SYNTHETIC BLEND OF LARVAL FRASS VOLATILES DETER OVIPOSITION OF THE BOX-TREE MOTH (*CYDALIMA PERSPECTALIS*)

B.P. MOLNÁR[(1)], Z. TÓTH[(2)], ZS. KÁRPÁTI[(1)]

[(1)] Department of Zoology, Plant Protection Institute, Centre for Agricultural Sciences, Hungarian Academy of Sciences, Herman O. 15. Budapest, H-1022, Hungary, molnar.bela.peter@agrar.mta.hu
[(2)] Lendület Evolutionary Ecology Research group, Plant Protection Institute, Centre for Agricultural Sciences, Hungarian Academy of Sciences, Herman O. 15. Budapest, H-1022, Hungary

ABSTRACT

Volatile stimuli emitted by an intact or herbivore-occupied host plant, non-host plants or the herbivore itself can have all influence final decision of females about where to lay eggs. Volatile substances surrounding larval excreted 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 structure elucidation, two aromatic derivates and one terpene alcohol have been identified to be physiologically active on the antennae of the adults: guaiacol, (±)-linalool and veratrol. In all compounds, antennal responses were found to be dose-dependent with EAG amplitudes being the highest at the highest dose levels. Single sensillum recordings on mated female antennae revealed that these frass compounds triggered 22 percent of the tested olfactory sensory neurons housed in trichoid sensilla. Behavioral bioassays indicated that the blend of these compounds had an oviposition deterrent effect on conspecific females.

Keywords: oviposition-deterrents, electrophysiology, larval frass, invasive pest, box tree moth.

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; Renwick and Radke 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*; Walker, 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 North-West Africa and Turkey (Kenis et al. 2013), and is a popular plant in public and private gardens and parks. 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 and has an unpleasant scent for 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 micro-extraction (SPME) to measure the time-related volatile profile changes of the fresh frass.

MATERIAL 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 ± 1ºC, 65 ± 5% RH, 16 h light 8 h dark photoperiod) to initiate a laboratory population. Larvae were kept in glass jars and fed on shoots of boxwood placed in a water container. When moths emerged potted boxwood plants were offed for egg laying, then leaves with eggs were placed into cylindrical glass jar, where 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. 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, 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 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 with PTFE tubes. Continuous, 1 l min^{-1} airflow was drawn through the setup. Volatiles were collected continuously for 4 hours using 1.5 mg activated charcoal adsorbent. 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 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. We used differently stored groups of frass (fresh, one, two, three days old at room temperature and six months old frozen) to compare their emissions to that of the vial-wick dispensers. SPME fiber (PDMS/CAR 0.53 mm, Supelco, Sigma-Aldrich, Bellefonte, PA, USA) was exposed into the sampling vial for five minutes 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).

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 by Molnár et al. (2015). Antennae were stimulated using stimulus air stream (2 l/min) directed into a constant, charcoal filtered, humidified air stream (1 l/min). The synthetic compounds were dissolved in mineral oil and 10 µl of the corresponding dilutions (0.1, 1, 10, 100, 1000 and 10 000 ng/µl) of the compounds were deposited on a filter paper (1 × 1 cm), which was then placed into a Pasteur-pipette and used as a stimulus cartridge. Responses for the mineral oil before and after each series of odor stimuli were averaged and subtracted from the absolute EAG amplitude.

Single sensillum recordings with sharp tungsten microelectrode were performed using standard equipment (Syntech) on trichoid sensilla following the procedure described by Kárpáti et al. (2013). 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 were applied on a filter paper disk (12.7 mm Ø) and placed into a Pasteur pipette. We used mineral oil as a control stimulus. The 0.5 sec stimuli (0.5 l/min) were delivered into the continuous air stream (1 l/min) using a stimulus controller (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). Helium was used as the mobile phase at 35 cm/s flow. One μl of samples were 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 were used with splitless mode for 1 min before septum purge to allow thermal desorption of the samples 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 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) decenyl acetate.

Chemicals and dispensers

Guaiacol (≥98%), (±)-linalool (97%) and veratrol (≥99%) 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. Volatile compounds were mixed in the same ratio as found in the natural frass volatile collection based on GC-MS quantitative analyzes. One ml 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 were placed into the cages, with three *B. sempervirens* 'Suffruticosa' plants offered for oviposition. 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. Positions of the three plants within the cages were rotated randomly on a daily basis. After three 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. 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.

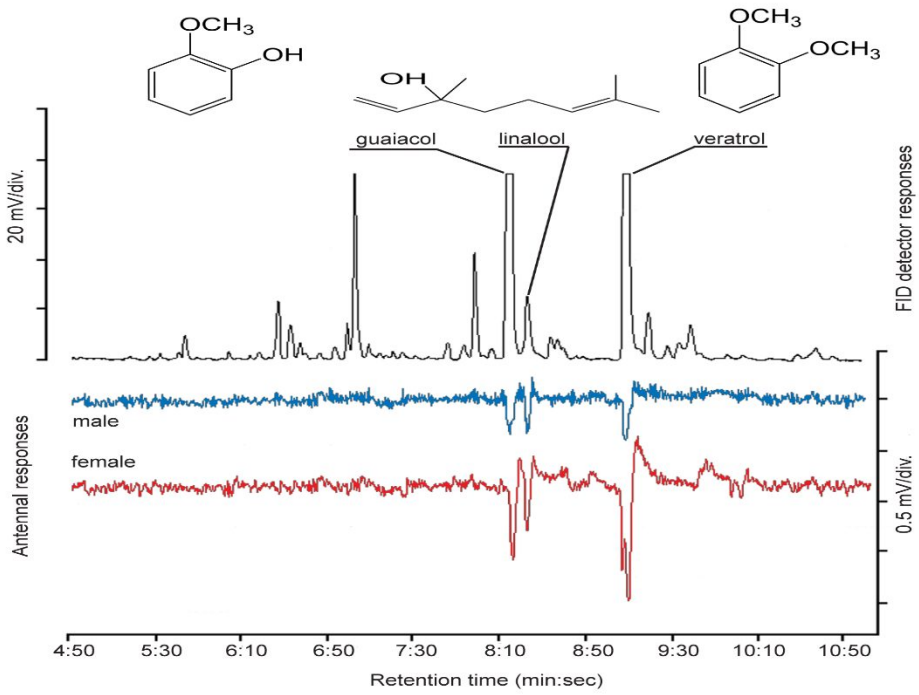
RESULTS

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 minute, respectively (Fig 1). Antennal active compounds were subsequently identified by GC-MS as guaiacol, (±)-linalool and veratrol.

Figure 1: Averaged recordings of coupled gas chromatographic with electroantennographic detection (GC-EAD) on *Cydalima perspectalis* antennae (n=6) with headspace volatile compounds of conspecific larval frass.



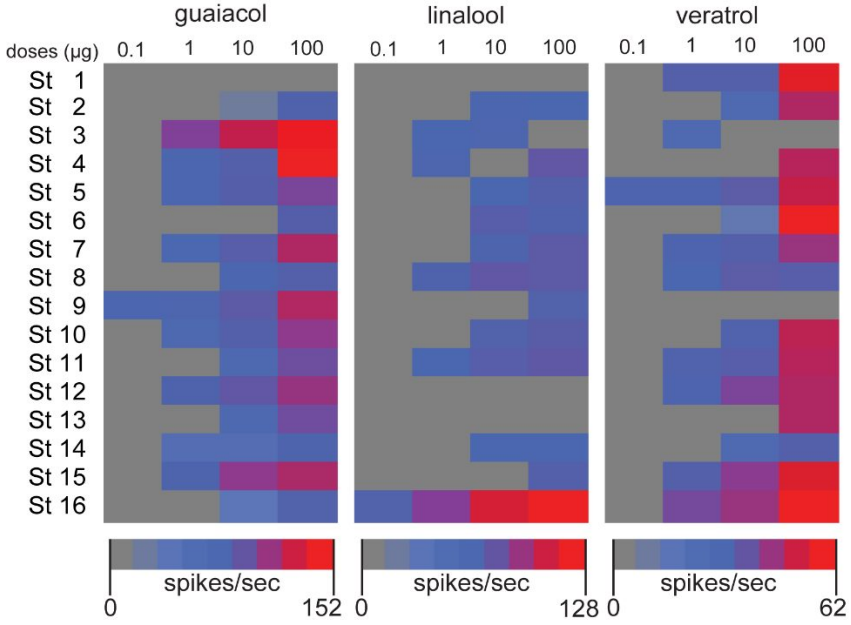
Dose-response tests

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: $F_{5,41}=222.97$, $P<0.001$) 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 $P\geq0.091$), 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 $P\geq0.652$), 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, $t=19.94$, $P<0.001$).

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 (Fig 2). 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 to 62 Hz. Thirteen neurons responded only to the higher doses (1, 10, 100 µg). In 3 cases, we found very sensitive neurons that responded to the lowest dose (0.1 µg) of guaiacol, (±)-linalool and veratrol (Fig 2, 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 2, St 1). Inhibitory responses (decreased spike frequency compared to spontaneous activity) of the OSNs were not found either during or after the stimulation onset.

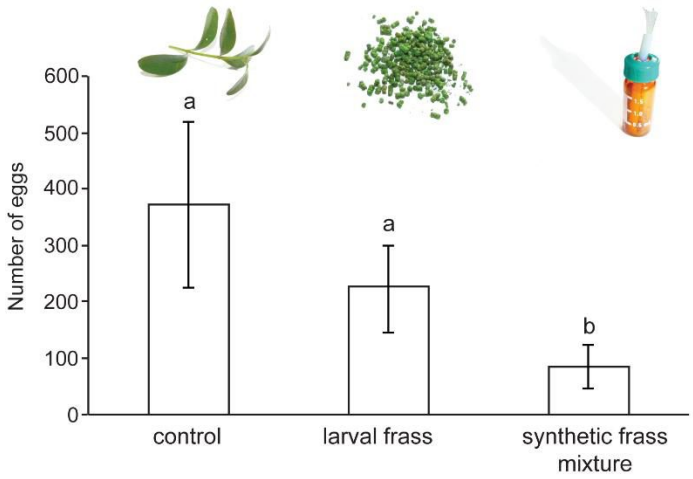
Figure 2: Dose dependent heatplot of single sensillum recordings on sensilla trichodea (St) of female *Cydalima perspectalis* antennae to three electrophysiological active larval frass compounds.



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 3). 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. 31 percent (parameter estimate: 0.69 [0.44-1.09]) on the plants with natural frass, and dropped by approx. 78 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 (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.

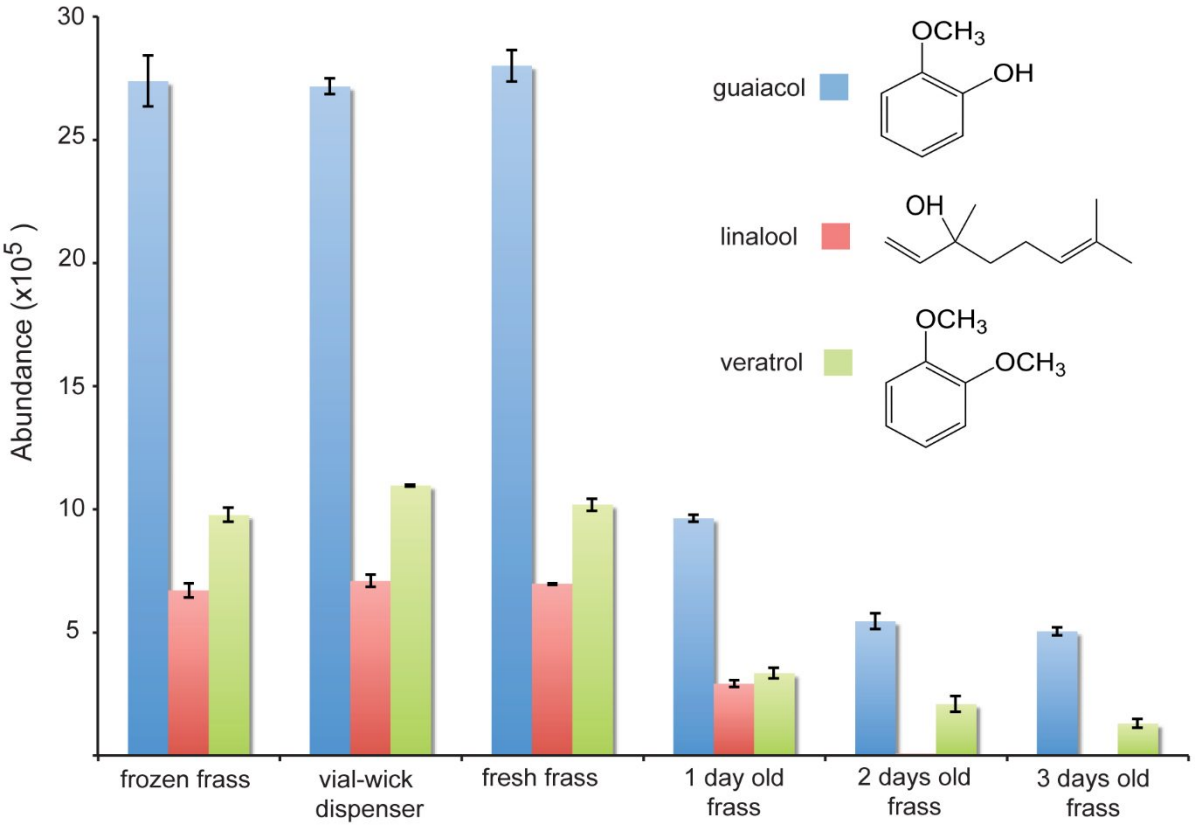
Figure 3: Results of *Cydalima perspectalis* three-choice oviposition bioassays. Number of eggs laid (mean ± SE) on potted boxwoods: untreated, treated with larval frass and treated with vial-wick dispenser loaded with synthetic mixture of the physiologically active frass compounds (guaiacol, (±)-linalool, veratrol).



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: $\chi 25=24.79$, $P<0.001$; (±)-linalool: $\chi 25=25.13$, $P<0.001$; veratrol: $\chi 25=27.08$, $P<0.001$; Fig 4). 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. 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 one day of exposure to the air, and became a fraction of the original amounts by the second day. Moreover, (±)-linalool was undetectable in the headspace of larval frass after three days of exposure.

Figure 4: 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 profile.



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 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) or aggregation pheromone components (Obeng-Ofori et al. 1994; Dillon et al. 2000), 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 hours and became undetectable on the third day. We propose that in our oviposition bioassay, the substantial loss of the active 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. 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).

CONCLUSION

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.

ACKNOWLEDGMENTS

This study was supported by the Hungarian Scientific Research Fund (NKFIH PD1041310) and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

REFERENCES

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. *Journal of Insect Physiology* 39:129–137.

Anderson P. and 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. 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.

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* 32:943–57.

Bruce, TJA., Wadhams, LJ., Woodcock, CM. 2005. Insect host location: a volatile situation. Trends Plant Sci 10:269–274.

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

Cardé, RT. and Willis, MA. 2008. Navigational Strategies Used by Insects to Find Distant, Wind-Borne Sources of Odor. *J Chem Ecol* 34:854–866.

de Bruyne, M., Baker, TC. 2008. Odor detection in insects: volatile codes. *J Chem Ecol* 34:882–97.

Dillon, RJ., Vennard, CT., Charnley, AK. 2000. Exploitation of gut bacteria in the locust. *Nature* 403:851.

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

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology*, 14(3):350-356.

Kárpáti, Z., Tasin, M., Cardé, RT. et al. 2013. Early quality assessment lessens pheromone specificity in a moth. *PNAS* 110:7377–7382.

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* 38–41.

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.

Leuthardt, FLG. and 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.
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.
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 41(11):997-1005.
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.
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* 20:2077–87.
Pinheiro, J., Bates, D., DebRoy, S. et al. 2015. nlme: Linear and nonlinear mixed effects models. R package version 3.1-121.
Reinhold, J. and Schumacher, J. 2013. Der Buchsbaum-Zünsler (*Cydalima perspectalis*) im Grenzach-Wyhlener Buchswald – Invasionschronik und Monitoringergebnisse. *Gesunde Pflanz* 65:1–6.
Reinecke, A. and Hilker, M. 2014. Annual Plant Reviews. John Wiley & Sons, Ltd, Chichester, UK.
Renwick, JAA. 1989. Chemical ecology of oviposition in phytophagous insects. *Experientia* 45:223–228.
Renwick, JAA. and 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. and Chew, FS. 1994. Oviposition Behavior in Lepidoptera. *Annu Rev Entomol* 39:377–400.
Rothschild, M. and Schoonhoven, LM. 1977 Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). *Nature* 266:352–355.
Schoonhoven, LM., van Loon, JJA., Dicke, M. 2005. Insect-Plant Biology: Paperback: Louis M. Schoonhoven - Oxford University Press. Oxford University Press, London.
Turlings, TC., Tumlinson, JH., Lewis, WJ. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251–3.
Valladares, G. and Lawton, JH. 1991. Host-Plant Selection in the Holly Leaf-Miner: Does Mother Know Best? *Journal of Animal Ecology* 60(1):227-240.
Vinson, SB. 1976. Host Selection by Insect Parasitoids. Annu Rev Entomol 21:109–133.
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.
Zakir, A., Sadek, MM., Bengtsson, M. et al. 2013. Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. *J. of Ecology* 101:410–417.
Zimmermann, O. and 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.