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Plant Volatiles Enhance Behavioral Responses of Grapevine Moth Males, *Lobesia botrana* to Sex Pheromone

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Abstract Plant volatiles play an important role in the lives of phytophagous insects, by guiding them to oviposition, feeding and mating sites. We tested the effects of different host-plant volatiles on attraction of *Lobesia botrana* males to the female-produced sex pheromone, in a wind tunnel. Addition of volatile emissions from grapevines or individual plant volatiles to pheromone increased the behavioral responses of *L. botrana* males over those to pheromone alone. At a low release rate (under-dosed) of pheromone, addition of (*E*)-β-caryophyllene, (*Z*)-3-hexenyl acetate, 1-hexanol, or 1-octen-3-ol increased all behavioral responses, from activation to pheromone source contact, while addition of (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-farnesene, (*Z*)-3-hexenol, or methyl salicylate affected only the initial behavioral responses. Dose–response experiments suggested an optimal release ratio of 1:1000 (sex pheromone: host plant volatile). Our results highlight the role of plant volatiles in the sensory ecology of *L. botrana*.

Keywords Pheromone response · Kairomone · Wind tunnel · *Vitis vinifera* · European grapevine moth · Tortricidae · Lepidoptera

Introduction

Host-plant volatiles can enhance both pheromone production and behavioral responses to pheromones in phytophagous insects (reviewed by Landolt and Phillips, 1997). Host-plant attractants have been extensively studied for *Lobesia botrana* (Lepidoptera: Tortricidae) females (Tasin et al., 2006, 2007; Anfora et al., 2009), but work focusing on the effects of plant volatiles on the behavior of *L. botrana* males is sparse. *L. botrana* uses a female-produced sex pheromone for mate finding. During the scotophase, male moths compete heavily for females, with males arriving at the calling female first most likely to achieve mating success. We hypothesized that host-plant volatiles could signal rendezvous sites by enhancing male behavioral responses to pheromone in *L. botrana*. For this, we studied the effects of host-plant volatiles on the attractiveness of the sex pheromone, and explored dose–re-sponse characteristics of behaviorally active plant volatiles.

Methods and Materials

Insects

Grapevine moth larvae were reared on artificial diet under a 16 L:8D photoperiod, 65% RH and 25°C during photophase, and 85% RH and 18°C during scotophase, as described in von Arx et al. (2011).

Test Compounds

Pheromone components (isomeric purities) used were: (*E,Z*)-7,9-dodecadienyl acetate (>97%), (*E,Z*)-7,9-dodecadien-1-ol (>94%), and (*Z*)-9-dodecenyl acetate (99.9%, Plant Research International, Wageningen, Netherlands), admixed at a ratio of 100:20:5 (Arn et al., 1988). Plant compounds tested were those shown to elicit antennal receptor cell responses in *L. botrana* males, or that had been identified as key semiochemicals in behavioral assays with *L. botrana* females (Tasin et al., 2007, von Arx et al., 2011): 1-hexanol,

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(*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, (*R*)-(+)-limonene, (*E*)-β-caryophyllene, (+)-terpinen-4-ol, and methyl salicylate (all >94%, Sigma-Aldrich, Buchs, Switzerland), 1-octen-3-ol (>97%, Merck, Munich, Germany), (*E*)-β-farnesene (>90%, Bedoukian Research, Danbury, CT, USA) and (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT, ~94%, Givaudan, Dübendorf, Switzerland). Ethanol (pro Analysis, Merck, Munich, Germany) was used as solvent.

Wind Tunnel

Experiments were carried out in a wind tunnel, equipped with a piezo sprayer (see von Arx et al., 2011 for the setup, dimensions and experimental conditions). Dose–response trials were performed with the ternary pheromone blend (0.01–100,000 pg.min^{-1}; data not shown) to establish optimal (1.0 pg.min^{-1}) and under-dosed pheromone release rates (0.1 pg.min^{-1}). In our first trial, pheromone (1.0 pg.min^{-1}) was presented in a grapevine volatile background (8 vine plants, *Vitis vinifera* cv. Solaris, placed between the charcoal filters and the perforated metal grid at the upwind end of the wind tunnel; plant height: 45±5 cm, wet mass of leaves: 20±2 g/plant). Then, we tested responses of males to single plant chemicals at a suboptimal dose (0.1 pg.min^{-1}) of pheromone. The suboptimal dose was used because behavioral responses of male *L. botrana*, in terms of takeoff (78%) and upwind flight (59%), were already high to the optimal pheromone rate (1.0 pg.min^{-1}) in the absence of plant volatiles. Single compounds were tested first at 100 pg.min^{-1}. Then, the 6 compounds that showed the strongest effect when added to pheromone were tested at additional release rates of 1.0 pg.min^{-1} and 10,000 pg.min^{-1}. Three-day-old males were presented individually to the odor source and scored for: (1) activation, (2) takeoff, (3) upwind flight, (4) passing the midline of the wind tunnel, and (5) odor source contact.

Statistical Analysis

Treatments were compared by fitting a generalized linear model (GLM) with a logit link function (logistic regression) to the behavioral responses, assumed to be binomially distributed (R 2.11.1). Analysis of deviance, based on the asymptotic $\chi 2$ distribution, was used to test whether flight responses were dependent on odor source ($P<0.05$), with treatment differences separated by Tukey- or Dunnett-contrasts.

Results

Grapevine volatile emissions increased the attractiveness of the optimal pheromone dose, with more males engaging in upwind flight and contacting the odor source to the combined odors than to the optimal pheromone dose alone (Table 1). The ten host-plant volatiles, tested individually at a release rate of 100 pg.min^{-1}, influenced flight behavior of grapevine moth males to under-dosed pheromone as follows: (1) (*E*)-β-caryophyllene, (*Z*)-3-hexenyl acetate, 1-hexanol, or 1-octen-3-ol increased the number of males undertaking all behavioral elements, (2) DMNT, (*E*)-β-farnesene, (*Z*)-3-hexenol, or methyl salicylate enhanced only initial behavioral elements, and (3) (*R*)-(+)-limonene or (+)-terpinen-4-ol had no effect on the attractiveness of the pheromone blend.

Host-plant volatiles also increased the attractiveness of under-dosed pheromone when tested at 1.0 and 10,000 pg. min^{-1} (Table 1). At 10,000 pg.min^{-1}, 1-hexanol or 1-octen-3-ol increased activation and take off rates, while (*E*)-β-caryophyllene enhanced the full behavioral repertoire to pheromone. At 1.0 pg.min, 1-octen-3-ol enhanced activation, while (*E*)-β-caryophyllene increased both activation and take-off. The amount of plant volatile released influenced male behavior, in general, with the number of moths displaying a given behavioral element higher at 100 pg.min than at 1.0 or 10,000 pg.min.

Discussion

We provide evidence that the perception of plant volatiles facilitates mate finding in *L. botrana*; plant volatiles admixed in a 1000:1 ratio with under-dosed pheromone attracted as many males as the optimal pheromone concentration. Thus, responses to low pheromone concentrations are increased when host-plant volatiles are perceived simultaneously. This simultaneous perception likely aids males to locate females and increases their chances of mating. The compounds, (*E*)-β-caryophyllene, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, methyl salicylate, and (*E*)-β-farnesene, have been found previously to enhance attraction of *Eupoecilia ambiguella* and *Cydia pomonella* to their respective sex pheromones (Yang et al., 2004; Schmidt-Buesser et al., 2009). Furthermore, 1-hexanol, (*E*)-β-caryophyllene, (*Z*)-3-hexenyl acetate, or 1-octen-3-ol attract grapevine moth males on their own when used at similar release rates as in the current study (von Arx et al., 2011). The lack of response, reported by Masante-Roca et al. (2007), of *L. botrana* males to grapevines could be due to variables associated with the plant material tested (e.g., grape variety, amount of plant material, cut vs. undamaged plants and the release rate of plant volatiles).

The compounds tested in our study are not unique to grapevine, raising the question of specificity of these signals. Although we tested plant compounds individually, one might expect multi-component blends to enhance attractiveness of under-dosed pheromone to a greater extent, based on our

Table 1 Effects of plant volatiles on the attractiveness of the female sex pheromone to *Lobesia botrana* males

		Behavioral responses[a]					
Treatments[b]	Release rate[c] ($pg.min^{-1}$)	Activation	Take off	Upwind	Midline	Contact	*N*
$P_{1.0}$ (optimal)+grapevine	not known	89^{***}	88^{***}	$75^{a\ ***}$	73^{***}	$71^{a\ ***}$	85
$P_{1.0}$	–	80^{***}	78^{***}	$59^{b\ **}$	59^{**}	$49^{b\ *}$	85
$P_{0.1}$+1-hexanol	1	61^{b}	58^{b}	40^{b}	40^{b}	35^{b}	80
	100	$78^{a\ **}$	$73^{a\ **}$	$61^{a\ **}$	$61^{a\ **}$	$55^{a\ *}$	80
	10,000	$77^{a\ ***}$	$59^{ab\ *}$	39^{b}	38^{b}	33^{b}	120
$P_{0.1}$+(*E*)-β-caryophyllene	1	$79^{a\ ***}$	$65^{a\ *}$	36^{b}	34^{b}	26^{b}	80
	100	$76^{a\ ***}$	$73^{a\ ***}$	$56^{a\ **}$	$56^{a\ **}$	$56^{a\ **}$	80
	10,000	$82^{a\ ***}$	$71^{a\ ***}$	$53^{a\ *}$	$52^{a\ *}$	$48^{a\ *}$	79
$P_{0.1}$+(*Z*)-3-hexenyl acetate	1	61^{b}	55^{b}	34^{b}	33^{b}	31^{b}	80
	100	$85^{a\ ***}$	$74^{a\ ***}$	$58^{a\ **}$	$53^{a\ *}$	$51^{a\ *}$	80
	10,000	63^{b}	58^{b}	34^{b}	34^{b}	25^{b}	80
$P_{0.1}$+1-octen-3-ol	1	$71^{b\ **}$	60^{b}	43^{a}	40^{a}	36^{ab}	80
	100	$89^{a\ ***}$	$79^{a\ ***}$	$56^{a\ **}$	$54^{a\ *}$	$50^{a\ *}$	80
	10,000	$66^{b\ *}$	$61^{b\ *}$	41^{a}	40^{a}	34^{b}	80
$P_{0.1}$+(*Z*)-3-hexenol	1	59^{b}	48^{b}	34^{b}	30^{b}	29^{b}	80
	100	$84^{a\ ***}$	$71^{a\ ***}$	$50^{a\ *}$	$50^{a\ *}$	44^{a}	80
	10,000	54^{b}	50^{b}	36^{ab}	36^{b}	30^{ab}	80
$P_{0.1}$+methyl salicylate	1	61^{b}	46^{b}	33^{b}	27^{b}	23^{b}	70
	100	$78^{a\ ***}$	$74^{a\ ***}$	49^{a}	49^{a}	46^{a}	80
	10,000	55^{b}	49^{b}	36^{ab}	34^{ab}	34^{ab}	80
$P_{0.1}$+(*E*)-β-farnesene	100	$74^{\ **}$	$66^{\ **}$	49	45	43	80
$P_{0.1}$+DMNT	100	$70^{\ **}$	59	38	34	31	80
$P_{0.1}$+(+)-terpinen-4-ol	100	60	45	33	33	33	40
$P_{0.1}$+(*R*)-(+)-limonene	100	60	45	30	28	20	40
$P_{0.1}$ (under-dosed)	–	49	45	34	34	33	80
EtOH (blank)	–	8	6	1	1	1	90

[a] Percentage of *L. botrana* males showing a particular behavioral response. *N* is the number of males tested for each treatment. Different letters assigned within a response indicate differences: (1) between responses to the two treatments with the optimal pheromone dose, and (2) among responses to the three release rates within a pheromone+plant volatile treatment (GLM, $P<0.05$). Asterisks among a behavioral response indicate differences between the under-dosed pheromone mixture ($P_{0.1}$) and the other treatments (GLM, $P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***; the ethanol control (blank) was not included in the analysis)

[b] Sex pheromone treatments consisted of a 3-component blend of *L. botrana* sex pheromone components (see text). The $P_{1.0}$ optimal dose (1.0 $pg.min^{-1}$) was presented in a background of grapevine plants and the $P_{0.1}$ under-dosed level (0.1 $pg.min^{-1}$) was presented in combination with the different release rates of individual plant compounds

[c] Release rate of plant volatile chemicals

finding that grapevine emissions had additive effects on moth attraction when added to the optimal pheromone dose (Ta-ble 1), and from experiments in which multi-component blends were more attractive to grapevine moths than simpler blends or single components (Tasin et al., 2007; von Arx et al., 2011). Because of the high responses of *L. botrana* males to the optimal pheromone dose, we explored the effects of single host-plant volatiles on pheromone attraction at an under-dosed level. Refining host-plant volatile mixtures, to add to the pheromone to improve attraction, could be the subject of future work.

With the exception of (*E*)-β-caryophyllene, a general pattern emerged from our dose–response experiment, of an optimal plant volatile release rate of 100 $pg.min^{-1}$ when using the under-dosed pheromone level. This fits well with release rates of compounds from grapevine inflorescences and berries (Tasin et al., 2006; Anfora et al., 2009). (*E*)-β-Caryophyllene increased attractiveness of pheromone over a wider range of release rates than the other compounds tested, indicating that grapevine moth males might be able to accommodate substantial variation in the amounts of some compounds, as shown for DMNT in the moth *Paral-obesia viteana* (Cha et al., 2011).

Our results indicate that some plant compounds attract both sexes of *L. botrana*. The three terpenes, (*E*)-β-caryophyllene, DMNT, and (*E*)-β-farnesene, as well as methyl salicylate and 1-octen-3-ol, were present in a headspace collection of grapevine that elicited upwind flight of mated females (Tasin et al., 2006). In a subsequent subtractive bioassay of this headspace collection, (*E*)-β-caryophyllene, DMNT, and (*E*)-β-farnesene were identified as essential components for eliciting upwind flight in mated *L. botrana* females (Tasin et al., 2007).

In summary, we demonstrated that responses of *L. botrana* males to sex pheromone are improved in the presence of plant compounds, and we provide evidence for the hypothesis that grapevine moth males use volatile host-plant cues in mate-finding. Future field experiments will test whether addition of plant volatiles to sex pheromone improve the efficiency of mating disruption techniques, perhaps by increasing the active range of dispensers.

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References

ANFORA, G., TASIN, M., DE CRISTOFARO, A., IORIATTI, C., and LUCCHI, A. 2009. Synthetic grape volatiles attract mated *Lobesia botrana* females in laboratory and field bioassays. *J. Chem. Ecol.* 35:1054–1062.

ARN, H., RAUSCHER, S., GUERIN, P., and BUSER, H.-R. 1988. Sex pheromone blends of three tortricid pests in European vineyards. *Agric. Ecosyst. Environ.* 21:111–117.

CHA, D., LINN JR., C. E., TEAL, P. E. A., ZHANG, A., ROELOFS, W. L., and LOEB, G. M. 2011. Eavesdropping on plant volatiles by a specialist moth: significance of ratio and concentration. *PLoS ONE* 6:e17033.

LANDOLT, P. J. and PHILLIPS, T. W. 1997. Host plant influences on sex pheromone behavior of phytophagous insects. *Annu. Rev. Entomol.* 42:371–391.

MASANTE-ROCA, I., ANTON, S., DELBAC, L., DUFOUR, M. C., and GADENNE, C. 2007. Attraction of the grapevine moth to host and non-host plant parts in the wind tunnel: effects of plant phenology, sex and mating status. *Entomol. Exp. Appl.* 122:239–245.

SCHMIDT-BÜSSER, D., VON ARX, M., and GUERIN, P. M. 2009. Host plant volatiles serve to increase the response of male European grape berry moths, *Eupoecilia ambiguella*, to their sex pheromone. *J. Comp. Physiol. A* 195:853–864.

TASIN, M., BÄCKMAN, A. C., BENGTSSON, M., VARELA, N., IORIATTI, C., and WITZGALL, P. 2006. Wind tunnel attraction of grapevine moth females, *Lobesia botrana*, to natural and artificial grape odour. *Chemoecology* 16:87–92.

TASIN, M., BÄCKMAN, A. C., CORACINI, M., CASADO, D., IORIATTI, C., and WITZGALL, P. 2007. Synergism and redundancy in a plant volatile blend attracting grapevine moth females. *Phytochemistry* 68:203–209.

VON ARX, M., SCHMIDT-BÜSSER, D., and GUERIN, P. M. 2011. Host plant volatiles induce oriented flight behaviour in male European grapevine moths, *Lobesia botrana*. *J. Insect Physiol.* 57:1323–1331.

YANG, Z. H., BENGTSSON, M., and WITZGALL, P. 2004. Host plant volatiles synergize response to sex pheromone in codling moth, *Cydia pomonella*. *J. Chem. Ecol.* 30:619–629.