Fein et al., 1982). Mated females that are attracted to apple fruit volatiles were also attracted to butyl hexanoate at two medium dosages tested. Females choosing this odour source moved quickly into the odour chamber baited with the single compound. Virgin females that do not discriminate fruit volatiles from a blank did not respond to the dosage range that
resulted bioactive for mated females. The only exception was found for the lowest dosage tested for which an elevated responsiveness similar to that of mated females was noted. This dosage was significantly attractive to virgin females. Our findings are compatible with the assumption that this carboxylic ester may exhibit an arrestant effect on virgin females down to a certain threshold value below which the females are clearly attracted into the semiochemical-baited odour chamber.

This is, to our knowledge, the first report on a host plant volatile attracting female *C. molesta*. Whether the compound is female specific or whether it also attracts males, similar to the pear alkyl ethyl and methyl esters of \((2E, 4Z)-2,4\text{-decadienoic acid}\) in *C. pomonella* (Knight and Light, 2001), remains to be investigated.

There is clear evidence that certain natural fruit volatile blends tested contain further constituents which are attractive to *C. molesta* females. Butyl hexanoate is a major compound emitted by mature apples. It is also present in the headspace of growing apple fruit (*Malus domestica* cv. Golden Delicious) in June, which is relatively early in the season, but not in the middle of the season from mid July to end of August (Hern and Dorn, in print). In the headspace of mature peach fruit, however, this compound was not identified among the 21 esters isolated (Narain et al., 1990). Consequently, the positive response to volatiles from mature peach fruit in mated female *C. molesta* must be due to further single or combined volatile compounds from the principle host plant. Butyl hexanoate and such further compounds hold promise for monitoring gravid *C. molesta* females which have a higher flight capacity than males (Hughes and Dorn, 2002). These females may disperse and colonise new fruit orchards (Yetter and Steiner, 1932; Steiner and Yetter, 1933). They are still capable of depositing a sizable number of eggs after the flight (Hughes and Dorn, 2002). As they cannot be caught by pheromone traps, the identified bioactive apple fruit component should be evaluated further as a candidate for monitoring mated female *C. molesta* immigrants.
6 General discussion

The general aim of this study was to generate basic knowledge for the set-up of a method to monitor *C. molesta* based on the capture of female moths using host plant volatiles. Specific aims were (1) to investigate a possible preference of *C. molesta* between peaches and apples (2) to identify host plant derived volatiles involved in the host finding of *C. molesta* (3) to test identified volatiles as potential attractants for female *C. molesta*.

The oriental fruit moth, *C. molesta*, is an oligophagous pest which mainly feed on a number of host plants in the family Rosaceae. When this study started, a major research question regarded a possible preference of *C. molesta* for apple, considered as a secondary host plant, as compared to peach, considered as a primary host plant. A correct interpretation of the host preference of *C. molesta* was considered useful for an appropriate strategy of pest management. A prerequisite to this research question was the investigation of the olfactory behaviour of *C. molesta* towards host plant volatiles.

To investigate the olfactory behaviour, the suitability of three different olfactometer designs was evaluated using green peach shoots as an odour source (Chapter 1). The green shoot of peach is commonly regarded as a preferred site for female oviposition, as it represents the food resource for larvae of the first generation. Results showed a strong attraction of female moths for plant odours. Additionally, a newly designed dual choice arena was the most suitable for my bioassays among the three olfactometers tested. A possible preference for apple or peach plant odours was then investigated with a number of bioassays comparing the attractiveness of both foliage and fruits for female moths (Chapter 2 and 3). Based on the olfactory response, female *C. molesta* showed the same level of preference for the two host plants. Further, host plant volatiles were identified using green peach shoots as an odour source. The natural blend emitted was collected from the headspace and analysed with GC-MS equipment (Chapter 2). Among the 22 volatile compounds
identified, a fraction containing short-chain compound resulted as bioactive for female *C. molesta*. An artificial mixture of three compounds identified in this fraction was as attractive as the fraction itself and the natural blend. In the final part of the study I tested the attractiveness of an apple fruit volatile, butyl hexanoate, for female *C. molesta* (Chapter 3). This substance, a carboxylic acid ester, attracted mated females at 2 medium dosages, and virgin females at the lowest out of 5 dosages tested.

*Dual choice arena: a smart design*

Previous investigations of the olfactory behaviour of *C. molesta* were limited to wind tunnel trials dealing with male response to sex pheromones (e.g. Willis and Baker, 1994, Valeur and Lofstedt, 1996, Rumbo and Vickers, 1997), with the exception of a pilot trial (Natale et al., 1999). Wind tunnel studies, though well established for males (e.g. Rumbo and Vickers, 1997) yielded only low responses in females of *Cydia* species. Therefore, the need for a promising set-up for laboratory observation of *C. molesta* olfactory behaviour was a challenge. My study evaluated the suitability of two classical olfactometer set-ups, testing individual insects, versus a newly developed dual choice set-up, testing groups of insects. All the three tools are suitable for the study of the olfactory behaviour. In fact, female *C. molesta* were attracted to host plant volatiles in the linear and the Y-tube olfactometers as well as in the dual choice arena. Nevertheless, the binary choice approaches have the advantage of demonstrating the preference of *C. molesta* females for peach odour over clean air, whereas the linear tube does not allow for an analysis of odour choice. The olfactory responses of individually- and group- tested females were relatively similar, even though a direct quantitative comparison of the results is not possible due to the different designs and different durations of the bioassays. However, a number of qualitative aspects are in favour of the dual choice arena. It allowed for differentiation of response between mated and virgin females. More mated than virgin
females responded to plant odour, but preference to odour as compared to clean air was similar. In addition it provides the test insect with a free choice of locomotory activity, including flight. The access from the large test chamber to the odour sources may have eliminated some of the random wandering that characterised female behaviour in the Y-tube. Further, periods of maximal choices of females exposed to the odours for several hours coincide with the circadian rhythm observed in *C. molesta* in the laboratory (Hughes and Dorn, 2002) as well as in the field (Roehrich, 1961), with maximum activity just before the onset of scotophase and during the first hours of the photophase. A major criticism to this set-up regarded the risk of backward movements of moths from the odour chamber, containing the plant organ, to the main test chamber where insects are released. This possibility would not preclude visual or tactile stimuli. However, a time course experiment indicated that moths do not move back to the test chamber once in the odour chamber, which rules out contact chemoreception as a cue for host choice under this experimental conditions. As all moths remain in an odour chamber once they had entered it, the observation can be limited to a final count at the end of the trial session. A final benefit of the dual choice arena in testing groups of insects is its working time effectiveness. Completion of an experiment requires only one fourth of the time needed for a Y-tube or a linear tube olfactometer experiment.

*Peach and apple plants: a similar preference for C. molesta*

Once olfaction was proved to be involved in the host habitat location behaviour of mated female *C. molesta*, a number of dual choice bioassays were carried out to investigate a possible preference between peach and apple plants. Female moths, which oviposit on foliage of peach and apple trees (Rothshild and Vickers 1991), were attracted to volatiles from shoots of both host plants. Similarly, mated females were attracted to volatiles from immature and mature fruits of both host plants, where the larvae feed by tunnelling. No discrimination between the volatiles from peach and
apple foliage and fruits was found. In contrast, virgin females were not significantly attracted to volatiles from immature and mature fruits of both host plants. The response to host plant volatiles, and more generally the interaction with the host plant, may differ according to the mating status of female moths. In previous works reviewing the evolution of host preference and oviposition behaviour in Lepidoptera, the choice of plant species was attributed to ovipositing females in the attempt to maximize larval survival and growth (e.g. Papaj and Rausher, 1987; Thompson and Pellrym, 1991). Virgin females, defined as non-ovipositing, would be therefore less attracted to host plants than mated females. The significance of olfactory stimuli in lepidopteran species is assumed to reflect their level of host specialization. Highly specialised species are expected to be dependent on olfaction, while this sensory modality is considered to be of low importance in highly polyphagous species (Ramaswamy, 1988). The host range in C. molesta is confined to plant species in the family Rosaceae, mostly in the genera Prunus and Pyrus, and one shrub, Eugenia myrianthus, from the family Myrtaceae, reflecting an intermediate level of specialization.

**Volatile identification and new approach to fractionation**

As no discrimination was found between foliage and fruits of the two host plants, peach foliage was chosen as odour source for identification of volatiles. Analysis of the volatile blend emitted by peach foliage revealed the presence of 22 compounds. A previous investigation (Horvat and Chapman, 1990) on leaf volatiles found only two compounds in sizable amounts which is possibly due to methodological differences. The major constituents were (Z)-3-hexen-1-yl acetate and (Z)-3-hexen-1-ol, accounting for 41 and 7% of the total quantity of volatiles in the headspace of shoot, respectively. Both compounds have also been reported from volatile collection of apple foliage (Bengtsson et al., 2001). They are categorised as green leaf volatiles and consist of a number of compounds of saturated or mono-unsaturated carbon
aldehydes, alcohols and acetates, which occur in all plants, but in very varying proportions in different species (Hansson et al., 1999). In a previous study, peach leaves were frozen, ground to a fine powder and solvent extracted (Horvat and Chapman, 1990). Here, analyses were provided with GC-MS previous thermal desorption of headspace volatiles from intact shoots. This yielded a larger number of quantifiable compounds without running the risk of including artefacts caused by oxidation in leaf homogenates. A new method of fractionation based on sorbent polymers which are described to trap different compounds within a given volatility range. This procedure is related to the so-called subtractive combination method which was defined as "subtracting fractions from the whole blend of compounds for bioassays" (Byers, 1992). The method started from headspace volatiles instead of crude extracts and subtracted all but one fraction instead of one single fraction from the total blend. It allowed identifying one fraction out of 5 tested with the same level of bioactivity as the natural blend. The bioactive fraction was supposed to contain short-chain compounds, from n-C6 to n-C8.

**Host plant derived attractants for female C. molesta**

An attractive effect on female *C. molesta* was found for a combination of two major green leaf volatiles, (Z)-3-hexen-1-yl acetate and (Z)-3-hexen-1-ol, plus benzaldehyde, out of the bioactive fraction. Antennal receptors of different lepidopteran species were stimulated in response to green leaf volatiles which included (Z)-3-hexen-1-yl acetate (reviewed by Visser, 1986; Bengtsson et al., 2001). This class of compounds, possibly in combination with further constituents of plant odours, are assumed to be involved in herbivore orientation to its host plant (van Tol and Visser, 2002). The unsaturated aldehyde, benzaldehyde, has also been detected during certain periods of the season in volatile blends from the apple tree (Bengtsson et al., 2001; A. Vallat and S. Dorn, unpublished). The ratios of these three compounds tested for the behavioural response of the moths reflect their ratio in the
natural blend. Two major compounds from a non-bioactive fraction did not elicit any response in *C. molesta* females. A combination of a minor constituent of the green leaf volatiles, *(E)-2-hexenal plus (Z)-3-hexen-1-ol*, was behaviourally inactive as well. Mixtures of compounds were also found to be attractive for other species. In the apple maggot fruit fly, *Rhagoletis pomonella* (Walsh), a seven-component mix was first reported to be attractive to sexually mature adults (Fein et al., 1982), and a later study identified an even higher effect for a five-component blend (Zhang et al., 1999). An attractive effect on female *C. molesta* was also found for a selected single constituent from apple fruit volatiles, butyl hexanoate. In fact, mated females were attracted to 2 medium dosages and virgin females at the lowest of 5 dosages tested. The highest dosages tested were behaviourally ineffective for females of both mating status. Recently found as a female-specific attractant for *C. pomonella* and patented as "Attractant for Codling Moth Females" (Dorn, S., and Hern, A., European Patent Application 00960658.3-2110, 2002), butyl hexanoate may acts as a generic attractant for both *C. molesta* and *C. pomonella* females. This ester could be used for a semiochemical-based monitoring of females of these two important congeneric tortricoids in apple orchards. Application of a single semiochemical against more than one pest has been hypothesised in the apple blotch leafminers, *Phyllonorycter crataegella* (Clemens) and *Phyllonorycter mespilella* (Hübner). As males of both species were reciprocally inhibited by *P. mespilella* pheromone, the development of a generic disruptive blend was hypothesised for pheromone-based control of *Phyllonorycter* leafminers in North American apple orchards (Ferrao et al., 1998). As female *C. molesta* were attracted both to a blend of peach top shoot volatiles and a single compound from apple fruit volatiles, it can not be excluded that further compounds from peaches or apples, singly or in combinations, may act as attractants for the oriental fruit moth.
Trapping females: a feasible challenge?

The results of my study demonstrate for first time, to my knowledge, that it is possible to attract female *C. molesta* using plant-derived volatiles from different host plants. Further investigations should evaluate the sex-specificity and the bioactivity of these compounds for trapping *C. molesta* females on a larger scale, paying also attention to the design of the trap. In fact, in addition to olfaction, vision might be included as a sensory modality for host habitat location and attraction as it is for other lepidopterans (Ramaswamy, 1988). An additional aspect to be evaluated is the wide variation of volatile blends released from the different host-plant of *C. molesta*. As a consequence, the response window to plant blends appears at present much wider than it is towards pheromone blend. Further, host plants continuously release large amounts of volatiles, which may play a strong competition versus kairomonal traps. On the other hand, a proper IPM strategy, in terms of optimal timing and placement of plant-derived attractants for monitoring, would overcome some of problems mentioned. In the assumption to use butyl hexanoate as a female moths attractant, for instance, baited traps should be placed when the competition with the natural source is sustainable. Butyl hexanoate is a major compound of apple fruits (Fein et al., 1982), which is released at different dosages during the course of the season (Hern and Dorn, in print). In addition, dosages and duration of release change with the different varieties of apples. A proper strategy for monitoring moths with butyl hexanoate in the apple fruit orchard *Malus domestica* cv. Golden Delicious, for example, should expect to place the kairomonal traps from mid July to end of August. In fact, fruits of that variety do not release butyl hexanoate in these two ranges of time (Hern and Dorn, in press). Further, it would consider using butyl hexanoate in a peach fruit orchard, as this compound is not released from peach fruit (Narain et al., 1990). In both the above-mentioned cases, the competition of the synthetic odour source versus natural odour sources may be favourable. In a similar way, using a synthetic mixture of (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol, and benzaldehyde in
an apple fruit orchard may also result as a proper strategy. Especially at the beginning of the season, when apple fruits are not yet there, these three compounds released from peach green shoots may have a favourable competition versus natural odour sources released from lignified apple shoots.

I am inclined to believe that, when all these factors will be investigated, plant volatiles, alone or coupled with pheromones, may be used as attractants for monitoring or to enhance the efficacy of mating disruption by pheromones. The combination of male and female attractants is expected to lead to control methods that will become far more efficient than conventional insecticides.
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Education

1999-2003 PhD study at the Swiss Federal Institute of Technology (ETH) Zürich, Switzerland; Institute of Plant Science, Applied Entomology.
1996-1999 Grant holder at the University of Bologna, Italy; Institute of Entomology. Collaborator in the EU Project AIR-3-CT94-1607, entitled: “Versatile Controlled Release Dispenser For Mating Disruption With Pheromones”.
1995 Civil service, Town hall of Castello di Serravalle, Italy.
1986-1994 Studies at the University of Napoli “Federico II” and the University of Bologna, and degree in Agricultural Science at the University of Bologna, Italy. Graduate thesis at the Institute of Entomology entitled: Comportamento delle larve di Diglyphus isaea (Walker) (Hymenoptera: Eulophidae) nei confronti dell'ospite Liriomyza trifolii (Burgess) (Diptera: Agromizidae).
1973-1985 Studies at the Scuole elementari, Scuole Medie, and Liceo Scientifico statali (Caserta, Italy).