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Response of female *Cydia molesta* (Lepidoptera: Tortricidae) to plant derived volatiles

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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.

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 (Rothschild & 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

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both odour sources were similarly attractive, the study focused on peach as the main host plant. The headspace volatiles of peach shoots were analysed 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.

Materials and methods

Insects and plants

Pupae of *C. molesta* were purchased from a commercial station in Italy (BioTechnologie B.T., Todi-Perugia, Italy). 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 × 30 × 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. The mated status of female moths was checked by dissection of the bursa copulatrix for the presence of a spermatophore at the end of each test. All female individuals used in the tests were found to have successfully mated. Moths were used only once and were not exposed to odour sources before the bioassay.

Three-year-old potted plants of peach *Prunus persica* L. Batsch cv. Redhaven and apple *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

Volatile collection

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^{-1} 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, 400 cc 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 h and were performed at 22°C, 60 ± 10% relative humidity.

Identification with coupled gas chromatography–mass spectrometry

Samples were analysed using a Hewlett Packard GC-MS instrument (GC 6890 mass selective 5973) equipped with a HP1, polydimethyl siloxane column with nominal film thickness 1 µm, 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^{-1}. 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^{TM}, 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^{-1}, 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^{-1} for 3 min. The desorption flow was kept at 30 ml min^{-1} 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^{-1} for all analyses and the split flow was 10 ml min^{-1}, 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 by 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^{-1} 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 h). The bioassays were carried out over a number of days. They started always 3 h 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 h, 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 chamber containing the odour was entered statistically more frequently 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. molesta* for one of the two host plants. Female *C. molesta* lay eggs on the foliage of top shoots, on the lower leaf surface in peaches and on the upper surface in apples (Rothschild & Vickers 1991). Top shoots of both peach and apple with 6 ± 1 leaves were used for the bioassay. The shoots were excised within 10 min before experiments, at the phenological stage defined as full leaf unfolding (Dierschke, 1970). The response of mated females to host plant volatiles was tested in three experiments in order to elucidate possible preference for: (i) peach shoot volatiles tested versus blank; (ii) apple shoot volatiles versus blank; and (iii) apple shoot volatiles versus peach shoot volatiles.

Female response to fractions

Fractions of volatiles emitted by excised peach shoots were used as odour sources in experiments investigating the attraction of mated female *C. molesta* to 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″ × 30 cm), *c.* 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$^{\mathrm{TM}}$). A tube sorbent was plugged between a 'plant chamber' and an 'odour chamber' of a 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$^{\mathrm{TM}}$, 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$^{\mathrm{TM}}$, 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$^{\mathrm{TM}}$, 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.
4. Tenax TA$^{\mathrm{TM}}$, 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 five 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 *n*-C6 to *n*-C8, major compounds in this range were used either singly or in combination as odour sources in experiments investigating the attraction of mated female *C. molesta* 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 behaviourally inactive fractions. Based on preliminary observations, disposable 0.5 µl Hirschmann microcapillary pipettes (Hirschmann®, Eberstadt, Germany) were baited with pure chemicals and immediately placed in the odour chamber. To obtain the required ratios between individual compounds, each compound was applied using an appropriate number of 0.5 µl microcapillaries. Quantities released over the trial period of 14 ± 1 h were determined gravimetrically using a microbalance set with a readability of 1 µg (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 analysed with generalized linear model using a Poisson distribution and a log link (Crawley, 1993).

Results

Chemical analysis

Twenty-two compounds were found in the headspace of excised peach shoots (table 1). Compounds ranged from *n*-C6 to *n*-C16. The major classes of compounds were

Table 1. Quantities and percentages of major volatile compounds released by peach shoots as observed in the analysis.

Peaks	Compounds	Carbons	Rt (min)	n	ng ± se	% ± se
1	valeric acid	5	5.90	14	0.46 ± 0.04	0.03 ± 0.1
2	(*E*)-2-hexenal^a	6	5.15	12	19.8 ± 0.3^b	0.42 ± 0.05
3	(Z)-3-hexen-1-ol^a	6	5.30	13	128.5 ± 4^b	6.67 ± 0.47
4	1-hexanol^a	6	5.60	13	1.93 ± 0.15	0.37 ± 0.02
5	benzaldehyde	7	7.54	15	116.4 ± 3^b	8.08 ± 2.57
6	benzonitrile	7	8.09	10	1.29 ± 0.24	0.08 ± 0.01
7	(Z)-3-hexen-1-yl acetate^a	8	8.74	15	613 ± 8	40.62 ± 1.25
8	methyl benzoate	8	10.53	15	0.71 ± 0.10	0.04 ± 0.01
9	benzylnitrile	8	11.44	11	0.79 ± 0.34	0.36 ± 0.08
10	methyl salicylate	8	12.55	15	2.00 ± 0.50	0.49 ± 0.04
11	nonatriene	9	11.02	13	1.21 ± 2.13	0.8 ± 0.64
12	*cis*-β-ocimene	10	9.57	15	3.61 ± 1.50^b	1.96 ± 0.12
13	3-carene	10	10.20	10	0.16 ± 0.03	0.01 ± 0.00
14	(Z)-3-hexen-1-yl butyrate^a	10	12.42	15	9.93 ± 1.13^b	2.25 ± 0.70
15	dodecane	12	12.69	13	1.12 ± 0.14	0.08 ± 0.01
16	(Z)-3-hexen-1-yl hexanoate	12	16.03	15	0.54 ± 0.32	0.12 ± 0.01
17	(Z)-3-hexen-3-yl benzoate	13	19.16	15	1.48 ± 0.50	0.40 ± 0.03
18	tetradecane	14	16.36	15	0.78 ± 0.10	0.17 ± 0.02
19	*trans*-caryophyllene	15	16.78	15	1.14 ± 0.13	0.34 ± 0.04
20	(*E*,*E*)-α-farnesene	15	17.26	14	0.19 ± 0.11	0.07 ± 0.01
21	β-farnesene	15	17.36	15	1.08 ± 0.28	0.32 ± 0.01
22	pentadecane	15	18.02	15	1.02 ± 0.64	0.20 ± 0.03

Rt, retention time; ^{a} green leaf volatile; ^{b} 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.

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 15 headspace samples analysed.

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) (fig. 1). There was no significant preference of female moths for peach shoot volatiles versus apple shoot volatiles (F-value = 1.07; DF = 1, 10; $P = 0.33$) (fig. 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 (fig. 2).

Female response to fractions

There was a significant difference between the four fractions of volatiles tested (F-value = 4.28; DF = 9, 29; $P < 0.05$) (fig. 3). The two fractions eluted with Tenax TA^{TM} and the Carbopak F^{TM}, 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^{TM} and Carboxen^{TM}, 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 1) 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, benzylnitrile and methyl salicilate. The subsequent bioassays were straight-

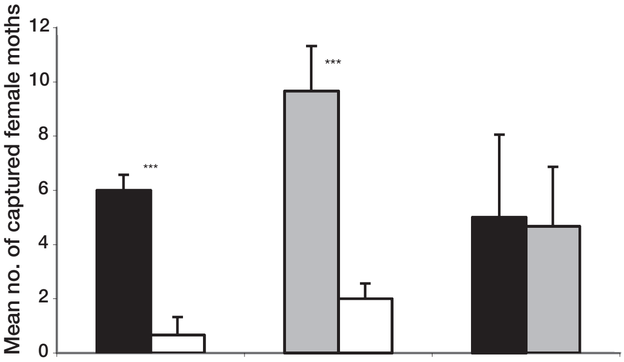

Fig. 1. Olfactory preference of mated female *Cydia molesta* exposed in a dual choice arena to excised peach shoot (■) vs. blank (□); excised apple shoot (▨) vs. blank (□); excised peach shoot (■) vs. excised apple shoot (▨). N = 3, 30 moths per replicate. (***$P < 0.001$; generalized linear model).

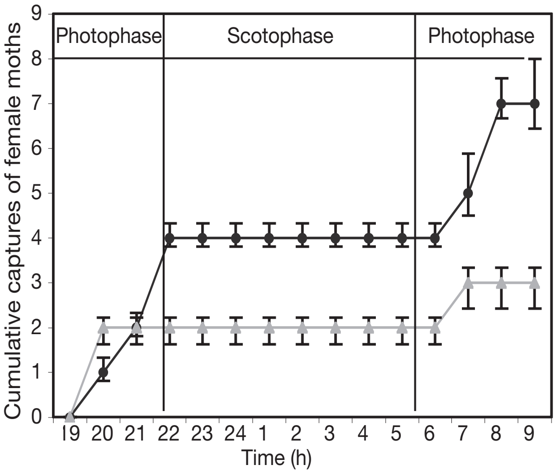

Fig. 2. Time course of cumulative captures of mated female *Cydia molesta* (dual choice arena bioassay) in the odour chamber containing a peach shoot (-●-) compared to a blank (-▲-).

forward, 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*)-α-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 2), *cis*-*β*-ocimene and (*Z*)-3-hexen-1-yl butyrate which were assumed to be behaviourally ineffective.

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 2) 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*-*β*-ocimene. There was a significant difference between the five combinations or single constituents tested (F-value = 2.43; DF = 9, 49; $P < 0.05$) (fig. 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; $P = 0.649$).

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 (Rothschild & 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, 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

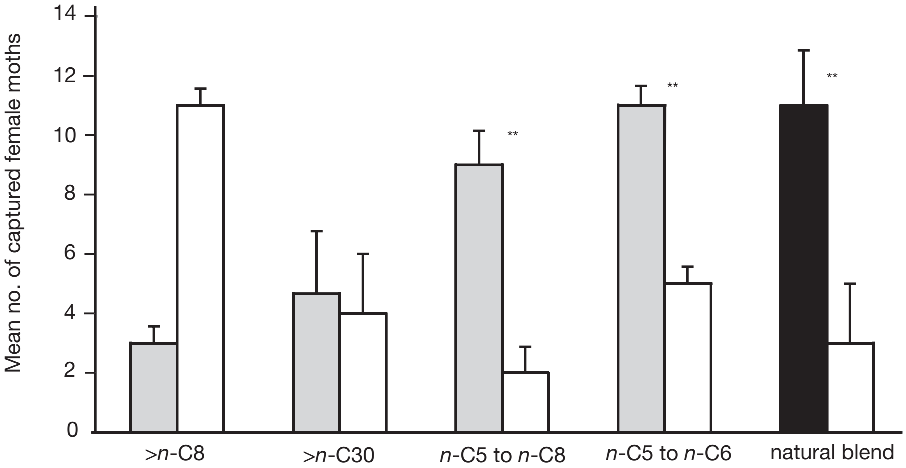

Fig. 3. Bioactivity of fractions of compounds (▨) with a different number of carbons compared to a blank (☐). Attraction of mated female *Cydia molesta* in a dual choice arena to a fraction of compounds with >8 carbons; fraction of compounds with >30 carbons; fraction of compounds of 5–8 carbons; fraction of compounds of 5–6 carbons; total emitted peach shoot volatiles (■). N = 3; 30 moths per replicate. (**$P < 0.01$; generalized linear model.)

Table 2. Artificial combinations or single constituents used in bioassays with chemicals.

Constituents		Ratio	Release (µg)
GLV	(Z)-3-hexen-1-yl acetate : (Z)-3-hexen-1-ol	4 : 1	1750 ± 0.32 : 175 ± 0.15
ALD	benzaldehyde	1	161 ± 0.07
MIX	(Z)-3-hexen-1-yl acetate : (Z)-3-hexen-1-ol : benzaldehyde	4 : 1 : 1	1750 ± 0.37: 175 ± 0.11 : 161 ± 0.08
EST	(Z)-3-hexen-1-yl butyrate	1	326 ± 0.27
TRP	*cis-β*-ocimene	1	221 ± 0.4

Ratios used reflect approximate ratios released from the peach shoots. To obtain the required initial ratios (µl : µl) between individual compounds, an appropriate number of 0.5 µl microcapillaries was used. Quantities released over the trial period were determined gravimetrically (mean ± standard error).

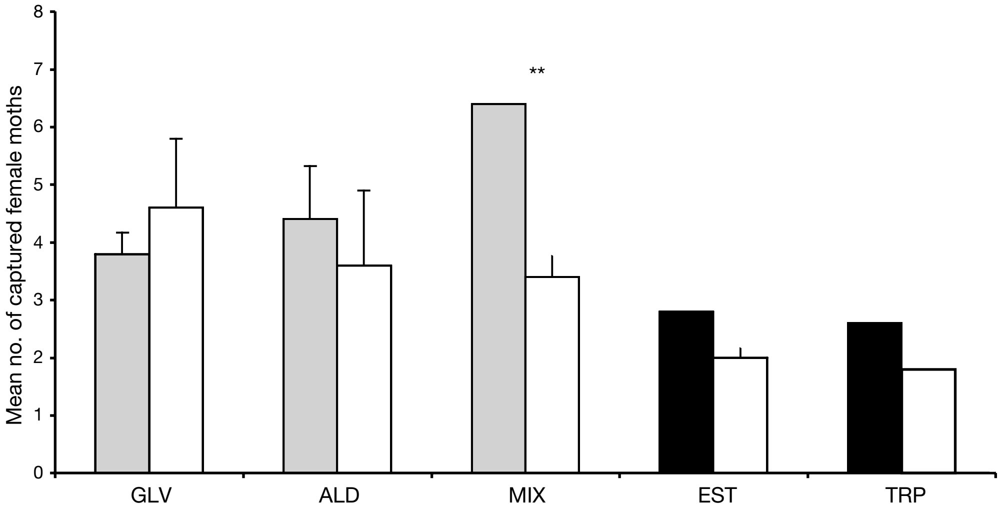

Fig. 4. Bioactivity of single or combinations of compounds (▨, bioactive fractions; ■, inactive fractions) as compared to a blank (☐). Attraction of mated female *Cydia molesta* in a dual choice arena to (Z)-3-hexen-1-yl acetate plus (Z)-3-hexen-1-ol (GLV), benzaldehyde (ALD), (Z)-3-hexen-1-yl acetate plus (Z)-3-hexen-1-ol plus benzaldehyde (MIX), (Z)-3-hexen-1-yl butyrate (EST), *cis-β*-ocimene (TRP). N = 5; 30 moths per replicate. (**$P < 0.01$; generalized linear model.)

headspace, respectively. Both compounds have also been reported from volatile collection of apple foliage (Bengtsson *et al.*, 2001). They are categorized 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* have been 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 with (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) (Diptera: Tephritidae) 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 analyse to what degree the bioactivity found for the mixture characterized 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 benefited 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.

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