**SCIENTIFIC NOTE**

# Behavioral and antennal responses of *Lobesia botrana* (Lepidoptera: Tortricidae) to volatiles from the non-host plant *Schinus molle* L. (Anacardiaceae)

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## ABSTRACT

*Lobesia botrana* (Lepidoptera: Tortricidae), the grapevine moth, is one of the major pest on grapes. Current control is based on spraying insecticides and/or mating disruption technique, using female sex pheromone, affecting only adult male's conduct. Behavioral active compounds as plants kairomones influence the interaction of phytophagous insects with plants, providing chemical cues to feed or lay eggs. Through electroantennography (EAG) and olfactory experiments, we investigated the role of volatiles of the non-host plant *Schinus molle* L. on behavior of *L. botrana*. Steam distilled essential oil (EO) from *S. molle* leaves was characterized by gas chromatography coupled to mass spectrometry (GC-MS). The most abundant compounds were limonene (17.61%), α-phellandrene (14.32%), β-caryophyllene (8.82%) and δ-cadinene (9.39%). Electrical responses of *L. botrana* at $1\times10^2$ μg mL$^{-1}$ EO were not different from control for females (0.81 mV) and males (1.22 mV). At $1\times10^3$ and $1\times10^4$ μg mL$^{-1}$ EO we found a significant increment for females to 1.28 and 1.57 mV, and 1.28 and 1.69 mV for males, respectively. In tube-Y olfactometer, at $1\times10^2$ μg mL$^{-1}$ EO the choice did not differ from the control for females (P = 0.7630) and males (P = 0.4054). Females were attracted to odor source at $1\times10^3$ μg mL$^{-1}$ (P = 0.0043) and at $1\times10^4$ μg mL$^{-1}$ (P = 0.0011). Males were attracted to the dose of $1\times10^3$ μg mL$^{-1}$ (P = 0.0278) and at $1\times10^4$ μg mL$^{-1}$ (P = 0.0017). We found that non-host plant volatiles elicited electrophysiological and behavioral responses and that *L. botrana* can discriminate between doses.

**Key words:** EAG, insect behavior, olfactometer, plant volatiles.

## INTRODUCTION

Plant volatiles are essential to phytophagous insects, providing relevant information on hosts search, feeding, egg laying and evading non-host plants (Bruce and Pickett, 2011; Dicke, 2015). Insects' mechanisms to recognize plants volatiles in both host and non-host commonly rely on blends of compounds at specific ratios (Bruce and Pickett, 2011). Non-host plant volatiles (NHPVs) may disrupt the olfactory process in finding the host by a masking, deterrent or repellent effect. The insect repellency elicited by NHPVs has been reported for several species (Zhang and Schlyter, 2010; Bruce and Pickett, 2011). The tea green leafhopper, *Empoasca vitis* (Hemiptera), was repelled by NHPVs of *Corymbia citriodora* (Myrtaceae), *Lavandula pinnata* (Lamiaceae) and *Rosmarinus officinalis* (Lamiaceae) (Zhang et al., 2014). The scolytid *Ips typographus* uses an active avoidance of specific volatiles as 1-hexanol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol, 3-octanol, and 1-octen-3-ol (Zhang and Schlyter, 2010). Furthermore, pheromone detection is also affected by NHPVs. Terpenoids as NHPVs disrupted male *Plutella xylostella* (Lepidoptera) ability to navigate on pheromone plume and female

calling behavior was adversely affected (Wang et al., 2016). The polyphagous European grapevine moth, *Lobesia botrana* (Lepidoptera), has shown attraction to host plant volatiles (kairomones) and to NHPVs. This may explain *L. botrana* behavioral plasticity (Ioriatti et al., 2011). *Lobesia botrana* displayed oviposition preference for the NHPVs (*S*)-(−)-perillaldehyde and isoegomaketone, released by *Perilla frutescens* plant (Cattaneo et al., 2014). NHPVs from *Tanacetum vulgare* (Asteracea) plants have shown inhibited oviposition and mating behavior, and adult longevity reduction on *L. botrana* (Gabel and Thiéry, 1994).

Pepper tree, *Schinus molle* L. (Anacardiaceae), native from the Andean region, has been used as an ancestral source in ethno-medicine (López et al., 2014; Abderrahim et al., 2018). Moreover, its essential oil, mainly from leaves and fruits, has revealed insecticidal and repellent activity to several pests (Wimalaratne et al., 1996; Benzi et al., 2009; Abdel-Sattar et al., 2010; Huerta et al., 2010). *Schinus molle* essential oil (EO) from fruits was an attractant to *Acyrthosiphon pisum* (Homoptera: Aphididae) (Kasmi et al., 2017), and the EO obtained from leaves was repellent to *Blatta orientalis* (Blattodea: Blatidae) (Deveci et al., 2010).

In this study, through electroantennographic and olfactory bioassays, the behavioral activity of females and males *L. botrana* in response to essential oil of *S. molle* was investigated. Gas chromatography coupled to mass spectrometry was used to identify volatile components of the essential oil.

## MATERIALS AND METHODS

**Insects**
Adults of *L. botrana* were obtained from a laboratory colony established at Instituto de Investigaciones Agropecuarias, INIA LA Cruz, Quillota, Chile. The colony is yearly refreshed and larvae are fed *ad libitum* on artificial diet (Southland Products Inc., Lake Village, Arkansas, USA) at 23 ± 1 °C and 16:8 h photoperiod with a 75% RH. To ensure virginity, at pupae stage the individuals are separated by sex in transparent plastic boxes until bioassays.

**Essential oil and chemical analyses**
In autumn 2017 we collected leaves from ornamental *S. molle* plants in Chillán, Ñuble Region, Chile; 289 g air-dried leaves were milled and boiled for 8 h to obtain the essential oil by hydrodistillation using a type Clevenger apparatus. We added $Na_2SO_4$ to remove water from samples and then were stored at 8 °C under complete darkness. An aliquot (1 mg mL$^{-1}$ diluted in hexane) of the essential oils (EO) was injected on a gas chromatographer equipped with a capillary column (Rxi-5ms: 5% diphenyl-95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 1.0 μm; Restek Corporation, Bellefonte, Pennsylvania, USA), coupled to a mass spectrometer (GC-MS QP2010 Plus, Shimadzu, Tokyo, Japan). Using helium as carrier gas (1.4 mL min$^{-1}$) the ionization was performed by electron impact (70 eV) and the acquisition in the mass range from 35 to 500 m/z. The GC oven was set up to 40 °C and then increased at the rate of 7 °C min$^{-1}$ until reach 250 °C. EO volatiles were identified by comparing the mass spectrum with NIST05 library (Standard Reference Data, NIST, Gaithersburg, Maryland, USA) and commercially available standards.

**Electroantennographic recordings**
We evaluated the sensitivity of *L. botrana* to volatiles from *S. molle* EO with an electroantennographic (EAG) bioassay. The recordings were achieved using a Syntech EAG system (Syntech, Hilversum, The Netherlands). Excised antennae from unmated adults of *L. botrana*, 2 to 4 d old, were mounted between glass electrodes filled with 0.1 M KCl and 0.1% polyvinylpyrrolidone (Ceballos et al., 2015). The antennae were extirpated above the scape with a scalpel. The distal segment was cut with dissection scissors to improve the connection. Three concentrations of EO in hexane (at $1 \times 10^2$, $1 \times 10^3$ and $1 \times 10^4$ μg mL$^{-1}$), were applied onto a filter paper strip (1 cm × 10 cm; Whatman N°1 paper), were delivered to the antennal preparation at 30 mL s$^{-1}$ for 2 s using a stimulus controller (CS-55, Syntech, Hilversum, The Netherlands) with a period of at least 60 s for recovery of antennal receptors. Data acquisition and analysis were carried out with EAG version 2014 software (Syntech, Hilversum, The Netherlands). Five antennae, from both males and females, were stimulated three times.

**Olfactometric bioassays**

The behavioral response of *L. botrana* (2 to 4 d old) to volatiles of *S. molle* was carried out using a glass Y-tube olfactometer (21 cm long with 3 cm id). Glass odor chambers (3 cm id and 15 cm high) were connected to the end of Y-tube arms to deliver the stimulus using filtered air 280 at mL min$^{-1}$ with a positive pressure air-pump. A volume of 10 μL of diluted EO ($1\times10^2$, $1\times10^3$ and $1\times10^4$ μg mL$^{-1}$) was loaded onto a filter paper strip ($1\times7.5$ cm), allowing 30 s to solvent evaporation, and then laid inside one odor chamber; hexane was used as control in the opposite odor chamber. One individual at a time was allowed to move freely inside the olfactometer for 6 min. The first choice, made by the insect trespassing beyond 6 cm from the intersection area of the Y-tube to any arm, was considered a positive election. The insects were tested once using a clean olfactometer under artificial fluorescent light (30-40 lux) at 23 ± 1 °C, 50 ± 3% RH (Cattaneo et al., 2014). Thirty replicates for each sex and EO concentration were conducted using a completely randomized experimental design.

**Statistical analysis**

The mean EAG amplitudes in response to hexane (control) and EO were compared by Student's t test ($P < 0.05$). To compare the EAG amplitudes elicited by the different concentration of EO volatiles, data were standardized by subtracting the average of the hexane-signal before and after the corresponding stimulus-signal and submitted to ANOVA followed by Tukey's HSD (honestly significant difference) test ($P < 0.05$) (Ceballos et al., 2015). The proportions of choice in the olfactometric bioassays were analyzed through Pearson's Chi-square test followed by Marascuilo's procedure for comparing multiple proportions ($P < 0.05$).

## RESULTS AND DISCUSSION

**Chemical characterization of essential oil of *S. molle***

Most volatile constituents of EO of *S. molle*, obtained by steam distillation of leaves, are terpenes mainly monoterpenes such as limonene (17.61%), α-phellandrene (14.32%), β-pinene (1.55%), β-myrcene (1.66%), sesquiterpenes as β-caryophyllene (8.82%), β-elemene (1.34%) and sesquiterpenoids δ-cadinene (9.39%), and α-amorphene (1.24%) (Table 1). Other studies on the chemical composition of EO from *S. molle* have shown the presence of limonene and

**Table 1. Chemical composition of volatile fraction of essential oil of *Schinus molle*.**

| Compound | Abundance | Insect (behavioral role)* | Reference |
|---|---|---|---|
| | % | | |
| Alcohol | | | |
| 3-Hexanol | 1.95 | | |
| Monoterpenes | | | |
| α-Pinene | 3.25 | *Cydia strobilella* (A) | Jakobsson et al., 2016 |
| | | *Chilasa epycides* (R) | Omura et al., 2006 |
| Camphene | 2.94 | *Papilio glaucus* (A) | Frankfater et al., 2009 |
| β-Pinene | 1.55 | *Cydia strobilella* (A) | Jakobsson et al., 2016 |
| β-Myrcene | 1.66 | *Cydia strobilella* (A) | Jakobsson et al., 2016 |
| α-Phellandrene | 14.32 | *Thyrinteina arnobia* (A) | Batista-Pereira et al., 2006 |
| *m*-Cymene | 3.39 | *Dysdercus cingulatus* (R) | Farine et al., 1992 |
| Limonene | 17.61 | *Lobesia botrana* (K) | Gabel et al., 1992 |
| | | *Thyrinteina arnobia* (A) | Batista-Pereira et al., 2006 |
| Sesquiterpenes | | | |
| 1,3,6-Heptatriene, 2,5,6-trimethyl | 1.55 | *Chilasa epycides* (R) | Omura et al., 2006 |
| β-Elemene | 1.34 | *Papilio paris* (R) | Omura et al., 2006 |
| | | *Papilio polytes* (R) | |
| β-Caryophylene | 8.82 | *Lobesia botrana* (A) | Tasin et al., 2005 |
| | | *Chilasa epycides* (R) | Omura et al., 2006 |
| Germacrene-B | 6.65 | *Papilio glaucus* (A) | Frankfater et al., 2009 |
| | | *Chilasa epycides* (R) | Omura et al., 2006 |
| Sesquiterpenoids | | | |
| α-Amorphene | 1.24 | *Atrophaneura aristolochiae* (A) | Honda and Hayashi, 1995 |
| δ-Cadinene | 9.39 | | |
| Elemol | 3.17 | *Trialeurodes ricini* (A) | Hussein et al., 2017 |

*Behavioral role based on the right column reference: (A) Attractant, (R) repellent and (K) kairomone.

α-phellandrene as common major constituents in leaves in range of 0.79% to 15.6% and 1.3% to 45.6%, respectively (Baser et al., 1997; Benzi et al., 2009; Santos et al., 2009; Deveci et al., 2010; Abderrahim et al., 2018). The composition presented in Table 1, responds to 78.8% of the total composition of the EO (1.08% w/w from 289 g DM).

**Electroantennographic responses of *L. botrana* to *S. molle* volatiles**
Volatiles compounds from EO of *S. molle* elicited a higher electrical amplitude on adults of *L. botrana*, except at the lowest dose evaluated. At $1\times10^2$ μg mL$^{-1}$ the EAG response elicited by the EO was not different from hexane (control) for females (t = 1.722; P = 0.096; CI$_{95\%}$ [-0.017; 0.200]) and males (t = 1.044; P = 0.305; CI$_{95\%}$ [-0.077; 0.237]) (Figure 1). However, at $1\times10^3$ μg mL$^{-1}$ we found a significant increment in the EAG responses for females (t = 3.057; P = 0.0050; CI$_{95\%}$ [0.066; 0.332]) and males (t = 3.444; P = 0.002; CI$_{95\%}$ [0.063; 0.087]). Similarly, the electrophysiological response at $1\times10^4$ μg mL$^{-1}$ was large for females (t = 2.099; P = 0.045; CI$_{95\%}$ [0.007; 0.659]) and males (t = 3.498; P = 0.002; CI$_{95\%}$ [0.123; 0.470]). Furthermore, the electrical amplitude registered from the antennas in response to the EO showed an increment with dose for both females (F = 53.3; P < 0.001; η$^2$ = 0.722) and males (F = 61.7; P < 0.001; η$^2$ = 0.746) (Figure 1).

**Behavioral response of *L. botrana* to *S. molle* EO volatiles**
In Y-tube olfactometer females and males of *L. botrana* were significantly attracted to *S. molle* volatiles, except at the lowest studied dose (Figure 2). At $1\times10^2$ μg mL$^{-1}$ the frequency of choice did not differ noticeably from the control for females (X2 = 0.09; P = 0.7630; CI$_{95\%}$ [0.0951; 0.3731]) and males (CI$_{95\%}$ [0.1418; 0.4445]; X2 = 0. 69; P = 0.4054). Females were notably attracted to odor source at $1\times10^3$ μg mL$^{-1}$ (CI$_{95\%}$ [0.4551; 0.7813]; X2 = 8.17; P < 0.0043) and at $1\times10^4$ μg mL$^{-1}$ (CI$_{95\%}$ [0.4878; 0.8077]; X2 = 10.67; P < 0.0011). Similarly, males were attracted to dose of $1\times10^3$ μg mL$^{-1}$ (CI$_{95\%}$ [0.4232; 0.7541]; X2 = 4.84; P = 0.0278) and at $1\times10^4$ μg mL$^{-1}$ (CI$_{95\%}$ [0.5212; 0.8334]; X2 = 9.85; P = 0.0017).

**Figure 1. Electroantennographic amplitude of *Lobesia botrana* (average and CI$_{95\%}$) to different dose of *Schinus molle* essential oil diluted in hexane.**
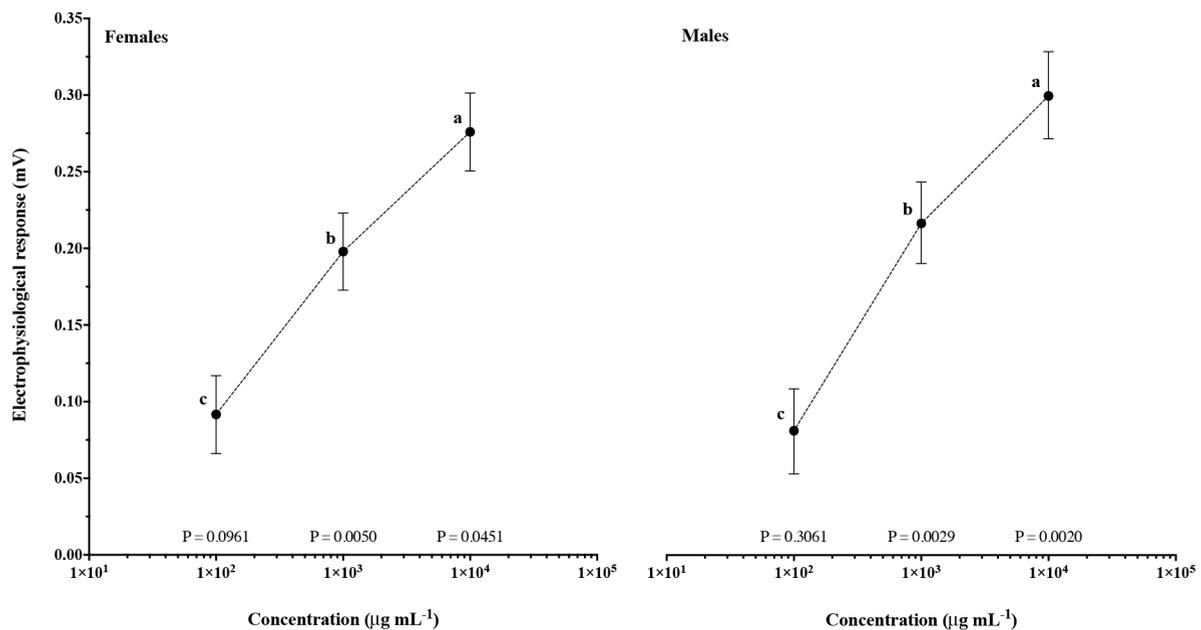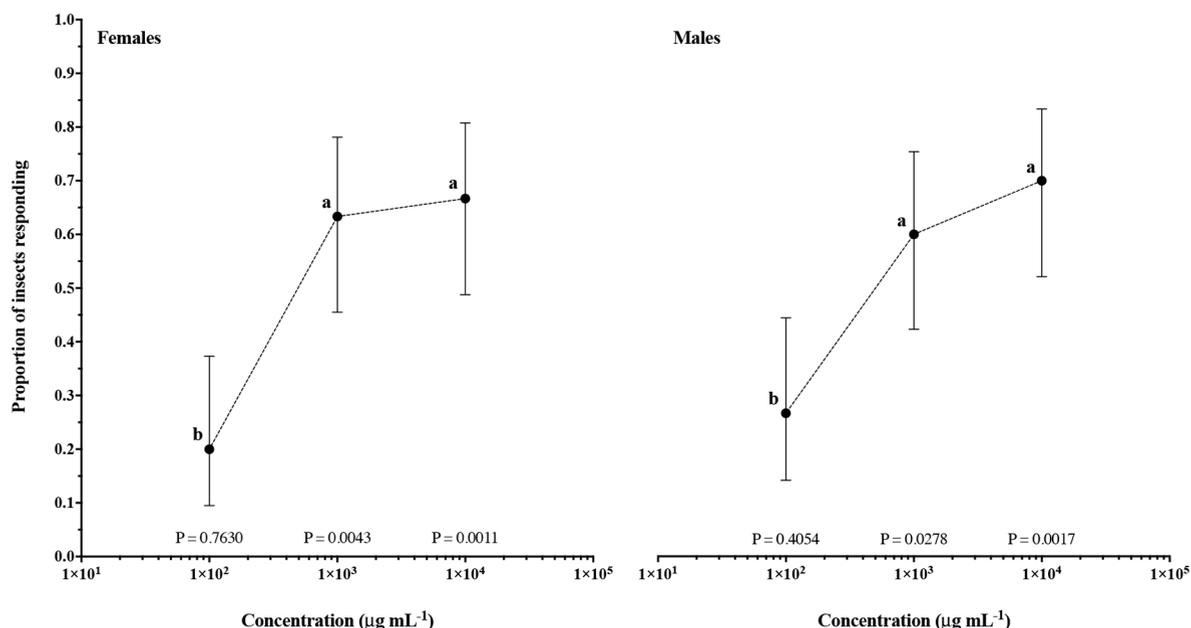


Means sharing a letter do not different significantly according Tukey's test (P < 0.05). Data were standardized by subtracting the average of the immediately prior and afterward controls to the essential oil signal.
P < 0.05, according Student's *t*-test, next to X-axe indicate significant differences between essential oil and hexane (control) for each dose.
CI$_{95\%}$: Confidence interval 95%.

**Figure 2. Behavioral response of *Lobesia botrana* (proportion and $CI_{95\%}$) to different dose of *Schinus molle* essential oil diluted in hexane.**



Proportions sharing a letter do not different significantly according Marascuilo's procedure (P < 0.05).
P < 0.05, according Chi-square test, next to X-axe indicate significant differences between essential oil and hexane (control) for each dose.
$CI_{95\%}$: Confidence interval 95%.

Most studies on chemical constituents of *S. molle* have been oriented to its medicinal use. However, insecticidal, repellent and fumigant activity for *S. molle* has been found for different herbivorous insects (Chirino et al., 2001; Ruffinengo et al., 2005; Abdel-Sattar et al., 2010; Deveci et al., 2010). On the other hand, the electroantennographic responses of *L. botrana* to host-plants volatiles have been extensively studied on *Daphne gnidium*, the putative ancestral host in Europe and *Vitis vinifera* (Tasin et al., 2005; Maher and Thiéry, 2006; von Arx et al., 2011). *Lobesia botrana* relation with non-host plants, mediated by volatiles compounds, has been studied on *Tanacetum vulgare* and *Perilla frutescens* (Cattaneo et al., 2014). The characterization of volatiles compounds emitted by *T. vulgare* and *P. frutescens* showed that *p*-cymene, *d*-limonene, α-thujene, α-thujone, β-thujone, thujyl alchohol, terpine-4-ol, Z-verbenol, piperitone, (*S*)-(−)-perillaldehyde and isoegomaketone were electrophysiological active on both males and females *L. botrana* (Gabel et al., 1992; Masante-Roca et al., 2007; Cattaneo et al., 2014). However, to our knowledge, literature on EO of *S. molle* and behavioral response of *L. botrana* seems insufficient. Our findings reveal that volatiles compounds from EO of *S. molle* stimulated olfactory receptors and induced electrophysiological responses on both females and males *L. botrana*. Additionally, we found a dose-dependent relationship between the amplitude of antennal deflection and the attraction behavior exhibited in the Y-tube olfactometer. Experiments on plant volatile perception by *L. botrana* allowed to find *E*-(β)-caryophyllene, *E*-(β)-farnesene and *E*-(4,8)-dimethyl-(1,3,7)-nonatriene (DMNT) as physiologically active compounds used as chemical cues in host finding and oviposition site selection (Tasin et al., 2006; Ioriatti et al., 2011).

## CONCLUSIONS

The volatile profile of the essential oil (EO) of *Schinus molle*, obtained by steam hydrodistillation of leaves, was electrophysiological and behavioral active on *Lobesia botrana*. At concentration of $1\times10^3$ and $1\times10^4$ µg mL$^{-1}$ EO was behavioral active to females and males *L. botrana*. Our results suggest that composition and compounds ratio in the volatile compounds profile are responsible for the attraction of females and males *L. botrana*.

## ACKNOWLEDGEMENTS

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