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Season-Long Volatile Emissions from Peach and Pear Trees *In Situ*, Overlapping Profiles, and Olfactory Attraction of an Oligophagous Fruit Moth in the Laboratory

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Abstract Insect herbivores that have more than one generation per year and reproduce on different host plants are confronted with substantial seasonal variation in the volatile blends emitted by their hosts. One way to deal with such variation is to respond to a specific set of compounds common to all host plants. The oriental fruit moth *Cydia* (= *Grapholita*) *molesta* is a highly damaging invasive pest. The stone fruit peach (*Prunus persica*) is its primary host, whereas pome fruits such as pear (*Pyrus communis*) are considered secondary hosts. In some parts of their geographic range, moth populations switch from stone to pome fruit orchards during the growing season. Here, we tested whether this temporal switch is facilitated by female responses to plant volatiles. We collected volatiles from peach and pear trees *in situ* and characterized their seasonal dynamics by gas chromatography–mass spectrometry. We also assessed the effects of the natural volatile blends released by the two plant species on female attraction by using Y-tube olfactometry. Finally, we related variations in volatile emissions to female olfactory responses. Our results indicate that the seasonal host switch from peach to pear is facilitated by the changing olfactory effect of the natural volatile blends being emitted. Peach volatiles were only attractive early and mid season, whereas pear volatiles were attractive from mid to late season. Blends from the various attractive stages shared a common set of five aldehydes, which are suggested to play an essential role in female attraction to host plants. Particular attention should be given to these aldehydes when

designing candidate attractants for oriental fruit moth females.

Keywords Oriental fruit moth · *Grapholita molesta* · Lepidoptera · Female olfactory attraction · Peach · Pear · Aldehyde · Invasive pest

Introduction

Plant-produced chemicals determine, to a great extent, the variety of host plants that can be exploited by insect herbivores (Berenbaum 1981; Ricklefs 2008). Among these chemicals, volatiles play a vital role in host plant choice by guiding herbivores to their hosts (Bruce et al. 2005). Herbivores with multiple generations per year that reproduce on different hosts are confronted with substantial variations in the volatile blends emitted by their different host plant species at particular periods in time, as well as by the same plant species across a growing season (Piñero and Dorn 2009; Tasin et al. 2010). Thus, the insects' odor-guided behavior must be adapted to respond to essential constituents of volatile blends and to the natural variations of these blends (Tasin et al. 2007; Cha et al. 2010; Najar-Rodriguez et al. 2010, 2011). One way to deal with variations in plant volatile blends is to respond to a specific set of compounds common to all host plants (Rajapakse et al. 2006; Leppik and Frérot 2012). Female insects are expected to tolerate a certain modulation of the ratios of these common compounds within distinct threshold values, as recently shown for two tortricid moth species (Cha et al. 2010; Najar-Rodriguez et al. 2010, 2011).

The behavioral effect of plant volatile constituents also can vary upon the context in which volatiles are perceived (Webster et al. 2010; Cunningham 2012). This is of particular relevance when distinct plant volatiles are used for

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monitoring pest insects. For instance, the pear-derived constituent ethyl (*E,Z*)-2,4-decadienoate is behaviorally more effective in attracting the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) when deployed in walnut orchards compared to when deployed in pear orchards (Light et al. 2001; Light and Knight 2005). When searching for behaviorally effective blends, it is particularly promising to search for suitable blends derived from several hosts rather than looking for single compounds derived from one of the herbivore's host plants. Thus, testing for similarities in volatile profiles across a selection of host species might lead to pinpointing a relevant group of volatiles that is crucial for attraction of the target insect across different environmental contexts.

An oligophagous species with multiple generations per year is the oriental fruit moth *Cydia* (= *Grapholita*) *molesta* (Busck) (Lepidoptera: Tortricidae). Originating from Asia (Rothschild and Vickers 1991; H. Kirk, S. Dorn and D. Mazzi, unpublished data), it became a major fruit-damaging pest globally (Mazzi and Dorn 2012). Its host range encompasses species within the family Rosaceae, mostly those from the genera *Prunus* and *Pyrus* (Rothschild and Vickers 1991). Its principal host is considered to be the stone fruit peach (*Prunus persica* L.) where it causes damage even at low population densities (Rothschild and Vickers, 1991), whereas the pome fruits pear (*Pyrus communis* L.) and apple (*Malus domestica* L.) are considered secondary hosts (Rice et al. 1972; Zhao et al. 1989; Rothschild and Vickers 1991). In some parts of their geographic range, populations show some seasonal dynamics in host plant choice, with switches from stone fruit orchards to pome fruit orchards in the growing season, mainly after peach harvest (Il'ichev et al. 2007; Myers et al. 2007). For instance, heavy infestations proceed from peach to pear and apple orchards in the Emilia-Romagna region in northern Italy from July onwards (reviewed by Pollini and Bariselli, 1993; Dorn et al., 2001; Melandri and Pasqualini 2004). Particularly, after peach harvest, pear orchards adjacent to peach orchards also become infested in southern Switzerland (Bovey 1979). Similarly, oriental fruit moth attacks were recorded on pear orchards adjacent to stone fruit orchards towards the end of the season in California, USA (Rice et al. 1972). In northern Jiangsu, China, where the oriental fruit moth completes five generations per year, it attacks peach orchards during the first three generations, whereas pear and apple orchards are infested from the third to the fifth generation (Zhao et al. 1989).

Studies of host plant choice in relatively specialized insect herbivores have demonstrated that this behavior is mediated, to a great extent, by chemical and taxonomic similarities across host plants. For instance, cluster analysis of insect herbivores feeding on 12 species of Umbelliferae plants in North America demonstrated that the insects distribute themselves according to similarities in host furanocoumarin chemistry (Berenbaum 1981). Molecular

phylogenies of the ancient and speciose genus *Blepharida* (Coleoptera: Chrysomelidae) and terpenoid chemical profiles of host plants used by these insects also showed that the historical patterns of plant-insect associations strongly correspond to the patterns of host chemical similarity (Becerra 1997). Furthermore, a portion of the community-wide distribution of Lepidoptera larvae among congeneric species of temperate broad-leaved trees recently has been shown to be directly related to similarities in the chemical composition of foliage (Ricklefs 2008). Two Rosacean hosts of the oriental fruit moth, peach and apple, attracted mated females by olfactory cues (Natale et al. 2003), with the level of attraction to various plant tissues from these tree species varying substantially over time (Piñero and Dorn 2009). We hypothesized that the switch from the primary host peach to a secondary host such as pear or apple could be facilitated by the presence of a common chemical signal, i.e., a common set of volatile compounds, that is emitted at similar ratios during the attractive stages of these Rosacean host plants.

Odor profiles released from apple trees at different phenological stages are well studied (Bengtsson et al. 2001; Hern and Dorn 2003; Vallat and Dorn 2005; Piskorski and Dorn 2010). However, little information is available on the seasonal dynamics of volatile emissions by peach and pear. Indeed, chemical knowledge is limited to volatiles identified from young peach twigs before bloom (Natale et al. 2003; Il'ichev et al., 2009) and from detached ripening and detached mature pear fruits towards and at the end of the season (Jennings et al. 1964; Light et al. 2001; Scutareanu et al. 2003; Lu et al. 2012). A comparison of the dynamics in volatile emission by different host plants of the oriental fruit moth could provide further insight into the behavioral relationship of this insect herbivore with its different hosts.

Here, we conducted laboratory bioassays to assess the olfactory preferences of mated oriental fruit moth females to odors emitted by peach as the primary host and pear as the secondary host, at different stages of tree phenology across an entire growing season. We characterized the headspace volatiles from peach and pear trees collected *in situ* over the season to test whether similarities in volatile blend compositions among host plants and growth stages correlate with female olfactory preferences. Finally, we related our new findings from peach and pear trees to previous basic data on volatiles collected throughout a growing season from apple trees (Vallat and Dorn 2005) in order to gain a synopsis on host-plant volatiles and insect interactions across a wider range of hosts of the oriental fruit moth including a related tortricid fruit pest.

Methods and Materials

Plant Material and Insects Peach (*Prunus persica* variety 'Red Heaven') and pear (*Pyrus communis* L. variety

‘Harrow Sweet’) plant material used in this study originated from an organic farm (46°23′43.73″N, 8°53′11.67″E; altitude: 357 m) in Airolo, Canton Ticino, southern Switzerland. Plant protection measures were confined to a single spray of copper in spring, which was applied to prevent fungal diseases.

The mated oriental fruit moth females used were obtained from a laboratory colony that was reared for no more than 10–12 generations at the ETH Applied Entomology Group (Najar-Rodriguez et al. 2012). The colony originated from individuals collected in peach orchards in northern Italy (Emilia-Romagna region). Neonate larvae were reared in glass Petri dishes containing an artificial diet based on soybean flour, agar, dry yeast, distilled water, sugar, Vanderzant vitamin mixture for insects, and preservatives (Najar-Rodriguez et al. 2013). The diet was devoid of host plant cues. Petri dishes were kept under L:D 16:8, 60 % RH, and 24 °C. Newly emerged adults were transferred to plastic containers (20×10×8 cm) in groups of about 10 females to 30 males to ensure mating. Water was provided *ad libitum*. Under these conditions, over 98 % of all females are expected to be mated within 2 d (Najar-Rodriguez et al. 2010).

Female Attraction to Volatiles from the Primary Host and a Secondary Host Attraction of mated oriental fruit moth females to the odors emitted by the primary host peach and the selected secondary host pear was tested in dual choice Y-tube olfactometer bioassays. To obtain natural host plant volatiles for bioassays, twigs (Table 1) were collected from trees growing in a close vicinity at 4 (for peach) or 5 (for pear) distinct phenological stages during the entire growing season of 2010: (1) flowering (April 16 and 29 for peach and pear, respectively), (2) post-flowering (May 25 for both peach and pear), (3) early fruiting (June 23 for both peach and pear), (4) mid-fruiting (August 4 for both peach and pear), and (5) late fruiting, i.e., just before harvest (September 1 for

pear). Pear twigs at the flowering stage contained almost three times more flowers compared with peach twigs at the same stage. We chose not to standardize the number of flowers across the two hosts by cutting off flowers to avoid the release of wound-associated volatiles. Peach fruits were harvested earlier than pear fruits, i.e., 2 wk after our mid-fruiting sampling, so peach material at the late fruiting stage was no longer available for either behavioral bioassays or volatile collection.

Twigs with similar numbers of leaves and flowers, or fruits devoid of any visible damage were excised from the trees around 2:00 p.m., and the stem ends were immediately covered with parafilm. Twigs then were placed inside clean plastic bags that contained wet cotton balls in order to prevent dehydration. All samples were placed immediately in a cooler and transported back to the ETH laboratory in northern Switzerland for testing on that same day.

The olfactometer consisted of a Y-shaped glass tube (diam: 2.5 cm; arm length: 23 cm; common arm length: 23 cm) connected to two tubular glass tubes (diam: 6 cm; length: 38 cm). Each of these tubes was, in turn, connected to a glass chamber (diam: 10 cm; length: 14 cm) in which the plant material was placed. Moistened, charcoal-filtered air was drawn into each of the two glass chambers and the Y-tube arms at a rate of 740±10 ml/min at the entrance. Airflow rates were always calibrated before the initiation of and also during experiments with an electronic flow meter (Agilent flow meter ADM 1000; Agilent Technologies, Centerville, DE, USA).

Bioassays were conducted in a dark room from 6 to 9 p.m. at 24–25 °C and 60–70 % relative humidity. This period in the diel rhythm of the oriental fruit moth corresponds to the time window when mated females show increased locomotory activity towards plant-derived volatiles (Natale et al. 2004). Single females were chosen at random and were brought into the experiment room 10 min before the start of the experiments to allow for acclimatization to the room conditions. A single female

Table 1 Morphological characteristics (mean ± SE) of peach and pear twigs from different phenological stages across an entire growing season that were used for determination of oriental fruit moth attraction in Y-tube olfactometer tests

Plant phenological stage	Host plant	Shoot length (cm)	No. of leaves	No. of flowers or fruits	Fruit diameter (cm)	Weight (g)
Flowering (April 16 / 29)	Peach	20.2±0.6	-	8.60±0.44	n/a	5.14±0.28
	Pear	16.6±0.4	32.85±4.06	23.00±2.60	n/a	14.14±1.27
Post flowering (May 25)	Peach	18.7±0.6	23.15±1.38	-	1.6±0.1	14.23±1.19
	Pear	19.4±0.9	39.80±2.49	-	1.1±0.1	13.38±0.82
Early fruiting (June 23)	Peach	23.1±1.0	15.65±1.78	1.80±0.12	3.2±0.1	43.25±2.65
	Pear	19.3±0.5	30.80±2.36	1.80±0.12	1.9±0.1	22.91±1.88
Mid-fruiting (August 4)	Peach	20.4±0.9	19.50±1.50	1.15±0.08	4.4±0.1	83.23±6.27
	Pear	16.0±0.7	29.95±2.22	2.10±0.07	3.8±0.1	101.13±3.06
Late fruiting (September 1)	Pear	18.7±0.7	20.20±2.03	1.30±0.11	4.8±0.1	117.87±7.75

was released at the entrance of the common arm of the Y-tube and exposed to a particular odor combination consisting of (a) clean air (blank) and (b) a plant sample (i.e., a peach or pear twig with a similar number of leaves and flowers or fruits). Once inside the Y-tube, the behavior of each female was observed for a maximum of 10 min. A 60-W red light bulb was placed above the olfactometer to allow for observation of female behavior during the experiment. A choice was considered to have been made if a female entered either arm and then crossed a score line drawn 3 cm from the intersection of the tube. By contrast, a choice was considered to have not been made if the female remained in the common arm of the Y-tube throughout the observation period (Bertschy et al. 1997).

All females tested were 4–5 d-old at the time of the experiments and were considered to be naïve, i.e., not previously exposed to natural or synthetic sources of host-related volatiles. A new plant sample was used for every 2 females tested. The positions of the glass tubes, as well as the positions of the two arms of the olfactometer, were systematically changed after testing 3–4 moths in order to avoid positional bias. Forty females were exposed at each sampling period to either peach or pear twigs. Females were tested on two to three consecutive days per period to account for daily differences in moth responsiveness. After each day, all parts of the olfactometer that came in contact with the moths were washed in a detergent solution, rinsed with acetone and hexane, and then oven-dried for at least 12 h at 120 °C.

Characterization of the Headspace Volatiles from the Primary and a Secondary Host We tested for similarities and differences in the volatile profiles emitted by peach and pear trees throughout the entire growing season of 2010 at the same 4 (for peach) or 5 (for pear) distinct phenological stages used for behavioral bioassays (Table 1). Ten 15-yr-old peach trees and ten 9-yr-old pear trees were selected within the same organic orchards that were used to obtain the plant material for bioassays. These designated trees were flagged for volatile sampling use throughout the growing season. All designated trees from the same species were growing in close vicinity in order to keep variation due to growing conditions minimal (Vallat and Dorn 2005).

Volatile collections were conducted for 6 h including the evenings, i.e., from 1 p.m. to 7 p.m., on sunny days without rainfall. Climatic data, including daily mean temperature, precipitation, and relative humidity, for each volatile-collection day were obtained from a nearby weather station in Biasca (46°21'N, 8°58'1E; altitude: 300 m), 8.8 km from the study site (www.meteoswiss.com). Mean daily temperature and relative humidity for the various sampling days were: 11.0 °C and 53 % (April 16); 16 °C and 66 % (April 29); 20 °C and 60 % (May 25); 16 °C and 64 % (June 23);

18 °C and 64 % (August 4); and 17 °C and 95 % (September 1). Mean monthly temperature and rainfall values recorded in the study area during the entire sampling period (1 April – 30 September) were 16.4 °C and 168.1 mm, respectively. These values were considered typical when compared to older (1959–2009) temperature (15.3 °C) and rainfall data (161.5 mm) available from the same weather station.

Volatile collection and analysis were based on the methods developed by Vallat and Dorn (2005) and Piskorski and Dorn (2010). To collect volatiles, a radial diffusive sampling system (Radiello model 120-2, Supelco, Buchs, Switzerland) was used. This system has low detection limits (Vallat and Dorn 2005), provides results that are as accurate as active sampling (Gallego et al. 2011), allows for efficient volatile collection without the need of heavy and encumbering pumping systems (Thammakhet et al. 2006), and minimizes plant manipulation and possible mechanical damage. Prior to sampling, sorbent cartridges (diam: 4.8 mm; length: 60 mm) containing Tenax TA (250±10 mg; particle size 20–35) were conditioned at 300 °C with a helium 5.0 (purity 99.99 %) flow of 60 ml min⁻¹ for 7 h. For storage and transport, the cartridges were enclosed in stainless steel tubes that were sealed with brass Swagelok caps (Arbor Ventil & Fitting, AG, Niederrohrdorf, Switzerland). On each sampling date, volatiles were sampled from one twig (approximately 15–20 cm in length) per tree (*N*=10 twigs/tree species) (Table 1). The sample contained leaves and flowers or fruits that were enclosed in a preconditioned plastic bag (Toppits®, Melitta GmbH, Egerkingen, Switzerland) together with a sorbent cartridge inserted in a diffusive body. The oven bag was preconditioned for at least 12 h in an oven heated up to 120 °C. Plant material was always chosen as homogeneously as possible, so that all twigs from the same phenological stage contained a similar number of leaves plus flowers or fruits (Table 1). Volatile samples from an empty plastic bag and from the air surrounding each orchard at a distance of approx. 30 m were collected as controls in parallel with each sampling. After sampling was completed, the sorbent cartridges were placed back into the stainless steel tubes, closed tightly, and taken back to the laboratory for analysis.

Gas chromatography/mass spectrometry (GC-MS) was used to analyze the qualitative and quantitative composition of the headspace volatiles. The volatiles were desorbed from the sorbent tubes with a Unity/Ultra thermal desorption (TD) system (Markes Int. Ltd., Llantrisant, UK) interfaced with a Hewlett-Packard 6890 GC-5976 MS (Hewlett-Packard Co., Palo Alto, CA, USA). The TD flow path was purged for 7 min prior to tube desorption. The tube was then desorbed for 12 min at 280 °C and refocused on a cold trap (Tenax TA/Carbograph 1TD) at -10 °C. The trap was desorbed for 7 min at 300 °C (carrier gas: helium (10 psi); transfer line temperature: 160 °C). The GC was equipped

with a retention gap (uncoated; 5 m×0.25 mm; Hewlett-Packard Co., Palo Alto, CA, USA) and a DB-5 ms column (30 m×0.25 mm; 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). The GC oven temperature was held for 5 min at 40 °C, then increased to 100 °C at 3 °C/min, up to 320 °C at 5 °C/min, and held at 320 °C for 4 min (transfer line temperature: 280 °C; ionization energy: 70 eV; ion source temp.: 230 °C; quadrupole temp.: 150 °C; mass range: 30–500 amu).

Chem Station Software (MSD Productivity Chem Station software, Agilent Technologies Inc., Santa Clara, CA, USA) was used for volatile identification and quantification. Identification was initially carried out by comparison of spectra to published mass spectrometry data. Only those volatiles that were present at higher amounts in the twigs' headspace than in the surrounding air were further analyzed. Linear retention indices (RIs) were calculated using a mixture of *n*-alkanes with chain lengths of C₇–C₄₀ as standards (Supelco Bellefonte, PA, USA). Calculated RIs were then compared with Adams (1995) and the NIST library. When possible, RIs were compared with those of available standard compounds of the highest analytical grade available.

For semi-quantification, cyclohexane (≥ 99.8 %, Sigma-Aldrich, Buchs, Switzerland) injected onto the sorbent tubes in the gas phase was used as internal standard. The internal standard was prepared as in Piskorski and Dorn (2010), based on the method described in Dewulf et al. (1999). The *Henry's* law coefficient for calculation was used from therein. Cyclohexane (0.235 mmol; 25.5 µl) was first dissolved in MeOH (100 ml). Then, aliquots of the solution (50 µl) were dissolved in H₂O (4.0 mL) in a series of 24.5 ml amber glass vials, which were tightly closed with Mininert valves (Sigma-Aldrich, Buchs, Switzerland) and incubated in a thermostatic chamber (Heraeus BK 6160 testing chamber, Thermo Fisher Scientific, Schwerte, Germany) at 25.0±0.1 °C for 24 h. Prior to desorption, aliquots of the gas phase (2.0 ml) from consecutive vials were injected onto the sorbent tubes using a gastight syringe together with a He flow of 60 mlmin⁻¹.

Statistical Analysis Multivariate analysis of variance (MANOVA) was performed to identify significant differences between volatiles (dependent variables) emitted by the peach and pear twigs at the different phenological stages sampled. Prior to analysis, volatile data were log₁₀ (X+1) transformed. If significant, one-way ANOVAs and Tukey HSD *post hoc* tests were further conducted to test for quantitative differences in concentrations of individual and total headspace volatiles emitted by peach and pear twigs at each phenological stage (JMP 8.0, SAS Institute Inc., Cary, NC, USA). Furthermore, principal component analysis (PCA) was applied to yield a 2D display of the multivariable set of data and to graphically determine

whether clustering of phenological stages occurred based on their overall volatile profiles (JMP, 9.0). If only a single compound was detected (as in the volatile blend collected from peach at the early fruiting stage), this blend was excluded from the analysis. PCA analysis also was applied to the volatile blends from apple trees collected in a commercial orchard that were previously characterized *in situ* across a full growing season (Vallat and Dorn, 2005). This study with apple basically used the same method for volatile collection and the same instruments for GC-MS analysis as the current study. Thus, potential differences between the current and this previous study should be largely confined to climatic and agronomic conditions prevailing at the time the studies were conducted.

Results of the behavioral bioassays were analyzed for preference (percentage of adults that made a choice between odor or clean air) and responsiveness (proportion of adults that made a choice). Individuals that did not make a choice were excluded from these analyses. First, *chi square* tests were carried out to test the null hypothesis of no preference for a particular blend. Then, unpaired-sample *t*-tests were carried out to compare responsiveness across odor combinations (Statistical software R, R Foundation for Statistical Computing, Vienna, Austria, www.R-project.org). The alpha value for each comparison was adjusted downward using the Benjamini and Hochberg procedure to correct for false discovery rates (type I errors) (Verhoeven et al. 2005).

Results

Female Attraction to Volatiles from the Primary Host and a Secondary Host The attraction of mated oriental fruit moth females to odors from the primary host (peach) and the selected secondary host (pear) distinctly changed across the growing season (Fig. 1). Female moths were attracted to volatiles from peach twigs collected at the flowering (April 16) ($\chi^2=19.70$, $N=37$, $P<0.001$) and early fruiting (June 23) ($\chi^2=6.43$, $N=35$, $P=0.001$) stages, but they were not significantly attracted to odors emitted by peach twigs at the post-flowering stage (May 25) or by fruit-bearing peach twigs at the mid-fruiting stage (August 4). Response to volatiles from pear twigs followed largely a reverse sequence, with females not being attracted to volatiles emitted by twigs at the flowering (April 29) and post-flowering (May 25) stages but attracted to volatiles emitted by fruit-bearing twigs later in the season, from early (June 23) ($\chi^2=6.74$, $N=38$, $P=0.001$) to mid- and late (August 4 and September 1, respectively) fruiting ($\chi^2=8.00$, $N=32$, $P<0.001$ for August 4 and $\chi^2=19.20$, $N=30$, $P<0.001$ for September 1). The most attractive tissues to the female moths were peach twigs at the flowering stage and pear twigs at the late fruiting stage ($t=3.88$, $df=14$, $P<0.001$ and $t=5.11$, $df=14$, $P<0.001$, respectively) (Fig. 1).

Fig. 1 Olfactory preference and responsiveness (i.e., percentage of females making a choice) of mated oriental fruit moth females to odors from either flower-bearing or fruit-bearing peach or pear twigs sampled from different phenological stages throughout an entire growing season. Tests were conducted in a Y-tube olfactometer. Clean air was used as control. $N=40$ females used for each dual choice. P -values based on *chi-square* tests: * = $P<0.05$; ** = $P<0.01$; ns = $P\geq 0.05$

Female responsiveness did not vary significantly across the season for either peach or pear, with the exception of the peach post-flowering stage when responsiveness was lower compared to any other phenological stage tested ($P<0.001$).

Characterization of the Headspace Volatiles from the Primary and a Secondary Host The volatile blends emitted by peach and pear twigs differed quantitatively, and to a lesser degree qualitatively, across the different phenological stages sampled throughout the season (Table 2). Quantitatively, total volatile emissions (taken as the sum of the concentration per twig (in ng) of individual volatile compounds) from flower- or fruit-bearing twigs differed between the hosts across the different phenological stages (MANOVA $F=2.21$, $P<0.001$) (Table 2 and Fig. 2). Total emissions from peach twigs were lower than those from pear twigs at all sampling dates (one-way ANOVA $F=13.53$, $df=1$; $P<0.001$ for post-flowering; $F=120.76$, $df=1$; $P<0.001$ for early fruiting; $F=5.73$, $df=1$; $P<0.001$ for mid-fruiting) except for the flowering stage, which yielded similar values for both plant species (one-way ANOVA: $F=0.04$, $df=1$; $P=0.830$) (Fig. 2). Seasonal dynamics in quantitative volatile emissions also were noted for each species. Total emissions from peach twigs were significantly higher at the flowering stage than at any other stage sampled, and were lower at the early fruiting stage than at any other stage sampled (one-way ANOVA: $F=907.79$, $df=3$; $P<0.001$) (Fig. 2). In contrast, total emissions from pear twigs increased almost linearly as the season progressed so that the lowest mean value was detected at the flowering stage and the highest mean value was detected at the late fruiting stage (one-way ANOVA: $F=362.78$, $df=4$; $P<0.001$) (Fig. 2). The exception was the mid-fruiting stage, when volatile emissions decreased to values similar to those detected at the flowering stage (one-way ANOVA: $F=0.77$, $df=1$; $P=0.391$). The relative contribution of individual compounds to the total blends also varied with tree phenology, which

further led to the quantitative differentiation of the blends emitted by the two plant species (Online Resource 1).

Qualitatively, a number of compounds are, to our knowledge, reported here for the first time from peach or from pear trees, with 1-undecanol being newly identified from both tree species (Online resource 2). A comparison between peach and pear volatiles revealed that although peach twigs at each phenological stage released a similar number of volatiles compared to pear twigs ($t=0.64$, $df=6$, $P=0.541$), the identity of the volatiles released by the two plant species differed across phenological stages (Table 2). While this led to qualitative differences in volatile blend composition, some clear overlap also became apparent. Specifically, six, four, one, and nine compounds were shared by both plant species at the flowering, post-flowering, early fruiting, and mid-fruiting stages, respectively (Table 2).

Relating Female Moth Attraction to Volatile Blend Composition Principal component analysis (PCA) clearly segregated the overall composition of the headspace volatile blends collected from the two plant species at the different phenological stages sampled (Fig. 3). Furthermore, PCA also segregated these volatile blends according to the behavioral effects they had on the oriental fruit moth females (Fig. 3). Volatile blends that attracted female moths were confined to two clusters: one comprised the volatile blends detected at the peach flowering, pear mid-fruiting, and pear late-fruiting stages, and the other comprised the volatile blend detected at the pear early fruiting stage. The blend from the peach early fruiting stage was excluded from this analysis for methodological reasons (as detailed in Materials and Methods). Non-attractive blends also clustered in two distinct groups: one comprised the volatile blend from the pear flowering stage and the other comprised the blends from peach and pear post-flowering stages and from peach mid-fruiting stage.

Interestingly, the composition of the blends from the attractive growth stages that clustered in one group, i.e.,

Table 2 Volatile compounds detected by TD-GC-MS in the headspace volatiles of peach and pear twigs from different phenological stages throughout an entire growing season. Concentration (in ng) per twig and compound ($\pm$ SE) is shown

Compound	RI	Flowering		Post-flowering			Early fruiting			Mid-fruiting			Late fruiting		
		Peach		Pear	P	Peach	Pear	P	Peach	Pear	P	Peach	Pear	P	Pear
<i>Alkanes</i>															
Heptane ^{a,s}	700	nd		nd	-	nd		-	nd		-	nd	220.3±16.7	-	358.4±47.4
Octane ^{a,s}	800	182.3±51.9		nd	*	nd		-	nd		-	nd	nd	-	nd
Decane ^{a,s}	1000	nd		nd	-	nd		-	nd		-	500.0±69.4	295.6±22.9	*	nd
<i>Alkenes</i>															
1-Heptene ^{a,s}	686	nd		nd		nd		-	nd		-	nd	322.1±41.6	-	822.9±173.2
1-Nonene ^{a,s}	888	34.2±9.2		37.5±4.9	ns	nd		-	nd		-	54.7±4.8	75.3±6.6	*	133.0±20.9
1-undecene ^{a,s}	1091	nd		nd	-	nd		-	nd		-	nd	nd	-	187.1±18.0
<i>Alcohols/phenols</i>															
3-Methyl-2-buten-1-ol ^{a,s}	724	nd		nd	-	nd		-	27.0±2.7		-	nd	nd	-	21.4±5.8
3-Methylbutan-1-ol ^{a,s}	728	nd		nd	-	nd		-	158.2±13.3		-	nd	nd	-	nd
2-Methylbutan-1-ol ^{a,s}	729	nd		nd	-	nd		-	nd		-	nd	nd	-	84.8±27.1
(Z)-3-Hexen-1-ol ^{a,s}	848	nd		103.1±36.4	-	nd		-	428.6±89.4		-	nd	227.2±77.0	*	355.7±143.7
Hexan-1-ol ^{a,s}	864	33.8±11.4		23.0±2.9	ns	nd		-	75.5±9.5		-	nd	32.3±5.3	-	55.5±7.2
Heptan-1-ol ^{a,s}	973	nd		nd		nd		-	nd		-	nd	nd	-	31.5±3.8
Phenol ^{a,s}	987	65.2±15.3		571.3±50.9	*	nd		-	nd		-	nd	trace	-	1553.7±133.5
Benzyl alcohol ^{a,s}	1035	69.0±12.1		120.7±8.8±27.1	*	276.1±27.1		-	nd		-	nd	241.7±27.7	-	183.3±19.0
1-Octanol ^{a,b,s}	1075	nd		nd	-	nd		-	nd		-	207.8±22.6	nd	-	trace
4-Methylphenol ^{a,s}	1084	nd		nd	-	nd		-	nd		-	nd	trace	-	133.0±13.3
1-Undecanol ^{a,s}	1381	4614.8±1348.7		3873.3±404.9	ns	nd		-	nd		-	nd	nd	-	3222.8±298.9
1-Tetradecanol ^{a,s}	1679	nd		542.7±104.3	-	nd		-	nd		-	nd	nd	-	nd
<i>Ketones</i>															
6-Methyl-5-hepten-2-one ^{a,s}	986	309.3±69.5		nd	-	nd		-	nd		-	nd	nd	-	nd
Acetophenone ^{a,s}	1062	140.6±78.5		nd	-	nd		-	nd		-	nd	nd	-	nd
α-Ionone ^{a,s}	1415	nd		nd	-	nd		-	nd		-	nd	368.0±29.1	-	222.9±23.5
Geranyl acetone ^{a,s}	1447	234.0±40.9		nd	-	577.2±57.0		-	nd		-	nd	nd	-	nd
<i>Aldehydes</i>															
Hexanal ^{a,s}	801	241.5±38.2		nd	-	nd		-	nd		-	nd	nd	-	193.5±18.0
(E)-Hex-2-enal ^{a,s}	845	nd		nd	-	nd		-	nd		-	nd	39.1±13.4	-	nd
Heptanal ^{a,s}	903	118.5±21.1		nd	-	nd		-	nd		-	nd	99.5±6.6	-	150.5±17.6
Benzaldehyde ^{a,s}	958	1663.5±274.2		nd	-	nd		-	nd		-	nd	764.5±136.5	-	907.2±125.9
Octanal ^{a,s}	1005	212.8±36.2		nd	-	nd		-	nd		-	nd	362.4±23.8	-	535.5±62.1
Nonanal ^{a,s}	1107	490.2±78.5		nd	-	nd		-	nd		-	nd	nd	-	3221.1±414.6
Lilac aldehyde A ^{a,g}	1138	nd		43.8±13.7	-	nd		-	nd		-	nd	nd	-	nd
Lilac aldehyde B ^{a,g}	1146	nd		57.5±20.3	-	nd		-	nd		-	nd	nd	-	nd
(E)-Non-2-enal ^{a,s}	1159	nd		nd	-	nd		-	nd		-	nd	nd	-	178.7±25.6
<i>Acids</i>															
Tetradecanoic acid	1771	75.4±25.9		nd	-	nd		-	nd		-	nd	nd	-	nd

Table 2 (continued)

Compound	RI	Flowering		Post-flowering		Early fruiting		Mid-fruiting		Late fruiting	
		Peach	Pear	P	Peach	Pear	P	Peach	Pear	P	Pear
Hexadecanoic acid ^{a,s}	1977	nd	nd	-	nd	nd	-	nd	430.9±211.2	-	nd
<i>Esters</i>											
Methyl 12-methylbutanoate ^{a,s}	770	nd	179.1±40.2	-	nd	nd	-	nd	nd	-	nd
(Z)-3-Hexen-1-yl acetate ^{a,s}	1008	45.2±6.8	606.1±169.9	*	3483.5±669.3	2382.0±575.8	ns	2132.9±354.9	1467.4±348.4	238.6±35.9	489.1±125.9
Isoamyl butyrate ^{a,s}	1059	nd	nd	-	nd	nd	-	nd	nd	-	167.8±21.9
Methyl salicylate ^{a,s}	1184	nd	87.1±15.5	-	319.4±27.7	477.4±71.0	ns	nd	1127.4±135.9	nd	136.5±23.2
Isobornyl acetate ^{a,s}	1280	nd	nd	-	nd	nd	-	nd	377.7±34.1	382.5±39.1	204.4±22.7
Methyl dodecanoate ^{a,s}	1525	nd	nd	-	nd	nd	-	nd	nd	-	916.4±196.0
Ethyl dodecanoate ^{a,s}	1595	nd	nd	-	nd	nd	-	nd	nd	-	1088.8±336.7
Benzyl benzoate ^{a,s}	1764	nd	nd	-	nd	nd	-	nd	607.0±113.8	-	325.6±88.3
<i>Terpenoids</i>											
α -Pinene ^{a,s}	926	nd	956.3±117.0	-	nd	479.3±103.8	-	nd	753.2±114.1	43.3±6.4	123.0±27.4
Camphene ^{a,s}	941	nd	nd	-	nd	nd	-	nd	nd	20.9±8.1	21.1±4.5
Sabinene ^{a,s}	966	nd	29.1±2.5	-	nd	nd	-	nd	nd	nd	nd
β -Pinene ^{a,s}	969	nd	112.3±19.4	-	nd	nd	-	nd	nd	30.6±2.4	43.4±7.0
3-Carene ^{a,s}	1002	nd	664.7±48.3	-	nd	nd	-	nd	nd	nd	29.7±4.1
Limonene ^{a,s}	1024	nd	544.8±44.1	-	nd	nd	-	nd	nd	150.3±15.8	130.6±12.7
(Z)-Ocimene ^{a,s}	1033	nd	nd	-	nd	2054.7±373.0	-	nd	1836.9±208.8	nd	nd
(E)-Ocimene	1045	nd	176.5±32.0	-	768.6±252.9	7288.8±1368.9	*	nd	8263.8±1214.2	674.1±542.6	405.9±146.9
(E,E)-2,6-Dimethyl-1,3,5,7-octatetraene	1126	nd	64.6±9.1	-	nd	873.4±193.8	-	nd	1565.4±242.8	114.3±22.8	nd
Citronellol ^{a,s}	1228	nd	nd	-	328.4±25.6	nd	-	nd	nd	nd	nd
β -Bourbonene ^{a,g}	1374	nd	173.9±30.2	-	nd	nd	-	nd	nd	318.5±29.9	nd
Caryophyllene	1405	nd	81.7±20.2	-	nd	nd	-	nd	nd	544.8±79.8	428.6±49.5
α -Farnesene	1504	nd	nd	-	nd	nd	-	nd	nd	trace	trace
Squalene ^{a,s}	2808	585.9±179.5	nd	-	225.1±18.8	230.4±8.4	ns	nd	nd	440.7±51.8	nd
<i>Aromatics</i>											
Methoxybenzene ^{a,s}	913	nd	nd	-	nd	82.4±9.4	-	nd	nd	21.7±4.4	34.4±4.7
Benzonitrile	986	trace	trace	-	trace	trace	-	trace	trace	trace	trace
p-Cymene ^{a,s}	1014	nd	nd	-	153.5±5.4	nd	-	nd	nd	nd	nd
Naphthalene ^{a,s}	1171	nd	nd	-	nd	nd	-	nd	nd	698.2±55.0	789.6±99.3

P-significance values based on one-way ANOVAs conducted for each single compound. * = $P < 0.05$, ns = non-significant, - = no pairwise comparison conducted

Ten samples used for all peach and pear phenological stages tested, except for peach flowering (6 samples used)

RI retention index

nd not detected

^a definitively identified

Source of standards: ^g from R. Clery (*Givaudan*, Dübendorf, Switzerland), ^s *Sigma-Aldrich* (Buchs, Switzerland)

Fig. 2 Comparison of the total volatile emissions, taken as the sum of the concentration (in ng) of all volatile compounds detected, ($\pm$ SE) from peach and pear twigs sampled from different phenological stages throughout an entire growing season. $N=10$ for all peach and pear phenological stages tested, except $N=6$ for peach flowering. P -values based on one-way ANOVAs conducted at each phenological stage: * = $P<0.05$; ** = $P<0.01$; ns = $P\geq 0.05$

peach flowering, pear mid-fruiting, and pear late fruiting, shared a number of compounds that were detected exclusively at these stages. These common volatiles comprise the aldehydes hexanal and nonanal (detected at both the peach flowering and pear late fruiting stages), and benzaldehyde, heptanal, and octanal (detected at the peach flowering, pear mid-fruiting, and pear late fruiting stages) (Table 2). Additional, shared volatile compounds detected in at least two of the three attractive stages comprise compounds from different chemical classes, including alkanes (heptane), alkenes (1-heptene), ketones (α -ionone), esters (benzyl benzoate), and aromatics (naphthalene), which were present at both mid- and late pear fruiting stages (Table 2). The volatile

blend from the pear early fruiting stage, although attractive, occupied a different position on the PCAs compared to the remaining attractive blends. The number of compounds detected in this attractive blend was exceptionally low, and, in particular, the aldehydes identified in the other attractive blends (although in most cases accounting for only 0.5 to 3 % of the total blends each (Online Resource 1)) were not detectable.

Similarities in Volatile Blends across Other Rosaceae Hosts of the Oriental Fruit Moth To detect common compounds in volatile blends from other secondary hosts of the oriental fruit moth, we carried out a PCA on a data set from the

Fig. 3 Score plot of the principal component analysis (PCA) of headspace volatiles from peach twigs (represented as circles) and pear twigs (represented as triangles) sampled at different phenological stages. Each single symbol represents a sample. $N=10$ for all peach and pear phenological stages tested, except $N=6$ for peach flowering. Equivalent plant phenological stages are represented with the same color. Stages attractive to oriental fruit moths represented as filled symbols and enclosed by open ovals. Non-attractive stages represented as open symbols

literature. It is the only suitable data set published and refers to volatiles from apple trees, chemically and behaviorally characterized throughout a full growing season. However, it uses a related tortricid fruit moth as a test species, the codling moth (Vallat and Dorn, 2005). PCA analysis segregated the overall compositions of these apple volatile blends according to their attraction to the codling moth (Online Resource 3). Blends from plant growth stages attractive to the codling moth clustered in two separate groups (Online Resource 3), and more than any other chemical class, aldehydes (hexanal, (*E*)-2-hexenal, heptanal, benzaldehyde, octanal, nonanal, and decanal), reflected exactly the same pattern (Online Resource 4).

Discussion

The changing volatile emissions *in situ*, which we characterized here for the first time for peach and pear trees over the season, coincide with the markedly changing behavioral responses of female oriental fruit moths. Whereas this oligophagous pest was attracted to volatiles from twigs of its primary host, peach, early and mid season, it was attracted only to its secondary host, pear, mid to late season. Interestingly, female-attracting growth stages of both host plant species share a common set of volatiles. From an ecological point of view, our results provide empirical evidence that some insects might deal with variations in plant volatile signals by responding to a specific set of compounds released by their host plants. From an applied point of view, our results pinpoint a group of compounds, which should be specifically considered when designing synthetic blends to be used for monitoring oriental fruit moth females in the field.

Female Attraction to Volatile Blends from the Primary and a Secondary Host Early in the season, only peach, not pear trees, offer a chemical environment that is attractive to the female moths, whereas later in the season female attraction shifts to volatiles emitted from pear fruit-bearing trees. Coinciding with our findings from the behavioral bioassay, a host shift of the oriental fruit moth from peach to pome fruit trees is documented for some regions across the globe, including southern Europe (Ivancich Gambaro 1978; Bovey 1979; Molnár 1992), where the strain used in this study was originally collected. The first two of its four to five generations are known to develop mainly on peach shoots (Ivancich Gambaro 1978; Pollini and Bariselli 1993). Correspondingly, it was in the first part of the season when the peach twigs attracted the female moths in our study. The third generation has been found to feed on both peach and pome fruits (Ivancich Gambaro 1978; Pollini and Bariselli 1993), and in accordance with this, we found that both

peach-fruit bearing and pear-fruit bearing twigs at the early fruiting stage were attractive to females. From the fourth generation on, the moth is solely found to develop on pome fruits in the field (Ivancich Gambaro 1978; Pollini and Bariselli 1993), and correspondingly, in our study, female moths were no longer attracted to peach towards the end of the season but remained attractive to pear for the remainder of the season. Taken together, these findings indicate that olfactory cues play a pivotal role in guiding mated oriental fruit moth females from the primary to the secondary host in the second part of the season, thus facilitating host switch. Further support for female olfactory attraction to the secondary hosts late in the season comes from two recent reports documenting female attraction to ripening apple fruits (Piñero and Dorn 2009) and to ripening and mature pear fruits (Lu et al. 2012).

Interestingly, volatiles emitted shortly after flowering from the primary host peach and from the secondary host pear (this study) or apple (Piñero and Dorn 2009) failed to attract mated oriental fruit moth females. Similarly, volatiles emitted from apple failed to attract codling moth females at the petal fall stage (Vallat and Dorn 2005). This consistent lack of attraction of these tortricid moths to their host trees shortly after bloom suggests an effective volatile-based, natural plant defense strategy (Vallat and Dorn 2005) against these key fruit herbivores at this crucial period for fruit development.

Common Volatiles and Attraction of Oriental Fruit Moth Females to its Hosts Although the volatile blends detected from peach and pear twigs across the season differed both qualitatively and quantitatively, attractive blends shared some common constituents, including four C₆–C₉ aliphatic aldehydes, i.e., hexanal, heptanal, octanal, and nonanal, and an aromatic aldehyde, benzaldehyde. These five compounds were emitted by the attractive peach and pear growth stages at similar relative ratios. Female oriental fruit moths have been shown to tolerate certain modulations of the ratios among the essential constituents in a volatile blend without losing their positive behavioral response (Najar-Rodriguez et al. 2010, 2011). Thus, our findings suggest that these aldehydes might essentially contribute to the successful peach and pear host location by oriental fruit moth females. Interestingly, these aldehydes were always present as minor constituents in the volatile blends that were attractive to the oriental fruit moth females, supporting the assumption that, for this species, minor components are essential for optimal insect attraction (Piñero and Dorn 2007).

Apple blends previously collected *in situ* at selected points across a growing season also contained the five aldehydes mentioned above (Vallat and Dorn 2005). Although these volatile compounds were present across phenological stages that were either attractive or non-attractive to codling moth females, attractive stages shared

similar relative concentrations of these compounds (Vallat and Dorn 2005). Furthermore, aldehydes were the chemical group that contributed most to the differentiation of the apple blends according to their attraction to the moths. The codling moth also is an oligophagous species whose larvae feed primarily on Rosaceae plants, with apples and pears being its main hosts (Wearing et al. 2001; Witzgall et al. 2008). Thus, aldehydes also might play a role in the attraction of the codling moth, and possibly of other oligophagous fruit moths of the same tribe Grapholitini, to Rosacean hosts.

Moving from basic ecology with natural volatile blends to application-oriented research with synthetic plant-derived blends, a recent meta-analysis underlined the importance of aldehydes, which appeared to be the most behaviorally effective chemical group in attracting female insects (Szendrei and Rodriguez-Saona 2010). For female tortricid moths, nonanal and benzaldehyde elicited a behavioral effect in the codling moth (Vallat and Dorn 2005), and one of these aldehydes each was included in synthetic blends recently reported to attract oriental fruit moth females (Piñero and Dorn 2007; Lu et al. 2012). Starting from volatile blends released by shoots of potted peach trees (Natale et al. 2003), benzaldehyde was among the essential constituents yielding an attractive synthetic blend (Piñero and Dorn 2007; Piñero et al. 2008). Starting from volatile blends released by detached ripening and detached mature pear fruits, nonanal also was found to be an essential constituent of attractive synthetic blends (Lu et al. 2012). Thus, these findings underline the importance of the presence of aldehydes in olfactory stimuli eliciting female attraction in the oriental fruit moth. They further indicate that various combinations of an aldehyde and volatile compounds from other chemical classes hold the potential to yield bioactive blends. Short-chain aldehydes might rapidly oxidize under ambient conditions (Larkin 1990; Marsden et al. 2008), suggesting that the addition of antioxidative agents might be required to increase the long-lasting effect of these aldehydes in synthetic blends. Developing different synthetic blends will be advantageous for future monitoring of the oriental fruit moth, as efficacy of synthetic blends can be context-specific, i.e., higher or lower depending on the host plant species in the orchard (Light et al. 2001; Light and Knight 2005; Lu et al. 2012).

In summary, we recommend paying particular attention to the aldehydes described here when designing female attractants for the oriental fruit moth. Furthermore, we suggest to consider different blend options containing plant-derived aldehydes for optimal results across different plant species ecosystems, and to explore multi-sensorial tools for field monitoring of this invasive pest.

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