J Pest Sci (2017) 90:873–885
DOI 10.1007/s10340-017-0837-0

ORIGINAL PAPER

Synthetic blend of larval frass volatiles repel oviposition in the invasive box tree moth, *Cydalima perspectalis*

Béla P. Molnár[1] · Zoltán Tóth[2] · Zsolt Kárpáti[1]

Received: 12 August 2016 / Revised: 18 January 2017 / Accepted: 27 January 2017 / Published online: 7 February 2017

Abstract Insects find their oviposition sites using visual, contact and olfactory cues. Volatile stimuli emitted by an intact or herbivore-occupied host plant, non-host plants or the herbivore itself can all influence the final decision of females concerning where to lay eggs. Volatile substances surrounding larval excreted pellets, i.e., frass of the invasive box tree moth (*Cydalima perspectalis* Walker) were collected, and the physiological activity was investigated by coupled gas chromatographic–electroantennographic detection. Based on structural elucidation, two aromatic derivates and one terpene alcohol were identified to be physiologically active on the antennae of the adults: guaiacol, (±)-linalool and veratrol. For all compounds, antennal responses were found to be dose dependent with EAG amplitudes being the highest at the highest dose levels. Females were also more sensitive to all three compounds compared to males. Single sensillum recordings on mated female antennae revealed that these frass compounds triggered 22% of the tested olfactory sensory neurons housed in trichoid sensilla. Behavioral bioassays indicated that the blend of these compounds had an oviposition-repellent effect on conspecific females: individuals laid significantly fewer eggs on boxwood plants equipped with dispensers loaded with the synthetic blend compared to those treated with natural frass or the control plants. This difference likely originated from the measured rapid changes in the volatile profile of larval excrement when exposed to the air at room temperature. Our findings have the potential to unravel the complex ecology of this invasive moth species characterized by rapid range expansion and extensive damage in Europe.

Keywords Oviposition preference · Frass volatiles · Electrophysiology · Single sensillum recording · Invasive pest · Crambidae

Key message

- The box tree moth (*Cydalima perspectalis*) is an invasive pest of boxwood in Europe. We investigated the oviposition-repellent effect of larval frass volatiles.
- Volatiles emanated by larval frass evoked antennal responses on both male and female antennae; specific compounds with this activity were identified as guaiacol, (±)-linalool and veratrol. These compounds were found to be detected by individual chemosensilla located on the antennae.
- A synthetic mixture of these volatiles, when applied on boxwood plants, significantly reduced the number of laid eggs demonstrating the blend's oviposition-repellent effect.

Communicated by M. Traugott.

Electronic supplementary material The online version of this article (doi:10.1007/s10340-017-0837-0) contains supplementary material, which is available to authorized users.

✉ Béla P. Molnár
molnar.bela.peter@agrar.mta.hu

[1] Zoology Department, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

[2] Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

Introduction

Insects use their olfactory system to find conspecifics, locate hosts or select suitable oviposition sites in a complex odor environment via the detection of signals with fine spatial–temporal resolution of signals (Cardé and Willis 2008; de Bruyne and Baker 2008; Reinecke and Hilker 2014). Phytophagous insects are able to accurately locate their host plants, despite that the plants are often being hidden among an array of other plants (Bruce et al. 2005). A females' choice of a suitable oviposition site is vital for survival of the progeny (Schoonhoven et al. 2005). According to the preference-performance hypothesis or the 'mother knows best' principle (Jaenike 1978; Courtney and Kibota 1990; Valladares and Lawton 1991), females preferentially oviposit on plants that maximize the survival and performance of their offspring, especially if the newly hatched larvae have limited or no capacity to move from their initial feeding site (Anderson and Löfqvist 1996).

Acceptance of a host plant by egg-laying females is based on the balance between positive and negative chemical stimuli for oviposition (Renwick and Chew 1994). Interestingly, the final decision is often taken on the basis of the absence of negative stimuli rather than the presence of attractants (Schöni et al. 1987; Renwick 1989). Volatiles emitted by larval frass (i.e., excreted indigestible waste) and herbivory-induced plant volatiles can act as kairomones and attract parasitoids and predators to the feeding larvae (Vinson 1976; Turlings et al. 1990; Thaler 1999; Bernays 2001; Kessler and Baldwin 2001). Moreover, these compounds may also serve as chemical cues for conspecific adults and have an oviposition-repellent effect as found in many Lepidopterans (Rothschild and Schoonhoven 1977; Renwick and Radke 1980, 1981; Hilker and Klein 1989; Anderson et al. 1993). These findings suggest that prior to oviposition, adult females can perceive chemical cues emanating from larval frass with their olfactory system and avoid laying their eggs on the plant already occupied to minimize food competition and cannibalism among larvae (Hilker and Klein 1989; Xu et al. 2006).

Box tree moth (BTM) (*Cydalima perspectalis*; Lepidoptera, Crambidae) is an invasive species feeding almost exclusively on plants belonging to the *Buxus* genus. This pest originates from Southeast Asia but has spread throughout Europe since 2007 (CABI 2016). BTM rapidly adapted to its new European host plant, the boxwood (*Buxus sempervirens* L.) and its varieties, even though this plant species does not occur naturally in the native range of this species (Leuthardt and Baur 2013; Leuthardt et al. 2013). This shrub is native to Southern and Western Europe from southern England to Northwest Africa and Turkey (Kenis et al. 2013), and is also a popular plant in public and private gardens and parks (Salvesen et al. 2009). Larvae of BTM feed on the leaves and bark of boxwood, causing them to dry out and die (Leuthardt and Baur 2013). Frass produced by the voracious larvae covers the plants due to the sticky larval webbing (Online Resource 1) and has an unpleasant scent to the human nose. Young larvae feed on the lower leaf surface whereas older larval stages feed inside the webbing and leave only midribs intact. With high infestation, larvae can completely defoliate the whole shrub leading to the death of the plant. Defoliation of boxwood in Southern and Western Europe has already initiated a change in the understory vegetation due to the increased exposure to sunlight, and it is likely that large areas of boxwood forests will disappear, affecting whole ecosystems in these regions (Kenis et al. 2013; Reinhold and Schumacher 2013).

In various moth species, larval frass has been found to have a deterrent or repellent effect on conspecific females, resulting in delayed oviposition or further searching for other, more suitable egg-laying sites (Anderson 2002). Larvae of BTM can overwinter at different larval stages (L2–L4 or even as mature instar) (She and Feng 2006; Nacambo et al. 2014), which leads to variation in the larval growth, pupation and emergence of adult moths in natural populations and results in the co-occurrence of different developmental stages, i.e., eggs, larvae and adults, during the reproductive season. In this study, we focused on exploring the repellent effect of the synthetic volatile larval frass blend derived from BTM and its physiological effect on adult moths. We first conducted headspace volatile collections from fresh larval frass taken from larvae, which were kept on *B. sempervirens*. Second, we tested if any of the volatile components of these extracts are physiologically active, i.e., can be detected by adult BTM, using coupled gas chromatography electroantennographic detection (GC–EAD) and identified the key compounds by gas chromatographic mass spectrometry (GC–MS). Third, we studied the neurophysiological responses of trichoid sensilla tuned to the identified larval frass volatile compounds using single sensillum recordings (SSR). Fourth, we performed oviposition bioassays to study potential difference in the behavioral effect of the synthetic frass blend and the fresh frass under laboratory conditions. Finally, we carried out solvent-free solid phase microextraction (SPME) to measure the time-related volatile profile changes of the fresh frass.

Materials and methods

Insects

Box tree moths were collected in an early larval stage from public gardens in different parts of Budapest, Hungary and kept in a climatic chamber (25 ± 1 °C, 65 ± 5% RH, 16 h light 8 h dark photoperiod) to initiate a laboratory population. Larvae were kept in cylindrical glass jars [internal diameter (ID) 20 cm, height 25 cm] and fed on 5–7, 40 cm long shoots of boxwood placed in a small water container. Pupae were then collected from the shoots and placed in mesh cages. When moths emerged, 30 cm high potted boxwood plants were offered for egg laying, and then leaves with eggs were placed into cylindrical glass jar, where 40 cm long boxwood shoots were offered for the hatching larvae to feed on.

Frass collection for oviposition bioassay

Frass was collected daily from fifth and sixth instar larvae feeding on boxwood shoots kept in a cylindrical glass jar (ID 20 cm, height 60 cm). Late instar larvae were used in order to get sufficient amounts of frass within a short time to avoid its desiccation. The collected frass was stored in airtight screw top vials in a freezer at −10 °C. In the oviposition bioassay, the used frass was at most 2 days old, after which it was replaced with fresh frass. In the 'natural frass' treatment group (see below), three netting bags (4 × 4 cm) per plants containing 3 g of frass were placed at different levels onto the potted boxwood.

Volatile collections of frass

Volatile collections were conducted from freshly collected larval excreta produced by fifth and sixth larval instars. Five grams of freshly collected larval frass was placed into a glass cylinder with quick-fit connections on both ends. The incoming air to the cylinder was filtered with charcoal and the other side was connected to a vacuum pump (Thomas G 12/02EB, Garder Denver Thomas GmbH, Fürstenfeldbruck, Germany) with PTFE tubes (ID 5 mm). Continuous, 1 l min^{-1} airflow was drawn through the setup. Volatiles were collected continuously for 4 h using 1.5 mg activated charcoal adsorbent (Brechbühler AG, Schlieren, Switzerland). Prior to that, the volatile collection filters were purified as described by Molnár et al. (2015). The adsorbed volatiles were eluted with 40 µl of *n*-hexane and kept at −40 °C. Subsequently, extracts were used for electrophysiological recordings (GC–EAD) and chemical identification (GC–MS).

Solvent-free headspace volatile collections with SPME fibers were also conducted in order to investigate stability and consistency of frass volatile components. Before each measurement, fibers were conditioned at 250 °C in the split/splitless injector of the GC–MS (HP Agilent 5890 GC and 5975 MS, Agilent Technologies, Palo Alto, USA) at split mode for 20 min. We used differently stored groups of frass (fresh, 1, 2, 3 days old at room temperature and 6 months old frozen) to compare their emissions to that of the vial-wick dispensers. For volatile samplings, either 5 g of larval frass or the vial-wick dispenser was placed in a glass vial (L: 80 mm, ID 20 mm) and was closed with aluminum foil and laboratory film (American Can, Greenwich, CT, USA) 5 min before sampling. SPME fiber (PDMS/CAR 0.53 mm, Supelco, Sigma-Aldrich, Bellefonte, PA, USA) was exposed into the sampling vial for 5 min at room temperature in five replicates.

Electrophysiological experiments (GC–EAD, EAG, SSR)

Coupled gas chromatographic–electroantennographic detection (GC–EAD) was performed according to the procedure described by Molnár et al. (2015). Briefly, antennae of 2-day-old mated females and 2-day-old unmated males were used to pinpoint the antennal active volatile components of frass. The GC was equipped with an HP-5 capillary column (30 m × 0.32 mm × 0.25 µm, J&W Scientific, Folsom, CA, USA) and was used in 'on-column' injection mode. The oven temperature was held at 50 °C for 1 min and then increased by 10 °C min^{-1} up to 230 °C. Helium was the carrier gas at a constant flow rate of 2.9 ml min^{-1}. The GC effluent was equally split to the FID (280 °C) and to the heated EAD port (220 °C). The EAD effluent was delivered into a stream of charcoal filtered and humidified air (1 l min^{-1}) and led to the antennal preparation. Two microliters of the volatile collection extract was injected into the GC. The antennal signal was pre-amplified by a factor of 10, converted to a digital signal by a high input impedance DC amplifier interface (IDAC-232, Syntech) and recorded simultaneously with the FID signal using GC-EAD2000 software (version 1.2.3, Syntech).

Dose responses to each identified compound were tested using electroantennography (EAG) on both male and female antennae. EAG responses were recorded from excised antennae of both sexes of BTM. The same instrument and technique were used to mount the antennae as described above. Antennae were stimulated using stimulus air stream (2 l/min) directed into a constant, charcoal filtered, humidified air stream (1 l min^{-1}). The synthetic compounds were dissolved in mineral oil (CAS 8042-47-5, Sigma-Aldrich), and 10 µl of the corresponding dilutions

(0.1, 1, 10, 100, 1000 and 10 000 ng/µl) of the compounds was deposited on a filter paper (1 × 1 cm), which was then placed into a Pasteur pipette and used as a stimulus cartridge. Stimulation time was 0.5 s long, and 1 min was allowed in-between stimuli for the antenna to recover. Responses for the mineral oil before and after each series of odor stimuli were averaged and subtracted from the absolute EAG amplitude. The compounds were tested on four each of male and female antennae in a random order.

Single sensillum recordings with sharp tungsten microelectrode were performed using standard equipment (Syntech) and following the procedure described by Kárpáti et al. (2013). Briefly, a 2–4-day-old mated female was inserted into a plastic pipette tip to immobilize the body. The head was protruded from the tip, and the antennae were placed on a microscopy glass slide covered with inert glue (Tanglefoot, Planet Natural Ltd., Bozeman, MT, USA). A sharpened tungsten wire reference electrode was inserted into the abdomen. To identify the sensitivity and selectivity of the olfactory sensory neurons (OSNs) responding to the frass volatile compounds, centrally located trichoid sensilla from the 9th–16th segments of the antennae were tested. The trichoid sensilla on the immobilized antenna were localized under a light microscope (Olympus BX51WI) at 750× magnification. The electrolytically sharpened tungsten-recording electrode was inserted into the base of the sensillum using a micromanipulator (DC-3K, Märzhäuser-Wetzlar Gmbh & Co Kg, Wetzlar, Germany). The extracellular analog signal was amplified 10× using a pre-amplification probe (Universal Single Ended AC/DC Probe PRS-1, Syntech). The amplified signal was filtered with 50–60 Hz suppression and sampled using integrated digital-analog converter (IDAC-4, Syntech). The antenna was continuously under a charcoal filtered, humidified air stream (1 l/min). Four different doses (0.1, 1, 10, 100 µg/ul) of the synthetic frass compounds were diluted in mineral oil (Sigma-Aldrich), and 10 µl of the solutions was applied on a filter paper disk (12.7 mm Ø; Schleicher & Schnell GmbH, Dassel, Germany) and placed into a Pasteur pipette. We used mineral oil as a control stimulus. The 0.5 s stimuli (0.5 l/min) were delivered into the continuous air stream (1 l/min) using a stimulus controller (CS-55, Syntech). The action potentials (spikes) were counted manually 0.5 s before and 0.5 s after the stimulus onset. The pre-stimulus spike number represents the spontaneous activity of the neuron. The spike frequency was calculated as the number of spikes during the stimulus time (0.5 s) minus the number of spikes before the stimulus onset (0.5 s) and expressed as the number of spikes per seconds.

Chemical identification

Samples were analyzed via GC–MS (HP Agilent 5890GC and 5975MS) operated in splitless injection mode and electron impact (EI) ionization mode at 70 eV, scanning m/z 29–400, at 2 scans/s. The GC was equipped with Rxi®-5Sil MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm, Restek, Bellefonte, PA, USA). Helium was used as the mobile phase at 35 cm/s flow. One microliter of samples was injected into the GC–MS with 220 °C injector temperature. The oven temperature was programmed from 50 °C (held for 1 min) at 10 °C/min up to 230 °C and held for 1 min.

For SPME samples, injector was used with splitless mode for 1 min to allow thermal desorption at 250 °C, the oven temperature was programmed: 50 °C, hold for 2 min, 10 °C/min up to 230 °C and hold for 1 min. Compounds were tentatively identified by matching their mass spectra with those in the MS Libraries (NIST 11 and Wiley), and they were verified by injection of synthetic references and were compared with the published Kovat's index (K_i) values. The quantity of each compound was calculated on the basis of the peak area and calibrated by comparing it with that of co-injected internal standard (10 ng/µl) *n*-decenyl acetate (CAS 112-17-4).

Chemicals and dispensers

Guaiacol (≥98%, CAS 90-05-1), (±)-linalool (97%, CAS 78-70-6) and veratrol (≥99%, CAS 91-16-7) were purchased from Sigma-Aldrich and were diluted in mineral oil (Sigma-Aldrich) for behavioral bioassay, EAG, SSR and *n*-hexane for verifications by GC–MS. Mineral oil is expansively used in preparing stock solutions for odor stimuli in electrophysiological practice such as EAG or SSR, because it is not known to be releasing any biologically relevant volatile compound (Andersson et al. 2012; Stensmyr et al. 2012). Additional mass spectroscopy measurement also confirmed that the volatile profile of mineral oil did not release any volatile compounds that could have interacted with the tested organic compounds or affected moths' oviposition behavior (data not shown). Volatile compounds were mixed in the same ratio as found in the natural frass volatile collection based on GC–MS quantitative analyzes. One milliliter of the synthetic blend was loaded in vial-wick dispensers (Zakir et al. 2013; Molnár et al. 2015) which contained 1 µg/µl guaiacol, 0.1 µg/µl linalool and 1 µg/µl veratrol. Prior to the experiment, the release rate of the dispensers was measured using SPME headspace collections analyzed by GC–MS.

Oviposition bioassay

Three-choice oviposition bioassays were conducted in parallel two screened cages (100 × 100 × 100 cm) in a climatic room (25 °C, 60% RH, 16:8 L:D). Fifteen males and fifteen females, which had emerged within 1 day, were placed into the cages, with three *B. sempervirens* 'Suffruticosa' plants offered for oviposition. The plants were obtained from a nursery garden, were of the same age and size, and were potted in 2-l nursery containers. This variety has dense foliage in spherical shape; the average height and width of the plants were 32.08 ± 1.62 cm and 28.65 ± 2.54 cm, respectively ($n = 7$). One of the three plants had three netting bags containing freshly collected frass ('plant with natural frass'; see details above). Another was equipped with three vial-wick dispensers, containing the blend of synthetic frass volatiles ('plant with synthetic frass'; see details above), while the third plant was the untreated control. Since mineral oil does not contain any physiologically or behaviorally relevant compounds (see above), plants in the control group were not equipped with dispensers loaded with mineral oil. Positions of the three plants within the cages were rotated randomly on a daily basis. Frass bags were replaced on the second day to avoid frass desiccation. After 3 days, the moths and the plants were removed and the eggs were counted on the plant leaves. The trials were repeated six times.

Statistical analysis

All statistical tests were performed in R 3.2.2 (R Core Team 2015). We used linear models to investigate the effect of sex and dose (both included as factors) on the antennal response in the identified volatiles. Antennal responses, expressed in millivolts, were calculated as the absolute EAG amplitudes of each chemical minus the averaged EAG amplitudes of the control (mineral oil) measured before and after each stimulus session (within sessions the testing order of the three chemical compounds were randomized). We applied linear mixed-effect model with restricted maximum likelihood estimation ('nlme' R package; (Pinheiro et al. 2015) to examine oviposition preference in gravid female moths and investigated whether individuals lay different number of eggs on the control plants, plants with natural frass and plants with synthetic frass (see above) during 3-day long trials. In this model, 'type of plants' was a fixed factor, whereas 'trial' was included as a random factor. The dependent variable (total number of laid eggs) was log-transformed to improve its fit to the normal distribution. Requirements of the fitted models were checked by plot diagnosis. Tukey HSD post hoc tests were performed to estimate the significance of between-group differences in the above models. We used Kruskal–Wallis tests with subsequent Games-Howell post hoc tests to compare the emitted amount of each volatile between frozen frass, vial-wick dispenser and differently aged frass samples. All tests were two-tailed with α set to 0.05.

Results

Electroantennography and structure elucidation of active compounds

Three compounds from the larval frass headspace collections elicited consistent and robust antennal responses from both male (between 0.282 ± 0.005 mV and 0.37 ± 0.004 mV; $n = 6$) and female (between 0.275 ± 0.003 mV and 0.52 ± 0.02 mV; $n = 6$) BTM antennae. Corresponding peaks in the FID trace eluted at 8.14, 8.21, 9.07 min, respectively (Fig. 1). Antennal active compounds were subsequently identified by GC–MS as guaiacol (2-methoxyphenol, CAS 90-05-1), (±)-linalool (3,7-dimethylocta-1,6-dien-3-ol, CAS 78-70-6) and veratrol (1,2-dimethoxybenzene, CAS 91-16-7).

Dose–response tests

For all three volatiles, the antennal responses of box tree moths were found to be dose-dependent [(±)-linalool: $F_{5,41} = 123.0$, $P < 0.001$; veratrol: $F_{5,41} = 165.70$, $P < 0.001$; guaiacol: $F_{5,41} = 222.97$, $P < 0.001$; Fig. 2] with EAG amplitudes being the highest at the highest dose levels. For (±)-linalool, there was no significant difference between 1 and 10 ng (estimated difference = 0.16 ± 0.23, $t = 0.72$, $P = 0.979$) and the 10 and 100 ng doses (0.15 ± 0.23, $t = 0.66$, $P = 0.986$). The 100 ng dose tended to differ from the 1 µg dose (0.67 ± 0.23, $t = 2.94$, $P = 0.057$), whereas the higher consecutive doses significantly differed from each other (1 µg vs. 10 µg: 1.38 ± 0.23, $t = 6.07$, $P < 0.001$; 10 µg vs. 100 µg: 2.22 ± 0.23, $t = 9.78$, $P < 0.001$). In veratrol, 1 µg dose elicited a similar amount of antennal response to all lower doses (all $P \geq 0.091$), whereas we found significant differences between the higher consecutive doses (1 µg vs. 10 µg: 1.08 ± 0.20, $t = 5.32$, $P < 0.001$; 10 µg vs. 100 µg: 3.20 ± 0.20, $t = 15.66$, $P < 0.001$). In guaiacol, a similar trend was seen as there was no significant difference between the consecutive lower doses (1 ng–1 µg; all $P \geq 0.652$), but higher doses of guaiacol induced stronger antennal responses (1 µg vs. 10 µg: 0.53 ± 0.12, $t = 4.52$, $P < 0.001$; 10 µg vs. 100 µg: 2.34 ± 0.12, $t = 19.94$, $P < 0.001$). Furthermore, sex also had a significant effect on the EAG amplitudes [(±)-linalool: $F_{1,41} = 35.72$, $P < 0.001$; veratrol: $F_{1,41} = 24.07$, $P < 0.001$; guaiacol:

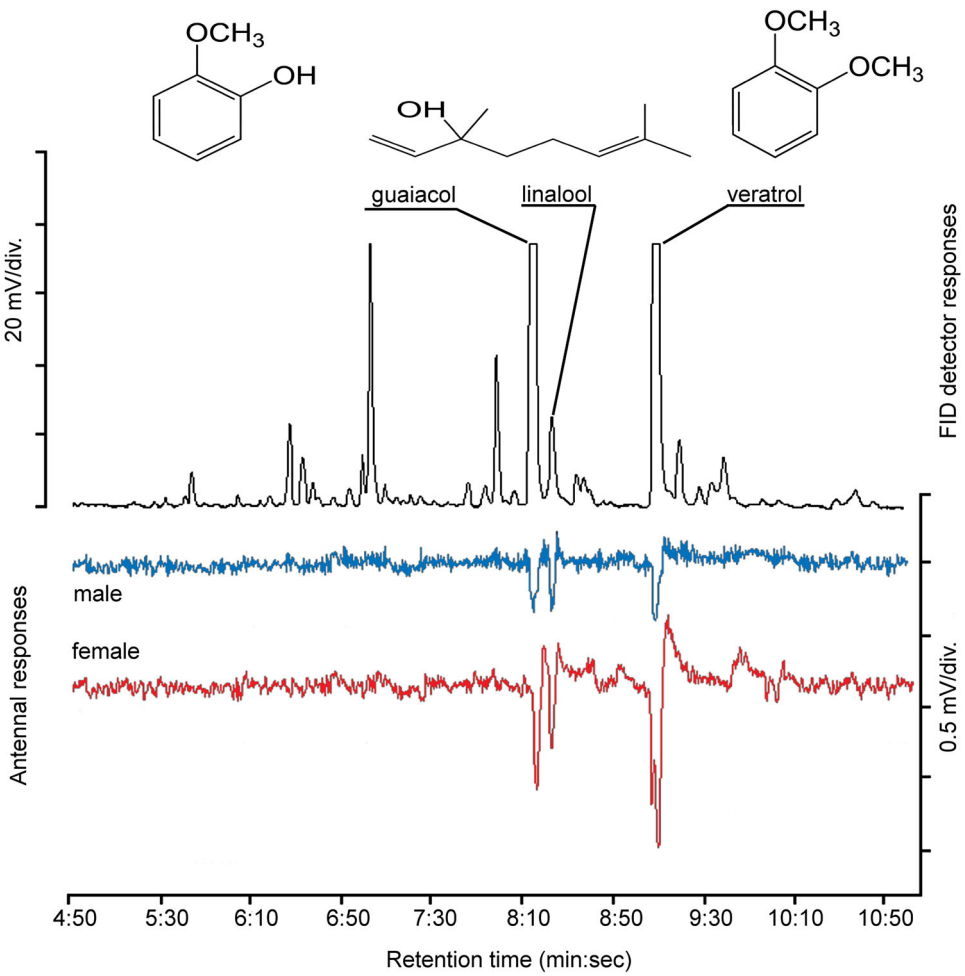

Fig. 1 Averaged recordings of coupled gas chromatographic with electroantennographic detection (GC–EAD). We tested the collected headspace volatile blend of conspecific larval frass on male and female antennae of *Cydalima perspectalis* ($n = 6$). Those compounds that elicited antennal responses were identified as guaiacol, (±)-linalool and veratrol using gas chromatography–ass spectrometry (GC–MS)

$F_{1,41} = 48.32$, $P < 0.001$; Fig. 2]; responses were significantly higher in female antennae compared to male antennae for all three compounds. At the highest cartridge dose, the responses to the various compounds ranged from 3.8 to 6.1 mV and from 4.2 to 6.7 mV on male and female antennae, respectively.

Single sensillum recordings

Only the sensilla trichodea responded to the tested odors. In total, 74 contacts were established on different sensilla trichodea in 21 mated females. Out of 74 recordings, only 16 sensilla responded to the tested single volatile compounds (Figs. 3, 4). In all cases based on the spike amplitudes, we found two sensory neurons housed in the sensillum, and for all cases, only one responded to the tested odors. The spontaneous activity of the tested neurons varied between 4 and 62 Hz. Thirteen neurons responded only to the higher doses (1, 10, 100 µg). In three cases, we found very sensitive neurons that responded to the lowest dose (0.1 µg) of guaiacol, (±)-linalool and veratrol (Fig. 3, St 5, St 9 and St 16, respectively). The tested neurons showed phasic-tonic responses to all stimuli. In 15 cases, the neurons were not compound specific and responded to more than one tested odor. We found only one sensillum, which responded specifically to veratrol (Fig. 3, St 1). Inhibitory responses (decreased spike frequency compared to spontaneous activity) of the OSNs were not found either during or after the stimulation onset.

Oviposition bioassay

Females laid significantly different numbers of eggs on the three types of plants during the trials ($F_{2,10} = 24.03$, $P < 0.001$; Fig. 5). Individuals laid most of their eggs (estimated number of eggs with 95% confidence interval: 224.80 [92.84–544.33]) on the control plants while the number of deposited eggs decreased by approx. Thirty-one percent (parameter estimate: 0.69 [0.44–1.09]) on the plants with natural frass, and dropped by approx. Seventy-eight percent (parameter estimate: 0.22 [0.14–0.34]) on the plants with synthetic frass. The applied post hoc test revealed that the differences between plants with synthetic frass and the other two types of plants were significant

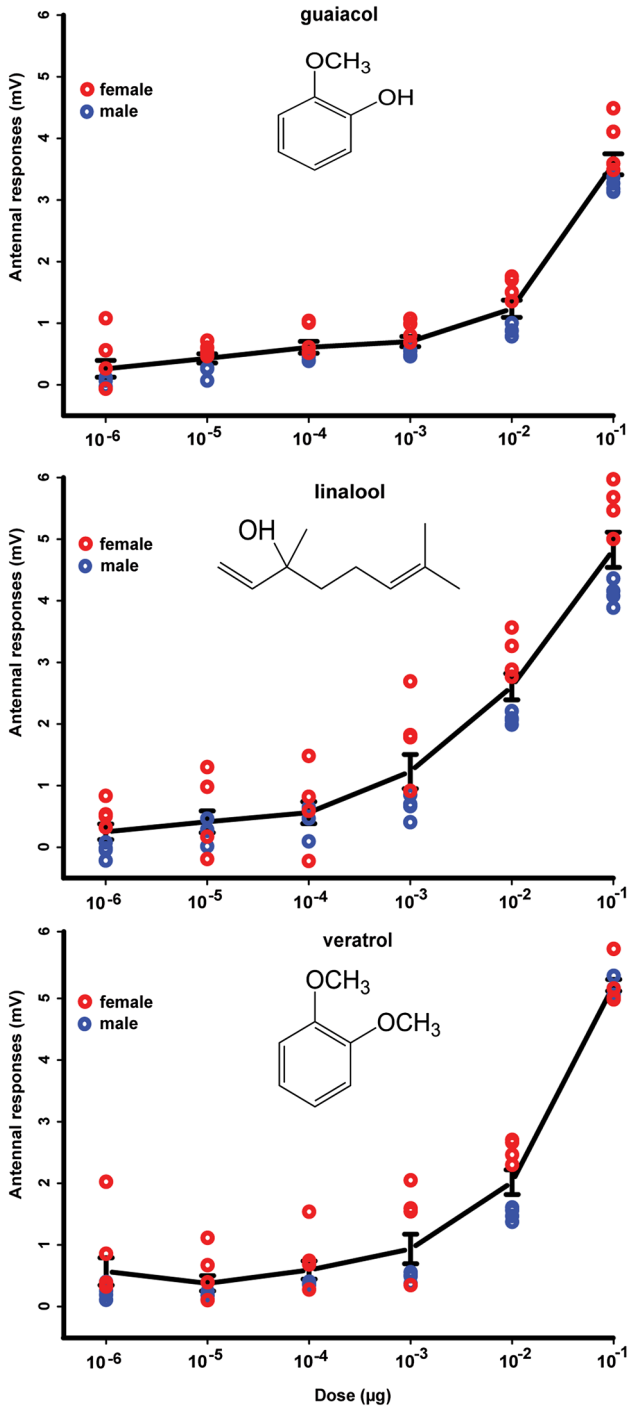

Fig. 2 Electroantennographic dose–response curves ($n = 4$, male and female *Cydalima perspectalis,* respectively). Mean ± SE values were calculated from the pooled data of the two sexes ($n = 8$), while *open circles* represent individual data points. Antennal responses to the three electrophysiologically active volatile frass compounds expressed in millivolts were calculated as the absolute EAG amplitudes of each compound, and the averaged EAG amplitudes of the control (mineral oil) measured before and after each stimulus pulse delivery were subtracted

(control vs. synthetic frass: $z = -6.64$, $P < 0.0001$; natural frass vs. synthetic frass: $z = -5.04$, $P < 0.0001$), while control plants and plants with natural frass did not differ from each other ($z = -1.61$, $P = 0.243$). The estimated random effect (given in SD ± 95% confidence interval) was 2.80 [1.71–7.15], which indicates considerable differences in the number of laid eggs between trials.

Investigation of the temporal changes in the volatile composition of larval frass

We found that the abundance of all three key compounds was significantly different between the examined differently aged frass groups (guaiacol: $\chi^2_5 = 24.79$, $P < 0.001$; (±)-linalool: $\chi^2_5 = 25.13$, $P < 0.001$; veratrol: $\chi^2_5 = 27.08$, $P < 0.001$; Fig. 6). Pairwise comparisons revealed that the amounts of emitted volatiles did not differ between the vial-wick dispenser and the fresh frass, and the fresh and frozen frass, although the amount of veratrol was found to be lower in the frozen frass compared to the vial-wick dispenser (Table 1). Nevertheless, these results indicate that these groups had very similar volatile compound profiles, especially when matched to those groups where frass was exposed to the air. The amount of all three volatiles considerably decreased even after 1 day of exposure to the air, and became a fraction of the original amounts by the second day (Fig. 6; Table 1). Moreover, (±)-linalool was undetectable in the headspace of larval frass after 3 days of exposure.

Discussion

In this study, we identified a synthetic blend from larval frass that can repel the oviposition by conspecific BTM females. GC–EAD, GC–MS and SSR analysis of volatile collections from larval frass revealed that guaiacol, (±)-linalool and veratrol are physiologically active compounds that can be detected by female and male antennae. Moreover, female antennae were found to be more sensitive to these compounds. In oviposition bioassays, females laid significantly fewer eggs on the boxwood plants treated with the synthetic mixture of frass compounds applied in vial-wick dispensers, whereas plants with natural frass did not differ from control plants. Headspace analyses of the natural frass revealed that the amount of some of the volatile compounds decreases quickly and thus the volatile composition of the physiologically and behaviorally active blend alters rapidly with time. These results suggest that adult BTMs are able to perceive volatiles from the larval

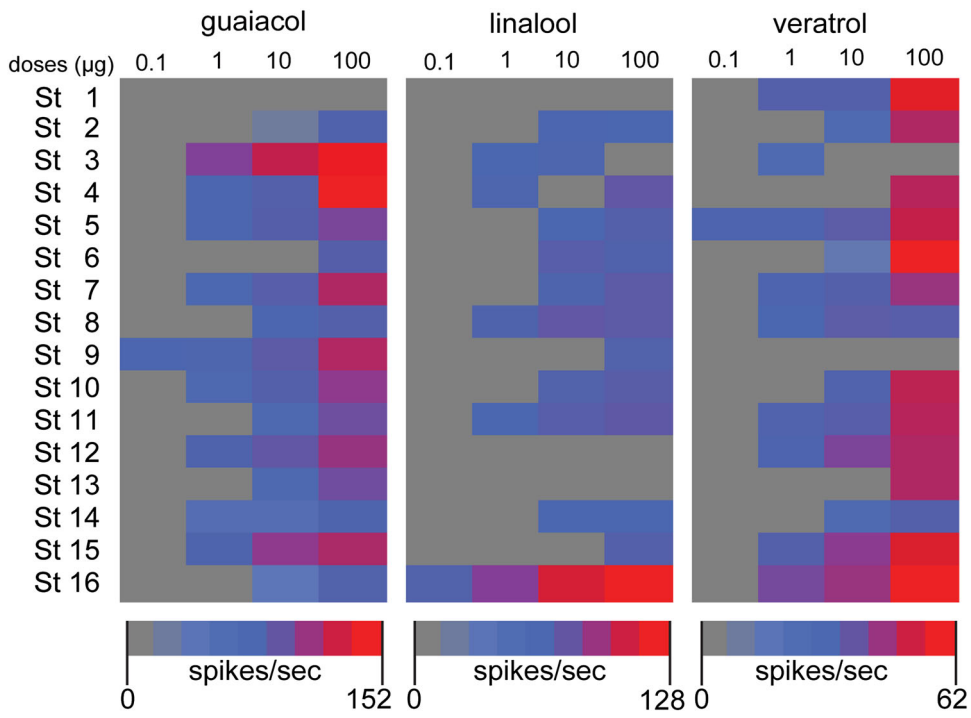

Fig. 3 Dose-dependent heatplot of single sensillum recordings on sensilla trichodea (St) of female *C. perspectalis* antennae to three active larval frass compounds. *Colors* indicate increasing response frequency from low (*blue*) to high (*red*), *gray* indicates no response. Ten-fold serial dilutions of neat compounds were tested. (Color figure online)

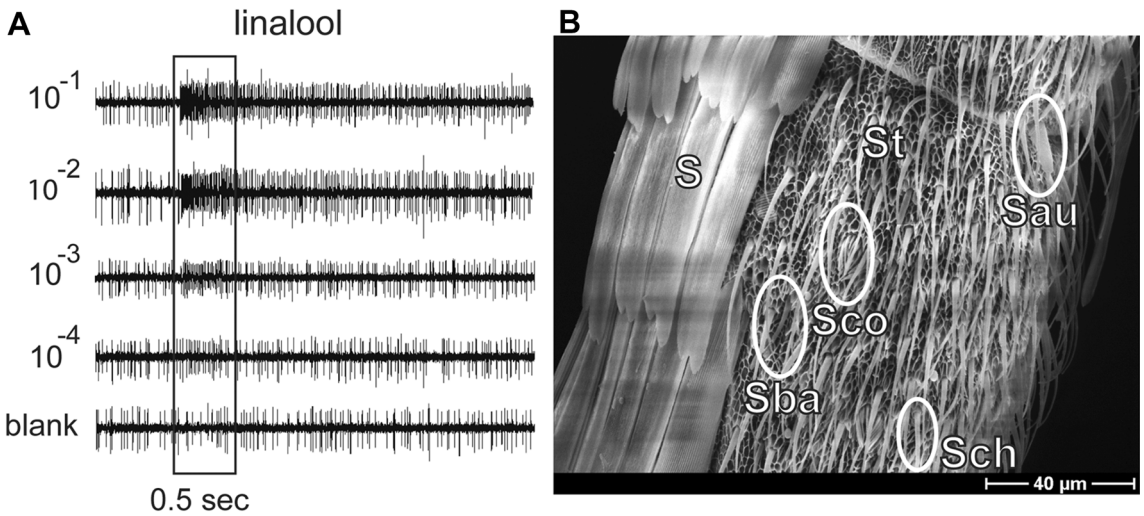

Fig. 4 **a** Typical response of OSN housed in trichoid sensillum to different dilutions of linalool on the antenna of a female *Cydalima perspectalis*. The *rectangular* indicates the stimulation period of 0.5 s. **b** Scanning electron microscopy image of the 6th flagellomere from the base of the female antenna of *C. perspectalis*. (S: scales, Sau: sensilla auricillica, Sco: sensilla coeloconica, St: sensilla trichodea, Sba: sensilla basiconica, Sch: sensilla chaetica

frass, but its repellent effect on gravid females is only temporal under natural circumstances. Therefore, when feeding larvae are not present, i.e., only old and dry larval frass would be present on the plant, and the females would be able to lay eggs again.

Although the identified compounds have previously been found to be present in insect frass as antifeedants (Borg-Karlson et al. 2006; Klein et al. 1990; Ramachandran et al. 1991) or aggregation pheromone components (Obeng-Ofori et al. 1994; Dillon et al. 2000; Fuzeau-Braesch et al. 1988), their oviposition-repellent effect has yet been overlooked. Unlike in previous studies on frass volatiles (e.g., Hilker and Klein 1989), we found that the amount of the compounds in the blend decreased dramatically after being exposed to the air and (±)-linalool was present only in a trace amount after 48 h and became undetectable on the third day. We propose that in our oviposition bioassay, the substantial loss of the active

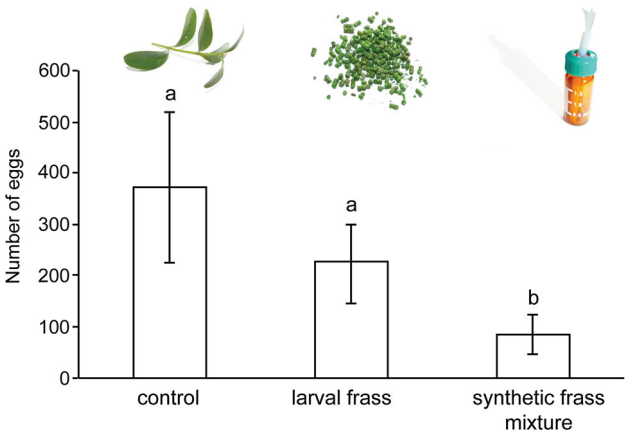

Fig. 5 Results of *Cydalima perspectalis* three-choice oviposition bioassays. Number of eggs laid (mean ± SE) on potted boxwoods: untreated, treated with larval frass and treated with vial-wick dispenser loaded with synthetic mixture of the physiologically active frass compounds (guaiacol, (±)-linalool, veratrol). *Different letters* indicate significant differences between treatment groups

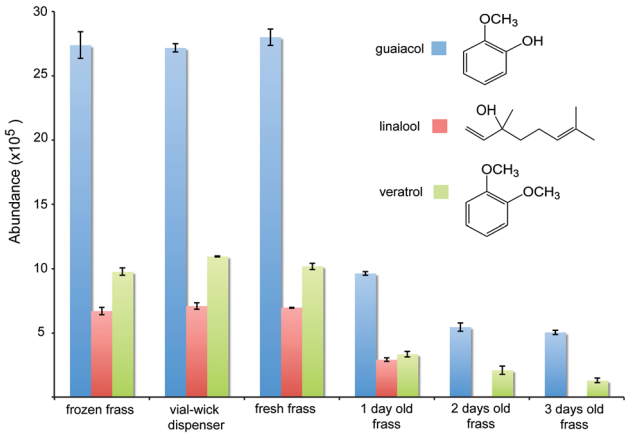

Fig. 6 Temporal changes in the abundance of the volatile compounds of larval frass of *Cydalima perspectalis* and frass mimic synthetic mixture (mean ± SE) using SPME sampling followed by GC–MS analyzes ($n = 5$). Amounts of the three volatile compounds drastically decreased with time at room temperature, whereas the vial-wick dispenser, fresh and frozen frass had similar volatile profiles

compounds from the blend may be responsible for the lack of the oviposition-repellent effect of the natural frass, as the bags containing natural frass were replaced on every second day. Thus, only the volatile blend continuously emitted from the dispensers, but not the natural frass, negatively affected females' oviposition. If so, females may, by detecting frass volatiles, receive up-to-date information about the host plants' occupancy level by conspecific larvae during their search for suitable oviposition sites. In accordance with this idea, Anderson et al. (1993) proved that a mixture of six compounds identified from larval frass of *S. littoralis* has a strong oviposition-deterring effect on conspecifics. However, if one of these compounds was excluded from the mixture, the deterrent-effect disappeared. Desiccation could be one of the main factors contributing to a decreasing release of volatile compounds from frass (Agelopoulos et al. 1995); however, other factors such as microbial activity or degradation by UV light may also play role. In BTM, the emergence of females can coincide with the occurrence of feeding larvae at which point considerable amounts of the larvae-produced frass accumulate in the larval webbing on the foliage (Online Resource 1). Volatiles emitted by fresh larval frass could signal to the females that a given host plant is already occupied by actively feeding larvae. BTM has no known parasitoids and is preyed upon by only a few predatory species in its invaded European range (Zimmermann and Wührer 2010; Wan et al. 2014), so its populations are limited only by the available amount of food sources (Wan et al. 2014; CABI 2016).

The chemical compounds of the larval frass may be derived from the host plant that larvae feed on, appearing as concentrated enzymatically produced breakdown products, or as microbial products of gut bacteria (Thibout et al. 1993). In the case of guaiacol and veratrol, we ruled out the direct plant origin and accumulation theory in BTM, since mass spectroscopy analyses of volatile collections from intact, mechanically damaged and larval-feeding damaged boxwood plants proved that these two compounds are absent in the host plant (Online Resource 2). However, (±)-linalool is emitted by the intact, mechanically and also larval-damaged boxwood (Online Resource 2, 3). Linalool is a common acyclic monoterpenoid floral scent compound produced by the flowers of many plant species (Pellmyr 1986; Dodson 1993; Pichersky et al. 1994; Crowell et al. 2002). However, this compound is also one of the most common herbivore-induced vegetative VOCs (Turlings and Tumlinson 1992; Paré and Tumlinson 1997; Kessler and Baldwin 2001). In this study, the enantiomeric emission of linalool from boxwood plant and frass produced by BTM larvae has not yet been clarified. In other moth species, enantiomers of linalool can have different effects on other moth species on feeding and oviposition behaviors (Reisenman et al. 2010, 2013), and (−)-linalool alone or together with other compounds has been shown to act as an oviposition-repellent volatile (Baldwin et al. 2002).

In addition to the EAG technique, where only summated antennal responses can be observed, we also conducted SSR recordings to better understand the sensitivity and selectivity of the individual olfactory units (sensilla) to the frass volatile compounds. The location of the chemosensilla perceiving oviposition-repelling pheromones and frass volatiles has been established in few insect species (Prokopy and Spatcher 1977; Klijnstra and Roessingh 1986; Hurter et al. 1987; Anderson et al. 1993). In BTM, we found both broadly and narrowly tuned OSNs responding

Table 1 Result of the applied Games-Howell post hoc tests for comparing the relative abundance of each volatile between differently aged frass groups

Pairwise comparisons	Guaiacol			(±)-Linaool			Veratrol		
	t value	*df*	*P*	*t* value	*df*	*P*	*t* value	*df*	*P*
Fresh—frozen	0.67	6.70	0.980	1.14	4.17	0.847	1.45	7.90	0.698
Fresh—vial-wick dispenser	1.49	6.09	0.681	0.60	4.26	0.985	3.81	4.32	0.091
Frozen—vial-wick dispenser	0.25	4.86	1.000	1.28	7.69	0.789	5.33	4.26	**0.029**
Fresh—1-day old	35.87	4.53	**<0.001**	39.33	4.99	**<0.001**	26.75	7.70	**<0.001**
Fresh—2-day old	40.46	5.96	**<0.001**	200.82	4.06	**<0.001**	24.54	7.42	**<0.001**
Fresh—3-day old	44.95	4.49	**<0.001**	201.81	4.00	**<0.001**	37.34	6.98	**<0.001**
Frozen—1-day old	21.99	4.21	**<0.001**	14.96	5.34	**<0.001**	23.34	7.33	**<0.001**
Frozen—2-day old	26.22	4.80	**<0.001**	28.61	4.00	**<0.001**	22.23	7.77	**<0.001**
Frozen—3-day old	27.71	4.19	**<0.001**	28.64	4.00	**<0.001**	32.74	6.53	**<0.001**
One-day old—vial-wick dispenser	59.89	5.80	**<0.001**	19.51	5.95	**<0.001**	45.57	4.48	**<0.001**
One-day old—2-day old	14.67	5.91	**<0.001**	29.84	4.01	**<0.001**	4.03	6.65	**0.041**
One-day old—3-day old	25.86	7.98	**<0.001**	29.93	4.00	**<0.001**	9.75	7.69	**<0.001**
Two-day old—3-day old	1.47	5.77	0.693	2.24	4.00	0.378	2.69	5.89	0.209
Two-day old—vial-wick dispenser	59.35	7.99	**<0.001**	37.14	4.00	**<0.001**	33.21	4.18	**<0.001**
Three-day old—vial-wick dispenser	76.20	5.66	**<0.001**	37.18	4.00	**<0.001**	69.89	4.72	**<0.001**

Significant *P* values are written in bold

to veratrol, guaiacol and (±)-linalool, which coincide with earlier findings in several insect species, where specialist and generalist OSNs responded to volatiles isolated from plant (Ignell and Hansson 2004; de Bruyne and Baker 2008; Andersson et al. 2009) and frass (Anderson et al. 1993, 1995). OSNs tuned to these three compounds have been confirmed in several insect species (Hansson et al. 1996; Ignell et al. 1998; Saïd et al. 2003; Hebets and Chapman 2000; Todd and Baker 1993; Bengtsson et al. 2009; Binyameen et al. 2014) and in a few cases their enantioselectivity was also verified (Ulland 2006).

In conclusion, our results indicate that the synthetic mixture of three larval frass compounds has a significant oviposition-repelling effect in BTM females and the composition of frass volatiles is likely to represent up-to-date chemical information for females about the suitability of occupied oviposition sites under natural conditions. We propose that future studies should investigate whether all three compounds are necessary to evoke the repellent effect on BTM females, whether the identified volatiles act additively or synergistically, or whether single compounds would be sufficient to repel egg-laying females. Also, the repellent effect of the identified volatile blend should be confirmed under field conditions. Ultimately, our results together with the proposed research venues may help to better understand the chemo-ecological characteristics of this invasive moth species, and pave way to the development of successful control methods for the preservation of boxwood populations in Europe.

Author contribution

PBM and ZK conceived and designed the experiments. PBM and ZK performed the experiments. PBM, ZT and ZK analyzed the data. PBM, ZT and ZKcontributed reagents/materials/analysis tools. PBM, ZT and ZK wrote the paper. All authors read and approved the manuscript.

Acknowledgements We are grateful to Pál Vági (Department of Plant Anatomy, Faculty of Science, Eötvös Loránd University, Budapest, Hungary) for his contribution in taking scanning electron microscopy images and to Kistie B. Brunsell for the linguistic corrections.

Funding This study was supported by the Hungarian Scientific Research Fund (OTKA, PD1041310), the Marie Curie Career Integration Grant (PCIG12-GA-2012-333980) and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences to ZK. During the study, ZT was supported by the 'Lendület' programme of the Hungarian Academy of Sciences (MTA, LP2012-24/2012) and a Hungarian Scientific Research Fund grant (OTKA, PD108938) and Postdoctoral Research Program of HAS (Grant No. SZ-029/2013).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human and animals The invertebrate insect species (box tree moth, *C. perspectalis*) used in the present study has a horticultural pest status and is not protected in Hungary. Therefore, individuals can be freely collected and used in laboratory experiments without permit or approval from the institutional ethics committee or national authorities under Hungarian law (348/2006, paragraph 10/3). Collecting sites were either owned by the research institute (Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest) or were publicly accessible; therefore, no permit was needed to access them. During experimentation, we avoided causing any unnecessary harm, suffering or distress to the study subjects. The insect collection was exclusively focused on the experimental species and did not involve endangered or protected species.

Informed consent Informed consent does not apply to these studies.

References

Agelopoulos NG, Dicke M, Posthumus MA (1995) Role of volatile inforchemicals emitted by feces of larvae in host-searching behavior of parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae): a behavioral and chemical study. J Chem Ecol 21:1789–1811. doi:10.1007/BF02033677

Anderson P (2002) Oviposition pheromones in herbivorous and carnivorous insects. In: Hilker M, Meiners T (eds) Chemoecology of insect eggs and egg deposition. Blackwell Verlag, Berlin, pp 235–256

Anderson P, Löfqvist J (1996) Oviposition deterrents from potato, wheat germ, larval frass, and artificial diet for *Agrotis segetum* (Lepidoptera: Noctuidae). Environ Entomol 25:6

Anderson P, Hilker M, Klein B, Schildknecht H (1993) Oviposition deterring components in larval frass of *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae): a behavioural and electrophysiological. Evaluation 39:129–137

Anderson P, Hansson BS, Löfqvist J (1995) Plant-odour-specific receptor neurons on the antennae of female and male *Spodoptera littoralis*. Physiol Entomol 20:189–198

Andersson MN, Larsson MC, Schlyter F (2009) Specificity and redundancy in the olfactory system of the bark beetle *Ips typographus*: single-cell responses to ecologically relevant odors. J Insect Physiol 55:556–567. doi:10.1016/j.jinsphys.2009.01.018

Andersson MN, Schlyter F, Hill SR, Dekker T (2012) What reaches the antenna? How to calibrate odor flux and ligand-receptor affinities. Chem Senses 37:403–420. doi:10.1093/chemse/bjs009

Baldwin IT, Kessler A, Halitschke R (2002) Volatile signaling in plant—plant—herbivore interactions: what is real ? Curr Opin Biotechnol 5:1–4. doi:10.1016/S-1369-5266(02)00263-7

Bengtsson JM, Wolde-Hawariat Y, Khbaish H et al (2009) Field attractants for *Pachnoda interrupta* selected by means of GC-EAD and single sensillum screening. J Chem Ecol 35:1063–1076. doi:10.1007/s10886-009-9684-7

Bernays EA (2001) Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. Annu Rev Entomol 46:703–727. doi:10.1146/annurev.ento.46.1.703

Binyameen M, Anderson P, Ignell R et al (2014) Identification of plant semiochemicals and characterization of new olfactory sensory neuron types in a polyphagous pest moth, *Spodoptera littoralis*. Chem Senses 39:719–733. doi:10.1093/chemse/bju046

Borg-Karlson A-K, Nordlander G, Mudalige A et al (2006) Antifeedants in the feces of the pine weevil *Hylobius abietis*: identification and biological activity. J Chem Ecol 32:943–957. doi:10.1007/s10886-006-9050-y

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10:269–274. doi:10.1016/j.tplants.2005.04.003

CABI (2016) *Cydalima perspectalis*. In: Invasive species compendium. http://www.cabi.org/isc/datasheet/118433. Accessed 1 Jan 2016

Cardé RT, Willis MA (2008) Navigational strategies used by insects to find distant, wind-borne sources of odor. J Chem Ecol 34:854–866. doi:10.1007/s10886-008-9484-5

Crowell AL, Williams DC, Davis EM, Wildung MR, Croteau R (2002) Molecular cloning and characterization of a new linalool synthase. Arch Biochem Biophys 405:112–121

Courtney SP, Kibota TT (1990) Mother doesn't know best: selection of hosts by ovipositig insects. In: Bernays EA (ed) Insect-plant interactions. CRC Press, Boca Raton, FL, pp 161–188

de Bruyne M, Baker TC (2008) Odor detection in insects: volatile codes. J Chem Ecol 34:882–897. doi:10.1007/s10886-008-9485-4

Dillon RJ, Vennard CT, Charnley AK (2000) Exploitation of gut bacteria in the locust. Nature 403:851. doi:10.1038/35002669

Dodson HEM (1993) Floral volatiles in insect biology. In: Bernays EA (ed) Insect-plant interactions. CRC Press, Boca Raton, pp 47–81

Fuzeau-Braesch S, Genin E, Jullien R et al (1988) Composition and role of volatile substances in atmosphere surrounding two gregarious locusts, *Locusta migratoria* and *Schistocerca gregaria*. J Chem Ecol 14:1023–1033. doi:10.1007/BF01018790

Hansson BS, Ochieng' SA, Grosmaitre X et al (1996) Physiological responses and central nervous projections of antennal olfactory receptor neurons in the adult desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). J Comp Physiol A. doi:10.1007/BF00222783

Hebets E, Chapman R (2000) Electrophysiological studies of olfaction in the whip spider *Phrynus parvulus* (Arachnida, Amblypygi). J Insect Physiol 46:1441–1448

Hilker M, Klein B (1989) Investigation of oviposition deterrent in larval frass of *Spodoptera littoralis* (Boisd.). J Chem Ecol 15:929–938

Hurter J, Boller EF, Städler E et al (1987) Oviposition-deterring pheromone in *Rhagoletis cerasi* L.: purification and determination of the chemical constitution. Experientia 43:157–164. doi:10.1007/BF01942834

Ignell R, Hansson B (2004) Insect olfactory neuroethology—an electrophysiological perspective. In: Thomas A. Christensen (ed) Methods in insect sensory neuroscience. CRC Press, New York, pp 319–347

Ignell R, Anton S, Hansson BS (1998) Central nervous processing of behaviorally relevant odours in solitary and gregarious fifth instar locusts, *Schistocerca gregaria*. J Comp Physiol A Sens Neural Behav Physiol 183:453–465. doi:10.1007/s003590050271

Jaenike J (1978) On optimal oviposition behavior in phytophagous insects. Theor Popul Biol 14(3):350–356. doi:10.1016/0040-5809(78)90012-6

Kárpáti Z, Tasin M, Cardé RT et al (2013) Early quality assessment lessens pheromone specificity in a moth. PNAS 110:7377–7382. doi:10.1073/pnas.1216145110

Kenis M, Nacambo S, Leuthardt FLG et al (2013) The box tree moth, *Cydalima perspectalis*, in Europe: horticultural pest or environmental disaster? Aliens Invasive Species Bull 33:38–41

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141–2144. doi:10.1126/science.291.5511.2141

Klein B, Schildknecht H, Hilker M, Bombosch S (1990) Oviposition-deterring compounds from larval frass of *Spodoptera littoralis*

(Boisd.). Zeitschrift für NaturforschungSection C Biosci 45:895–901
Klijnstra JW, Roessingh P (1986) Perception of the oviposition deterring pheromone by tarsal and abdominal contact chemoreceptors in *Pieris brassicae*. Entomol Exp Appl 40:71–79. doi:10.1111/j.1570-7458.1986.tb02157.x
Leuthardt FLG, Baur B (2013) Oviposition preference and larval development of the invasive moth *Cydalima perspectalis* on five European box-tree varieties. J Appl Entomol 137:437–444. doi:10.1111/jen.12013
Leuthardt FLG, Glauser G, Baur B (2013) Composition of alkaloids in different box tree varieties and their uptake by the box tree moth *Cydalima perspectalis*. Chemoecology 23:203–212. doi:10.1007/s00049-013-0134-1
Molnár BP, Tóth Z, Fejes-Tóth A et al (2015) Electrophysiologically-active maize volatiles attract gravid female European Corn Borer, *Ostrinia nubilalis*. J Chem Ecol. doi:10.1007/s10886-015-0640-4
Nacambo S, Leuthardt FLG, Wan H et al (2014) Development characteristics of the box-tree moth *Cydalima perspectalis* and its potential distribution in Europe. J Appl Entomol 138:14–26. doi:10.1111/jen.12078
Obeng-Ofori D, Torto B, Njagi PG et al (1994) Fecal volatiles as part of the aggregation pheromone complex of the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). J Chem Ecol 20:2077–2087. doi:10.1007/BF02066244
Paré PW, Tumlinson JH (1997) De novo biosynthesis of volatiles lnduced by lnsect herbivory in cotton plants. Plant Physiol 11:1161–1167
Pellmyr O (1986) Three pollination morphs in *Cimicifuga simplex*; incipient speciation due to inferiority in competition. Oecologia 68:304–307
Pichersky E, Raguso RA, Lewinsohn E, Croteau R (1994) Floral scent production in Clarkia (Onagraceae) (I. Localization and developmental modulation of monoterpene emission and linalool synthase activity). Plant Physiol 106:1533–1540
Pinheiro J, Bates D, DebRoy S et al (2015) nlme: Linear and nonlinear mixed effects models. R package version 3.1-121
Prokopy RJ, Spatcher PJ (1977) Location of receptors for oviposition-deterring pheromone in *Rhagoletis pomonella* flies. Ann Entomol Soc Am 70:960–962. doi:10.1093/aesa/70.6.960
R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
Ramachandran R, Norris DM, Phillips JK, Phillips TW (1991) Volatiles mediating plant-herbivore-natural enemy interactions: soybean looper frass volatiles, 3-octanone and guaiacol, as kairomones for the parasitoid *Microplitis demolitor*. J Agric Food Chem 39:2310–2317. doi:10.1021/jf00012a044
Reinecke A, Hilker M (2014) Annual plant reviews. Wiley, Chichester
Reinhold J, Schumacher J (2013) Der Buchsbaum-Zünsler (*Cydalima perspectalis*) im Grenzach-Wyhlener Buchswald – Invasionschronik und Monitoringergebnisse. Gesunde Pflanz 65:1–6. doi:10.1007/s10343-013-0292-7
Reisenman CE, Riffell JA, Bernays EA, Hildebrand JG (2010) Antagonistic effects of floral scent in an insect-plant interaction. Proc R Soc B 277:2371–2379. doi:10.1098/rspb.2010.0163
Reisenman CE, Riffell JA, Duffy K et al (2013) Species-specific effects of herbivory on the oviposition behavior of the moth *Manduca sexta*. J Chem Ecol 39:76–89. doi:10.1007/s10886-012-0228-1
Renwick JAA (1989) Chemical ecology of oviposition in phytophagous insects. Experientia 45:223–228. doi:10.1007/BF01951807
Renwick JAA, Chew FS (1994) Oviposition behavior in Lepidoptera. Annu Rev Entomol 39:377–400. doi:10.1146/annurev.en.39.010194.002113
Renwick JAA, Radke CD (1980) An oviposition deterrent associated with frass from feeding larvae of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Nocturidae). Environ Entomol 9:318–320
Renwick JAA, Radke CD (1981) Host plant constituents as oviposition deterrents for the Cabbage Looper, *Trichoplusia ni*. Entomol Exp Appl 30:201–204. doi:10.1111/j.1570-7458.1981.tb03099.x
Rothschild M, Schoonhoven LM (1977) Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). Nature 266:352–355. doi:10.1038/266352a0
Saïd I, Tauban D, Renou M et al (2003) Structure and function of the antennal sensilla of the palm weevil *Rhynchophorus palmarum* (Coleoptera, Curculionidae). J Insect Physiol 49:857–872. doi:10.1016/S0022-1910(03)00137-9
Salvesen PH, Kanz B, Moe D (2009) Historical cultivars of *Buxus sempervirens* L. revealed in a preserved 17th century garden by biometry and amplified fragment length polymorphism (AFLP). Eur J Hortic Sci 74:130–136
Schöni R, Städler E, Renwick JAA, Radke CD (1987) Host and non-host plant chemical influencing the oviposition behavior of several herbivorous insects. In: Labeyrie V, Fabres G, Lachaise D (eds) Proc 6th Int Symp Insect-Plant relation. Dordrecht, pp 31–36
Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-plant biology: paperback. Oxford University Press, London
She D-S, Feng F-J (2006) Bionomics and control of *Diaphania perspectalis* (Walker). J Zhejiang Univ Sci Technol 26:47–51
Stensmyr MC, Dweck HKM, Farhan A et al (2012) A conserved dedicated olfactory circuit for detecting harmful microbes in Drosophila. Cell 151:1345–1357. doi:10.1016/j.cell.2012.09.046
Thaler JS (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399:686–688. doi:10.1038/21420
Thibout E, Guillot JF, Auger J (1993) Microorganisms are involved in the production of volatile kairomones affecting the host seeking behaviour of *Diadromus pulchellus*, a parasitoid of *Acrolepiopsis assectella*. Physiol Entomol 18:176–182. doi:10.1111/j.1365-3032.1993.tb00465.x
Todd IL, Baker TC (1993) Response of single antennal neurons of female cabbage loopers to behaviorally active attractants. Naturwissenschaften 80:183–186. doi:10.1007/BF01226381
Turlings TC, Tumlinson JH (1992) Systemic release of chemical signals by herbivore-injured corn. Proc Natl Acad Sci USA 89:8399–8402
Turlings TC, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251–1253. doi:10.1126/science.250.4985.1251
Ulland S (2006) Discrimination between Enantiomers of Linalool by Olfactory Receptor Neurons in the Cabbage Moth *Mamestra brassicae* (L.). Chem Senses 31:325–334. doi:10.1093/chemse/bjj036
Valladares G, Lawton JH (1991) Host-Plant Selection in the Holly Leaf-Miner: Does Mother Know Best? J Anim Ecol 60:227. doi:10.2307/5456
Vinson SB (1976) Host selection by insect parasitoids. Annu Rev Entomol 21:109–133. doi:10.1146/annurev.en.21.010176.000545
Wan H, Haye T, Kenis M et al (2014) Biology and natural enemies of *Cydalima perspectalis* in Asia: Is there biological control potential in Europe? J Appl Entomol 138:715–722. doi:10.1111/jen.12132
Xu H, Li G, Liu M, Xing G (2006) Oviposition deterrents in larval frass of the cotton boll worm, *Helicoverpa armigera*

(Lepidoptera: Noctuidae): chemical identification and electroantennography analysis. J Insect Physiol 52:320–326

Zakir A, Sadek MM, Bengtsson M et al (2013) Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. J Ecol. doi:10.1111/1365-2745.12041

Zimmermann O, Wührer B (2010) Initial investigations on the ability of the indigenous larval parasitoid *Bracon brevicornis* to control the box-tree pyralid *Diaphania perspectalis* in Germany. DGaaE-Nachrichten 24:25–26