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Attraction of Female Grapevine Moth to Common and Specific Olfactory Cues from 2 Host Plants

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Abstract

In herbivorous insects with more than 1 host plant, attraction to host odor could conceptually be mediated by common compounds, by specific compounds released by each plant or by combinations of common and specific compounds. We have compared the attraction of female grapevine moth, *Lobesia botrana*, with specific and common (shared) odors from 2 different plants: a wild host (*Daphne gnidium*) and a recently colonized host (*Vitis vinifera*). Odor blends eliciting female attraction to *V. vinifera* have previously been identified. In this study, olfactory cues from *D. gnidium* were identified by electroantennographic detection and chemical analysis. The attraction of mated females to synthetic odor blends was then tested in a wind tunnel bioassay. Female attraction was elicited by a blend of compounds released by both from *D. gnidium* and *V. vinifera* and by 2 blends with the compounds released specifically from each host. However, more complete odor blends of the 2 plants elicited stronger attraction. The common compounds in combination with the specific compounds of *D. gnidium* were the most attractive blend. This blend was tested with the common compounds presented both in the ratio emitted by *D. gnidium* and by *V. vinifera*, but there was no difference in female attraction. Our findings suggest that specific as well as common plant odor cues play a role in *L. botrana* host recognition and that there is plasticity in attraction to partial blends. The results are discussed in relation to mechanisms behind host odor recognition and the evolution of insect–plant associations.

Key words: *Daphne gnidium*, electrophysiology, host recognition, insect–plant interaction, *Lobesia botrana*, plant volatiles, *Vitis vinifera*

Introduction

Phytophagous insects have in the course of evolution become associated with a particular plant or a constellation of plant species to the exclusion of others. Heritable changes in insects' plant recognition mechanism are proposed as the primary event in the evolution of insect–plant associations (Schoonhoven et al. 2005 and references therein). The most important source of information in this recognition process is presumably chemical ad physical plant cues (Ehrlich and Raven 1964; Städler 1992; Renwick and Chew 1994; Becerra 1997; Menken and Roessingh 1998). Evolutionary change in insects' host-plant range is suggested to be mediated by chemical similarity between old and new hosts or even restricted to plants with phytochemical similarity because of lack of adequate selectable variation (Jermy 1993; Menken and Roessingh 1998; Agosta 2006; Rajapakse et al. 2006).

Many phytophagous insects are attracted by host-plant odor, but the mechanism behind this chemical recognition is not understood. Plant odors are complex blends, composed of many compounds of diverse chemical structures. The compounds have different systematic distributions; some are common for many different plant genera, whereas others are taxonomically restricted (Knudsen et al. 1993). Fraenkel (1959) suggested that insects use specific compounds for host-plant recognition. Visser (1986) reasoned that, in addition to specific plant odor components, the ratio between general compounds offered the specificity needed. Recently, Bruce et al. (2005) argued that the ratio between

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ubiquitous plant volatiles should be seen as the most prevalent mechanism mediating host-plant recognition.

In insects with nondispersive larval stages, the host plant (i.e., larval source of food) is selected by the ovipositing female. The grapevine moth, *Lobesia botrana*, is polyphagous on a wide range of plant families (Stoeva 1982). Females are attracted to and lay their eggs on several plants, but flax-leaved daphne, *Daphne gnidium*, is among the wild hosts (Thiery and Moreau 2005 and reference therein). Another host plant is grapevine, *Vitis vinifera*, on which *L. botrana* is a severe pest. The adaptation of *L. botrana* to grapevine is considered to be recent. Intense damage in vineyards has been recorded only since the beginning of the 20th century (Marchal 1912; Balachowsky and Mesnil 1935).

A recent wind tunnel study has shown attraction of mated *L. botrana* females to a synthetic blend of 10 compounds identified in headspace from *V. vinifiera* grape clusters (Tasin, Backman, Bengtsson, Varela, et al. 2006). These 10 compounds elicited electrophysiological responses in moth antennae and are therefore likely to be involved in odor recognition of this host. By identifying compounds in the headspace of *D. gnidium*, we could in our study compare female attraction with synthetic odor cues from a wild host (*D. gnidium*) with the volatiles released by a recently colonized host-plant species (*V. vinifera*). Conceptually, attraction could be mediated by the ratio of common compounds, by specific compounds, or by combinatorial blends with partly interchangeable compounds and ratios. In this context, common and specific are referred to the compounds released only by the host plants of *L. botrana*. Previous studies have analyzed phylogenies of phytophagous insects and taxonomy/chemical similarity of host plants to establish the importance of plant chemistry on herbivore host shifts (Agosta 2006 and references therein). We argue that understanding the mechanism behind host-plant recognition is another important key to reveal the role of plant chemistry in the evolution of insect–plant interactions.

Materials and methods

Insects

A culture of *L. botrana* was maintained in the laboratory on a semiartificial diet. Wild larvae were collected from grapevine plants and yearly introduced in the colony to avoid inbreeding. The rearing was maintained under a reverse 18:6 h L:D photoperiod, with scotophases from 12:00 to 18:00. Insects of both sexes emerged in Plexiglass cages and mated. Attraction of mated females to synthetic odors was tested in the wind tunnel using 2- to 3-day-old females. Only females laying eggs were used in the bioassay.

Chemicals

The synthetic compounds used were 4,8-dimethyl-1,3(*E*),7-nonatriene (89% purity; a gift from Prof W. Francke, Hamburg University, Germany), 2-ethyl-1-hexanol (99.6%; Sigma–Aldrich, Milan, Italy), (*E*)-β-caryophyllene (82.9%; Sigma–Aldrich), methyl salicylate (99%; Sigma–Aldrich), 1-octen-3-ol (octenol; 98%; Akros, NJ), linalool oxide furanoids (97%; racemic mixture; Fluka, Buchs, Switzerland), linalool oxide pyranoids (98%; Nippon Terpene, Tokyo, Japan), (±)-linalool (97%; Fluka), (*E*)-β-farnesene (92.4%; Bedoukian Research Inc., Danbury, United States), and (*E*,*E*)-α-farnesene (97.3%; Firmenich, Geneva, Switzerland), (*Z*)-3-hexenyl benzoate (97%; Sigma–Aldrich), benzothiazole (95%; Sigma–Aldrich), and ethyl benzoate (99%; Fluka).

Collection of volatiles

Daphne gnidium plants were collected from a field in Fiumicino, Italy. Headspace collections were made from cut branches with leaves and from cut shoots with both leaves and flowering blossoms (500 g). The plant material was placed in a polyacetate cooking bag (45 × 55 cm, Toppits, Melitta, Sweden), and the bag was sealed around the plant stems with a polypropylene rope. Air was drawn through the bag at 150 mL/min and over an adsorbing filter (50-mg Porapak Q cartridge, Sigma–Aldrich) for 24 h (Tasin et al. 2005). The adsorbing filter was eluted with 300 μL of redistilled hexane (Sigma–Aldrich). Samples were sealed in glass micropipettes and stored at –18 °C until use. Three collections from each phenological stage were pooled before analysis.

Electrophysiological and chemical analysis

Headspace volatiles were identified on a Perkin–Elmer Auto-System XL gas chromatograph (60-m × 0.32-mm × 0.5-μm DB-Wax fused silica column, J&W Scientific Inc., Folsom, CA; temperature programed from 60 °C [3 min] at 8 °C/min to 220 °C [20 min]) coupled to a Perkin–Elmer TurbomassGold high-resolution mass spectrometer with an ionization potential of 70 eV. Helium (1.2 mL/min) was used as carrier gas. Splitless injection (2 μL) was used. The temperature of the injector was 250 °C (Tasin, Backman, Bengtsson, Varela et al. 2006). Transfer line was set at 220 °C under partial vacuum. These temperatures were chosen according to the properties of both analytes and column. All compounds were identified by comparison with mass spectra and retention times of the respective synthetic standards on 2 columns (gas chromatography–mass spectrometry [GC–MS] and gas chromatography with electroantennographic detection [GC–EAD], see below).

GC–EAD

GC–EAD analyses were done on an HP 5890 GC (30 m × 0.32 mm × 0.5-μm HP-INNOWax column, Agilent, Palo Alto, CA) programed from 60 °C (3-min hold) at 8 °C/min to 220 °C (10-min hold) coupled with a Syntech electroantennogram detector (Hilversum, The Netherlands). The antenna was cut off from the head of the moth and suspended between 2 glass electrodes filled with Beadle-Ephrussi Ringer solution (Bjostad 1998.). Electrodes were connected to a high-impedance DC amplifier (Syntech, The Netherlands). The mounted antennae

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were placed into the air stream carrying the volatiles eluting from the GC. Five successful recordings with the same sample of volatiles were evaluated. Compounds eliciting responses in all the 5 runs were regarded as active. To confirm the antennal activity of identified volatiles, GC–EAD runs were performed with a mixture of synthetic plant compounds (Table 1).

Wind tunnel assay

Behavioral assays were done in a wind tunnel earlier described by Tasin et al. (2005). Synthetic volatile compounds were delivered by means of a piezoelectric sprayer (El-Sayed et al. 1999; Gödde et al. 1999). Dilutions were fed by a microdialysis high-precision pump driven syringe, at a rate of 10 μL/min, into a glass capillary with an elongated tip. Vibration of the capillary at ultrasonic frequency (ca. 100 kHz), by means of a piezoceramic disc, dispersed the solution into microdroplets that evaporated within few centimeters from the capillary tip.

Because no obvious interaction was observed between moths within a tested group, as indicated earlier by Tasin et al. (2005) and by Masante-Roca et al. (2007), we tested moths only in groups. One hour before the end of the photophase, batches of 10 mated females were placed in cylindrical plastic containers (20-cm × 12-cm ID). The cylinders were closed with gauze on 1 side and with a solid lid on the other. A cylinder was placed on a holder at the downwind end of the wind tunnel, with the gauze facing the downwind end of the tunnel. The lid was then removed, allowing the moths to fly spontaneously upwind. Moth behavior was scored 1) for upwind oriented flight in the center of the tunnel and 2) for arrival at the odor source within 5 cm, after upwind oriented flight over 180 cm. Females responding to the stimulus were removed from the tunnel. The behavior of each batch was recorded for 20 min. Two batches of females were tested per day. Attraction to each odor blend was tested with 4 batches of females on different days. Females were used only once in the bioassay.

Synthetic blends

Compounds eliciting antennal responses in female *L. botrana* were formulated in blends for the wind tunnel experiments.

Table 1 Chemical and Electrophysiological Analysis of *D. gnidium* headspace

Compound	Branch with leaves[a] (%)	Flowers and leaves[a] (%)	EAD activity[b] (μV; N = 4)
Monoterpenes			
(E)-Linalool oxide (furanoid)	—	2.4	20 ± 16
(Z)-Linalool oxide (furanoid)	—	6.8	23 ± 5
Linalool	1.7	24.2	58 ± 17
Unidentified 1	0.5	0.8	9 ± 3
(*E*)-Linalool oxide (pyranoid)	—	28.4	10 ± 12
(*Z*)-Linalool oxide (pyranoid)	—	12.4	38 ± 5
Unidentifed 3	trace	1.2	10 ± 8
Sesquiterpenes			
β-Caryophyllene	1.2	3.3	6 ± 5
(*E,E*)-α-Farnesene	5.2	1.9	19 ± 3
Unidentified 2	2.7	0.4	—
Benzenoids			
Ethyl benzoate	81.2	16.0	8 ± 5
Methyl salicylate	5.7	1.3	48 ± 10
Benzothiazole	0.22	0.1	10 ± 11
(*Z*)-3-Hexenyl benzoate	1.6	0.6	30 ± 8
Unknown			
Unidentifed 4	—	0.1	5 ± 6

Only compounds eliciting antennal responses are shown. Headspace were collected on Porapak Q filters and subsequently desorbed by solvent.
[a]Proportions based on total amount of compounds eliciting antennal responses. The average amount of linalool collected from 500 g of shoots with leaves and from 500 g of shoots with leaves and flowers were 0.32 and 6.2 μg/h respectively.
[b]Antennal response (±SD) to flowers and leaves collection analysed by coupled gas-chromatography/electroantennodetection. Identity of compounds was confirmed by comparison of mass spectrum and retention time with those of the respective synthetic standards. The antennal response of unidentified 2 was not quantified due to co-elution with methyl salicylate.

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Five blends of synthetic compounds emitted by *D. gnidium* and *V. vinifera* were prepared in ratios given as sprayed release in Figure 2. A common blend (C) was formulated to contain the 6 volatiles identified in both the headspace of *D. gnidium* shoots and flowers and in the headspace of unripe *V. vinifera* grapes. In this blend, compounds were added according to the ratio measured in *V. vinifera* headspace (data from Tasin, Backman, Bengtsson, Ioriatti, et al. 2006). Two blends were prepared with compounds identified exclusively in respective plant: a daphne-specific (DS) blends with identified compounds added in the relative ratio released by *D. gnidium* shoots and flowers (see Table 1) and a grape-specific (GS) blend with compounds added in the ratio released by *V. vinifera* grapes (data from Tasin, Backman, Bengtsson, Ioriatti, et al. 2006). A daphne blend (DS + C) was formulated by adding the DS blend to the common blend and a grape mimic blend (GS + C) by adding the GS blend to the common blend. In addition, a corrected daphne blend (DS + C′) was prepared where the compounds present in blend C were added according to the ratios identified in headspace from *D. gnidium* flowers plus leaves (see Table 1). The total amount of compounds was kept the same in blend DS + C and DS + C′. All synthetic compounds were diluted in redistilled ethanol (99% purity), and this solvent was also used as blank stimuli.

Statistics

The number of insects orienting to the plume and landing at the source was submitted to a generalized linear model using R software (R Development CoreTeam 2005). Treatments were separated by contrasts.

Results

Electrophysiological and chemical analysis

Fifteen compounds in headspace collections from *D. gnidium* elicited antennal responses in mated *L. botrana* females (Figure 1). Identification of these compounds (Table 1) showed 10 volatiles released by both leaves and flowers. Four monoterpenes were only present in headspace collected from a shoot with flowers (*E*- and *Z*-isomers of linalool oxide furanoid and pyranoid). The most frequent compound eliciting an antennal response was ethyl benzoate, making up to 16% of the identified headspace from *D. gnidium* shoot with flowers and leaves and 81% from leaves alone. Response to an *E,E*-α-farnesene was detected in both leaves and leaves with flowers. In GC–EAD analysis of 10 ng of synthetic compounds, antennal responses were strongest to (*Z*)-3-hexenyl benzoate, benzothiazole, and (*Z*)-linalool oxide pyranoid (Table 1). Four compounds eliciting antennal responses were not identified (Table 1).

The identified compounds in *D. gnidium* flower headspace were compared with electrophysiologically active compounds previously identified in volatile release from *V. vinifera* (Tasin, Backman, Bengtsson, Ioriatti, et al. 2006). Six identified compounds were released from both plant species, 5 compounds were found exclusively in *D. gnidium*, and 4 compounds were specific for *V. vinifera* (see Figure 2).

Bioassay

The attraction of mated *L. botrana* females to synthetic blends of plant volatile compounds was studied in the wind tunnel.

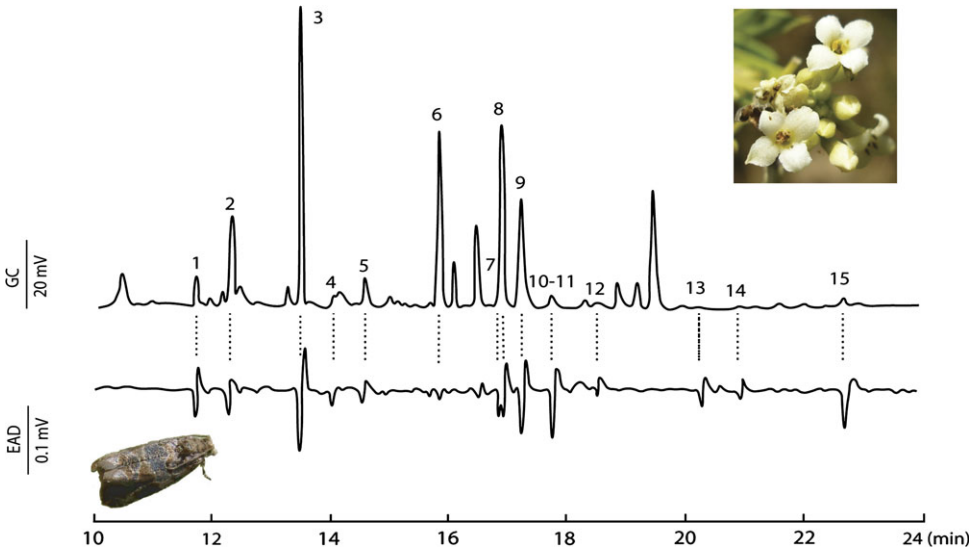

Figure 1 Simultaneously recorded GC–EAD analysis of volatiles collected from full blossoming flowers and leaves of *Daphne gnidium*. The upper trace represents Flame Ionization Detector response and the lower the antennal responses (EAD) of female *Lobesia botrana*. Activity was found to the following compounds: 1) (*E*)-linalool oxide furanoids, 2) (*Z*)-linalool oxide furanoids, 3) (±)-linalool, 4) unidentified, 5) (*E*)-β-caryophyllene, 6) ethyl benzoate, 7) (*E*,*E*)-α-farnesene, 8) (*E*)-linalool oxide pyranoid, 9) (*Z*)-linalool oxide pyranoid, 10) methyl salicylate, 11) unidentified, 12) unidentified, 13) benzothiazole, 14) unidentified, 15) (*Z*)-3-hexenyl benzoate.

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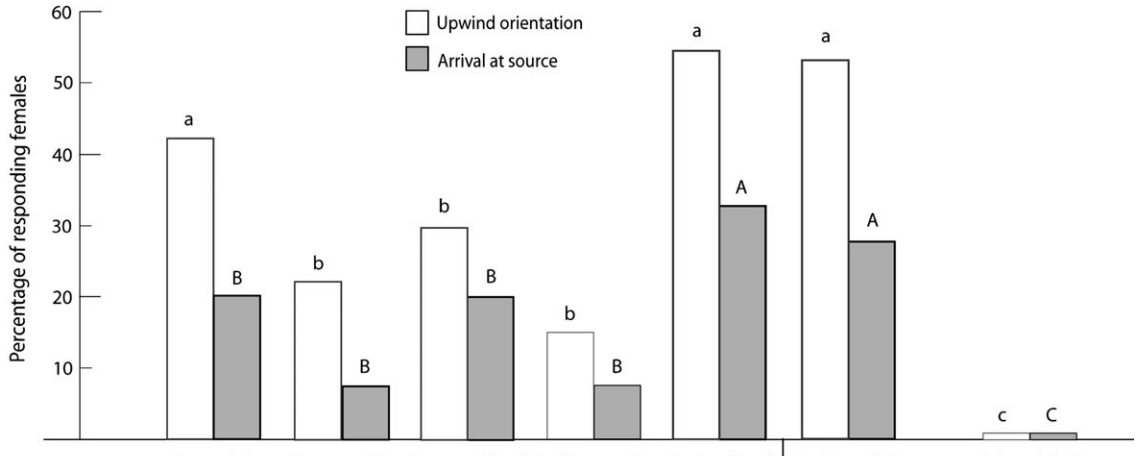

compound	release rate (ng/h)	%	Grape mimic (GS + C)	Grape specific (GS)	Common blend (C)	Daphne specific (DS)	Daphne blend (DS + C)	Daphne mimic (GS + C′)	%
(E)-DMNT	26.9	76.9	●	●	○	○	○	○	
(E)-linalool oxide (furanoid)	2.7	7.7	●	○	●	○	●	●	28.8
1-octen-3-ol	6.0	17.1	●	●	○	○	○	○	
(Z)-linalool oxide (furanoid)	2.7	7.7	●	○	●	○	●	●	28.8
2-ethyl-hexan-1-ol	2.9	8.3	●	●	○	○	○	○	
linalool	2.6	7.4	●	○	●	○	●	●	100.0
(E)-β-caryophyllene	35.0	100.0	●	○	●	○	●	●	13.9
(E)-β-farnesene	3.6	10.3	●	●	○	○	○	○	
(E,E)-α-farnesene	24.0	68.6	●	○	●	○	●	●	7.2
methyl salicylate	1.6	4.6	●	○	●	○	●	●	5.6
ethyl benzoate	12.7	36.3	○	○	○	●	●	●	67.8
(E)-linalool oxide (pyranoid)	19.7	56.3	○	○	○	●	●	●	88.2
(Z)-linalool-oxide (pyranoid)	6.3	18.0	○	○	○	●	●	●	28.3
benzothiazole	0.1	0.3	○	○	○	●	●	●	0.5
(Z)-3-hexenyl benzoate	0.5	1.4	○	○	○	●	●	●	2.5

Figure 2 Attraction of mated *Lobesia botrana* females in the wind tunnel to synthetic blends of plant compounds identified from *Daphne gnidium* headspace. Females were scored for upwind orientation (white bars) and arrival at the source within 5 cm (black bars). Bars capped with the same letter are not statistically different (Generalized Linear Model; upwind orientation $F_{6,28} = 10.72$; $P < 0.001$, source contact $F_{6,28} = 6.3$; $P = 0.003$). No female upwind attraction was recorded to blank stimulus.

Identified compounds from *D. gnidium* and *V. vinifera* eliciting female antennal responses were used as stimuli. Female upwind orientation and arrival at the source within 5 cm ($n = 40$) were compared between the blends: DS, GS, common compounds (C), DS + C, and GS + C. All these 5 blends stimulated female upwind orientation flight and close range source contact (Figure 2). More females showed upwind orientation flight to the more complex blends, DS + C and GS + C, than to the incomplete blends (Figure 2). The DS + C blend, with all the 11 compounds identified in *D. gnidium* headspace, was the most powerful attractant. This blend elicited 55% of the females to lock on to the odor plume and 33% to arrive at the source within 5 cm. The number of females arriving at the DS + C blend was significantly higher than to the GS + C blend or the incomplete blends (Figure 2).

In the common blend (C), compounds were released in the ratio identified from *V. vinifera*. As a consequence, the compound ratio in the DS + C blend did not reflect the ratio emitted by *D. gnidium*. Therefore, female attraction was tested to an additional blend (DS + C′), where the compounds present in blend C were added according to the ratios identified in *D. gnidium* headspace. The most dominating compounds of blend DS + C were (*E*)-β-caryophyllene (35%), (*E*)-linalool oxide pyranoid (20%) and (*E*,*E*)-α-farnesene (24%). Blend DS + C′ was dominated by linalool (29%), (*E*)-linalool oxide pyranoid (26%), and ethyl benzoate (20%). However, there were no differences in number of females locking on to the plume or arriving at the source between these 2 blends. No female attraction to blank stimuli (ethanol) was observed.

Discussion

The compounds in *V. vinifera* and *D. gnidium* headspace that elicited antennal responses in female *L. botrana* antennae were only partly overlapping. This result indicates that *L. botrana* has the physiological capacity to detect both

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common and specific compounds from 2 different host plants. Our wind tunnel bioassay showed that mated *L. botrana* females were attracted to synthetic blends with only common or only specific compounds from 2 host plants. Still higher attraction was obtained when the specific compounds were added to the blend of common compounds (Figure 2). The mechanism behind olfactory host-plant recognition is under debate. Fraenkel (1959) suggested that insects use specific compounds for host-plant recognition. Visser (1986) argued that, in addition to specific plant odor components, the ratio between general compounds offered the specificity needed. Bruce et al. (2005) argued that the ratio between ubiquitous plant volatiles should be seen as the most prevalent mechanism mediating host-plant recognition. The compounds classified as specific for *V. vinifera* and *D. gnidium* in our study are by no means unique for these plants. However, the compounds in the DS blend have not been identified in other studies of grapevine headspace (Buchbauer et al. 1994; Rosillo et al. 1999; Sonego et al. 2002; Boido et al. 2003; Tasin et al. 2005; Cha et al. 2008). Because these daphne compounds increase female attraction, general volatiles released by all plants cannot be the only odor cues mediating host recognition in *L. botrana*. Similarly, moths of the closely related species *Cydia pomonella* are attracted to a single compound that is abundant in the headspace of one host plant but is not identified in the headspace of another (Light et al. 2001; Landolt et al. 2007). Host races of *Rhagoletis pomonella* flies have been shown to be mostly attracted to unique mixtures of volatiles from their natal hosts, where compounds from the races′ different hosts were only partly overlapping (Zhang et al. 1999; Nojima, Linn, Morris, et al. 2003; Nojima, Linn, and Roelof 2003). In addition, a synergistic effect between host-specific compounds and general green leaf odors is recognized (see, e.g., Guerin et al. 1983; Pinero and Dorn 2007). Behavioral results therefore argue against a recognition mechanism primarily based on general odors.

As argued by Visser (1986) and Bruce et al. (2005), the ratio between released plant compounds offers specific recognition cues. This has been shown in attraction of the Colorado potato beetle, *Leptinotarsa decemlineata*, where the ratio between general green leaf volatiles was important for the behavior (Visser and Avé 1978). The attraction of *L. botrana* females to 3 synthetic grapevine volatiles has previously also been shown to be ratio dependent (Tasin, Backman, Bengtsson, Ioriatti, et al. 2006). In contrast to these results, female attraction to the ratio-corrected daphne mimic and the daphne mimic with a disparate ratio did not differ in our study (Figure 2). A possible explanation is that compound ratio is less important for host recognition in a more complex host mimic than in a 3-component blend. In nature, background odor from plants surrounding the host will interfere with the recognition process. Perhaps is the number of compounds used for host recognition in phytophagous insects adjusted continuously to minimize the signal-to-noise ratio? However, the ratio of shared compounds in the uncorrected daphne blend was copied from the grapevine mimic. It is possible that the different sets of ratios from the 2 hosts are interchangeable but that the same compounds in a third ratio would not attract the females. To study the behavioral role of blend proportion and blend complexity with compounds shared by host and non–host plants would thus be of great interest in *L. botrana*.

Grapevine has recently been included in the host range of *L. botrana*, whereas *D. gnidium* is considered among the wild host plant (Marchal 1912; Balachowsky and Mesnil 1935; Stoeva 1982; Thiery and Moreau 2005). Host transfer by means of colonization is suggested as the predominant mode for evolution of host associations in phytophagous insects (Futuyma and Slatkin 1983; Miller and Wenzel 1995). In this process, heritable change in the insects' plant recognition is proposed as the primary event (Städler 1992; Renwick and Chew 1994). New plants might be incorporated in the host range because the compounds important for attraction are also present in the new one (Menken and Roessingh 1998). The compounds from *V. vinifera* and *D. gnidium* were partly overlapping, but exclusive compounds from either host increased the attraction to the blend of shared compounds. However, mated *L. botrana* females showed highest attraction to the most complete odor mimic of the supposed wild host (Figure 2). From an evolutionary point of view, it would be interesting to know if the compounds identified in *V. vinifera* but not in *D. gnidium* are released from other hosts of *L. botrana*.

The plasticity in host recognition/acceptance is suggested to be very important in insect speciation (Dethier 1982; Jermy 1984; Jaenike and Papaj 1992; Berlocher and Feder 2002; Linn et al. 2003, 2005; Tasin et al. 2007). Our results suggest a complex recognition system in a polyphagous herbivore. Mated *L. botrana* females were attracted not only to common compounds from 2 hosts but also to the specific compounds of either host. Highest attraction was elicited when the specific compounds were added to the common ones. This observed plasticity might, in combination with plant abundance and larval suitability, be very important for the forming of new insect–plant interactions.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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