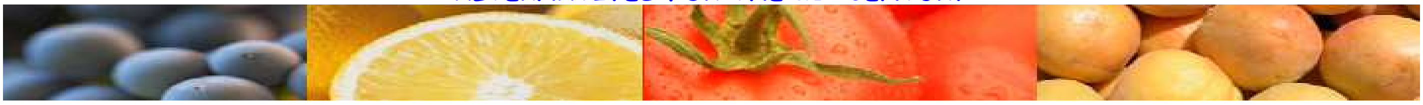

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Plant odours attractive to Lobesia botrana mated females

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SUMMARY

In laboratory experiments, we identified and quantified volatiles emitted by inflorescences and berries of two grape varieties (Trebbiano and Sangiovese) and examined the effects of the volatiles on oviposition by the grapevine moth Lobesia botrana; compared to Trebbiano, Sangiovese is relatively more susceptible to L. botrana infestations. Chemical and electrophysiological analysis indicated only quantitative differences between the volatiles released by the two varieties. In a dual-choice oviposition bioassay based only on volatile cues, females did not show any preference between the two varieties. The six major components of the odour profiles that were GC-EAD-active to female antennae included: (S)-(-)-limonene, 4,8-dimethyl-1,(E)3,7-nonatriene, (±)-linalool, (E)-caryophyllene, (E,E)-α-farnesene, and methyl salicylate. At the beginning of the berry touch phenological stage, their proportions were about 10:0.6:0.4:0.5:0.9:0.6 in Trebbiano and 10:1:0.4:1.5:0.4:0.3 in Sangiovese. A six-component synthetic lure (with the proportion 10:1:1:1:1:1, which approximated the ratio of components released by both varieties) was used in further laboratory oviposition bioassays; depending on its dosage, the synthetic lure either attracted or repelled oviposition. In a release-capture experiment conducted in a field cage covering two grapevine rows, the synthetic lure was more attractive than a grape cluster or a blank control and stimulated oviposition on the vegetation near the lure. The results indicate that L. botrana uses olfactory cues to select oviposition sites and that an artificial lure, containing the major volatiles released by two grape varieties, may be useful in monitoring female numbers in the field.

KEY WORDS: grapevine moth, female behaviour, oviposition bioassay, plant volatiles, field cage.

INTRODUCTION

Nowadays, the management of Lobesia botrana (Denis & Schiffermüller) (Lepidoptera Tortricidae), the primary insect pest of commercial viticulture in the Mediterranean regions, is mainly based on insecticidal control, even though preventive strategies, such as pheromone mating disruption, are increasingly and effectively used in Area Wide Projects (Ioriatti et al., 2008).

In recent years great attention has been drawn to the study of *L. botrana* females behaviour before the egg laying.

The knowledge of the chemical factors that drive the female in the host finding and host acceptance is of great importance for two main reasons: a) more precise forecasting of the egg laying in the field; b) possible use of those chemicals (attractants or repellents) in the manipulation of the females behaviour (monitoring, push-pull, lure & kill, etc..).

Fermented fruit juices have been and are currently considered useful for monitoring numbers of both sexes of L. botrana but their use is time-consuming because the captured insects must be handled, sorted, and identified (Thiery et al., 2006).

L. botrana females are attracted to essential oil of rosemary (Rosmarinus officinalis L.) in an olfactometer (Katerinopoulos et al., 2005), and to tansy flowers (Tanacetum vulgare L.): the steam-distillate extract from tansy flowers and a synthetic blend based on that extract consistently attracted females in a field cage (Gabel et al., 1992).

Susceptibility to oviposition by L. botrana females, as well as larval fitness, differs among varieties of Vitis vinifera L. (Fermaud, 1998; Snjezana, 2004; Moreau et al., 2006).

Finding a good site for oviposition is a challenging task for female grapevine moths, and their decision has a critical consequence for the life history of the offspring. According to recent reports, L. botrana females use contact cues to evaluate the suitability of substrates for oviposition (Maher et al., 2006); moreover, they are attracted from long distances to odours either released from grape clusters (Tasin et al. 2005, 2006a, 2006b, 2007; Masante-Roca et al. 2007) or grape leaves and flower buds (Masante-Roca et al. 2007).

In a very recent paper, Tasin et al. (2010) demonstrate that Daphne gnidium and Grapevine share a number of volatile compounds and that the highest attraction of L. botrana mated females in the wind tunnel is elicited when the specific compounds of D. gnidium are added to the blend.

Preliminary laboratory bioassays have shown that odours emitted from grape bunches of different varieties are able to drive the oviposition at a short range of both L. botrana females (Tasin et al., 2008; Tasin et al., 2009) and the related species Eupoecilia ambiguella (Hübner) (Lepidoptera Tortricidae) (Anfora et al., *2008). To verify this hypothesis, we studied how L. botrana oviposition was affected by the emission of grape clusters of the varieties Trebbiano and Sangiovese. These varieties, which are commonly grown in Italy and especially in the Tuscany region, were selected because they differ in susceptibility to L. botrana oviposition. As a matter of fact; Sangiovese was shown to be more susceptible than Trebbiano, mainly during the second flight of the year (Bagnoli, 1990).*

To date, most behavioural studies on grapevine moth females have been carried out in the laboratory with fresh and synthetic material. On the contrary, no data are available on the attractiveness of synthetic plant volatiles to females under field conditions, where the odour strongly competes with the canopy background (Cha et al., 2008).

In our study, a lure blended with the six major electrophysiologically active volatile compounds released by either Sangiovese or Trebbiano was first tested in the laboratory for its attractiveness to L. botrana females and its effect on oviposition. Then, traps baited with this mimic were used in a field cage placed in a vineyard, and the effect on the female behaviour was evaluated in terms of trap captures and oviposition preference. Details of this reasearch have been recently published on Journal of Chemical ecology (Anfora et al. 2009).

MATERIALS AND METHODS

Moths used in this study were selected from a colony of L. botrana reared on artificial diet. During the experiment, wild larvae were periodically collected and added to the colony to reduce inbreeding.

Grape clusters of Sangiovese and Trebbiano varieties (V. vinifera) were taken from an experimental vineyard at Pisa University. The grape clusters were collected at five different phenological stages (stage 17, fully developed inflorescences; stage 23, full flowering; stage 33, beginning of berry touch; stage 35, beginning of ripening; and stage 38, berries ripe for harvest) (Eichhorn and Lorenz, 1977). Inflorescences or grape clusters were immediately placed in a polyacetate bag and volatiles collected according to the procedure of Faccoli et al. (2008). Three to four collections from different groups of inflorescences or grape clusters were done for each variety in each phenological stage. For stage 33 (beginning of berry touch), volatiles of the two varieties were also collected directly in the field using intact grape clusters attached to the plants. This was done to determine whether there were qualitative and/or quantitative differences in volatiles collected in the laboratory versus the field.

Chemical and electrophysiological analyses were performed using methods described by Anfora et al. (2005) and Faccoli et al. (2008).

Formulation of synthetic lure

Based on the chemical and electrophysiological data, a synthetic lure was prepared with (S)-(-)-limonene, (E)-DMNT, (±)-linalool, (E)-caryophyllene, (E,E)-

α-farnesene, and methyl salicylate. The relative proportions of these volatiles change during the season according to grape phenological stage. Because stage 33 corresponds to the second flight of L. botrana, the lure was formulated based on the proportions released from grapes at stage 33. Stage 33 grape volatiles were found in the follow ratios: 10:0.6:0.4:0.5:0.9:0.6 [(S)-(-)-limonene:(E)-DMNT:(±)-linalool:(E)-caryophyllene:(E,E)-α-farnesene:methyl salicylate] from Trebbiano and 10:1:0.4:1.5:0.4:0.3 from Sangiovese. Because the components and their ratios were similar for the two varieties at stage 33, the synthetic lure was prepared with the six components in the approximate and simplified proportion 10:1:1:1:1:1 [(S)-(-)-limonene:(E)-DMNT:(±)-linalool:(E)-caryophyllene:(E,E)-α-farnesene:methyl salicylate], with the aim to mimic both varieties.

Field-cage experiment

A release-capture experiment in a field cage was conducted in June and July 2008 in a thin-meshed (mesh size 2 mm) net tunnel (length 30 m, width 4 m, median height 2.5 m) covering two grapevine rows (guyot training system) of a Chardonnay vineyard at the IASMA-FEM Research Centre (San Michele all'Adige, Italy). The canopy height was 2.5 m, with 0.8 m between vines and 3 m between rows. A piece of mesh was used to divide the tunnel into two 15-m-long cages. All the grape clusters within the cages were removed to reduce competition with natural odours. In each cage, three replicates of three differently baited traps (nine traps total per cage) were installed 1 m above the ground along the two rows: four traps were in one row and five were in the other row, with 3 m separating traps in the same row. In preliminary field trials, different trap models were evaluated for their suitability to catch females (data not shown). The traps employed consisted of square sticky base (30 x 30 cm) with a clear plastic cylindrical screw-top jar (8 cm diameter x 11 cm height) in the middle. Thirty holes (1.5 ± 0.2 mm ID) in the side wall of each jar allowed volatiles to escape. The screw top of each jar was fixed to the sticky base. The jar was unscrewed from its top to introduce the bait.

Three baits or treatments were compared: grape mimic, a cut grape bunch (phenological stage 33, Chardonnay from the same rows used in the experiment), and a blank. For the grape mimic, the solution (15 mg diluted in an equal volume of light mineral oil [Sigma-Aldrich]) was impregnated in a rubber septum (Wheaton, 20 mm straight plug stopper). Mated females (N = 140) were released every second week between the rows of each cage, for a total of three releases per cage. The releases alternated between cages so that only one cage received females in any week. Grape mimic baits were replaced at each release. The number of females captured on the sticky base of each trap was determined daily for the first week after release. Four weeks after the first release in each cage, L. botrana larvae were counted on grapevine shoots and leaves within 50 cm of each trap. Counting lasted 5 minutes/replicate and presence of larvae developing in shoots and leaves was ascertained.

Statistical analyses

The software used for the statistical analyses was KyPlot 5.0 (Kyenslab Inc., Tokyo, Japan).

For the field-cage experiment, nonparametric one-way ANOVA (Kruskal-Wallis test) followed by the Bonferroni-Dunn post-hoc multiple comparison test was used to assess differences in the attractiveness of the baits. Parametric one-way ANOVA followed by the Tukey post-hoc multiple comparison test was used to assess the effect of baits on the level of pest infestation; homogeneity of variance had been previously determined with Levene's test.

For more details about materials and methods see Anfora et al. (2009).

RESULTS AND DISCUSSION

Chemical and electrophysiological analyses

Antennae of grapevine moth mated females responded to 23 compounds from headspace collections of the two grape varieties. The two odour profiles perceived by mated females had a high degree of similarity (see Anfora et al., 2009).

For both varieties, several terpenes, released in all the phenological stages and frequently in large amounts, elicited the highest antennal responses. The most abundant terpenes were (E)-DMNT at stage 17 and (S)-(-)-limonene from stage 33 to stage 38. (E)-DMNT, (S)-(-)-limonene, (E,E)-α-farnesene, (±)-linalool, (E)-caryophyllene, and methyl salicylate were always collected during the three grape phenological stages when L. botrana oviposition occurs in nature (stages 17, 33, and 35).

Field-cage experiment

The recapture rate in the field-cage experiment was approximately 10% (83 individuals caught vs. 840 released). Traps baited with grape mimic caught significantly more mated L. botrana females than traps baited with one grape cluster or unbaited traps, and traps baited with grape clusters captured more females than unbaited traps.

The numbers of grapevine moths larvae on shoots and leaves near the traps significantly differed with bait ($F=59.0$; $df=15$; $P<0.001$).

Two main goals motivated our research on olfactory communication between plant and moth: to understand the role of olfactory cues in the selection of oviposition sites by *L. botrana* and to develop a synthetic lure based on host-plant volatiles for monitoring female numbers in the field. Our first hypothesis was that olfactory cues could greatly affect the selection of oviposition sites among different host-plant genotypes. The characterization of the different sensory cues released by grapevine genotypes differing in tolerance to grapevine moth could allow the development of innovative methods for manipulating *L. botrana* behaviour. This hypothesis was not confirmed by our results. The two varieties chosen for their different susceptibility to *L. botrana* did not show significant differences in the odour profiles (volatiles released), and the volatiles from these two varieties elicited similar antennal responses. Moreover, the two odour profiles did not differ in their ability to attract ovipositing females in a dual-choice bioassay (see Anfora et al., 2009).

In our study we measured the headspace volatiles of the grape clusters because we were interested in identifying the odour cues guiding not only long-range attraction and host recognition but also oviposition; in the field under the climatic conditions of the northern-central Italian regions, oviposition by *L. botrana* occurs on grape clusters at the phenological stages 17, 33, and 35 (Anfora et al., 2007). In formulating a synthetic lure that would attract mated females, we included grape-cluster volatiles that elicited both consistent electrophysiological responses by *L. botrana* and that were released in high quantities during all the critical phenological stages of the two grape varieties. Thus, we have selected a subset of the GC-EAD active compounds consisting of the ubiquitous (S)-(-)-limonene, (E)-DMNT, (±)-linalool, (E)-caryophyllene, (E,E)-α-farnesene, and methyl salicylate with the proportion 10:1:1:1:1:1.

In our case, the ratio of the key active volatiles, such as the most abundant (S)-(-)-limonene, was similar in both varieties at phenological stage 33 and therefore cannot explain differences in their susceptibility to grapevine moth. The lower susceptibility expressed under natural conditions by Trebbiano vs. Sangiovese might be due to factors such as the asynchrony between susceptible phenological stage and moth flight (Bagnoli, 1990), the ecological fitting of the varieties and the influence of other sensory cues (Maher et al., 2006; Moreau et al., 2006).

Our synthetic lure attracted *L. botrana* egg-laying females both in the laboratory bioassay and in the field-cage experiment. Moreover, the large number of *L. botrana* larvae detected near traps baited with synthetic lure in the field bioassay corroborates our hypothesis that *L. botrana* uses olfaction to select oviposition sites. It is noteworthy that, in the absence of grape bunches in the field cages, *L. botrana* females deposited their eggs on grapevine shoots and leaves, and that the larvae seemed capable of developing on such organs. This is in agreement with the reported attractiveness of various grapevine plant parts to *L. botrana* in wind tunnel experiments (Tasin et al. 2005, Masante-Roca et al. 2007). Based on our results, we conclude that *L. botrana* uses a specific ratio of common volatiles emitted by grape clusters both to find hosts and to select oviposition sites. In this way, the behaviour of *L. botrana* is similar to that of other tortricids (Wearing & Hutchings, 1973; Suckling et al., 1996; Grant et al., 2007).

The lesser attractiveness of cut grape cluster bait vs. synthetic grape mimic bait could be due to a lower quantity of volatiles released by the former bait, but this was not investigated in the present study. However, because the attractiveness of the grape mimic recorded in the field cage was quite weak, we assume that its attractiveness was reduced by the vineyard background odour.

In a given environment, each plant is capable of synthesizing ten to hundreds of primary and secondary metabolites, with diverse biological properties and functions, that can create complex backgrounds able to disrupt insects from selecting acceptable host plants or synthetic blends. Consequently, an artificial lure for capturing females must be able to compete with background odours. Furthermore, the approximate ratio of compounds in the blend, based on our specific extraction conditions, along with the dispensers' characteristics, are likely to not exactly fit with the natural emissions of grapes affecting negatively the attractiveness of the lure (Heat et al., 1986; Tasin et al., 2006b, Cha et al., 2008).

In conclusion, our study demonstrates that *L. botrana* uses host volatiles to select oviposition sites and that formulation of synthetic lures can be useful for monitoring female numbers and activity in the field.

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REFERENCES

Anfora G, Tasin M, Bäckman AC, De Cristofaro A, Witzgall P, Ioriatti C (2005) Attractiveness of year-old polyethylene Isonet sex pheromone dispensers for Lobesia botrana. Ent Exp Appl 117: 201-207

Anfora G, Schmidt S, Ioriatti C (2007) Morfologia e biologia. Pp 22-31 in Anfora G (eds) Le tignole della vite. SafeCrop

Anfora G, Tasin M, Bäckman AC, Leonardelli E, De Cristofaro A, Lucchi A, Ioriatti C (2008) Olfactory responses of Eupoecilia ambiguella (Hübner) (Lepidoptera Tortricidae) females to volatiles from grapevine. IOBC wprs Bull 36: 233-236

Anfora G, Tasin M, De Cristofaro A, Ioriatti C, Lucchi A, (2009) Synthetic grape volatiles attract mated Lobesia botrana females in laboratory and field bioassays. J. Chem. Ecol. 35: 1054-1062

Bagnoli B (1990) Incidenza delle infestazioni da artropodi e difesa dei vigneti in Toscana. La Difesa delle Piante 13 (3-4): 89-112

Cha DH, Nojima S, Hesler SP, Zhang A, Linn CE, Roelofs WL, Loeb GM (2008) Identification and field evaluation of grape shoot volatiles attractive to female grape berry moth (Paralobesia viteana). J Chem Ecol 34: 1180-1189

Eichhorn KW, Lorenz DH (1977) Phänologishche Enwicklungsstadien der Rebe. Nachrichtenbl Dtsch Pflanzenschutzdienstes (Braunschweig) 29:119–120

Faccoli M, Anfora G, Tasin M (2008) Responses of the Mediterranean Pine Shoot Beetle Tomicus destruens (Wollaston) to pine shoot and bark volatiles. J Chem Ecol 34: 1162-1169Fermaud M (1998) Cultivar susceptibility of grape berry cluster to larvae of Lobesia botrana (Lepidoptera: Tortricidae). J Econ Entomol 91: 974-980

Gabel B, Thiéry D, Suchy V, Marion-Poll F, Hradsky P, Farkas P (1992) Floral volatiles of Tanacetum vulgare L. attractive to Lobesia botrana Den. et Schiff. females. J Chem Ecol 18: 693-701

Grant GG, Guo J, McDonald L, Coppens MD (2007) Oviposition response of spruce budworm (Lepidoptera: Tortricidae) to host terpenes and green leaf volatiles. Can Entomol 139: 564-575

Heath RR, Teal PEA, Tumlinson JH, Mengelkoch LJ (1986) Prediction of release ratios of multicomponent pheromones from rubber septa. J Chem Ecol 12: 2133-2143

Ioriatti C, Lucchi A, Bagnoli B (2008) Grape areawide pest management in Italy. Pp 208-225 in Koul O, Cuperus GW, Elliott N (eds) Areawide pest management: theory and implementation. CABI

Katerinopoulos HE, Pagona G, Afratis A, Stratigakis N, Roditakis N (2005) Composition and insect attracting activity of the essential oil of Rosmarinus officinalis. J Chem Ecol 31: 111-122

Maher N, Thiery D, Städler E (2006) Oviposition by Lobesia botrana is stimulated by sugars detected by contact chemoreceptors. Physiol Entomol 31: 14-21

Masante-Roca I, Anton, S, Delbac L, Dufour M-C, 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

Moreau J, Benrey B, Thiery D (2006) Assessing larval food quality for phytophagous insects: are the facts as simple as they appear? Funct Ecol 20: 592-600

Snjezana H (2004) Susceptibility of some grapevine cultivars in area of vineyards of Podgorica on the attack of European grape berry moth - Lobesia botrana Den. & Schiff. (Lepidoptera, Tortricidae). Acta Horticulturae 652: 355-358

Suckling DM, Karg G, Gibb AR, Bradley SJ (1996) Electroantennogram and oviposition responses of Epiphyas postvittana (Lepidoptera: Totricidae) to plant volatiles. New Zeal J Crop Hort 24: 323-333

Tasin M, Anfora G, Ioriatti C, Carlin S, De Cristofaro A, Schmidt S, Bengtsson M, Versini G, Witzgall P (2005) Antennal and behavioural responses of grapevine moth Lobesia botrana females to volatiles from grapevine. J Chem Ecol 31: 77-87

Tasin M, Bäckman AC, Bengtsson M, Ioriatti C, Witzgall P (2006a) Essential host plant cues in the grapevine moth. Naturwissenschaften 93: 141-144

Tasin M, Bäckman AC, Bengtsson M, Varela N, Ioriatti C, Witzgall P (2006b) Wind tunnel attraction of grapevine moth females, Lobesia botrana, to natural and artificial grape odour. Chemoecology 16: 87-92

Tasin M, Bäckman AC, Coracini M, Casado D, Ioriatti C, Witzgall P (2007) Synergism and redundancy in plant volatile blend attracting grapevine moth females. Phytochemistry 68: 203-209

Tasin M, Anfora G, Bäckman AC, Ioriatti C, De Cristofaro A, Pozzolini E, Leonardelli E, Lucchi A (2008) Volatile from grape drive the oviposition of Lobesia botrana at short distance. IOBC wprs Bull 36: 351-353

Tasin M, Anfora G, Leonardelli E, Ioriatti C, Lucchi A, De Cristofaro A, Pertot I (2009) A bioassay-based approach for the evaluation of host-plant cues as oviposition stimuli in grapevine moth. IOBC wprs Bull 41: 83-86

Tasin M, Bäckman AC, Anfora G, Carlin S, Ioriatti C, Witzgall P (2010) Attraction of female grapevine moth to common and specific olfactory cues from two host plants. In press on Chemical Senses

Thiéry D, Retaud P, Dumas-Lattaque L (2006) Piégeage alimentaire de l'eudémis de la vigne: un outil intéressant et performant pour la description de la dynamique des vols zédépontes. Phytoma 592: 27-30

Wearing CH, Hutchings RFN (1973) Alfa-farnesene, a naturally occurring oviposition stimulant for the codling moth Laspeyresia pomonella. J Insect Physiol 19: 1251-1256