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Leafroller-induced grapevine volatiles attract heterospecific adults *Lobesia botrana*

Comentado [CG1]: Alternative title: "Leafroller-induced plant volatiles attract the heterospecific moth *Lobesia botrana*"

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Abstract

Plants release volatiles in response to caterpillar feeding. These herbivore-induced plant volatiles ~~that~~ (HIPVs) ~~attracts~~ natural enemies of the herbivores ~~and~~ repel or attract conspecific adult herbivores ~~in~~ a tri-trophic interaction which has been considered to be an indirect plant defence against herbivores. Recently, we have demonstrated the attraction of male and female *Lobesia botrana* (Lepidoptera: Tortricidae) to a blend of phenylacetonitrile and acetic acid, two compounds identified as ~~herbivore-induced plant volatiles~~ (HIPVs) in heterospecific apple – leafroller interactions. ~~Thus,~~ ~~the~~ ecological basis of our finding was not clearly understood. ~~Accordingly~~ ~~Thus~~, this work was undertaken to further investigate ~~HIPVs~~ ~~(HIPVs interactions among heterospecific leafroller species.)~~ To this end, we explored the effect of ~~in~~ grapevine – leafroller interaction ~~and~~ HIPVs released by the larvae of one grape tortricid study on the response of adults of the heterospecific ~~adult the~~ European grapevine moth, *L. botrana* ~~to these~~ ~~volatiles~~. We sampled headspace volatiles ~~compounds emitted~~ from uninfested grape ~~leaves~~ ~~vine~~ ~~trees~~, and from grape ~~leaves~~ ~~vine~~ ~~trees~~ infested with larvae of the generalist ~~herbivore the~~ grapevine leafroller moth, *Sparganothis pilleriana* (Lepidoptera, Tortricidae), ~~larvae using~~ ~~headspace collection~~ and analyzed them by gas chromatography/mass spectrometry. Infested grape ~~leaves~~ ~~trees~~ uniquely released three compounds (phenylacetonitrile, 2-phenylethanol and indole) that ~~were~~ ~~are~~ not released from healthy ~~trees~~ ~~grape leaves~~. ~~A caged field trial was carried out to compare~~ ~~the~~ attractiveness of male and female *L. botrana* to these three compounds plus acetic acid was assayed in field-caged trials. ~~Nine different lure composition blends was formulated,~~ comprising a full factorial set ~~of presence/absence~~ of the three compounds, each with acetic acid, plus a blank lure as control, were tested. ~~Of the lure compounds,~~ only lures containing phenylacetonitrile had a significant effect on trap catches, with catches which were

generally higher in the presence of phenylacetonitrile in the blend than in lures containing 2-phenylethanol, indole or blank traps. In contrast, the presence of 2-phenylethanol and indole did not substantially affect ~~trap~~ catches relative to controls, comparing traps with blank traps. ~~These~~ Our results confirm our hypothesis that phenylacetonitrile is released during grapevine infestation with leafrollers. ~~and~~ In addition, adult *L. botrana* can detect these compounds, which confirms, suggesting that more complex tritrophic interactions can be heterospecific across the same trophic level. ~~The~~ In other words, our results indicate that not only conspecific adults but also heterospecific adults s can exploit HIPVs ~~compounds of host plants~~.

KEYWORDS

Caterpillar-induced plant volatiles, phenylacetonitrile, 2-phenylethanol, indole, acetic acid, *Lobesia botrana*, *Sparganothis pilleriana*, grape, tri-trophic interaction

INTRODUCTION

Insect herbivory often changes the emission of volatile organic compounds of plants (Dicke and Baldwin 2010; Hare 2011). Natural enemies of insect herbivores such as predators and parasitoids may be attracted to these altered plant odours and this signal can increase their ability to locate their herbivore hosts (Alborn et al. 1997; Yoshinaga et al. 2010). This tri-trophic relationship has been hypothesised as an indirect plant defence strategy that recruits natural enemies to incapacitate the herbivores. In contrast, feeding by caterpillars on plants can result in contrasting behaviour by conspecific adult herbivores: in some plant species, volatiles induced by caterpillar feeding repel conspecific adult herbivores (De Moraes et al. 2001; Signoretti et al. 2012), while odours of other plant species infested with caterpillars can attract conspecific adults (El-Sayed et al. 2016, Suckling and El-Sayed 2017; El-Sayed et al. 2018). The advantages of the attraction of conspecific adult herbivores for both the herbivores and the plants are unclear. El-Sayed et al. (2016) suggested that herbivore-induced plant volatiles (HIPVs) signals might indicate a lower plant resistance hence more suitable host plants to herbivores. For example, plant resistance has been linked to the higher production of secondary compounds, mainly monoterpenes and other compounds, in response to herbivory (Pare and Tumlinson 1999). An increase in the emission of monoterpenes and other compounds from infested plants could cause lower concentrations of these compounds in the foliage resulting in lower resistance to herbivore feeding and benefit herbivores (Trowbridge et al. 2014).

In horticulture ecosystems, apple trees uniquely release several compounds, including acetic acid, benzyl alcohol, phenylacetonitrile, indole, 2-phenylethanol, and (E)-nerolidol, only when infested by larvae of light brown apple moth (LBAM), *Epiphyas postvittana* (Walker), the eye-spotted bud moth (ESBM), *Spilotea ocellana* (Denis & Schiffermüller), and the oblique-banded

leafroller (OBLR), *Choristoneura rosaceana* (Harris), and the pandemis leafroller (PLR), *Pandemis pyrusana* (Kearfott) (El-Sayed et al. 2016; Suckling and Suckling 2017, El-Sayed et al. 2018). El-Sayed et al. (2016) found that a binary blend of either the three HIPVs, phenylacetonitrile and acetic acid, or 2-phenylethanol and acetic acid, attracted a significant number of conspecific male and female adult LBAM in New Zealand. Further investigation with other leafrollers (Tortricidae) in North America, including the ESBM, OBLR and PLR revealed similar responses. Male and female adults of ESBM were most attracted to a blend of phenylacetonitrile and acetic acid while male and female adults of OBLR and PLR were most attracted to a blend of 2-phenylethanol and acetic acid (El-Sayed et al. 2016; Suckling and El-Sayed 2017; El-Sayed et al. 2018). In the same trials, the general predator, the common green lacewing, *Chrysoperla plorabunda* (Fitch) was also attracted to a ternary blend of phenylacetonitrile and 2-phenylethanol + acetic acid attracted (El-Sayed et al. 2018).

Recently, the caterpillar-induced plant volatiles from the apple-leafroller system was tested in two vineyards in Spain and Hungary for their attractiveness to the European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Tortricidae: Olethreutinae) (El-Sayed et al. 2019). As seen for Tortricinae species, a binary blend of phenylacetonitrile + acetic acid attracted significantly more male and female *L. botrana* to traps than acetic acid or blank lures. However, in this work, the ecological basis for the attraction of *L. botrana* to HIPVs found in the apple-leafroller system was not clearly understood. Therefore, this study was conducted to ~~investigate~~determine the HIPVs released from grapevine leaves ~~in response to~~infestation with leafroller larvae and the response of adult leafrollers moths to these volatiles. The leafrollers selected for ~~this study~~HIPV induction is *Sparganothis pilleriana* (Denis & Schiffermüller) (Tortricidae: Tortricinae) whose larvae attack grape leaves and share ~~the same~~ habitat with *L. botrana*. We have analysed the headspace of healthy

grape leaves and grape leaves infested with *S. pilleriana* larvae and identified the unique compounds produced only by infested leaves. Besides, we have conducted field caged trapping trials in ~~the~~ conventional vineyard~~s~~ to investigate the direct responses of adult male and female *L. botrana* to HIPVs identified in the grapevine-leafrollers system. This work was expected to elucidate the ecological basis underlying the attraction of *L. botrana* to HIPVS in the apple-leafroller system previously reported (reference). It will also shed the light about similarities in HIPVs between the apple-leafrollers ~~system~~ and grapevine- leafrollers system~~s~~.

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MATERIALS AND METHODS

Plants and insects

All volatile collection and caged trial were conducted at the Julius Kühn-Institute (JKI) experimental vineyard in Siebeldingen, Germany. Naturally infested grape with the grapevine leafrollers. *S. pilleriana* were chosen based on visual observation prior to volatile collection. Plant infested with at least one or more larvae were selected for sampling. Healthy leaves were selected from neighbouring plants. Infestations were characterized by the presence of feeding shelters either by the roll in a single leaf or seal or fold two leaf surfaces together by larval web. Infestation was confirmed by the presence of the larvae in the feeding shelter.

Chemicals

The chemical purity of the compounds used in the field experiments was as follows: Glacial acetic acid (99%), phenylacetonitrile (99%), 2-phenylethanol (99%), and indole (99%). Glacial

acetic acid was stored at ambient temperature while all other compounds were stored at -20°C until used. All compounds were purchased from Sigma-Aldrich (MO, USA).

Air entrainment of volatiles emitted from uninfested and grapevine infested with grapevine leafroller larvae

Volatile collections from infested grapevine trees ('variety') with grapevine leafroller larvae and uninfested grapevine trees were conducted in the field in Siebeldingen, Germany, using a dynamic headspace collection method, where air containing the odour was absorbed by a sorbent filter that was then extracted with hexane. Intact tree branches with either grapevine leaves infested with leafroller larvae or uninfested leaves were enclosed in a polyester oven bag (Glad NZ®, 35 cm × 50 cm). A charcoal-filtered airstream was pulled over the enclosed leaves at 0.5 L/min, and the headspace volatiles were collected for 24 h on an adsorbent filter containing 50 mg of Tenax-GR 35/60 (Alltech Associates Inc.) in a 60 mm long × 6 mm diameter glass tube. For the collection of control samples, a charcoal-filtered airstream was pulled through an empty oven bag in the same vineyards. Samples were sealed and shipped in dry ice to The New Zealand Institute for Plant & Food Research Limited (PFR) facility for Gas Chromatography/Mass Spectrometry (GC/MS) analysis. At the PFR laboratory, the Tenax filters were extracted with 0.5 mL of n-hexane (AnalaR BDH, Laboratory Supplies, Poole, UK). A sub-sample of 100 µL was reduced to 10 µL at ambient temperature under a stream of argon and 1 µL of the concentrated extract was injected in the GC/MS. Six volatile collection samples of

infested leaves, six uninfested leaves and six control samples were sampled. Different plants were used for each sample.

Analysis of Air-Entrainment Samples by GC/MS

The concentrated extracts of the air-entrainment samples were analyzed using GC/MS (Varian 3800 GC coupled to a Varian 2200 MS). Helium was used as the carrier gas (1 mL min^{-1}), and injections were splitless for 0.6 min. Transfer line and ion trap temperatures were 250 and 180°C , respectively. The GC injector temperature was set at 220°C , and the oven ramp was 40°C for 2 min, $4^{\circ}\text{C min}^{-1}$ to 240°C , hold for 10 min, and then $15^{\circ}\text{C min}^{-1}$ to 260°C , using a VF-5 MS capillary column ($30 \text{ m} \times 0.25 \text{ mm}$ inner diameter $\times 0.25 \text{ }\mu\text{m}$ film; and a polar $30 \text{ m} \times 0.25 \text{ mm i.d.} \times 0.5 \text{ }\mu\text{m}$, VF23-MS capillary column; Varian, Inc., Walnut Creek, CA, USA). The spectra were recorded at an ionization voltage of 70 eV over a mass range mass-to-charge (m/z) of 20 to 499. Kovats retention indices (KI) were calculated for the compounds (El-Sayed 2020) (Table 1). Structural assignments of the compounds were made by comparing their mass spectra with MS library (NIST 2017), as well as by comparison with Kovats retention indices published in the literature (El-Sayed 2020). Identification of some volatiles was confirmed by comparison with authentic samples.

Field Caged Experiment

A field caged trial was carried out to compare three different lure combinations (**P1** lure: containing Phenylacetonitrile, **P2** lure: containing Indole, **P3** lure: containing 2-phenylethanol) for their attractiveness to male and female *L. botrana*. In all, nine lure compositions were formulated, comprising a full factorial set of presence/ absence of the three components. Each combination except the no compound blank also included acetic acid (Table 1). This trial was

carried out in an experimental vineyard at JKI site in Siebeldingen, Germany, using three field cages. Each cage was set up with three traps, each loaded with a different lure composition, with the traps, each placed in a different corner. The lures tested in each cage were determined using a resolvable row-column design with 'row' for each cage and 'corner' for each position. The design was Latinized by rows. A complete replicate (3 cages) was run on each of three days. The design was generated with CycDesign 5.1 (VSN International Ltd 2013). This trial is a choice test, with each of the nine lures representing a choice. It was not possible to use all nine lures within a cage, so this test is not a true choice between all of the lures, but instead, a choice between the three lures in each cage. Each cage had a unique combination of three of the 9 lures. No combination of the three lures was used more than once. Thus, of the 84 possible combinations of three lures from 9 treatments, only twelve were used. In each of the runs, fifty insects (50:50, male:female) were released into each cage. Delta transparent traps (Trifolio-M GmbH, Lahnau - Germany) with sticky bases were used in the experiment. Sticky bases were removed after 48 hours and the number of insects caught on each trap was recorded.

Electroantennographic (EAG) Bioassay

Synthetic compounds were diluted in n-hexane, except for indole which was diluted in absolute ethanol. Dilutions were ~~made~~ 10-fold, from 10^{-1} to 10^{-5} , which roughly corresponds to 100µg, 10 µg, 1 µg and 100 ng/µl. 10 µl of test stimuli were loaded on a piece of filter paper (Whatman #1, 1.5 cm², pre-cleaned with n-hexane). After 30-sec solvent evaporations, the filter paper was inserted into a 1-ml disposable plastic pipette tip. Between uses stimulus cartridges were stored in glass vials closed with screw-cap lids. A given pipette cartridge was not puffed more than 8 times and they were disposed at the end of the day. Antenna were stimulated first with solvent

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control followed by the test stimuli in random order, from the lowest dose (1 µg) to the highest dose (1 mg) ~~for~~in each of them. Male and females 3 to 5 days of age were immobilized with CO₂ and introduced in a 200-µl disposable pipette tip with the antennae exposed. The head and the base of the antennae were ~~fixed~~restrained with melted dental wax. Electrodes were made with platinum filaments inserted in drawn-glass capillary tubes that were filled with saline solution (0.2M KCl). One electrode was inserted into the eye (reference electrode) and the other contacted the most distal segments of the antennae (recording electrode). A 0.5 L/min flow of charcoal-filtered and humidified air blew continuously over the insect preparation. Each stimulation (i.e., "puff") generated by a stimulus controller (CS-55, Syntech, Germany) was delivered for 0.5 s at 0.2 l/min with an interstimulus interval of at least 30 s. The signal was pre-amplified (10x gain), high-pass filtered at 0.1 Hz, digitized and analyzed (Syntech, Buchenbach, Germany). Only one antenna per insect (N = 8 per sex) was used and each antenna was stimulated with all test odorants.

Statistical Analysis

Field cage experiment. For each cage, the number of insects not caught on the traps were calculated. These numbers were included as a fourth choice for each cage. The data were first analysed with a Poisson-gamma hierarchical generalized linear model (Lee et al. 2006), with logit links, which allows the inclusion of random effects, each included with the gamma distribution. The fixed effects (Lure, including factors for the Lure components; and cage total) were included with a Poisson distribution. Inclusion of the cage total leads to the percentage of insects within a cage being assessed rather than the counts, similarly to the Poisson log-linear model for multinomial data (McCullagh and Nelder 1989). Random effects were assessed with a χ^2 test of the change in deviance on dropping a term, as implemented in Genstat's HGRTEST

procedure (GenStat Committee 2015). The importance of the lure components (main effects and all interactions) was assessed similarly, with the HGFIXED procedure. Of the random effects, only Replicate was found to be important, so was included in the final model.

Percentages and associated standard errors are technically very difficult to obtain from the multinomial model, particularly when random effects are included. Therefore, the data for each of the lures and those not on a trap were then analysed using a standard binomial generalized linear model (McCullagh and Nelder 1989) with a logit link with no random effects, and with the dispersion set to that for the multinomial log-linear model, with dispersion set to that from a multinomial model with no random effects fitted. These models were used to obtain approximate confidence limits for the estimated percentages. The predicted percentages and associated confidence limits were obtained on the logit scale, and back transformed. The estimates were then rescaled to be as if each lure was run in each cage. The analyses were all carried out with Genstat (Payne et al. 2017).

EAG bioassay. The maximum negative EAG potential (mV) generated by each puff was the analyzed parameter. Four-term log-logistic models [`drc()` in the library `drc` of R, (Ritz et al., 2015)] were fitted to each individual dose-response curve to estimate the dose at which the curve was at half height (i.e., the expected dose 50 or ED50). ANOVA models [`glm()` in R, (R Core Team, 2018)] tested the effect of concentration, sex and test compound on maximum EAG

response and ED50. Pairwise tests were performed with [the R package emmeans\(\)](#) (Lenth, 2018).

RESULTS

Analysis of Air-Entrainment Samples by GC/MS Volatiles

Analysis of the headspace of uninfested and infested grapevine trees indicated quantitative and qualitative differences in odour profiles. We identified a total of 33 compounds in the headspace of uninfested grapevine trees, and 36 compounds in the headspace of infested grapevine trees (Table 2). The infestation of grapevine trees with *S. pilleriana* larvae resulted in a change in the ratio of the compounds emitted from infested grapevine trees (Table 2). Trans- β -ocimene and cis-ocimene were the main compounds representing 87% and 70% of total volatiles produced in the headspace of uninfested and infested grapevine trees respectively. The amount of (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (Z)- β -Farnesene, Germacrene-D, (+)-Valencene, (Z,E)- α -Farnesene, α -Farnesene produced from the infested leaves were significantly much higher than uninfested leaves (Table 2). In addition to quantitative differences, a minor qualitative difference was observed between infested and uninfested plants. three compounds (phenylacetonitrile, 2-phenylethanol, and indole) were present only in the headspace of infested grapevine trees.

Phenylacetonitrile, 2-phenylethanol and indole were tested in the field trials. In this study, we could not verify the presence of acetic acid because of the limitations of the technique used to collect headspace volatiles. However, since acetic acid was produced only from the infested apple trees and was critical for the attraction of other related leafrollers in our previous work (El-Sayed et al. 2016), we hypothesized it would be also important for this species. The selection of the four compounds phenylacetonitrile, 2-phenylethanol, indole and acetic acid for

the field test was based on chemical analysis conducted in this work and our results from the previous study (El-Sayed et al. 2016). On the other hand, Suckling and El-Sayed (2017) showed that changing the ratio of HIPVs does not affect the response of adult herbivores. Accordingly, in this study compounds were tested at an equal ratio in multicomponent blends.

Field Caged Experiment

The total number of male and female caught (and thus total not caught) in each cage varied noticeably between the four runs, with 40, 59, 65 and 90% not caught in runs 1 to 4 respectively. Overall, around 40% of insects were caught in any trap ($p < 0.001$ to compare with those not caught). Of those caught on the traps (Table 3), the percentage on the blank trap was very low (0.5%; $p < 0.001$ comparing with the ~35% caught on traps with lures). Of the lure components, only P1 had a noticeable effect ($p = 0.046$ when comparing traps with P1 to those without), with catches generally higher in the presence of phenylacetonitrile in the blend. In contrast, the presence of P2 or P3 did not substantially affect the catch ($p = 0.296$ for P2 and 0.813 for P3). In addition, the presence of P2 or P3 did not substantially alter the effect of adding P1 ($p > 0.2$ for all interactions). It was not possible to determine the exact number of male and female caught in each treatment because of the low-quality insects which make it difficult for sex identification. However, for those that have been identified, the ratio of male/female was around 50/50 in all treatments tested.

Electroantennographic Bioassay

n-hexane generated negligible voltages (mean $\pm$ SEM, 0.3 ± 0.08 mV in males and 0.25 ± 0.11 mV in females). Average responses to the 1 mg dose of test compounds ranged between 1.97 mV (phenylacetonitrile, females) and 0.3 mV (indole, males) (Figure 1). Female antennae

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responded more strongly to test stimuli than male antenna (ANOVA, $df=1$, $F=18.16$, $P<0.001$). Odorant effect was marginally significant in the ANOVA ($df=3$, $F=2.77$, $P=0.042$) but no significant differences between odorant pairs could be detected. Sigmoidal functions could be fitted to all the dose-response curves except for indole in males, which showed an atypical decrease in response as the concentration increased. Comparison of the ED50s of the other 3 compounds revealed no significant sex effect (ANOVA, $df=1$, $F=0.3485$, $P=0.5636$) ~~but a or significant~~ odorant effect (ANOVA, $df=2$, $F=14.4834$, $P=0.2402$) effects. ~~so that the antennae were more sensitive to~~ The estimated ED50s for 2-phenyl ethanol, phenylacetonitrile, and acetic acid phenylacetonitrile than to 2-phenyl ethanol, while acetic acid fell in between (ED50s, were 7434.2602 ± 263.619 μ g, 78.41 ± 32.44 μ g and 134.41 ± 24.52 μ g ~~and 78.41 ± 32.44 μ g,~~ respectively).

DISCUSSION

Qualitative differences in the emission of volatile organic compounds (VOCs) were observed between grapevine trees infested with grapevine leafroller moth larvae and uninfested grapevine trees. Infested plants uniquely produce three VOCs, phenylacetonitrile, 2-phenylethanol, and indole, that were not present in uninfested plants. Although grapevine and apple belong to two different families, Vitaceae and Rosaceae respectively, these three HIPVs were also produced by apple trees infested by other leafrollers including, OBLR, ESBM, LBAM and PLR larvae (El-Sayed et al. 2016; El-Sayed et al. 2018). Acetic acid was reported in the headspace of apple trees infested with LBAM larvae (El-Sayed et al. 2016). In our previous study, when these HIPVs were tested individually, few insects were caught in traps, and only a combination of aromatic

HIPVs and acetic acid attract male and female herbivores (El-Sayed et al. 2016). For that reason, we did not test phenylacetonitrile, 2-phenylethanol, and indole individually in this study. In the caged trial conducted in Germany, only blends contain phenylacetonitrile caught a significant number of males and females *L. botrana* than other blends without phenylacetonitrile. In contrast, the presence of 2-phenylethanol, and indole did not significantly enhance the attraction compared to acetic acid alone or synergized or antagonized the response to phenylacetonitrile. Similarly, a binary blend of phenylacetonitrile + acetic acid was the most attractive blend to ESBM males and females (El-Sayed et al. 2016). In contrast, a binary blend of 2-phenylethanol + acetic acid was the most attractive blend for female OBLR (El-Sayed et al. 2016). In between these two species, a binary blend of phenylacetonitrile + acetic acid, or 2-phenylethanol + acetic acid were equally attractive to PLR males and females (Suckling and El-Sayed 2017, El-Sayed et al. 2018). Also, similar to our results, a combination of these two compounds + acetic acid did not result in a significant increase in the numbers of males and females captured in these species (El-Sayed et al. 2016; Suckling and El-Sayed 2017, El-Sayed et al. 2018).

About 35% of the total insects released in the field cage were caught in the traps baited with HIPVs compounds. The majority of these insects were caught in traps baited with blends containing phenylacetonitrile. These results provide further support to the previous finding reported by El-Sayed et al (2019) that a blend of phenylacetonitrile + acetic acid was attractive to male and female *L. botrana*. In addition, in this study, we have demonstrated the production of these compounds from grape leaves infested with leafrollers. This might provide an explanation of the ecological basis underlying the attraction of adults *L. botran* to these compounds. In our previous work in apple-leafrollers interaction, we have proposed that the attraction of the adult leafrollers to conspecific HIPVs signal might indicate a lower plant

resistance hence more suitable host plants to herbivores. This study provides further dimension in insect-plant interaction, that not only adult herbivore can detect conspecific HIPVs but also heterospecific can detect HIPVs of other herbivores possibly to gain information about host suitability. Thus, HIPVs chemical signal in horticulture ecosystem act as universal chemical signal exploited at various level of multitrophic interaction including conspecific and heterospecific adult herbivore as well as natural enemies. Because of the physical status of insects caught in sticky bases, it was not possible to sex all insects. However, for those we were able to sex, the ratio was around 50:50 male: female. In the previous studies (El-Sayed et al. 2019), the ratio of male to females was around to 50:50, while in other studies the ratio of females was higher than male (El-Sayed et al. 2018). For *L. botrana*, we did not determine the mating status of females, but our result with other tortricid showed that both mated and virgin females were attracted to these compounds (El-Sayed unpublished).

The catch of males and females *L. botrana* in traps baited with 2-phenylethanol + acetic acid was not significant from traps baited with acetic acid alone. Similarly, in our previous trials in Spain and Hungary, the attraction to this blend was not significant from traps baited with acetic acid alone (El-Sayed et al. 2019). In contrast, a blend of 2-phenylethanol + acetic acid was attractive to males and females *L. botrana* in field trapping trials (Tasin et al. 2018; Larsson Herrera et al. 2020). Few possibilities might explain the disparity between the results in our study and the other two studies. One possible explanation is because of the geographical variation in the response of various populations of EGVM to HIPVs compounds. For example, we have observed strong variation in the response of OBLR to HIPVs in various location in North America, where it is very attractive in the western coast and not working at all in the eastern coast (El-Sayed unpublished data). 2-phenylethanol was detected in the headspace of

grape leaves infested with *S. pilleriana* larvae and also is produced in grape berries inoculated with various bacteria (Tasin et al 2018). In grape berries inoculated with microorganisms, 2-phenylethanol is produced in the microbial transformation of L-phenylalanine, during the microbial fermentation process (Hua and Xu 2011). 2-phenylethanol can be further oxidized to phenylacetaldehyde and then further transformed to phenylacetate. All these compounds were found in grape berries inoculated with microorganisms (Tasin et al 2018). On the other hand, we could not find phenylacetaldehyde and phenylacetate in grape leaves infested with *S. pilleriana* larvae. In black poplar leaves, both phenylacetonitrile and 2-phenylethanol are biosynthesized from the precursor L-phenylalanine by CYP79/CYP71 enzymes with phenylacetaldoxime as an intermediate (Irmisch et al. 2015).

In this study and our previous studies, we have identified a common HIPVs from apple and grapevine trees infested with leafrollers larvae, which attracted conspecific, heterospecific adult herbivores. Our current finding, together with our recently published results with other species (El-Sayed et al. 2016; Suckling and El-Sayed 2017), indicates that this phenomenon is widespread among leaf-feeding moths. Thus, providing a new pathway for the identification of potent kairomone for female of major agricultural pests and demonstrates the potential of HIPVs as a new tool in pest management. What are the advantages for conspecific and heterospecific adult herbivores of being attracted to infested plants? Infested plants might be more suitable oviposition sites for females because plant resistance may be much lower and survival rate would be higher for offspring than with healthy uninfested plants (Williams and Myers1984). There is no doubt that natural enemies, hyperparasitoids, conspecific herbivores and

heterospecific herbivores are exploiting HIPVs for their benefits in complex heterogeneously structured natural systems.

All HIPVs identified in this study elicited EAGs from both male and female antennae, and although there was a trend for 2-phenylethanol to produce the largest responses, EAG response in both sexes. All test compounds produced statistically similar EAG responses and their dose-response curves had similar ED₅₀s. However, the dose at half curve height (ED₅₀s) was significantly lower for 2-phenylethanol than for phenyl acetonitrile in both sexes, so the antenna of *L. botrana* appears to be most equally sensitive to phenyl acetonitrile all the tested odorants. Recently, Perez-Aparicio et al (2019) investigated the EAG response of male and female *L. botrana* to various host plant volatiles. Methyl salicylate elicited the highest response in females while (E)- β -farnesene elicited a higher response than several other plant odorants in both sexes (Perez-Aparicio et al., 2019). Both compounds were found in both infested and healthy grape leaves in our study. However, the amount (E)- β -farnesene present in the infested grape leaves was significantly higher than in healthy leaves. Future experiments will include testing these two EAG active compounds, in combination with the active blend identified in this study. The EAG response to HIPVs was much higher in female compared to the male response. This agrees with the presence of a larger proportion of plant-sensitive sensilla in females than in males (De Cristofaro et al., 2008; Perez-Aparicio et al., 2019).

The HIPVs reported in this study can be used to monitor and control these important tortricid pests in conventional apple orchards, while monitoring the abundance and manipulate the distribution of natural enemies in organic apple orchards to improve the efficacy of IPM. Extension of the phenomenon of the attraction of HIPVs to conspecific leaf-feeding herbivore to heterospecific fruit-feeding frugivore was not obvious or expected. However, *L. botrana* does

feed on different vegetative parts including inflorescences, developing fruit and mature fruit (Ioriatti et al. 2011). Our findings suggest that the phenomenon of attraction of herbivores to HIPVs may be extended across species and subfamily boundaries. In this study, adult *L. botrana* were attracted to binary mixtures of certain HIPV compounds together with acetic acid. We anticipate in the future, a more complex blend that incorporates other compounds will provide further improvements to the current blend. Consequently, Currently, we are working on testing more complex mixtures of odourants to improve trap catches with HIPVs. All compounds identified as HIPVs in this study are widely commercially available, which would make area-wide implementation feasible. This kairomone could be used to monitor females within mating disruption plots. Our preliminary trials showed potential application in pest management. Further work with this attractant should involve as trap efficiency and active space. Also, potential combinations with sex pheromones can be investigated. It seems likely that these new tools will be amenable to research and development of control tactics targeting several Tortricidae pests.

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Table 1. Components used in each of the lures

Lures	Phenylacetonitrile	Indole	2-phenylethanol	Quantities (μl)			AA (mL)
	P1	P2	P3	P1	P2	P3	
B 1	+	-	-	100	0	0	0.3
B 2	-	+	-	0	100	0	0.3
B 3	-	-	+	0	0	100	0.3
B 4	+	+	-	50	50	0	0.3
B 5	+	-	+	50	0	50	0.3
B 6	-	+	+	0	50	50	0.3
B 7	+	+	+	33	33	33	0.3
B 8	-	-	-	0	0	0	0.3
B 9	-	-	-	0	0	0	0

Table 2. Relative amounts (% $\pm$ SEM) of the compounds identified in the headspaces of grapevine trees infested with *Sparganothis pilleriana* larvae and uninfested grapevine trees

Compound	RI value ¹	% ²	
		Infested	Uninfested
cis-3-Hexenyl acetate	994	0.32 (0.07)	0.20 (0.12)
Hexyl acetate	1002	0.05 (0.02)	0.03 (0.03)
(-)-Limonene	1012	0.13 (0.05)	0.15 (0.08)
cis-Ocimene	1029	23.77 (3.90)	39.55 (9.23)
trans- β -Ocimene	1040	47.01 (35.93)	48.83 (40.64)
DMNT	1091	0.35 (0.11)	0.12 (0.07)
Linalool	1095	1.20 (0.55)	1.39 (1.16)
2-phenylethanol	1115	0.80 (0.35)	nd
DMNT	1113	1.64 (1.39)	2.41 (1.09)
(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	1119	0.42 (0.25)	0.94 (0.77)
(4E,6E)-2,6-Dimethyl-2,4,6-octatriene	1126	1.17 (0.53)	1.01 (0.71)
Phenylacetoneitrile	1142	1.06 (0.23)	nd
Ethyl benzoate	1165	0.04 (0.01)	0.01 (0.01)
Terpinen-4-ol	1173	0.03 (0.01)	0.04 (0.04)
(E)-3-Hexenyl butyrate	1186	0.26 (0.06)	0.13 (0.14)
Methyl salicylate	1192	0.05 (0.01)	0.03 (0.02)
Hexyl butyrate	1193	0.43 (0.17)	0.37 (0.33)
α -Terpineol	1195	0.24 (0.10)	0.25 (0.25)
Decanal	1206	0.10 (0.01)	0.08 (0.02)
(3Z)-3-Hexenyl 2-methylbutanoate	1230	0.08 (0.02)	0.04 (0.05)
cis-3-Hexenyl isovalerate	1236	0.13 (0.04)	0.04 (0.05)
Hexyl isovalerate	1243	0.05 (0.01)	0.04 (0.05)
Indole	1288	3.69 (3.10)	nd
(-)- β -Bourbonene	1378	0.30 (0.09)	0.11 (0.06)
β ELEMENE	1390	0.12 (0.04)	0.08 (0.05)
Caryophyllene	1417	2.84 (0.54)	2.13 (1.20)
Germacrene D	1429	0.09 (0.02)	0.04 (0.04)
Humulene	1455	0.72 (0.15)	0.42 (0.31)
(Z)- β -Farnesene	1470	1.63 (1.69)	0.02 (0.03)
GERMACRENE-D	1485	0.85 (0.30)	0.14 (0.16)
(+)-Valencene	1498	1.32 (1.11)	0.10 (0.11)
(Z,E)- α -Farnesene	1514	1.87 (1.13)	0.32 (0.39)
α -Farnesene	1529	6.67 (5.48)	0.57 (0.24)
trans-Nerolidol	1577	0.30 (0.15)	0.23 (0.16)
Hexyl benzoate	1594	0.08 (0.04)	0.06 (0.07)
(-)-Caryophyllene oxide	1597	0.19 (0.08)	0.10 (0.07)

¹ Kovats Retention Index (VF5-MS capillary column)

² Percentage of total volatiles produced is given as the mean area of the GC peaks followed by the standard error (n = 6).

nd: not detected

Table 3. Estimated percentage catch on traps with each lure type (95% confidence limits)

Lure	P1	P2	P3	%
B1	+	-	-	6.5 (4.0,10.1)
B2	-	+	-	3.0 (1.8,5.0)
B3	-	-	+	4.0 (2.4,6.5)
B4	+	+	-	4.7 (2.8,7.5)
B5	+	-	+	6.0 (3.7,9.4)
B6	-	+	+	4.2 (2.5,6.8)
B7	+	+	+	4.3 (2.6,7.0)
B8	-	-	-	3.2 (1.9,5.3)
B9	-	-	-	0.5 (0.3,0.9)
not on trap				63.7 (49.6,75.8)

Figure Legends

Figure 1. Electroantennogram responses of *Lobesia botrana* antennae to 4 odorant stimuli.

Symbols and error bars show the mean and SEM (they have been toggled along the x-axis for the sake of clarity). Curves represent log-logistic models fitted to the mean EAG responses, except for indole in males (dotted line) which could not be modelled.

Figure 1