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Synergism and redundancy in a plant volatile blend attracting grapevine moth females

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Abstract

A flight tunnel study was done to decipher the behavioral effect of grape odor in grapevine moth *Lobesia botrana*. A blend of 10 volatile compounds, which all elicit a strong antennal response, attracts mated grapevine moth females from a distance, by upwind orientation flight. These 10 grape volatiles are in part behaviorally redundant, since attraction to a 3-component blend of β-caryophyllene, (*E*)-β-farnesene and (*E*)-4,8-dimethyl-1,3,7-nonatriene was not significantly different from the 10-component blend. Blending these three compounds had a strong synergistic effect on female attraction, and omission of any one compound from this 3-component blend almost abolished attraction. It was nonetheless possible to substitute the three compounds with the other grape volatiles which are perceived by the female antenna, to partly restore attraction. Several blends, of varying composition, elicited significant attraction. The observed behavioral plasticity in response to grape volatile blends probably reflects the variation of the natural plant signal, since females oviposit on different grape varieties, in different phenological stages.

Keywords: *Vitis vinifera*; Vitaceae; Grapevine; Attraction bioassay; Wind tunnel; Plant volatiles; Kairomone; Odor signal; Host finding; *Lobesia botrana*

1. Introduction

Plant volatiles serve a diversity of functions. They protect plants against environmental stress factors, and mediate biotic interactions with other plants, beneficial and herbivorous microorganisms and arthropods, and with avian and mammalian foragers (Kesselmeier and Staudt, 1999; Isman, 2000; Pichersky and Gershenzon, 2002; Engelberth et al., 2004; Bruce et al., 2005; Goff and Klee, 2006). Grapevine *Vitis vinifera* provides a particularly fascinating example for these multiple functions of volatile organic compounds. Grape-derived volatiles afford essential flavor compounds, which determine the varietal character and the sensory quality of grapevines, and are also thought to serve as direct defense against fungal pathogens and insect herbivores. Last but not least, insect herbivores and predators exploit grapevine volatiles as long-range signals for host location (Vancanneyt et al., 2001; Kulakiotu et al., 2004; Van Den Boom et al., 2004; Swiegers et al., 2005; Lund and Bohlmann, 2006).

The principle of insect attraction to their plant hosts by volatile compounds is well established. The challenge is now to identify the volatile signals which allow insects to discriminate suitable larval hosts from the background chemical environment, and which guide upwind orientation flights towards plants for oviposition. Knowledge of the chemicals and the behavioral mechanisms involved in host recognition is fundamental for the study of plant–insect interactions, including host-race formation and

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sympatric speciation, and is also a crucial input for the development of novel insect control techniques (Berlocher and Feder, 2002; Bengtsson et al., 2006). Grapevine moth *Lobesia botrana* (Den. & Schiff.) (Lepid., Tortricidae), an economically most important grape insect in the Palearctic region, serves as model species for the direct and indirect use of plant volatiles in insect control.

Grape berries and leaves release a hundred and more volatiles (Schreier et al., 1976; Marais, 1983; Wirth et al., 2001; Girard et al., 2002; Tasin et al., 2005). Grapevine moth females are attracted by grape odor for egg laying on flower buds and berries in different phenological stages (Bovey, 1966; Thiery, 2005). The antennae of grapevine moth females specifically respond to grape volatiles, and the processing of plant odor signals in the antennal lobe, the olfactory center in the insect brain, is under study (Masante-Roca et al., 2005; Tasin et al., 2005). Recently, it has been shown that a blend of 10 synthetic grape volatiles, which elicited an antennal response, attracts as many grapevine moth females by upwind orientation flights as a bunch of green grapes, or headspace collections made from the same grapes (Tasin et al., 2006b). This blend could be reduced to three compounds, (*E*)-β-caryophyllene, (*E*)-β-farnesene and (*E*)-4,8-dimethyl-1,3,7-nonatriene, without significant loss of behavioral activity (Tasin et al., 2006a).

The purpose of this study was to further explore the olfactory perceptual space which accommodates host finding in grapevine moth. Wind tunnel experiments were done with the grape volatiles producing a strong antennal response, to determine which compounds evoke upwind flight behavior. Another question was if host attraction is encoded by a unique volatile blend, or whether the components of an attractant blend can be replaced with other grape volatiles.

2. Results

2.1. Subtractive bioassay

The starting point of this wind tunnel study was a blend of 10 grape volatiles, which had been shown to attract grapevine moth females *L. botrana* (Tasin et al., 2006b). The most abundant compounds in this blend were (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), β-caryophyllene and (*E*,*E*)-α-farnesene, while DMNT and 1-octen-3-ol (octenol) elicited the strongest antennal response (Fig. 1). This 10-component blend elicited upwind flights over 120 cm, from the release cage towards the source, in 45% of the test females, and 21% of the females approached the source, after 180 cm upwind flight from the release cage (blend *A* in Fig. 2). The females did not respond to a blank stimulus, consisting of ethanol only.

A series of tests was done to identify the compounds which were essential for female attraction. Blend *A* was arbitrarily split into blends *B* through *E*. Upwind flight and landing response of grapevine moth females towards

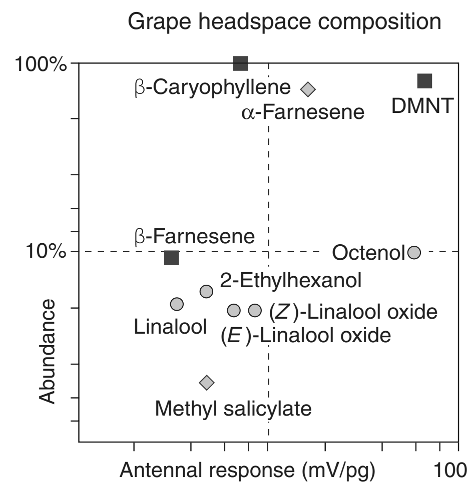

Fig. 1. Proportion of volatile compounds in grape *V. vinifera* headspace which elicited a consistent antennal response in grapevine moth *L. botrana* females (Tasin et al., 2006b).

B, *C* and *E* were not significantly different from blend *A*. Blend *D*, containing compounds which are rather typical for grape, linalool and its furanoid oxides and octenol, did not attract females to the source (Fig. 2).

One by one, the components of blend *E* were subtracted to produce the 4-component blends *F* through *K* (Fig. 2). Omission of DMNT (blend *G*) gave a significant reduction of the number of females responding. Fewer females engaged in upwing flights and approached the source after subtraction of either β-caryophyllene, (*E*)-β-farnesene, (*E*,*E*)-α-farnesene or methyl salicylate, but the difference between blends *F*, *H*, *I* and *K* and blend *E* was not significant. Last not least, the 3-component blend *L* of β-caryophyllene, (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*)-β-farnesene, in a 100:78:9 ratio, was not significantly different from the response to the 10-component grape mimic *A* (Fig. 2).

Further reduction of blend *L* to the corresponding 2-component blends *O*, *R* and *T* resulted in a substantial decrease in female response (Fig. 3), confirming an important role of β-caryophyllene, DMNT and also (*E*)-β-farnesene in female upwind attraction.

2.2. Substitution bioassay

Comparison of blends *B* and *C* with *L* showed that β-caryophyllene, DMNT and β-farnesene could be substituted with other compounds (Fig. 2). A series of experiments was thus done to determine whether compounds of the 3-component blend *L* could be replaced by other compounds eliciting an antennal response (Figs. 1, 3).

Linalool and its oxides (blend *M*), or octenol and 2-ethyl-1-hexanol (blend *N*) were added to the 2-component blends *O*, *R* and *T* (Fig. 3). Blend *R* did not attract any females to the source, until *M* or *N* were added, to afford blends *S* and *C*. More females responded to *P* and *Q*, compared to blend *O*. However, attractancy of blends *P*, *Q*, *S*, and *C* was not entirely restored, compared to the 3-component blend *L*