Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12362

Sexual differences in electrophysiological and behavioral responses of *Cydia molesta* to peach and pear volatiles

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Accepted: 22 July 2015

Key words: oriental fruit moth, Lepidoptera, Tortricidae, male, female, host plant, wind tunnel, field trial, Rosaceae

Abstract

The oriental fruit moth (OFM), *Cydia* (= *Grapholita*) *molesta* (Busck) (Lepidoptera: Tortricidae), is a serious invasive pest. The stone fruit peach, *Prunus persica* (L.) Batsch, is its primary host, and the pome fruit pear, *Pyrus bretschneideri* Rehder (both Rosaceae), is its secondary host. Electrophysiological and behavioral responses of *C. molesta* females and males to peach shoot and pear fruit volatiles were compared in laboratory and field bioassays. Based on gas chromatography–electroantennographic detection (GC–EAD) activity, 13 compounds in the headspaces of peach and pear volatiles elicited female antennal responses. Of these, eight compounds also elicited male antennal response. Four lures were developed based on male and female EAD responses to pear and peach-derived volatile organic compounds (VOCs). More males than females were captured for all four lures during field trials, even in traps with lures that emitted VOCs based on female EAD responses. Lures based on female EAD responses to pear fruit VOCs consistently caught more females than lures based on male EAD responses to pear fruit VOCs in either peach or pear orchards. Peach shoot VOC lures based on female EAD responses did not attract more females than lures based on male EAD response to peach shoots. The two pear-derived VOC lures were highly attractive to both sexes in peach orchards, whereas conversely, the two peach-derived VOC lures showed stronger attraction in pear orchards. Seasonal population monitoring indicated both sexes made inter-orchard flights during the late peach- and pear-fruiting periods. A possible hypothesis that could explain different response profiles in females and males and seasonal migration for herbivores with multiple generations per year is discussed.

Introduction

The oriental fruit moth (OFM), *Cydia* (= *Grapholitha*) *molesta* (Busck) (Lepidoptera: Tortricidae), presumably originating from north-west China, has become widely distributed throughout the world (Rothschild & Vickers, 1991). Until the late 1970s the species was considered to be oligophagous and only to damage stone fruit in Western Europe (Bovey, 1979). In recent years, however, OFM damage to pome fruit orchards has been widely reported in Asia, Europe, Australia, and America (Rothschild & Vickers, 1991; Dorn et al., 2001; Myers et al., 2006; Il'ichev et al., 2009; Lu et al., 2012). In North China, peach shoots and pear fruits are the primary OFM host substrates early and late in the growing season (Lu et al., 2012), and this may result in movement from stone fruit orchards to pome fruit orchards (Zhao et al., 1989; Yang & Liu, 2010; Lu et al., 2012).

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The flight of female *C. molesta* significantly exceeds that of males (Hughes & Dorn, 2002). Females are directly responsible for infestations by making inter-orchard flights and laying eggs on pear fruits (Yetter & Steiner, 1932; Steiner & Yetter, 1933), so this study focuses on the interaction between adult OFM response to host plant-derived volatiles with emphasis on a comparison between female and male responses.

Olfactory cues for OFM females from both shoots and fruits of peach and apple have been studied extensively (Natale et al., 2003, 2004; Piñero & Dorn, 2007, 2009; Najar-Rodriguez & Dorn, 2013). Excised peach shoots emitting (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, and benzaldehyde at a 4:1:1 ratio was attractive to mated OFM females (Natale et al., 2003). Volatiles emitted from peach and apple fruits were also attractive to mated females in a dual choice arena (Natale et al., 2004). Behavioral experiments have identified (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenal, benzonitrile, and benzaldehyde, emitted by peach shoots, to act in a synergistic manner, resulting in a highly attractive odor mixture to female OFM (Piñero & Dorn, 2007). Recent studies pay particular attention to the seasonal dynamics of volatile emissions from different hosts. Piñero & Dorn (2009) reported that *C. molesta* females selected shoots, twig with leaves, and fruits of peach and apple trees at different times of the season. Najar-Rodriguez & Dorn (2013) reported volatile emissions from in situ peach and pear trees over an entire season, along with their olfactory attractiveness to *C. molesta* females. Some studies have been conducted to indicate high attractiveness of particular odor combinations to female OFM in the laboratory (Piñero et al., 2008), but field data are lacking. For the few studies that report field responses of OFM to lures, there seems to be no correspondence between the laboratory and field findings. For example, Il'ichev et al. (2009) carried out headspace analysis of volatiles from the intact young shoot tips of peach. They found that a mixture of (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, and (*E*)-β-farnesene at a ratio of 1:2:2 was the best attractant, but only to OFM males, no females were captured in the field. More recently, based on female OFM responses recorded using gas chromatography–electroantennographic detection (GC–EAD), the profile of bioactive volatiles emitted from immature and mature pear fruit were characterized (Lu et al., 2012) and reported two pear-derived multi-component attractants that can be used for monitoring both sexes of oriental fruit moth in the field. Subsequently, they carried out comparative analyses of peach and pear fruit volatiles attractive to both OFM sexes, and evaluated the attractiveness of volatile mixtures mimicking peach and pear fruit volatiles to OFM males and females (Lu et al., 2014). Despite using mixtures containing volatiles attractive to females in the laboratory, male captures were still higher under field conditions in these studies.

These results raised two important questions. Why are more male OFMs than females captured by traps with lures emitting plant-derived volatile organic compounds (VOCs) attractive to females in the laboratory? What is the difference in the electrophysiological and behavioral responses of both sexes to peach and pear volatiles between the laboratory and field bioassays? The answers should help to elucidate the behavioral mechanisms underlying higher male catches when using volatiles selected on the basis of female electroantennography (EAG) responses.

The objectives of the study were (1) to identify and analyze the VOCs from young peach shoots [*Prunus persica* (L.) Batsch] and pear fruits [*Pyrus bretschneideri* Rehder (both Rosaceae)] attractive to OFMs, and (2) to compare the responses of male and female OFM to peach shoot and pear fruit volatiles using laboratory and field bioassays.

Materials and methods

Insects

Larvae were collected from infested shoots of peach, *Prunus persica* L. Batsch 'Wuyuexianbiangan', in an experimental orchard at the Institute of Forestry and Pomology (IFP), Beijing Academy of Agriculture and Forestry (BAAF), Beijing, China (39°58′N, 116°13′E) in late June. These larvae were maintained in a climatic chamber at 24 ± 1 °C, 65–70% r.h., and L16:D8 photoperiod, with the photophase starting at 05:00 hours. Until the third instar, larvae were mass-reared on apple, *Malus domestica* L. Borkh. 'Hongfushi' (Rosaceae), in a glass container (27 cm diameter, 13 cm high) and then transferred to smaller individual glass containers (2.5 cm diameter, 8 cm high) until eclosion. A bell-shaped glass container (6- and 15-cm-diameter openings, 41 cm high) was used to raise the adults, and covered with fine nylon mesh at both sides. A 15% (wt/vol) honey solution was provided on water-soaked cotton via a hole pierced through the mesh in the small side of container. The moths were reared in the laboratory for three generations before testing. For electrophysiological experiments (GC–EAD), 2- to 3-day-old females and males were used. For the wind-tunnel bioassay, mated female and male moths were selected randomly. Mated moths were obtained by placing groups of ca. 20 newly emerged females together with 30 males in the same cage for two photoperiodic cycles to ensure mating. Each adult moth was used in only one assay and moths were not exposed to synthetic odor sources before the bioassay.

Chemicals

Tetradecane (99% purity), pentadecane (99%), hexadecane (99%), octadecane (99%), nonadecane (99%), (*Z*)-3-hexen-1-ol (98%), butyl butanoate (98%), (*Z*)-3-hexenyl acetate (98%), benzaldehyde (99%), methyl salicylate (99%), (*E*)-β-ocimene (60%), and (*E,E*)-α-farnesene (49%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The remaining farnesene fraction consisted of mainly (*E*)-β-farnesene (26%), (*Z*)-β-farnesene (18%), and (Z,*E*)-α-farnesene (7%). We used this mixture of farnesene isomers for our EAG and field studies and refer to it as 'farnesene'. Heptadecane (99%), 1-hexanol (99%), 2-ethyl-1-hexanol (99%), nonanal (97%), hexyl acetate (99%), and racemic linalool (97%) were obtained from Fluka Production (Buchs, Switzerland). Ethyl butanoate (99%) and butyl acetate (99%) were bought from Acros Organics (Morris Plains, NJ, USA). Ethyl hexanoate (98%) and hexyl butanoate (98%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Compounds that did not elicit antennal responses, and for which no standards were available, were tentatively identified using the NIST-database (Tasin et al., 2005).

Plant materials

Ten-year-old plants of peaches and pears were cultivated in an experimental orchard at the IFP. The peach variety used was flat peach (FP), 'Wuyuexianbiangan'. The pear variety used was Jimi (JM). Both varieties are commonly grown in the Beijing area. No insecticides or any specific treatment against OFM were used in the orchard during the tests.

Collection of VOCs from detached plant tissues

Peach shoots (ca. 30 cm long) were cut from selected trees and sealed with liquid paraffin (Fluka Production), before being transferred to the laboratory nearby (Anfora et al., 2009). Plant material was used for volatile collection or wind-tunnel assays within 20 min after cutting. Pear and peach fruits were healthy and were picked from the experimental orchard trees when fruits of the corresponding varieties were ripe in the Beijing area (Cao et al., 2000, 2006; Jiang et al., 2002).

A push–pull system was used to collect headspace VOCs. Peach shoots (ca. 1 500 g) and pear fruits (ca. 1 500 g) were placed into a 2 l glass jar for extraction. Air aspirated with a vacuum pump (Qianxi Air, Beijing, China) and filtered through an activated charcoal filter was passed through the jar at 300 ml per min, and finally through a sorbent cartridge (Porapak Q, 50 mg, 80/100 mesh; Supelco, Bellefonte, PA, USA). The sorbent cartridges were held between plugs of glass wool in a glass tube (10 cm long, 0.5 cm inner diameter). VOC samples were collected for 8 h at 24 ± 1 °C and 65–70% r.h.

Volatiles were desorbed by eluting the sorbent cartridge with 500 µl hexane (HPLC grade, Sigma-Aldrich) at room temperature. Five samples each were collected from peach shoots and ripe pear fruits. The samples were analyzed by GC–EAD and gas chromatography–mass spectroscopy (GC–MS). For quantitative analyses, 0.5 µg ethyl pentanoate (99%; Fluka Production) was added as an internal standard to each sample. Preliminary analyses showed that this compound was not detectable in the headspace VOCs of the peach or pear varieties studied here. Sample volumes were reduced to 50 µl using a slow stream of nitrogen and then analyzed. If not used immediately, extracts were sealed in glass vials and stored at −18 °C until used.

GC–MS

Headspace VOCs of peach shoots and pear fruits were analyzed with an Agilent 5973 MS coupled to an Agilent 6890 N GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a polar DB-WAX-fused silica column or a non-polar DB-5-fused silica column (both 30 m × 0.25 mm i.d., 0.25 µm film; J&W Scientific, Folsom, CA, USA). The temperature program was as follows: 50 °C for 1 min, then increasing 3 °C per min to 120 °C, then increasing 10 °C per min to 240 °C, and finally held at 240 °C for 10 min. Windows NT/MASS Spectral Search Program software (version 1.7) was used for data analysis. Injections were made in the splitless mode. Helium was used as the carrier gas (1.0 ml per min). For electron impact (EI) mass spectra, the ionization voltage was 70 eV, and the temperatures of the ion source and of the interface were 230 and 280 °C, respectively. The emission current was 34.6 µA. Identification of VOCs was verified by comparison with authentic samples.

GC–EAD

A micromanipulator assembly (MP-15) was connected to a stimulus controller (CS-55; both Syntech, Hilversum, The Netherlands). All signal sources were connected to a serial data acquisition interface (IDAC-4; Syntech). The antennae of OFM males and females were excised using micro-scissors. A few segments from the tips of antennae were clipped off and mounted on the antenna holder with two metal electrodes using conductive gel (Spectra 360; Parker Laboratories, Fairfield, NJ, USA), and then the electrode holder was inserted into the EAD probe. Testing began after a relative stable baseline had been achieved. The outlet of the GC column was split in a 2:1 ratio between a cut antenna and the flame ionization detector (FID). The mounted antenna was positioned in the charcoal-filtered and humidified air stream that carried the

VOCs from the GC column. The antennal and FID signals were amplified and recorded simultaneously using Syntech software (GC-EAD 32, version 4.4). Each sample was tested with six antennae, each derived from a different moth. Identities of EAD-active compounds were verified by comparison of mass spectra and retention times with those of synthetic standards.

The VOCs were analyzed with an Agilent 6890 N GC with a flame ionization detector, coupled with a Syntech EAD. Column type and oven temperature program were the same as in the GC–MS analysis. Nitrogen was used as the carrier gas (1.0 ml per min).

Field experiment

Based on the results of the GC–EAD analyses, VOCs from the peach shoots and pear fruits that elicited antennal responses in female and male OFMs were used to formulate four separate blends for the field tests. The four synthetic blends derived from the two host plants were evaluated in adjacent orchards of the two host crops from the time of late peach fruiting to late pear fruiting.

The four VOC blends were as follows: (1) Sh-F (shoot-female): VOCs from peach shoots to which females had positive EAD responses; (2) Sh-M (shoot-male): VOCs from peach shoots to which males had positive EAD responses; (3) Fr-F (fruit-female): VOCs from pear fruits to which females had positive EAD responses (Lu et al., 2012); and (4) Fr-M (fruit-male): VOCs from pear fruits to which males had positive EAD responses. The four blends of synthetic compounds were prepared in the same ratios of GC–EAD-active VOCs as emitted by the corresponding plant tissue (Table 1). These were prepared using 100 mg of the most abundant compound and adding the others in the same proportion as in the naturally occurring volatile blend.

Goblet-shaped rubber septa (10 mm deep, 6 mm i.d.) with a maximum volume of 400 µl (Shunyi Rubber, Beijing, China) were used to disperse the VOC blends. This volume was sufficient for all the VOC blends in our study. The VOC blends were prepared 1–2 h prior to their use in field bioassays. For each VOC blend, rubber septa were filled and then fixed upward on the bottom of a sticky delta trap. Traps were placed in the field at dusk, when OFM flight intensity peaks, for all the treatments. We deployed the rubber septa with solutions directly in the trap, before the mixture solution was fully impregnated into the rubber septa. All odor blends were deployed in this way (Lu et al., 2012). Unbaited traps (HPLC-grade hexane; Sigma-Aldrich) and OFM sex-pheromone lures (Geruibiyuan Technology, Beijing, China) were used as controls.

Table 1 Components, their amounts loaded on rubber septa (mg), and their ratios for the blends used in the field experiment. The blends are based on female (F) or male (M) EAD response to detached peach (*Prunus persica* cv. Wuyuexianbiangan) shoots (Sh) and mature pear (*Pyrus bretschneideri* cv. Jimi) fruits (Fr)

Compound[1]	Sh-F	Sh-M	Fr-F[2]	Fr-M
Alcohols				
1-Hexanol			1	1
(*Z*)-3-Hexen-1-ol	4			
Aldehydes				
Nonanal			1	
Esters				
Ethyl butanoate			100	100
Butyl acetate			70	70
Ethyl hexanoate			7	7
Hexyl acetate			5	5
(*Z*)-3-Hexenyl acetate	100	100		
Hexyl butanoate			1	1
Benzenoids				
Benzaldehyde	4			
Terpenoids				
(*E*)-β-Ocimene	85			
Linalool	1			
Farnesene[3]			4	4

[1]In order of elution during gas chromatography on a polar DB-Wax-fused silica column.
[2]The blends of Fr-F were based on headspace VOC composition from detached mature pear fruits (Lu et al., 2012).
[3]Farnesene = mixture of (*E*,*E*)-α-farnesene (49%), (*E*)-β-farnesene (26%), (*Z*)-β-farnesene (18%), and (Z,*E*)-α-farnesene (7%).

The field test was conducted in a 7-ha peach orchard and a 6.5-ha pear orchard at the IFP with ca. 30–50 m open space between the orchards. Both orchards had a history of OFM infestation. Two trials were conducted, each of 2 weeks: 5–18 August (Field experiment 1) and 5–18 September (Field experiment 2) 2013. These coincided with peak OFM activity in the Beijing area. Sticky delta traps (35 × 20 × 20 cm) were used to trap the insects. In each orchard, the trials were carried out in a randomized block design containing six replicate blocks. In each of the six blocks, the different treatments were repeated only once. Blocks were at least 120 m apart. Traps were installed ca. 1.5 m above the ground and were separated by at least 30 m to minimize interference between traps.

The traps were monitored twice weekly, captured OFMs were transferred to the laboratory, and their number and sex were recorded. The data of captures over each 2-week period were pooled.

Wind-tunnel assay

The wind-tunnel tests were designed to test for sexual differences in flight ability toward the four VOC blends.

Males and females were used in the wind-tunnel assay. The laboratory wind tunnel was 1.6 m long, 0.5 m wide, and 0.5 m high. A fan at the upwind end generated a steady airflow into the tunnel, set at 0.3 m s^{-1} at the point of release of moths. The light intensity in the tunnel was ca. 250 lx. The room was kept at 23 ± 2 °C and 50–70% r.h.

Four VOC blends were prepared in proportions of GC–EAD-active VOCs found in the natural blends emitted by the corresponding varieties (Table 1). The predominant VOC in the mixture was dosed at 0.5 mg. The VOCs were diluted with hexane (HPLC grade; Sigma-Aldrich). Preliminary tests in the wind tunnel showed that these concentrations were adequate to elicit moth responses. The septum loaded with one of the blends was placed on a holder at the upwind end in the center of the tunnel, 10 cm from the upwind end. Individual moths were scored for the following behaviors: (1) departure from the release cage and flight upwind; (2) arrival within 10 cm of the VOC source; and (3) landing on the source. We categorized each moth as the behavior associated with the furthest distance traveled within 20 min.

Tests began 2 h before the beginning of the scotophase and lasted 3 h. Moths were sexed and were then transferred into the test room 2 h before the experiments. Batches of 10 moths were placed in a small, screened metal release cage (7 cm diameter, 9 cm high) with a side door through which the air from the wind tunnel could flow. The release cage was placed on a holder at the downwind end of the tunnel, 30 cm above its floor and ca. 140 cm from the VOC source. The door, facing the upwind end of the tunnel, was opened to allow the moths to leave the cage. Each batch of 10 mated moths was tested for 20 min, and 6 batches of moths were used per day. Each VOC blend was tested with nine batches of moths, each on a different day. Individual moths were tested only once.

Data analysis

Mean numbers of OFM males and females captured in traps baited with each VOC blend in the field and the performance scores of OFM females and males to the VOC source in the wind tunnel were analyzed by one-way ANOVA. Tukey's multiple range test was used to test for significant differences among VOC blends in the mean numbers of OFMs captured ($\alpha = 0.05$). Unpaired sample t-tests were used to test for significant differences between sexes in three flight behaviors in the wind-tunnel assay. All data were analyzed with the statistical program SPSS v. 16.0 (SPSS, Chicago, IL, USA).

Results

Characterization of the headspace volatiles from peach shoots and pear fruits

Compounds belonging to various chemical classes were identified from peach shoots: hydrocarbons, alcohols, aldehydes, esters, benzene derivatives, and terpenoids (Table 2). Seven components—octadecane, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, benzaldehyde, methyl salicylate, (*E*)-β-ocimene, and linalool—were characteristic of peach shoots and absent from ripe pear fruits. Thirteen components—tetradecane, 6-methyl-octadecane, 1-hexanol, 2-ethyl-1-hexanol, 2-methyl-1-hexadecanol, (*E,E*)-α-farnesene, and seven esters—were characteristic of ripe pear fruits and absent from peach shoots. In particular, (*Z*)-3-hexenyl acetate and ethyl butanoate were the most abundant VOCs identified in peach shoots and pear fruits, respectively.

Female and male antennal responses to VOCs from peach shoots and pear fruits

From detached peach shoots, (*Z*)-3-hexenyl acetate elicited antennal responses in both sexes (Figure 1A and B), whereas (*Z*)-3-hexen-1-ol, benzaldehyde, (*E*)-β-ocimene, and linalool only elicited responses in females. From mature pear fruits, nonanal elicited only responses in females, and 1-hexanol, ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, hexyl butanoate, and (*E,E*)-α-farnesene elicited antennal responses in both sexes (Figure 1C and D).

Thirteen compounds from the headspace VOCs of detached peach shoots and mature pear fruits elicited antennal responses in OFM females: 1-hexanol, (*Z*)-3-hexen-1-ol, ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, hexyl butanoate, (*Z*)-3-hexenyl acetate, nonanal, benzaldehyde, (*E*)-β-ocimene, linalool, and (*E,E*)-α-farnesene (Figure 1A and C). Of these, eight compounds elicited antennal responses in males: 1-hexanol, ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, hexyl butanoate, (*Z*)-3-hexenyl acetate, and (*E,E*)-α-farnesene (Figure 1B and D).

Based on male and female EAD responses to pear- and peach-derived VOCs the following four lures were developed: (1) Sh-F: (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, benzaldehyde, (*E*)-beta-ocimene, and linalool (ratio 4:100:4:85:1); (2) Sh-M: (*Z*)-3-hexenyl acetate; (3) Fr-F: 1-hexanol, nonanal, ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, hexyl butanoate, and farnesene (1:1:100:70:7:5:1:4); and (4) Fr-M: 1-hexanol, ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, hexyl butanoate, and farnesene (1:100:70:7:5:1:4).

Table 2 Quantities of volatile compounds collected in the headspace of peach (*Prunus persica* cv. Wuyuexianbiangan) shoots and mature pear (*Pyrus bretschneideri* cv. Jimi) fruits: mean (± SD) amount (ng 100 g^{-1} plant material h^{-1}) and relative amount (expressed relative to the most abundant compound, set to a value of 100)

	Peach		Pear	
Compound	Amount	Relative amount	Amount	Relative amount
Hydrocarbons				
Tetradecane*			1.50 ± 0.11	<1
Pentadecane*	0.81 ± 0.10	1	2.05 ± 0.25	1
Hexadecane*	0.72 ± 0.07	1	3.07 ± 0.28	1
Heptadecane*	0.62 ± 0.13	1	1.70 ± 0.20	1
Octadecane*	0.29 ± 0.03	<1		
6-Methyl-octadecane			1.41 ± 0.12	<1
Nonadecane*	0.30 ± 0.10	<1	1.32 ± 0.10	<1
Alcohols				
1-Hexanol*			2.95 ± 0.25	1
(*Z*)-3-Hexen-1-ol*	3.15 ± 0.63	4		
2-Ethyl-1-hexanol*			0.73 ± 0.23	<1
2-Methyl-1-hexadecanol			0.52 ± 0.28	<1
Aldehydes				
Nonanal*	0.34 ± 0.15	<1	0.48 ± 0.22	<1
Esters				
Ethyl butanoate*			302.11 ± 13.00	100
Butyl acetate*			212.32 ± 11.29	70
Butyl butanoate*			0.93 ± 0.18	<1
Ethyl hexanoate*			19.75 ± 4.25	7
Hexyl acetate*			13.75 ± 2.25	5
(*Z*)-3-Hexenyl acetate*	71.45 ± 3.50	100		
Hexyl butanoate*			3.25 ± 0.25	1
Hexyl hexanoate			0.53 ± 0.23	<1
Benzenoids				
Benzaldehyde*	3.15 ± 0.14	4		
Methyl salicylate*	0.52 ± 0.07	<1		
Terpenoids				
(*E*)-β-Ocimene*	61.02 ± 2.66	85		
Linalool*	0.33 ± 0.06	<1		
(*E,E*)-α-Farnesene*			12.54 ± 2.23	4

Compounds marked with an * had been conclusively identified by comparison of spectra and retention times with those of an authentic standard. Compounds in bold face type elicited antennal responses in GC–EAD experiments. Compounds within each class were listed according to retention times on a polar DB-Wax-fused silica column.

Field experiment 1

The first field tests were carried out during the late peach-fruiting and early pear-fruiting stages, respectively. Based on the number of male moths trapped with the control sex pheromone, it seems a larger OFM population was present in the peach orchard at this time than in the pear orchard (Figure 2A and B).

In peach orchards, lures that contained pear-derived VOCs (Fr-F, Fr-M) caught significantly more OFM males and females than the control or the lures that contained peach-derived VOCs (Sh-F, Sh-M) (Figure 2A; one-way ANOVA: males: $F_{4,25} = 105.80$; females: $F_{4,25} = 43.59$, both $P<0.01$). Fr-M did not catch more males or females than Fr-F, and Sh-M did not catch more males than Sh-F (Figure 2A). Even though the Sh-F blend was based on female response, no more females were caught than with Sh-M (Figure 2A).

In the pear orchard, Sh-M caught more OFM males than the control and the other lures, but there were no significant differences among Sh-F, Fr-M, and Fr-F blends (Figure 2B; $F_{4,25} = 33.67$, $P<0.01$). All four blends were equally attractive to females (Figure 2B).

Field experiment 2

During the peach harvest and the late fruiting stage for pear, a significantly larger OFM population was present in the pear orchard than in the peach orchard, based on the number of male moths trapped with the control sex

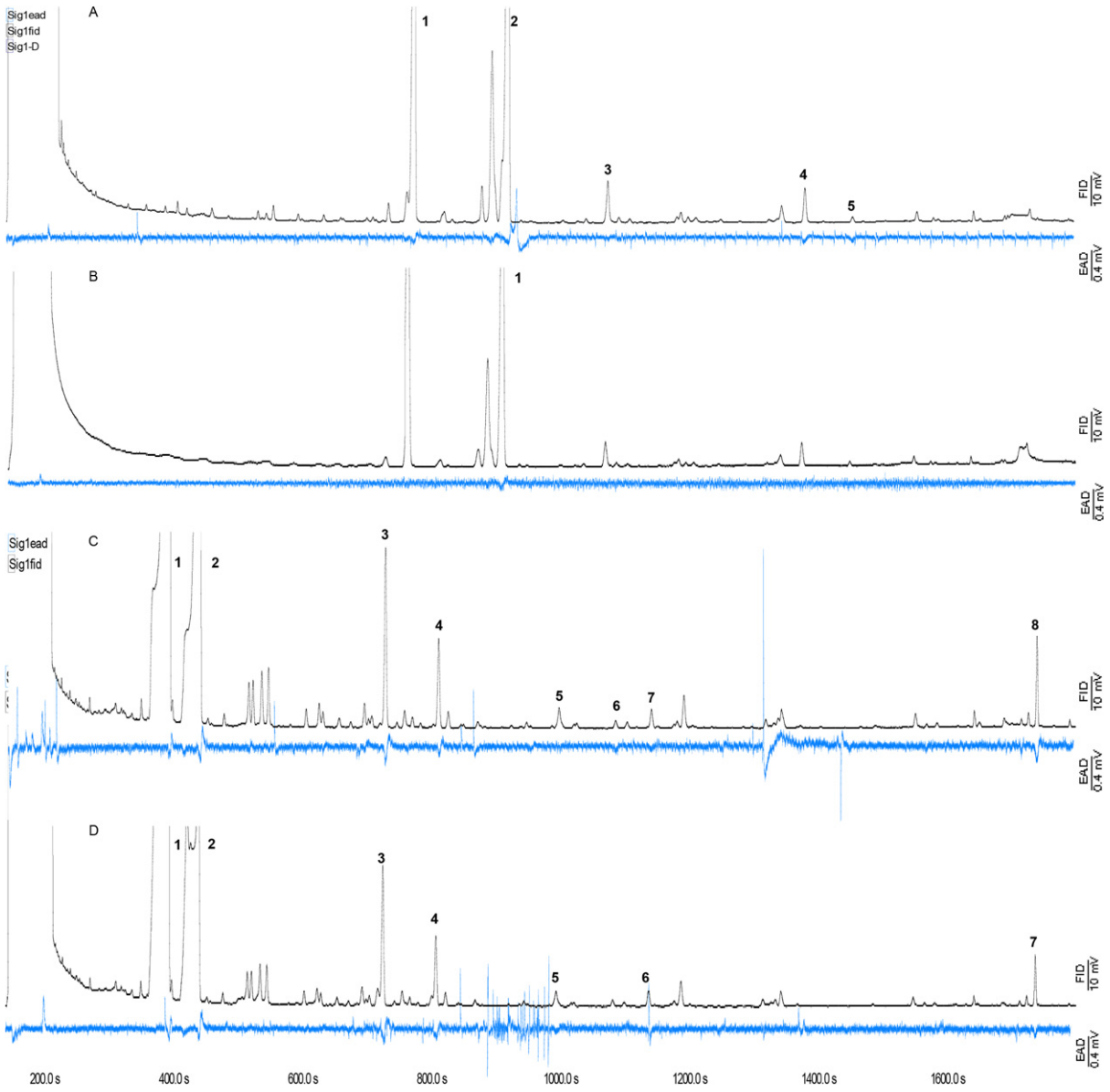

Figure 1 Simultaneously recorded *Cydia molesta* (A, C) female and (B, D) male GC–EAD responses to VOCs collected from (A, B) detached shoots of peach variety Wuyuexianbiangan and (C, D) mature fruits of the pear variety Jimi using a polar DB-WAX capillary column. In each panel, the upper trace indicates the flame ionization detector responses (FID), the lower trace the antennal responses (EAD). EAD-active compounds found consistently in six tests were (A) (*E*)-β-ocimene (1), (*Z*)-3-hexenyl acetate (2), (*Z*)-3-hexen-1-ol (3), benzaldehyde (4), linalool (5); (B) (*Z*)-3-hexenyl acetate (1), (C) ethyl butanoate (1), butyl acetate (2), ethyl hexanoate (3), hexyl acetate (4), 1-hexanol (5), nonanal (6), hexyl butanoate (7), (*E,E*)-α-farnesene (8); (D) ethyl butanoate (1), butyl acetate (2), ethyl hexanoate (3), hexyl acetate (4), 1-hexanol (5), hexyl butanoate (6), (*E,E*)-α-farnesene (7).

pheromone (Figure 2C and D). In the peach orchard, much fewer OFM were present based on the monitoring by sex pheromone-baited traps, as almost no moths were caught in the traps. In the pear orchard, lures that contained peach-derived VOCs (Sh-F, Sh-M) caught significantly more OFM males than the control and the lures that contained pear-derived VOCs (Fr-F, Fr-M) (Figure 2D; one-way ANOVA: $F_{4,25} = 65.67$, $P<0.01$). Although the Sh-F blend was based on female responses, Sh-M, Fr-F, and Sh-F blends caught significantly more females than Sh-F in pear orchards. (Figure 2D; $F_{4,25} = 47.38$, $P<0.01$).

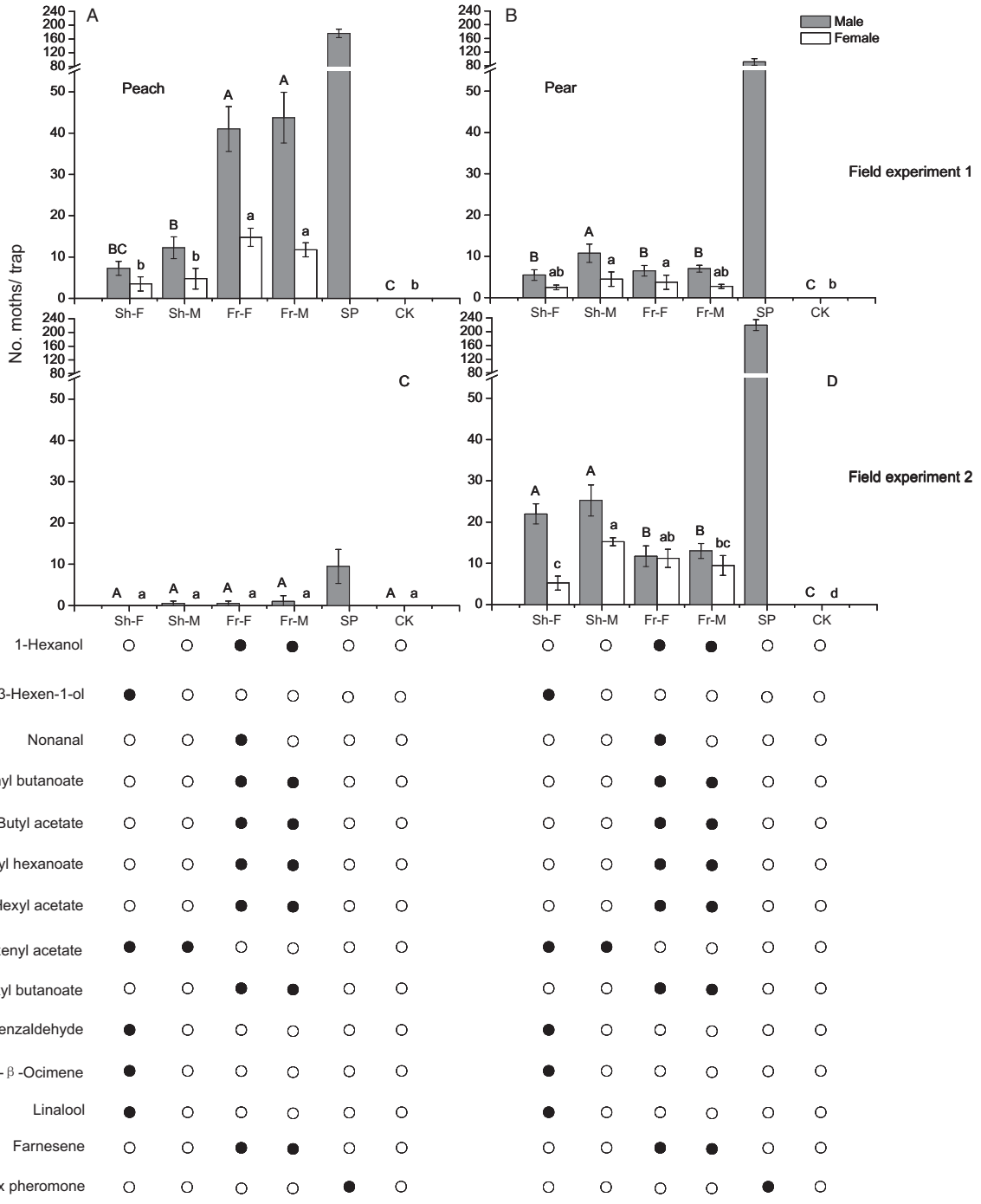

Figure 2 Mean (± SD; n = 6) total number of *Cydia molesta* males and females captured in traps in (A, C) peach and (B, D) pear orchards, during (A, B) 5-18 August (Field experiment 1) and (C, D) 5-18 September (Field experiment 2). Each trap was baited with a rubber septum containing synthetic VOC blends corresponding to those emitted by peach shoots (Sh-F, Sh-M), pear fruits (Fr-F, Fr-M), a hexane control (CK), and sex pheromone (SP). The ratios of the constituent components are given in Table 1. Each lure contained 100 mg of the most abundant component. Means within a panel capped with different letters (capital for males, lower case for females) are significantly different (one-way ANOVA followed by Tukey's multiple comparison test: $P<0.05$).

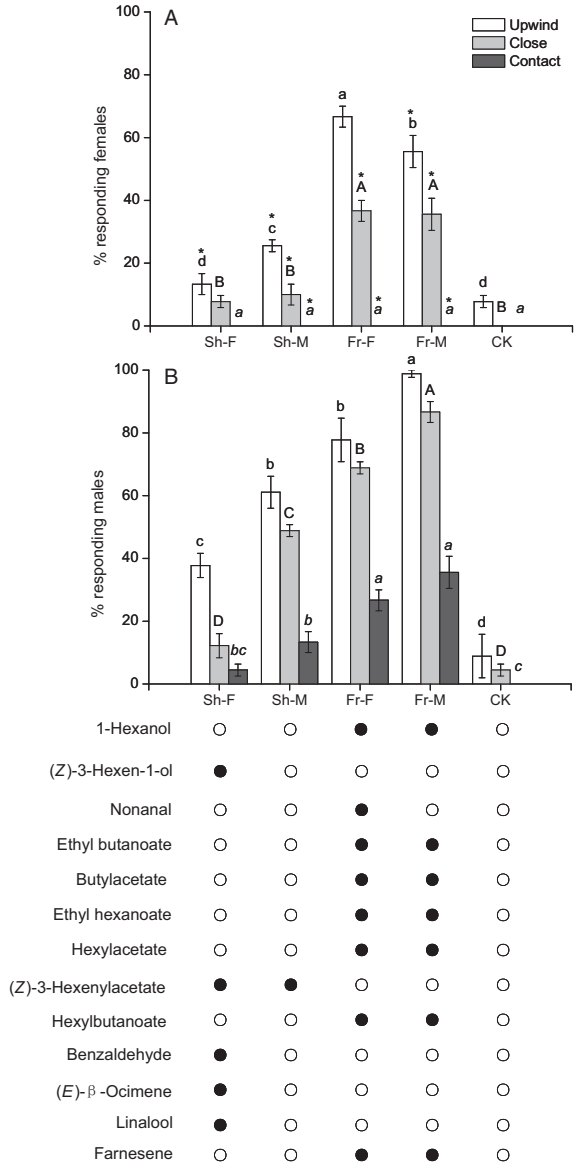

Figure 3 Mean (± SD) attraction (%) of mated *Cydia molesta* (A) females and (B) males in a wind tunnel to synthetic VOC blends mimicking the headspace of detached shoots of peach variety Wuyuexianbiangan, mature fruits of pear variety Jimi, and a hexane control (CK). The ratios of the constituent components are given in Table 1. Farnesene = mixture of (*E*,*E*)-α-farnesene (49%), (*E*)-β-farnesene (26%), (*Z*)-β-farnesene (18%), and (Z,*E*)-α-farnesene (7%). Means within a panel with the same color capped with different letters are significantly different (one-way ANOVA followed by Tukey's multiple comparison test: $P<0.05$). Moths were scored for orientation (upwind), flight to within 10 cm of the VOC source (close), and landing on the VOC source (contact). Differences between sexes in the three flight behaviors were analyzed by unpaired-sample t-tests (*$P<0.05$).

Wind-tunnel bioassays

All four blends stimulated upwind flight in both females and males (Figure 3A and B), but only males reached and contacted the source (Figure 3B). Males were more strongly attracted than females to the synthetic VOC blends. In the laboratory, pear-derived lures were more attractive to both sexes than peach-derived lures. The Fr-F blend proved to be the most attractive to females in the wind tunnel: 67% of the females flew upwind (one-way ANOVA: $F_{4,10} = 183.43$) and 37% arrived within 10 cm of the source ($F_{4,10} = 83.11$, both $P<0.01$), but none landed on the source (Figure 3A). Similarly, Fr-M was most attractive to males in the wind tunnel: 99% of the males flew upwind ($F_{4,10} = 130.22$), 87% arrived within 10 cm of the source ($F_{4,10} = 510.10$), and 36% landed on the source ($F_{4,10} = 64.57$, all $P<0.01$) (Figure 3B). Although the Sh-F blend was based on female responses, no more females than males flew upwind and arrived within 10 cm of the source (Figure 3A).

Discussion

Female oriental fruit moths represent a more serious threat to adjacent orchards than males, because their flight exceeds that of males (Hughes & Dorn, 2002), and because females lay the eggs that develop into the damaging larvae. Previous studies mainly focused on the interaction between adult OFM females and host plant-derived volatiles (Natale et al., 2003, 2004; Piñero & Dorn, 2007, 2009; Najar-Rodriguez & Dorn, 2013). Most recently, the discovery that mixtures containing volatiles attractive to females in the laboratory could capture more males under field conditions stimulated the comparison of responses of male and female OFM to host plant-derived volatiles.

We found that EAD-active compounds from the headspaces of pear fruits were similar for females and males—eight and seven compounds, respectively—indicating a similar chemosensory system of OFM male and female antennae. In the field, male captures significantly outnumbered female catches, even in traps with eight-compound lures based on female responses to pear fruit VOCs (Fr-F).

Responses to host plant-derived volatiles were also found to differ between the sexes. The blend without nonanal (Fr-M) was more attractive to males than the blend containing nonanal (Fr-F), suggesting that nonanal acts as a repellent or attraction-inhibitor for OFM males under field conditions. Nonanal has been characterized as an oviposition deterrent for female codling moth, *Cydia pomonella* (L.) (Yokoyama & Miller, 1991). Other studies also suggested a sex difference in

response to semiochemicals. For turnip moths, *Agrotis segetum* (Denis & Schiffermüller), Hansson et al. (1989) characterized the pheromone and plant volatile perception in males and females by EAD and single sensillum techniques and found female receptors are specialized for plant volatile reception and are insensitive to pheromones. In contrast, the specialized pheromone receptors on male antennae are sensitive not only to pheromones but also to plant volatiles. In addition, males have specialized plant volatile receptors (Hansson et al., 1989). Codling moth males and females were found to differ in the number of olfactory receptor neurons in the antennae (Bäckman et al., 2000).

Overall, the behavioral response of males toward host plant-derived volatiles both in the laboratory and in the field was stronger than that of females, demonstrating goal-directed male orientation toward sex pheromone sources. Some authors have suggested that males use plant VOCs to distinguish environments where they can find females more easily (Ansebo et al., 2004; Il'ichev et al., 2009). Females on the other hand need to consider more factors, such as flight mode, response profile, or flight strategy toward host plants under field conditions. The flight mode of males may be different from that of females. In our wind-tunnel study, females were attracted by synthetic lures, but the performance of males exceeded that of females especially when approaching the source. The orientation mechanisms of males flying in search of females are adapted to locate a point-source of sex pheromone. By contrast, females searching for suitable oviposition sites might not be as strongly attracted to point sources of plant VOCs, and therefore trap design could have a large influence on their ability to attract females. Similar results were found for the codling moth, an important pest of apple (Coracini et al., 2004). Female codling moths have frequently been observed to fly upwind over several meters toward branches with green apples, but contacted the apple less frequently, which suggests that the females employ a different search strategy than males, especially at close range, looking for a suitable oviposition site (Witzgall et al., 1999). Also, females may locate oviposition sites based on both chemical and visual cues. The greatest flight activity of mated and unmated male and female OFMs occurred during the first hour of dusk, when light intensity decreased from 3 750 to 57 lx, suggesting that the females use optical cues to locate oviposition sites (Hughes & Dorn, 2002). Similar results have been found in grape berry moth, *Paralobesia viteana* (Clemens), a crepuscular species, which oviposits less in the absence of light (Clark & Dennehy, 2002). The physical structure and low volatility of VOCs present on fruit surfaces could also affect female performance. In our study, OFMs always laid eggs on the smooth surface of waxed paper in the laboratory. The sticky base of the traps might repel female landing.

As predicted, the VOC blend based on male responses to peach shoot VOCs (Sh-M) caught more males than the blend based on female responses to the same source (Sh-F); however, the blend based on female responses (Sh-F) did not attract more females than the male response-derived blend (Sh-M). Of the VOCs from peach shoots, (*Z*)-3-hexenyl acetate elicited antennal responses from both sexes, whereas the other four components, (*Z*)-3-hexen-1-ol, benzaldehyde, (*E*)-β-ocimene, and linalool, only elicited responses from females. Field assays indicated the other four components were redundant in attracting not only males but also females. We hypothesize that volatiles collected from cut young peach shoots may differ from the headspace of intact peach shoots. This could greatly affect the olfactory orientation of OFMs. In our study, beside a hydrocarbon, six components—(*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, benzaldehyde, methyl salicylate, (*E*)-β-ocimene, and linalool—were characteristic of detached peach shoots. Natale et al. (2003) found 22 compounds in the headspace of excised peach shoots. (*Z*)-3-Hexen-1-ol, (*Z*)-3-hexenyl acetate, benzaldehyde, methyl salicylate, and (*Z*)-β-ocimene were also detected in their study. Il'ichev et al. (2009) analyzed intact young shoot tips of potted peach trees by GC-MS. They found (*Z*)-3-hexenyl acetate and (*Z*)-β-ocimene in the headspace of living peach shoots, but (*Z*)-3-hexen-1-ol, benzaldehyde, and methyl salicylate were not detected. We deduce that (*Z*)-3-hexen-1-ol, benzaldehyde, and methyl salicylate are characteristic of detached peach shoots and may be induced by the artificial cutting of shoots. These volatile components are associated with plant stress caused by manual damage and could indicate a low-quality host or a non-host. Active avoidance of non-host odors and even nutritionally unsuitable hosts appears to be an important part of the insect host location process.

In conclusion, our study suggested similarities in the chemosensory system of OFM male and female antennae, but also differences in responses were found between the sexes. Further study is needed to identify the volatiles from intact peach shoots that attract OFM males or females. Both, males and females could disperse into secondary host orchards between the late peach-fruiting and late pear-fruiting stage. The two pear-derived VOC blends (Fr-F and Fr-M) caught significantly more OFM males and females than the other blends in peach orchards; conversely, the two peach-derived blends were more attractive

than the other blends in pear orchards. Together, the blends derived from one plant species should be used in orchards of the other species. This may be valuable for designing further candidate attractant blends for *C. molesta*. In addition, we should seek to better understand the developmental differences between the sexes of *C. molesta* and among its host crops and explore better pest management approaches based on optimizing the peach–pear planting system in the field.

Acknowledgements

We are grateful to Editage (Cactus Communications, Trevose, PA, USA) for language improvement. This work was supported by Beijing Natural Science Foundation (grant no. 6142015), Beijing Young Elite Project (grant no. 2013D009046000002), Fundamental Research Funds for the Central Universities (grant no. YX2015-09), National Natural Science Foundation of China (grant nos. 31570643, 31270693, 81102747), and Public Welfare Project from the Ministry of Agriculture of China (grant no. 201103024).

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