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# Antennal responses of black plum sawfly (*Hoplocampa minuta*) to European plum (*Prunus domestica*) flower volatiles

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## RESEARCH ARTICLE

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**ABSTRACT**

Black plum sawfly (*Hoplocampa minuta*) is an important pest species of plum (*Prunus domestica*). In organic plum orchards, the yield loss caused by *H. minuta* larval damage can reach almost 100% occasionally. Adults feed on pollen and nectar of the plum flower; therefore, we hypothesize that, besides visual cues, also olfaction plays an important role in habitat and host finding. To understand the chemical communication mediated by flower volatiles to black sawflies, we investigated the chemical signals released from plum flowers, which can trigger the peripheral physiological responses of adult sawflies. First, using gas chromatography coupled with electroantennography (GC-EAD), we selected 18 physiologically active compounds from the headspace volatile collection of plum flowers, which triggered the *H. minuta* male and female antennae. Subsequently, we determine the volatilome of plum flower and identified those compounds, which elicited physiological responses, using gas chromatography coupled with mass spectrometry (GC-MS). These antennally active components in flower volatiles could be candidates for potential kairomone, which could later be used for attracting males and females of *H. minuta* and could contribute to developing pesticide-free, effective monitoring and lure and kill strategy against this pest.

**KEYWORDS**

*Hoplocampa minuta*, GC-EAD, GC-MS, flower volatiles, *Prunus domestica*

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## INTRODUCTION

Sawflies cause serious damage in plum, apple and pear orchards in Europe and North America (Caruso and Cera, 2004; Graf et al., 1996; Liston et al., 2019; Nagy, 1960). Among many sawfly species in Europe, the black plum sawfly (*Hoplocampa minuta* Christ., 1791) is one of the most important pests of plum (*Prunus domestica* L., Rosaceae). In organic plum orchards, the yield loss caused by *H. minuta* varies between 36 and 96% (Andreev and Kutinkova, 2010; Caruso and Cera, 2004; Liston et al., 2019; Oroian et al., 2009; Rozpara et al., 2010).

*H. minuta* is a monophagous, univoltine species feeding exclusively on plum fruit. The larvae and the quiescent prepupae in cocoons overwinter 5–20 cm below ground. Pupation takes place approximately one month before the adult emergence. Adults emerge during the flowering period of the plum (phenological stage: 55–69 on the BBCH-scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie)) (Friedrich and Buchter-Weisbrodt, 1996; Meier, 2018; Nagy, 1960). The adults are active at daylight and feed on pollen and nectar of the flowering plum. After mating, females lay 50–70 eggs singly under the epidermis of the flower sepal using their saw-like ovipositor (Nagy, 1960). During the early fruit development (phenological stage: BBCH 70–72), the hatched larvae enter into the fruit. During the feeding, larvae can damage several neighboring fruits. Later, the damaged, unripe fruits, contaminated with larval excrement, fall off from the tree. The plant protection strategy against this species is solely based on chemical insecticide treatment. The most appropriate time of the treatment is the petal fall of the plum flower, which coincides with the larval hatch (Bovien and Stapel, 1940; Leski, 1960). Although these insecticides are applied to target *H. minuta*, their use in plum orchards can have implications for non-target insects, such as bees and other beneficial insects.

The aim of the ecosystem-based integrated pest management (IPM) is long-term prevention of pests, which incorporates a deep knowledge of the biology and ecology of the targeted pest (Flint, 2012; Lefebvre et al., 2015). To date, only a white sticky trap-based monitoring system is available to detect and monitor the existence of *H. minuta* adults in plum orchards (Blaisinger, 1975; Kárpáti et al., 2021; Tamošiūnas et al., 2014). However, understanding the chemical communication between *H. minuta* and its host plant, plum (*P. domestica*), helps to develop a kairomone-based, species-specific environmentally friendly pest control strategy. As an example, ethyl (2*E*, 4*Z*)-2,4-decadienoate has already been demonstrated to be an effective species-specific kairomone, which attracts both male and female codling moths (*Cydia pomonella*) (Light et al., 2001). Therefore, the objective of this study was to identify the chemical structure of the plum flower volatile compounds, which are detected by the *H. minuta* male and female antenna, using gas chromatography coupled with electroantennography (GC-EAD) and gas chromatography coupled with mass spectrometry (GC-MS).

## MATERIAL AND METHODS

### Insects

Adult *H. minuta* males and females were collected from an organic plum orchard located in Nagykovácsi, Pest county, Hungary (GPS coordinates: 47.575018, 18.897893) during the plum flowering period. An insect collecting net with an extended handle was used to catch adults during their flower visit.

## Volatile collection

Headspace volatile collections were conducted from intact plum (*P. domestica*, var. Besztercei NM 122) flowers in the full blooming stage (BBCH 65). Flowers were enclosed in a plastic oven bag (Alufix GmbH, Wr. Neudorf, Austria) on the stem of the plum tree. The bag was tightly closed with cable ties around the stem and using a vacuum pump (Thomas G 12/02 EB, Garder Denver Thomas, Fürstenfeldbruck, Germany) charcoal filtered continuous airflow at 0.8 L $min^{-1}$ was drawn through using inert PTFE tubing. Volatiles were continuously collected for 4 hours using SuperQ adsorbent (80/100 mesh, Altech, Deerfield, IL, USA), packed in a glass tube (ID: 4 mm). Before experiments, adsorbent filters were purified sequentially with methanol (purity: 99.8%; Reanal, Budapest, Hungary), a mixture of methanol and chloroform (chloroform purity: 99.9%; Reanal) (3:1), acetone (purity: 99.5%; Reanal) and dichloromethane (purity: 99.9%; Reanal). Filters were subsequently dried with nitrogen (Linde, Dunaharaszti, Hungary; purity: 99.999%) flow and heated for 2 hours at 70 °C (Kunert et al., 2009). Headspace volatiles was eluted with 200 µl of dichloromethane. The extracts were concentrated to 40 µL under nitrogen stream and kept at −40 °C until used for GC-EAD recordings and GC-MS chemical identification. To exclude the possibility of contaminations, a system blank volatile collection without flower was also made.

## Electrophysiology

The GC-EAD system consisted of a 6890N gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with an HP-5 column (J&W, 30 m × 0.32 mm, 0.25 µm film thickness; Agilent Technologies Inc.), with a split to FID and an effluent conditioning assembly (Ockenfels Syntech, Kirchzarten, Germany) carrying the effluent to an electroantennographic detector. Injections were made in splitless mode (230 °C). The temperature program was as follows: the initial temperature was held at 50 °C for 1 min, then the heating rate was 10 °C $min^{-1}$ to 230 °C, which final temperature was held for 10 min. Helium was used as carrier gas (flow: 2.0 ml $min^{-1}$). The GC was controlled and data acquisition was performed by an Agilent ChemStation Program (version Rev. A. 10.02). For electroantennography recordings, the head of the insect was incised. The head and the tip of the antennae were placed between two glass capillary electrodes (1.17 mm i.d.), filled with ringer solution (Ephrussi and Beadle, 1936) and equipped with silver wires for transmitting the electric signal to the pre-amplifier. Connections were established with the help of MP15 micromanipulators. Electric signals were pre-amplified by an IDAC2 amplifier and analyzed by Syntech GC-EAD 2014 v. 1.2.5. software (Ockenfels Syntech, Kirchzarten, Germany). Four-four *H. minuta* male and female GC-EAD recordings were made.

## Identification of volatile constituents

The GC-MS measurements were carried out on an Agilent 6890 GC (Agilent Technologies Inc.) coupled to an Agilent 5973 mass selective detector, operated in scan mode. Helium was used as a carrier gas with a flow rate of 1 mL $min^{-1}$ in constant flow mode. One µL of the sample was injected in splitless mode and purge flow was 20 mL $min^{-1}$ after 1 min. For GC separation, an HP-5 MS UI (30 m × 0.25 mm × 0.25 µm, J&W, Agilent) column was installed, and the GC was programmed as follows: 40 °C hold for 1.0 min, increased by 10 °C $min^{-1}$ to 270 °C and held

for 10 min. For MS detection EI ionization was used with a standard 70 eV energy. The auxiliary heater was set to 290 °C, the MS source to 250 °C and the MS quad to 150 °C. For identification, the scan event was set to monitor *m*/*z* 35–400 with a scan speed of 2 scan/s. The system was served by a ChemStation software D.01.02.16 (Agilent Technologies Inc.). Compounds were tentatively identified by matching their mass spectra with those in the MS Libraries (NIST 11 and Wiley) and the identification was verified by comparison with Kováts index (Ki) values published and calculated.

## RESULTS

Using GC-MS, we separated and identified 60 chemical compounds from the headspace volatile collection of the European plum flower (Table 1). We also detected two additional compounds, which were chemically not identified (Table 1). The 10 most abundant compounds were: methyl salicylate (15.09%), (*E*)-cinnamyl alcohol (13.27%), beta-linalool (11.5%), (*Z*)-beta-ocimene (9.74%), lilac aldehyde A (9.25%), lilac aldehyde B (7.83%), benzaldehyde (6.94%), (*Z*)-cinnamyl alcohol (5.85%), decanal (3.51%) and nonanal (3.15%) (Table 1). Using GC-EAD, we recorded 18 volatile compounds from the plum flower volatile collection, which were antennally active; i.e. either female or male antenna of *H. minuta* responded to these compounds: hexanal (1), (*Z*)-3-hexen-1-ol (2), benzaldehyde (3), 6-methyl-5-heptene-2-one (4), (*Z*)-3-hexenyl acetate (5), (*Z*)-beta-ocimene (6), beta-linalool (7), nonanal (8), lilac aldehyde B (9), lilac aldehyde A (10), lilac aldehyde D (11), methyl salicylate (12), lilac alcohol A (13), lilac alcohol D (14), (*Z*)-cinnamyl alcohol (15), (*E*)-cinnamaldehyde (16), (*E*)-cinnamyl alcohol (17), alpha-farnesene (18) (Fig. 1). Of these compounds, hexanal, (*Z*)-3-hexen-1-ol, (*Z*)-beta-ocimene, (*Z*)-cinnamyl alcohol and alpha-farnesene activated only female antennae (Fig. 1). Compounds, which elicited a response from male antennae, also activated female antennae (Fig. 1).

## DISCUSSION

Revealing the elements of chemical communication between flowers and insects, in the case of pests specialized to be attracted to flowers, are essential to developing an environmentally friendly, non-pesticide-based pest management strategy. To develop a flower odor-based attracting method against *H. minuta*, the monophagous pest of plum (*P. domestica*), the first step is to pinpoint plum flower volatile compounds, which are detected by the male and female antenna. The second step is to identify the chemical structure of those, antennal active compounds. In this study, using GC-EAD recordings, we found 13 compounds, which activated both male and female antennae. Additionally, we identified 5 compounds, which elicited antennal response only from female antennae (Fig. 1). Besides floral attractants for females, these additional compounds may be involved in finding an appropriate oviposition site. The GC-MS identification results indicate that the 18 active compounds belong to the groups of aldehydes, alcohols, ketones, esters and monoterpenes (Table 1). Based on our knowledge, this is the first study to identify antennally active plum flower volatiles in the *Hoplocampa* genus. In the same, Tenthredinidae family, however, Kehl et al. (2010) identified several *Salix fragilis* flower-emitted volatiles, which elicited EAD responses from leaf-galling sawfly (*Pontania proxima*) female antenna.

*Table 1.* Volatile organic compounds identified in headspace collections from the European plum flower (*Prunus domestica*). Relative area is expressed as a percentage relative to total volatile compounds

| Compound | RT | RI | Relative area (%) | EAD |
|---|---|---|---|---|
| 1-octene | 3.45 | 791 | 0.03 | |
| Hexanal | 3.50 | 802 | 0.04 | 1 |
| Octane | 3.56 | 816 | 0.04 | |
| (*Z*)-3-hexen-1-ol | 4.25 | 858 | 0.22 | 2 |
| (*Z*)-2-hexen-1-ol | 4.38 | 865 | 0.07 | |
| p-xylene | 4.52 | 875 | 0.38 | |
| 1-nonene | 4.81 | 890 | 0.10 | |
| Nonane | 4.94 | 900 | 0.69 | |
| Benzaldehyde | 5.91 | 960 | 5.40 | 3 |
| 6-methyl-5-heptene-2-one | 6.23 | 986 | 0.84 | 4 |
| 1,2,3-trimethyl benzene | 6.43 | 993 | 0.22 | |
| (*Z*)-3-hexenyl acetate | 6.53 | 998 | 1.59 | 5 |
| Benzyl alcohol | 7.02 | 1,033 | 0.50 | |
| (*E*)-beta-ocimene | 7.04 | 1,048 | 0.90 | |
| (*Z*)-beta-ocimene | 7.23 | 1,049 | 7.59 | 6 |
| Allo-ocimene | 7.30 | 1,041 | 0.50 | |
| (*Z*)-linalool oxide (furanoid) | 7.59 | 1,067 | 0.85 | |
| (*E*)-linalool oxide (furanoid) | 7.65 | 1,084 | 0.57 | |
| Beta-linalool | 8.08 | 1,100 | 8.96 | 7 |
| Nonanal | 8.13 | 1,104 | 2.45 | 8 |
| (*E*)-4.8–dimethyl–1,3,7-nonatriene | 8.26 | 1,116 | 1.57 | |
| Benzyl cyanide | 8.65 | 1,140 | 0.12 | |
| Lilac aldehyde B | 8.73 | 1,197 | 6.10 | 9 |
| 4-oxoisophorone | 8.77 | 1,147 | 1.89 | |
| Lilac aldehyde A | 8.86 | 1,197 | 7.21 | 10 |
| Lilac aldehyde D | 9.07 | 1,197 | 2.21 | 11 |
| 1-nonanol | 9.12 | 1,168 | 0.50 | |
| Epoxylinalool | 9.17 | 1,198 | 0.28 | |
| Methyl salicylate | 9.56 | 1,191 | 11.76 | 12 |
| Lilac alcohol A | 9.62 | 1,211 | 0.59 | 13 |
| Decanal | 9.66 | 1,204 | 2.74 | |
| Lilac alcohol C | 9.77 | 1,251 | 1.07 | |
| Alpha-limonene diepoxide | 9.84 | 1,294 | 0.49 | |
| (*Z*)-cinnamaldehyde | 9.91 | 1,258 | 0.67 | |
| Lilac alcohol D | 9.98 | 1,251 | 0.65 | 14 |
| Hydrocinnamic alcohol | 10.05 | 1,232 | 2.13 | |
| (*Z*)-3-decen-1-ol | 10.39 | 1,266 | 0.11 | |
| (*Z*)-cinnamyl alcohol | 10.48 | 1,275 | 4.56 | 15 |
| 1-decanol | 10.59 | 1,272 | 0.62 | |
| (*E*)-cinnamaldehyde | 10.68 | 1,272 | 2.05 | 16 |
| (*E*)-cinnamyl alcohol | 11.15 | 1,315 | 10.34 | 17 |
| 2-undecenal | 11.88 | 1,363 | 0.40 | |
| Hydrocinnamyl isobutyrate | 11.96 | 1,408 | 0.40 | |
| *m/z* 56,57,71,89,141, 173 | 12.02 | | 0.16 | |

*(continued)*

**Table 1.** Continued

| Compound | RT | RI | Relative area (%) | EAD |
|---|---|---|---|---|
| Cinnamyl acetate | 12.19 | 1,440 | 0.10 | |
| Tetradecane | 12.34 | 1,413 | 0.33 | |
| Dodecanal | 12.46 | 1,411 | 0.24 | |
| *m/z* 55,67,79,91,93,119,161 | 12.61 | | 0.17 | |
| Methyl azelaaldehydate | 12.74 | 1,429 | 0.16 | |
| Beta-caryophyllene | 12.77 | 1,420 | 0.06 | |
| (*E*)-cinnamyl acetate | 12.83 | 1,446 | 0.41 | |
| Geranylacetone | 12.95 | 1,458 | 0.38 | |
| 2,6,10-trimethyltetradecane | 13.12 | 1,519 | 0.10 | |
| (*Z*,*E*)-α-Farnesene | 13.50 | 1,491 | 0.66 | |
| Pentadecane | 13.62 | 1,500 | 1.07 | |
| Alpha-farnesene* | 13.68 | 1,509 | 1.92 | 18 |
| (*Z*)-3-hexenyl benzoate | 14.55 | 1,570 | 0.71 | |
| Hexadecane | 14.82 | 1,600 | 0.47 | |
| Heptadecane | 15.97 | 1,700 | 0.42 | |
| Benzyl benzoate | 16.81 | 1,765 | 0.31 | |
| Eicosane | 18.09 | 2,000 | 1.52 | |
| Henicosane | 20.13 | 2,100 | 0.40 | |

RT: Retention Time, RI: Retention Index, EAD: compounds elicited EAD responses, numbers correspond to Fig. 1 EAD responses. * Unknown isomer of alpha-farnesene.

Our 18 identified, physiologically active compounds, are typical flower volatiles identified also from other flowers, which have a function in chemical communication in other insect taxa (El-Sayed, 2022). Additionally to the 18 antennal active components, we also identified most of the volatile components from the collected plum flower headspace, with the exception of two (Table 1). Using a comparable volatile collection method, Baraldi et al. (1999) also found similar compounds in plum headspace; however, our identification shows additional and different volatile components from the headspace of the European plum flower. These differences could be due to the different plum varieties or the condition and timing of the volatile collection.

Adults of different *Hoplocampa* species feed on nectar and pollen for energy and protein sources, respectively (Nagy, 1960). Therefore, to locate the flower, as a nectar and pollen source, *H. minuta* may use these components, which electrophysiologically activated both male and female antennae. Volatile organic components emitted by flowers and leaves are generally important kairomones for herbivores to locate their host plant (Bruce et al., 2005; Finch and Collier, 2000). Although the EAD responses themselves cannot differentiate the attractive and repulsive components, we hypothesize that these antennally active flower compounds, in a multi-component blend, play a role to attract males and females in field conditions.

Based on our knowledge, this is the first study showing the antennal active volatile compounds from the European plum flower, which elicit electrophysiological responses in male and female *H. minuta* antennae. Future studies should focus on the behavioral responses of male and female *H. minuta* to these compounds both in laboratory and field conditions. Further, the identification of these antennal active flower compounds might pave the way for the development of feeding attractant lures, which could both attract males and females in the plum orchards.

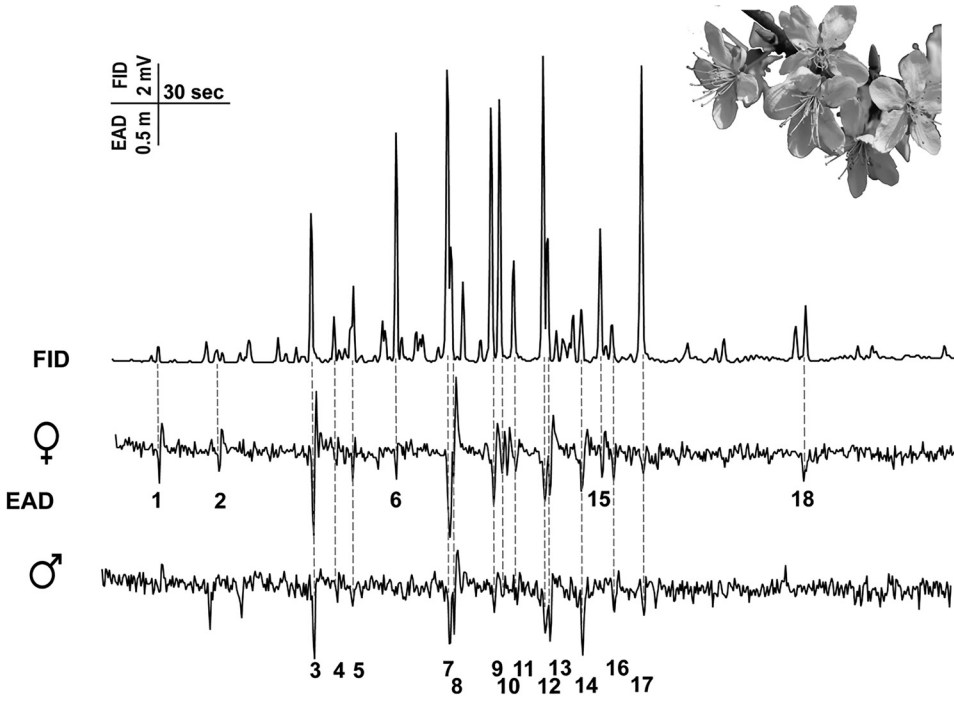


*Fig. 1.* Electrophysiological responses of *Hoplocampa minuta* male and female antenna to headspace volatile compounds from the European plum flowers (*Prunus domestica*) as analyzed by gas chromatography coupled with electroantennography (GC-EAD). Top, GC trace (FID); middle: female antennal signal (EAD); bottom, male antennal signal (EAD). hexanal (1), (*Z*)-3-hexen-1-ol (2), benzaldehyde (3), 6-methyl-5-heptene-2-one (4), (*Z*)-3-hexenyl acetate (5), (*Z*)-beta-ocimene (6), beta-linalool (7), nonanal (8), lilac aldehyde B (9), lilac aldehyde A (10), lilac aldehyde D (11), methyl salicylate (12), lilac alcohol A (13), lilac alcohol D (14), (*Z*)-cinnamyl alcohol (15), (*E*)-cinnamaldehyde (16), (*E*)-cinnamyl alcohol (17), alpha-farnesene (18)

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