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FLORAL VOLATILES OF *Tanacetum vulgare* L. ATTRACTIVE TO *Lobesia botrana* DEN. ET SCHIFF. FEMALES

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Abstract—The European grapevine moth (EGVM), *Lobesia botrana*, is a major pest of grapes in Europe. Females are attracted to a nonhost plant: tansy (*Tanacetum vulgare* L.), which is a common weed in Slovakian vineyards. A steam distillate extract of tansy flowers was analyzed by means of a GC-EAG technique to screen constituents detected by the olfactory receptors of EGVM females. From more than 200 GC peaks, nine peaks corresponding to monoterpenoids released an EAG response in more than 70% of the females (N = 15): *p*-cymene, *d*-limonene, α-thujene, α-thujone, β-thujone, thujyl alcohol, terpinene-4-ol, (*Z*)-verbenol, and piperitone. The steam distillate of tansy as well as a synthetic blend of identified compounds released consistent attraction in a field cage. The use of nonhost plants and host plant odors in integrated pest management is discussed.

Key Words—*Lobesia botrana*, *Vitis vinifera*, *Tanacetum vulgare*, tansy,

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grapevine, Lepidoptera, Tortricidae, Compositae, plant-insect relationships, olfaction, allelochemicals, terpenoids, coupling GC-EAG.

INTRODUCTION

The role of host plant odors mediating different behaviors has received much attention for monophagous and polyphagous insects. It has been suggested that host-specific molecules as well as more commonly occurring compounds are used by phytophagous insects to identify their host plants (Finch, 1980; Visser, 1986; Masson and Mustaparta, 1990).

The European grapevine moth (EGVM), *Lobesiabotrana* Den. et Schiff., represents one of the most important pests of vineyards in Europe. This insect is considered a polyphagous insect restricted to a narrow range of plants belonging to different families (Stoeva, 1982). Olfaction seems to play an important role in its colonization patterns. The attraction of adults has been observed in response to fermented wine and fruit odors (Feytaud and Bos, 1914; Roussel and Vonderheyden, 1964). In nature, EGVM females are strongly attracted to tansy, *Tanacetum vulgare* L. (Gabel, 1992). This attraction appears to be sexually selective, since males are seldom observed on this plant, and has been attributed to volatiles emitted by the flowers (Gabel et al., 1991). Tansy is not considered to be a host plant of the EGVM nor a rendezvous site for mating (Gabel, 1992).

Tansy is a wild species of Compositae growing in temperate regions. In Slovakian vineyards, the absence of herbicide usage favors the natural occurrence of this plant within vineyard rows and at the borders. More than 30 chemotypes of tansy have been reported worldwide according to the major constituents belonging to monoterpenes and sesquiterpenes (Nano et al., 1979; Holopainen et al., 1987).

In this paper, we report on the detection of tansy flower volatiles by EGVM females and the attractiveness of a reduced mixture of these volatiles. We also discuss the potential use of tansy and nonhost plant odors in integrated control programs.

METHODS AND MATERIALS

Plant Extract. Flowers of tansy (without the pedicel) were collected along vineyard borders and stored at −15°C. Tansy flowers were steam distilled using a distillation-extraction device modified after Likens and Nickerson (Pharmacopea Bohemoslovaca, 1987). Flowers (440 g) were extracted in 1 liter of distilled water. The solvent-free fraction corresponding to the first 2 hr of distillation was collected and used for the experiments.

Insect Material. A laboratory strain of EGVM from INRA Pont-de-la-Maye, annually infused with wild insects from Bordeaux vineyards (France), was reared on a semisynthetic diet under controlled conditions (16:8 hr light–dark; 22°C ± 1°C; 60% relative humidity). Two-day-old, mated females were used for electrophysiological recordings.

Gas Chromatography–Electroantennography Coupling. A gas chromatograph (GC) was equipped with an on-column injector, a 5-m-long deactivated fused silica megabore precolumn, a 30-m-long fused silica megabore DB-5 column (5% phenyl-, 95% methyl-pyloxane), a deactivated Y-shaped fused silica outlet splitter heated at a constant temperature (250°C), and a flame ionization detector (FID) (split ratio = $\frac{2}{3}$ towards the antenna) (see Thiéry et al., 1990, for details). The carrier gas was helium. The temperature was raised linearly at 5°C/min from 40°C up to 250°C. Molecules eluted from the column were blown into a humidified stream of purified air: air speed = 20 cm/sec, flow diameter = 0.8 cm. The relative concentration of each constituent was expressed as a percentage of the sum of all the peaks after subtraction of the solvent peak area.

Electroantennograms (EAG) were recorded from 15 females. Isolated heads of EGVM were mounted between the recording electrode covering the tip of the antenna and the indifferent electrode inserted in the clypeus. EAG and GC signals were stored on a microcomputer using custom software. GC peaks that elicited an EAG response in more than four individuals were subsequently chemically identified.

Identification. Identification of chemicals was performed by GC–mass spectrometry (GC: HP 5890II; MS: HP5971A). Identifications were based on electron impact ionization (EI, 70 eV). The mass spectra obtained were compared to spectra from the NBS Database and confirmed by injection of standard chemicals when available.

Bioassay. The attractiveness of the steam distillate and of a blend of the chemicals identified through the GC-EAG analysis were tested in a field cage with an artificial population of EGVM. A row of vine grapes (Cabernet Sauvignon cultivar) was covered under a tunnel made of P17 tissue (polypropylene, 0.17 mm thickness, SODOCA S.A.R.L., France) (length = 12 m, height = 2.5 m, width = 3 m). Five traps designed for EGVM females (Gabel, 1990) were used: one control, two baited with tansy extract, and two baited with the synthetic blend. The traps were placed evenly within the row, 1 m above the ground. Exchangeable trapping plates were coated with Bird Tanglefoot glue (Tanglefoot Co., Michigan). Males (N = 738) and mated females (N = 592) were released in the cage. The number of insects recaptured was counted each morning during five consecutive days. The odors were released from a glass

TABLE 1. MOLECULES RELEASING EAG IN MORE THAN 11 OF 15 *Lobesia botrana* FEMALES[a]

Molecule	Amount (%)	EAG response (in mV ± SD)
p-Cymene	0.27	0.16 ± 0.05
d-Limonene	0.03	0.19 ± 0.08
α-Thujene	0.28	0.20 ± 0.08
α-Thujone	1.05	0.33 ± 0.16
β-Thujone	78.34	0.67 ± 0.28
Thujyl alcohol	0.64	
Terpinen-4-ol	0.75	0.29 ± 0.07
(Z)-Verbenol	4.33	0.30 ± 0.10
Piperitone	6.35	0.25 ± 0.10

[a] Identifications by GC-MS (electron impact ionization), mass spectra compared to reference spectra (NBS Database) and confirmed by injection of standards.

tube (1 cm diam.) filled with either the extract of tansy (1 ml) or a blend of monoterpenes (1 ml) in the proportions expressed in Table 1. The monoterpenes used in the synthetic blend were of GC standard quality: *p*-cymene, thujyl alcohol, piperitone, terpinen-4-ol (INRA, Dijon, France), *d*-limonene and (Z)-verbenol (Fluka), isomers of thujone (Pharmaceutical Faculty, Bratislava, CSFR). α-Thujene was not available.

RESULTS AND DISCUSSION

Steam distillation of tansy flowers yielded an extract in which over 200 peaks were detected by the GC analysis. The GC-EAG method was used as a screening procedure to locate the most active fractions and compounds of this complex blend. Only 24 GC peaks released measurable EAG responses in 27% of the females. Nine peaks elicited responses in 70% of the females (Figure 1). These peaks are monoterpenes: *p*-cymene, *d*-limonene, α-thujene, α-thujone, a blend of β-thujone and thujyl alcohol, terpinen-4-ol, (Z)-verbenol, and piperitone.

EAG responses closely matched the corresponding GC peaks (Figure 1). This relationship was true for the major peak (β-thujone) where the EAG response was maintained until end of the peak tail (Figure 1). Responses were observed during the release of thujyl alcohol, which occurred while the antenna was still recovering from the stimulation of β-thujone. Improved separation or classical EAG studies are needed to confirm if thujyl alcohol stimulates the olfactory receptors of EGVM females. Both major and minor compounds released EAG

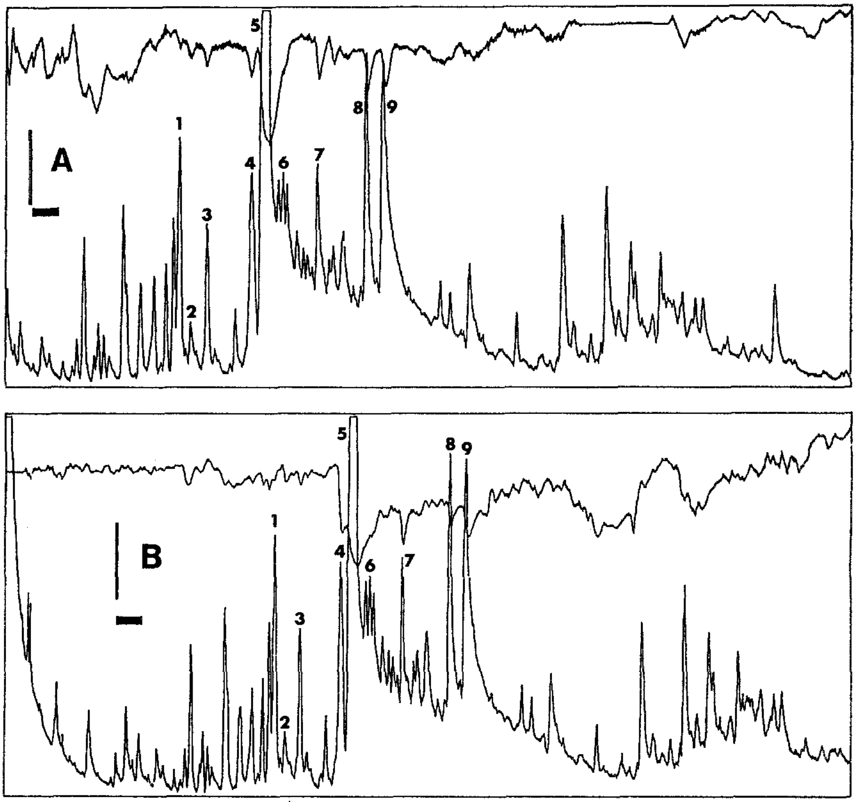

FIG. 1. GC-EAG recordings of two females of *Lobesia botrana* in response to tansy extract. Upper trace = EAG, lower trace = FID signal. 1 = *p*-cymene, 2 = *d*-limonene, 3 = α-thujene, 4 = α-thujone, 5 = β-thujone, 6 = thujylalcohol, 7 = terpinen-4-ol, 8 = (Z)-verbenol, 9 = piperitone. Horizontal bar = 100 sec; vertical bars—A: 1 mV, B: 2 mV.

responses in EGVM females (Table 1). The detection of chemicals at low concentrations within plant extracts suggests an important biological activity of these substances.

The biological activity of eight of the nine monoterpenes [*p*-cymene, *d*-limonene, α-thujone, a blend of β-thujone and thujyl alcohol, terpinen-4-ol, (Z)-verbenol, and piperitone] identified in the tansy flowers were compared to the steam distillate (Figure 2). A total of 28.4% of the released females were recaptured in the traps (2.6% in the control, 15.5% with the steam distillate, and 10.3% with the synthetic blend). Low temperature (13.5°C) and high wind speed (15 m/sec) reduced the effectiveness of the traps during the first night. We assumed that tansy odors were effective only against females. For this reason

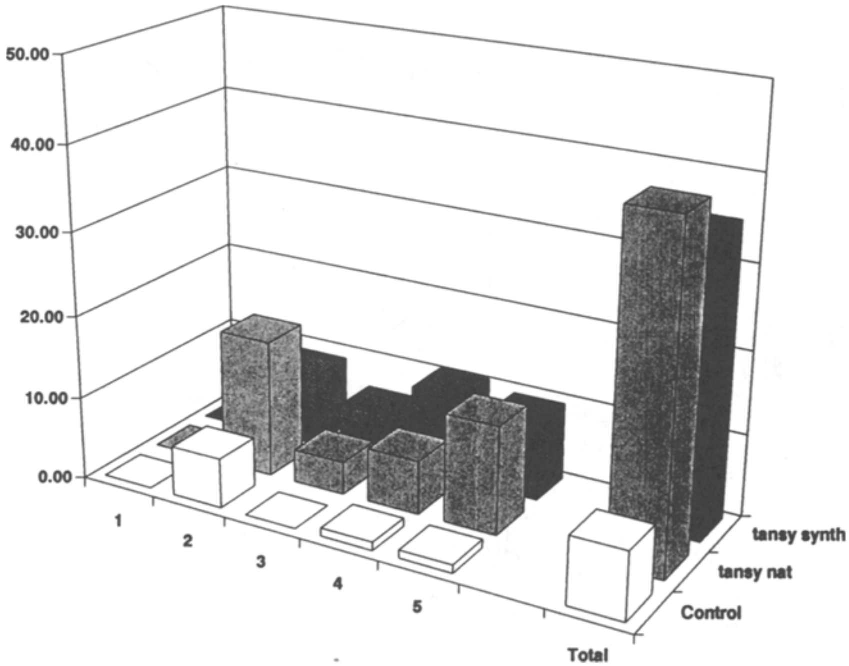

FIG. 2. Daily number of *Lobesia botrana* females caught in two different odorant traps (5 days): white bars = control, grey bars = tansy steam distillate, striped bars = synthetic blend.

we have used a type of trap especially designed to catch females (Gabel, 1990). However, males (22%) were also recaptured, possibly attracted by sexual pheromones emitted from the trapped females (Gabel and Stockel, 1988). Catching females with tansy extracts confirmed previous reports on the attractiveness of natural populations of EGVM females by tansy plants (Gabel, 1992). At the concentration used, our synthetic blend was almost as attractive as the steam distillate. α-Thujene was lacking in the synthetic blend used. The role of this chemical as well as different concentrations of the reduced blend have now to be tested.

Terpenic compounds have been tested as possible trapping agents against EGVM by Stellwaag and Götz (1937), but their results were inconclusive mainly for methodological reasons. Except for thujone, most of the monoterpenes used in our trapping experiments are present in flower or leaf extracts of grapevines (Schreier et al., 1976). These data suggest that such molecules are involved in olfactory recognition of plants by EGVM females.

Other volatile compounds, such as general green leaf volatiles, might contribute to the olfactory recognition in the EGVM. Chemical analysis of various grapevine cultivars reported that green odor volatiles were emitted from leaves and immature grapes [e.g., 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexenal] (Kepner and Webb, 1956; Wildenradt et al., 1975; Schreier et al., 1976; Shimizu and Watanabe, 1981). Such chemicals are produced at low concentration in Compositae flowers (Rudlof, 1963; Etiévant et al., 1984; Flath et al., 1985; Souleles and Stamatakou, 1991). In our experiments, we could not demonstrate whether the females detect these substances. Other distillation methods should be used to extract and concentrate such chemicals.

The attraction of EGVM females towards a nonhost plant on which oviposition and feeding was never observed raises an exciting ecological question. Olfactory detection of major terpenoids of tansy suggests that females might search for tansy-specific chemicals. However, the exact role of tansy in the biology of EGVM is not yet understood. Tansy flowers and nectar might provide a food source needed by females for egg maturation (Stoeva, 1982). Tansy has long been used for various pharmaceutical and toxicological purposes (Chandler et al., 1982; Dembitskii et al., 1984). It is therefore possible that the occurrence of females on tansy flowers is related to pharmacophagy as demonstrated in the Lepidoptera (Boppré, 1984).

Nonhost plant odors can interfere with the perception of the particular blend used by an herbivore and modify its orientation (Thiéry and Visser, 1986, 1987). Tansy has been reported to alter colonizing patterns of different insect species. Observations made on the flea beetle, *Phyllotreta cruciferae*, indicated that tansy inhibits adult colonization (Latheef and Ortiz, 1984). Essential oils of tansy have also been proposed as repellents against aphids in orchards (Dembitskii et al., 1984) and Colorado potato beetle (Schearer, 1984). In the cabbage worm, *Pieris rapae*, tansy increases the number of eggs laid on crop plants, whereas in the cabbage looper, *Trichoplusia ni*, the effect was the reverse (Latheef and Ortiz, 1983). In the case of EGVM, the odor produced by tansy could be used to (1) interfere with the orientation of females to their host, (2) lure females outside the vineyards, (3) monitor the population dynamics, or (4) directly control the population by mass trapping. The reduced blend of monoterpenes that evoked significant electrophysiological and behavioral activity for the EGVM, must now be tested for its behavior-evoking activity in larger scale experiments in vineyards.

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