

Bioactive Volatile Compounds from *Penicillium digitatum*-Infected Apples: Oviposition Attractants for Yellow Peach Moth *Conogethes punctiferalis* (Lepidoptera: Crambidae)

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Abstract

Plant-associated microbes critically shape the dynamics of plant- and insect-associated communities. In previous studies, we reported that the yellow peach moth *Conogethes punctiferalis* (YPM) preferred to *Penicillium digitatum*-infected apples (PDA) for oviposition. However, the underlying mechanisms remains unclear. In the present study, the behavioral and physiological experiments were conducted to determine how *P. digitatum* affects the oviposition selection of YPM females via altering host plant volatile organic compounds (VOCs). YPM females were attracted to and laid more eggs on PDA than on fungi non-infected apples (NIA), mechanically damaged apples (MDA), and *P. digitatum* in potato dextrose agar medium (PPD). Four-arm olfactometer assays further confirmed that odors in PDA were responsible for the attractiveness of YPM females. PCA of the 38 VOCs revealed a clear separation of PDA from NIA, MDA, and PPD, including methyl 2-methylbutyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane that were specifically emitted by PDA. Moreover, when *P. digitatum*-induced specific VOCs in individual or synthetic blends consisting of methyl 2-methylbutyrate, butyl caprylate, or *n*-tetradecane were added to apples, a significant selection rate of YPM females choosing apples occurred, suggesting that these three specific VOCs acted as predominant olfactory signals for YPM females to PDA. Taken together, the microbe *P. digitatum* was an important driver of the interactions between YPMs and host plants by altering plant volatiles. These findings may form the basis for developing attractant baits for field trapping YPMs in the future.

Key Message

1. The plant-associated microbes can be a direct or indirect driver for the interactions between host plants and herbivorous insects.
2. Mated YPM females preferred *P. digitatum*-infected apples (PDA) to fungi non-infected apples (NIA), mechanically damaged apples (MDA), and *P. digitatum* in potato dextrose agar medium (PPD).
3. A projection to principal component analysis (PCA) based on the absolute contents of all detected volatiles showed a clear separation between PDA and NIA, MDA, PPD. Furthermore, there were 5 specific odorants from *P. digitatum*-induced VOCs, including methyl 2-methylbutyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane.
4. Mated YPM females were attracted to apples with individual or synthetic blends, including methyl 2-methylbutyrate, butyl caprylate, and *n*-tetradecane.
5. Taken together, plant-associated microbes (*P. digitatum*) changed the host selection behavior of YPMs via affecting host plant volatiles.

Introduction

Plant-associated microbes are widely reported as important but overlooked drivers of host plant-herbivorous insect interactions, either direct effects of plant-associated microbes on herbivorous insects via the ingestion of microbes and/or microbial metabolites or indirect effects of plant-associated microbes via altering the host plant biochemistry (Eberl et al. 2018; 2019; 2020). The impacts of plant-associated microbes on herbivorous insects could further cascade up and down multiple trophic levels in the arthropod community at spatial scales ranging from patterns within single host plants to entire landscapes (Tack and Dicke 2013). Consequently, it is meaningful to investigate tripartite interactions among plant-associated microbes, host plants, and herbivorous insects for improving our knowledge on the ecology and evolution of plant-microbe-insect interactions and designing more effective management strategies to control herbivorous insects in agroecosystems.

Plant-associated microbes exhibit diverse effects on the herbivorous insects' foraging behavior, such as the location and selection of host plants, including detrimental, beneficial, and neutral (Kopper et al. 2004; Witzgall et al. 2012). Furthermore, the changes in host plant volatile organic compounds (VOCs) caused by plant-associated microbes infection could be responsible for the alteration of herbivorous insects' foraging behavior (Groen et al. 2016; Rizvi et al. 2016; Grunseich et al. 2019). For example, the bacteria on the egg-surface, such as *Providencia* sp. and *Klebsiella* sp., increase the relative content of β -caryophyllene in host plant VOCs, and result in deterring the oviposition of *Bactrocera dorsalis* (Li et al. 2020). Interestingly, a recent study shows that *Lymantria dispar* L. are attracted to volatiles from rust spores (*Melampsora laricipopulina*) (Eberl et al. 2018), suggesting that VOCs emitted by both host plants and the microbes themselves are important for establishing tripartite interactions among herbivorous insects, host plants, and plant-associated microbes (Tasin et al. 2012; Fernandez-Conradi et al. 2018). Our previous results also found that mated YPM females preferred to *Penicillium* fungi-infected apples, and the components and proportions of apples VOCs were changed by *Penicillium* fungi-infection (Shi et al. 2019; Guo et al. 2022), triggering us to explore which components of VOCs from *Penicillium*-infected apples or *Penicillium* itself were crucial for mediating the foraging preference of YPM females to *Penicillium*-infected apples.

The YPM is a generalist herbivorous insect feeding on a broad range of host plants, such as apples, corns, and so on (Li et al. 2015). However, the current strategies of interrupting their normal mating with sex pheromones are useless for mated YPM females. Other strategies, including plant-derived attractants and/or repellents as allelochemicals that selectively manipulate the behavior of YPM females, demand for the integrated pest management of YPMs (Xiao et al. 2012; Luo and Honda 2015a). Therefore, further understanding the principle of chemical ecology about the effects of plant-associated microbes on YPMs might be meaningful for developing attractants based on bioactive host plant VOCs to trap YPM females. Keeping the above in view, we determined the effect of *P. digitatum* on the VOCs of apples, and the cascading effects on the oviposition selection of mated YPM females. Our specific objectives were to determine (1) the oviposition selection and behavioral responses of YPM females among the fungi non-infected apples (NIA), mechanically damaged apples (MDA), *P. digitatum*-infected apples (PDA), and *P. digitatum* in potato dextrose agar medium (PPD); (2) the differences of VOCs from NIA, MDA, PDA, as well as PPD; (3) which VOCs were key components for affecting oviposition behavior of YPMs.

Material And Methods

Insects

A colony of YPMs was established and had been maintained for about 25 generations on maize in climate incubators (RTOP-B, Zhejiang Top Instrument Co., Ltd.) at $23 \pm 1^\circ\text{C}$, RH $75 \pm 2\%$, 16L/8D photoperiod, and 3,500 lux light intensity (Guo et al. 2021). Adult moths were provided with 5%–8% honey solution after emergence. Apples covered with gauze pieces were provided for the oviposition of mated YPM females in the cage.

Fungal culture

The *P. digitatum* isolated from orange fruits were purified according to the method described in Guo et al. 2022. PDA medium (7 mm diameter) with fully grown *P. digitatum* was also prepared and incubated at 30°C for 6 d before behavioral assays.

Apple treatments

Fungi non-infected apples (NIA): Apple (*Malus pumila*, Red fuji variety) fruits with uniform size (7–9 cm diameter) and shape were bought from the supermarket of Beijing University of Agriculture (Beijing, China) and were stored at 4°C in a refrigerator for further experiments.

Mechanically damaged apples (MDA): Apple fruits with uniform size were firstly sterilized using 75% alcohol for 1 min, and then 1% sodium hypochlorite for 3 min, at last washed with sterilized distilled water under the horizontal-laminar airflow clean bench. Two holes (7 mm diameter) at the opposite sides of each apple were punched and immediately stuffed using sterilized fungus-free potato dextrose agar medium.

***P. digitatum*-infected apples (PDA):** Apple fruits with uniform size were firstly sterilized using 75% alcohol for 1 min, and then 1% sodium hypochlorite for 3 min, at last washed with sterilized distilled water under the horizontal-laminar airflow clean bench. Two holes were punched as MDA, and then stuffed using potato dextrose agar medium with fully grown *P. digitatum*.

After treatments, each apple was placed into a sterilized plastic box ($25 \times 18 \times 12$ cm) and incubated at 30°C for 2, 4, 6, and 8 d before used for following behavioral assays. Considering the fact that the apples infected by *P. digitatum* for 8 d or longer would become rotten in the following days, the apples infected for 6 d were, therefore, used in the later experiments.

Oviposition behavioral experiments

To test the effects of *P. digitatum* on the oviposition behavior of mated YPM females, four treatments (NIA, MDA, PDA, and PPD) were simultaneously offered in a wood-frame cage ($35 \text{ cm} \times 27 \text{ cm} \times 25 \text{ cm}$) with plastic gauzes on side walls to allow the oviposition of mated YPM females according to the methods reported in Guo et al. (2022). Each experiment was replicated 20 times with a total number of 200 females. The egg numbers on each sheet were counted separately and the data were statistically treated on the basis of average number of eggs by 10 females.

Four-arm olfactometer experiments

A four-arm olfactometer was used to test behavioral responses of mated YPM females to the odors from NIA, MDA, PDA, and PPD according to the method described in Guo et al. (2022). The behavioral response was classified as a choice if the moth passed over 1/3 length of the arm associated with one of four odors and stayed there for more than 30 sec. Conversely, no-choice was assigned if the tested moth remained in the common arm for 3 min. A total of 211 individual moths were tested. The selection rate in the four-arm olfactometer experiment was defined as the number of females that made a selection for the odor divided by the total number of females that made a selection for any odors offered simultaneously.

VOCs collection and analysis

Five apples of each treatment (NIA, MDA, PDA) and correspondingly similar size of PPD that were placed into a $48.2 \text{ cm} \times 59.6 \text{ cm}$ oven bag respectively (Reynolds Kitchens, Richmond, VA, USA) were used to collect VOCs according to dynamic headspace collection method reported by Guo et al. (2022). After collection, the trapped volatiles were eluted using chromatography-grade *n*-hexane (99.9%) and then were analyzed using an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5975 Mass Spectrometer (MS). The procedures for the GC-MS analysis were the same as described in Du et al. (2016) with the exceptions that the GC was equipped with a DB-5MS column ($60 \text{ m} \times 0.25 \text{ mm} \times 0.15 \mu\text{m}$, Agilent, USA) rather than a HP-5MS column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) and the injector temperature was 250°C other than 210°C . Following injection, the column temperature was maintained at 37°C for 6 min, followed by an increase in temperature of $2^\circ\text{C}/\text{min}$ to 70°C for 5 min, and then an increase of $5^\circ\text{C}/\text{min}$ up to 200°C , at last maintained at 200°C for 5 min. Compounds were tentatively identified by comparing mass spectra with NIST Standard Reference Database 98 (Agilent Technologies, Palo Alto, CA, USA). Compounds were quantified by their total ion abundance relative to that of the internal standard (*n*-nonyl acetate).

Electroantennogram assays

Five specific VOCs from PDA, including methyl 2-methyl butyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane, were chosen for electroantennogram (EAG) measurements. Four concentrations (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} (v/v)) of five individual compounds were prepared. All chemicals (purities $\geq 95\%$) were purchased from commercial companies, which methyl 2-methyl butyrate and styrene were from J & K Chemical Ltd. (Shanghai, China), methyl caproate, butyl caprylate, and *n*-tetradecane from TCI Development Co., Ltd. (Shanghai, China). The test solutions were made by firstly dissolving 100 μL of compounds into 900 μL of laboratory-grade mineral oil, and then serially diluted with mineral oil to the desired concentrations. The test solutions were stored at -20°C for further analyses.

EAG recordings were performed on 3- to 4-day-old mated YPM females that the moths at this stage were eggs-loading and sensitive to signals used for oviposition location (Belmain et al. 2002). The method of EAG recordings was the same as that described by Du et al. (2016). Stimulus was delivered and

tested in increasing doses on the antennae of mated YPM females with mineral oil and *n*-hexanol being used as control and standard stimuli, respectively. EAG test was run for a variable number of replicates per day, and each compound at each concentration was tested on 15 antennae. In each test, the control and standard stimuli were applied subsequently after four successive stimulations. Normalization was achieved by dividing the peak EAG amplitude of the test puff with the average EAG amplitude of the two nearest standard stimulations after subtracting the amplitude recorded in response to the mineral oil.

Y-tube olfactometer experiments

The preference of mated YPM females to apples with or without five specific VOCs (methyl 2-methylbutyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane) from PDA was tested using Y-tube olfactometer. For Y-tube olfactometer assays, apples with and without five specific VOCs from PDA (the amount was referred to the concentrations in fungi-infected apple fruits (Table S1)) were separately placed into the chambers of the Y-tube. The test procedure was similar to that in our previous study (Guo et al. 2022). Each individual moth was used only once, and totally 80 mated female moths were tested for each treatment. The selection rate in the Y-tube olfactometer experiment was defined as the number of females that made a selection for apples with exogenous compounds divided by the total number of females that made a selection between apples with and without exogenous compounds.

Statistics analyses

Data obtained from oviposition selection experiments, behavioral assay in four-arm olfactometers, EAG tests, and the absolute content of host plant VOCs were subjected to analysis of variance (ANOVA) using Tukey-HSD test. The data of Y-tube olfactometer experiments were analyzed using non-parametric *Chi*-square analysis (Females with no choice were excluded from statistical analyses). Quantification of volatiles measured as the absolute content of each compound was analyzed using principal component analysis (PCA) using the software program SIMCA P+ 11.0 (Umetrics AB, Umeå, Sweden). All statistics except the PCA analysis were performed using the SPSS16.0 statistical software. Graphs were generated in the program of Graphpad Prism 9.0.

Results

Oviposition selection

In order to assess the effects of *P. digitatum* on the oviposition selection of mated YPM females, the apples infected with *P. digitatum* for 2, 4, 6, and 8 d were simultaneously provided to allow the oviposition selection of mated YPM females. The results showed that the number of eggs on the PDA (8 d) was significantly higher than those on the PDA (2 and 4 d), and was larger (but not significant) than that on the PDA (6d) ($F_{3,79} = 11.699, P < 0.01$; Fig. 1A). When NIA, MDA, PDA, and PPD were simultaneously offered in a cage to allow for oviposition, the average number of eggs laid by 10 mated YPM females (173.2) was significantly higher on PDA than those on MDA (22.3), NIA (22.2), and PPD (5.7) ($F_{3,79} = 111.413, P < 0.01$; Fig. 1B), respectively.

The behavioral responses of mated YPM females were tested in a four-arm olfactometer. The selection rate of mated YPM females to the odor of PDA (42.03%) was significantly higher than those to the odor of NIA (17.91%), the odor of MDA (24.17%), and the odor of PPD (15.89%) ($F_{3,19} = 18.948, P < 0.01$; Fig. 1C), suggesting that mated YPM females preferred to the odor of PDA.

VOCs profiles

In total, 38 volatile compounds were detected from the emissions of NIA, MDA, PDA, and PPD, including 24 compounds in NIA, 22 compounds in MDA, 24 compounds in PDA, and 1 compound in PPD (Fig. S1, Table 1). The results showed that α -farnesene was the most abundant compound in NIA, MDA, and PDA. Compared with MDA and NIA, five compounds, including methyl 2-methylbutyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane, were specifically detected in PDA. Only one compound, ethyl butyrate, was found in PPD (Table 1).

A principal component analysis (PCA) based on the absolute content of the 38 compounds was then performed to determine the major sources of variations in the four treatments (NIA, MDA, PDA, and PPD). The biplot depicted by graphical PCA expounded the first two principal components (PCs) with an explication of 70.61% of the total variance in the four treatments. The first component (PC1), which explained 50.68% of the total variance, was clearly isolated NIA. The second component (PC2) accounted for 19.93% of total variance (Fig. 2A). Further analyses indicated that the major loadings of PC1 were for *n*-butyl butyrate (-0.995), isoamyl 2-methylbutyrate (-0.995), hexyl hexanoate (-0.989), amyl hexanoate (-0.989) and major loadings of PC2 were for styrene (0.887), ethyl octanoate (0.887), α -farnesene (0.868), ethyl caproate (0.865). And then, PC scores were subjected to one-way ANOVA. The results showed that NIA was significantly higher than PDA and NIA in PC1 scores and PDA had the highest RF2 scores among four treatments (Fig. 2B and 2C).

EAG and behavioral responses of mated YPM females to volatile compounds

Five specific VOCs emitted from PDA, including methyl 2-methylbutyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane, were selected for EAG and behavioral tests using synthetic pure compounds. Five compounds all triggered significant EAG responses (with normalized EAG more than 0.5) of mated YPM females and the EAG responses showed a dose-dependent relationship with concentrations increasing from 10^{-4} to 10^{-1} (v/v) (Fig. 3). Furthermore, the behavioral responses indicated that the five individual compounds all showed the strongest attractiveness to the mated YPM females at 10^{-2} (v/v) (Fig. 4).

To address the preference of mated YPM females to fungi-infected apples, we further tested the behavioral response of mated YPM females to apples with and without *P. digitatum*-induced specific VOCs including individual and the mixed blend of five different compounds using Y-tube olfactometer (Fig. 5, Table S1). For individual compounds, mated YPM females preferred to apples with methyl 2-methylbutyrate (the selection rate of 66.25%, $\chi^2 = 8.45$), butyl caprylate (the selection rate of 61.25%, $\chi^2 = 4.05$), and *n*-tetradecane (the selection rate of 66.25%, $\chi^2 = 8.45$). However, mated YPM females had significant repellence to apples with styrene (the selection rate of 37.5%, $\chi^2 = 5.00$). For mixed compounds, mated YPM females had significant preference to apples with methyl 2-

methylbutyrate and butyl caprylate (the selection rate of 62.5%, $\chi^2 = 5.00$), methyl 2-methylbutyrate and *n*-tetradecane (the selection rate of 63.75%, $\chi^2 = 6.05$), butyl caprylate and *n*-tetradecane (the selection rate of 70%, $\chi^2 = 12.8$), styrene, methyl 2-methylbutyrate, and *n*-tetradecane (the selection rate of 62.5%, $\chi^2 = 5.00$), methyl 2-methylbutyrate, methyl caproate, and butyl caprylate (the selection rate of 67.5%, $\chi^2 = 9.80$), styrene, methyl 2-methylbutyrate, methyl caproate, and butyl caprylate (the selection rate of 61.25%, $\chi^2 = 4.05$), methyl 2-methylbutyrate, methyl caproate, butyl caprylate, and *n*-tetradecane (the selection rate of 61.25%, $\chi^2 = 4.05$), styrene, methyl 2-methylbutyrate, methyl caproate, butyl caprylate, and *n*-tetradecane (the selection rate of 63.75%, $\chi^2 = 6.05$).

Discussion

Plant-associated microbes and herbivorous insects often co-occur on the same host plant. It has indicated that plant-associated microbes have significantly cascading effects on host preference of herbivorous insects via affecting host plant VOCs (Fernandez-Conradi et al. 2018; Grunseich et al. 2019). In the present study, *P. digitatum* infection changed the VOCs profile of apple fruits, including five specific VOCs that were methyl 2-methylbutyrate, styrene, methyl caproate, butyl caprylate, and *n*-tetradecane, and consequently attracted the oviposition of YPM females. These findings implied that the roles of plant-associated microbes should be taken into account in the interactions between YPMs and host plants.

Plant associated microbes could alter the oviposition and foraging behaviors of subsequent herbivorous insects. However, there are no uniform effects of plant-associated microbes on insects' behavior. For example, *Botrytis cinerea* has an avoidance response of *Lobesia botrana* for laying eggs on the grape plants (Tasin et al. 2012). On the contrary, YPM females laid more eggs on PDA than on NIA and MDA, as well as than on PPD in the current study. Furthermore, the number of eggs laid by YPM females increased along *P. digitatum* infection time. The positive effects of plant-associated microbes on the preference of herbivorous insects for host plants have also been reported in other studies (Cardoza et al. 2002; 2003), confirming the important roles of plant-associated microbes in the host selection of herbivorous insects. Moreover, a meta-analysis of 1113 case studies gathered from 101 primary papers suggests that the concept of tripartite interactions among host plants, plant-associated microbes, and herbivorous insects is dependent on microbes lifestyle (biotrophic or necrotrophic pathogens), herbivorous insects feeding guild (sap-sucking or chewing insects), and the spatial scale of the interaction (local or distant) (Fernandez-Conradi et al. 2018), suggesting that a quantitative estimate of the overall effects of *P. digitatum* on the selection and oviposition of YPMs remains to be comprehensively explored in the further experiments.

Chemical communication is an ancient and ubiquitous channel to mediate species interactions, and host plant VOCs is defined as olfactory cues in host location, recognition and selection of herbivorous insects. As same in YPMs, the YPM females were attracted to and laid eggs on artificial substrates that released host plant odors (Luo and Honda 2015a, b; Du et al. 2016). In the present study, the VOCs profile of PDA was significantly different from NIA, MDA and PPD. Furthermore, mated YPM females had an obvious preference for PDA odors to NIA, MDA, and PPD odors in the four-arm olfactometer, which was not only in line with the oviposition preference of YPM females for PDA, but also further indicated the potential role of host plant VOCs for the preference of YPM females. Moreover, our experiments revealed a clear separation between VOC profiles of PDA (including five *P. digitatum*-induced specific VOCs) versus MDA or NIA via PCA analysis, implying that these specific VOCs could be served as signals for the oviposition and foraging behaviors of YPMs to PDA. These results are agree with recent studies that host plant VOCs could be frequently altered by plant-associated microbes' infection and consequently have impacts on the host selection of herbivorous insects (Groen et al. 2016; Rizvi et al. 2016; Grunseich et al. 2019). Thus, it is not uncommon for herbivorous insects to employ fungi-induced kairomones for host location.

Some certain specific VOCs are emerged as attractants in the oviposition and foraging behavior of herbivorous insects (Turlings and Erb 2018). For example, styrene is reported to be a spoilage marker of decayed apples after infection by *Penicillium expansum* and elicits strong electrophysiological antennal activity for *Ips typographus* at very low levels (Kim et al. 2018, 2019; Schiebe et al. 2019). As in our study, styrene was specifically emitted in a relatively ample amount from PDA and triggered significant EAG responses. However, mated YPM females showed obvious repellence to the styrene-supplemented apples in Y-tube olfactometer assay, which is the same as a previous study that styrene significantly reduces pine weevils' attraction to cut pieces of Scots pine twigs (Azeem et al. 2013). Indeed, YPM females exhibited significant preference to apples with three other *P. digitatum*-induced specific VOCs, including methyl 2-methylbutyrate, butyl caprylate, and *n*-tetradecane, individually or together, suggesting that it was the mixed blends, but not one specific VOCs, that served as olfactory cues for the host orientation and oviposition selection of YPM females to PDA. This is consistent with the evidence that changes in the overall composition and relative ratios of the host plant VOCs make plants co-infested by *Nilaparvata lugens* (Stål) and *Chilo suppressalis* (Walker) unattractive to *Anagrus nilaparvatae* females (Hu et al. 2020). One possible explanation is that, compared with individual components, the quantitative as well as qualitative differences in the blend of plant VOCs have a significant effect on herbivorous insects' behaviors (Bruce and Pickett 2011). Collectively, these *P. digitatum* induced specific VOCs functioned together as olfactory cues for the interactions between YPM females and apples.

In summary, a battery of experiments were carried out to gain further insight into the hypothetical roles of the plant-associated microbes (*P. digitatum*) in mediating the host plant location and oviposition selection of YPMs via host plant VOCs. Current study found that YPM females preferred to *P. digitatum*-infected apples for oviposition. Odors from *P. digitatum*-infected apples were responsible for the attractiveness of YPM females as demonstrated in the four-arm olfactometer assay. Furthermore, three of five *P. digitatum*-induced specific VOCs, including methyl 2-methylbutyrate, butyl caprylate, and *n*-tetradecane, might serve as key olfactory cues for YPM females to *P. digitatum*-infected apples via PCA analysis and Y-tube olfactometer assay. These findings shed light on the underlying mechanisms of the attraction of YPMs by *P. digitatum*-infected apples, and might form the basis for the development of attractant formulations for field trapping YPM moths. In the future, field experiments will be carried out to prove the semiochemical roles of individuals as well as blends of *P. digitatum*-infected apple volatiles in attracting YPMs.

Declarations

Author Contributions

DYL, YZG, and GHG conceived the idea, designed the experiments, and directed the implementation. APP and MSZ performed the experiments. MSZ, APP, GHG, and DYL conducted statistical analyses. ZMZ contributed to the conception and design of the study. YZ prepared the materials and lab facilities necessary for this work. GHG and DYL wrote the first draft of the manuscript, and YZG contributed to the subsequent manuscript development. All authors read and approved the final manuscript.

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Conflict of interest All authors declare that there are no conflicts of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present investigation.

Informed consent All authors of this manuscript accepted that the paper is submitted for publication in the Journal of Pest Science, and reported that this paper has not been published or accepted for publication in another journal, and it is not under consideration by another journal.

Data Availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

References

1. Azeem M, Rajarao GK, Nordenhem H, Nordlander G, Borg-Karlson AK (2013) *Penicillium expansum* volatiles reduce pine weevil attraction to host plants. *J Chem Ecol* 39(1):120–128. <https://doi.org/10.1007/s10886-012-0232-5>
2. Belmain SR, Simmonds MS, Blaney WM (2002) Influence of odor from wood-decaying fungi on host selection behavior of deathwatch beetle, *Xestobium rufovillosum*. *J Chem Ecol* 28(4):741–754. <https://doi.org/10.1023/a:1015284625697>
3. Bruce TJ, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects-finding the right mix. *Phytochemistry* 72(13):1605–11. <https://doi.org/10.1016/j.phytochem.2011.04.011>
4. Cardoza YJ, Alborn HT, Tumlinson JH (2002) In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. *J Chem Ecol* 28(1):161–174. <https://doi.org/10.1023/a:1013523104853>
5. Cardoza YJ, Lait CG, Schmelz EA, Huang J, Tumlinson JH (2003) Fungus induced biochemical changes in peanut plants and their effect on development of beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae. *Environ Entomol* 32:220–228. [https://doi.org/10.1043/0046-225X\(2003\)032\(0220:FIBICIP\)2.0.CO](https://doi.org/10.1043/0046-225X(2003)032(0220:FIBICIP)2.0.CO)
6. Du YL, Zhang JX, Yan ZG, Ma YQ, Yang MM, Zhang ZY, Zhang MZ, Qin L, Cao QQ (2016) Host preference and performance of the yellow peach moth (*Conogethes punctiferalis*) on chestnut cultivars. *PLoS One* 11(6):e0157609. <https://doi.org/10.1371/journal.pone.0157609>
7. Eberl F, Bobadilla MFD, Reichelt M, Hammerbacher A, Unsicker SB (2020) Herbivory meets fungivory: insect herbivores feed on plant pathogenic fungi for their own benefit. *Ecol Lett* 23(7):1073–1084. <https://doi.org/10.1111/ele.13506>
8. Eberl F, Hammerbacher A, Gershenson J, Unsicker SB (2018) Leaf rust infection reduces herbivore-induced volatile emission in black poplar and attracts a generalist herbivore. *New Phytol* 220(3):760–772. <https://doi.org/10.1111/nph.14565>
9. Eberl F, Uhe C, Unsicker SB (2019) Friend or foe? The role of leaf-inhabiting fungal pathogens and endophytes in tree-insect interactions. *Fungal Ecol* 38:104–112. <https://doi.org/10.1016/j.funeco.2018.04.003>
10. Fernandez-Conradi P, Jactel H, Robin C, Tack AJM, Castagneyrol B (2018) Fungi reduce preference and performance of insect herbivores on challenged plants. *Ecol* 99(2):300–311. <https://doi.org/10.1002/ecy.2044>
11. Groen SC, Jiang S, Murphy AM, Cunniffe NJ, Westwood JH, Davey MP (2016) Virus infection of plants alters pollinator preference: A payback for susceptible hosts? *PLoS Pathog* 12(8):e1005790. <https://doi.org/10.1371/journal.ppat.1005790>
12. Grunseich JM, Thompson MN, Aguirre NM, Helms AM (2019) The role of plant-associated microbes in mediating host-plant selection by insect herbivores. *Plants (Basel, Switzerland)* 9(1):6. <https://doi.org/10.3390/plants9010006>
13. Guo H, Meng L, Zhang M, Ren Z, Qin X, Du Y. (2021) Binding specificity of OBPs in the yellow peach moth *Conogethes punctiferalis* (Guenée). *J Appl Entomol* 145(10):1001–1014. <https://doi.org/10.1111/jen.12922>
14. Guo HG, Han CY, Zhang AH, Yang AZ, Qin XC, Zhang MZ, Du YL (2022) *Penicillium* fungi mediate behavioral responses of the yellow peach moth, *Conogethes punctiferalis* (Guenée) to apple fruits via altering the emissions of host plant VOCs. *Arch Insect Biochem Physiol* e21895. <https://doi.org/10.1002/arch.21895> Epub ahead of print.
15. Hu X, Su S, Liu Q, Jiao Y, Peng Y, Li Y, Turlings TC (2020) Caterpillar-induced rice volatiles provide enemy-free space for the offspring of the brown planthopper. *elife* 9:e55421. <https://doi.org/10.7554/eLife.55421>
16. Kim HW, Lee SM, Seo JA, Kim YS (2019) Effects of pH and cultivation time on the formation of styrene and volatile compounds by *Penicillium expansum*. *Molecules* 24(7):1333. <https://doi.org/10.3390/molecules24071333>
17. Kim SM, Lee SM, Seo J, Kim Y (2018) Changes in volatile compounds emitted by fungal pathogen spoilage of apples during decay. *Postharvest Biol Technol* 146:51–59. <https://doi.org/10.1016/j.postharvbio.2018.08.003>

18. Kopper BJ, Klepzig BJ, Raffa KF (2004) Components of antagonism and mutualism in *Ips pini*-fungal interactions: Relationship to a life history of colonizing highly stressed and dead trees. *Environ Entomol* 33(1):28–34. <https://doi.org/10.1603/0046-225X-33.1.28>
19. Li DY, Ai PP, Du YL, Sun SL, Zhang MZ (2015) Effects of different host plants on the development and reproduction of yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Crambidae). *Austral Entomol* 54(2):149–153. <https://doi.org/10.1111/aen.12105>
20. Li HJ, Ren L, Xie MX, Gao Y, He MY, Hassan B, Lu YY, Cheng DF (2020) Egg-surface bacteria are indirectly associated with oviposition aversion in *Bactrocera dorsalis*. *Curr Biol* 30(22):4432–4440. <https://doi.org/10.1016/j.cub.2020.08.080>
21. Luo ZX, Honda H (2015a) Olfactory and biophysical assessment of the oviposition stimulating potential of host and non-host plants for the yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Crambidae). *Appl Entomol Zool* 50(2):183–189. <https://doi.org/10.1007/s13355-014-0320-9>
22. Luo ZX, Honda H (2015b) Function of plant odors in oviposition behaviors of the yellow peach moth *Conogethes punctiferalis* (Lepidoptera: Crambidae). *Appl Entomol Zool* 50(3):347–353. <https://doi.org/10.1007/s13355-015-0341-z>
23. Rizvi SZ, Raman A, Wheatley WM, Cook G (2016) Oviposition preference and larval performance of *Epiphyas postvittana* (Lepidoptera: Tortricidae) on *Botrytis cinerea* (Helotiales: Sclerotiniaceae) infected berries of *Vitis vinifera* (Vitales: Vitaceae). *Insect Sci* 23(2):313–25. <https://doi.org/10.1111/1744-7917.12191>
24. Schiebe C, Unelius CR, Ganji S, Binyameen M, Birgersson G, Schlyter F (2019) Styrene, (+)-trans-(1R,4S,5S)-4-thujanol and oxygenated monoterpenes related to host stress elicit strong electrophysiological responses in the bark beetle *Ips typographus*. *J Chem Ecol* 45(5–6):474–489. <https://doi.org/10.1007/s10886-019-01070-8>
25. Tasin M, Knudsen GK, Pertot I (2012) Smelling a diseased host: Grapevine moth responses to healthy and fungus-infected grapes. *Anim Behav* 83(2):555–562. <https://doi.org/10.1016/j.anbehav.2011.12.003>
26. Tack AJM, Dicke M (2013) Plant pathogens structure arthropod communities across multiple spatial and temporal scales. *Funct Ecol* 27:633–645. <https://doi.org/10.1111/1365-2435.12087>
27. Turlings TCJ, Erb M (2018) Tritrophic Interactions Mediated by Herbivore-Induced Plant Volatiles: Mechanisms, Ecological Relevance, and Application Potential. *Annu Rev Entomol* 63:433–452. <https://doi.org/10.1146/annurev-ento-020117-043507>
28. Witzgall P, Proffitt M, Rozpedowska E, Becher PG, Andreadis S, Coracini M, Lindblom TU, Ream LJ, Hagman A, Bengtsson M, Kurtzman CP, Piskur J, Knight A (2012) “This is not an apple”-yeast mutualism in codling moth. *J Chem Ecol* 38(8):949–957. <https://doi.org/10.1007/s10886-012-0158-y>
29. Xiao W, Matsuyama S, Ando T, Millar JG, Honda H (2012) Unsaturated cuticular hydrocarbons synergize responses to sex attractant pheromone in the yellow peach moth, *Conogethes punctiferalis*. *J Chem Ecol* 38(9):1143–1150. <https://doi.org/10.1007/s10886-012-0176-9>

Tables

Table 1: VOCs were identified^a in headspace collections from four treatments^b.

No.	Compounds	CAS	Retention time (min)	Relative content (%) ^c				Absolute content (µg/µL) ^d		
				NIA	MDA	PDA	PPD	NIA	MDA	PDA
1	Ethyl propionate	105-37-3	4.36	—	2.63±1.17 ³	—	—	—	2.75±1.36	—
2	<i>n</i> -Propyl acetate	109-60-4	4.43	—	0.36 ¹	—	—	—	0.39	—
3	2-Methyl-1-butanol	137-32-6	5.10	—	0.94±0.27 ²	0.43±0.11 ³	—	—	0.74±0.01	0.58±0
4	Methyl 2-methylbutyrate	868-57-5	6.34	—	—	0.39±0.10 ⁴	—	—	—	0.56±0
5	Ethyl butyrate	105-54-4	7.56	—	9.57±2.19 ³	2.68±0.22 ⁵	100.00±4.17 ³	—	10.58±3.95 ^b	3.94±0
6	<i>n</i> -Propyl propionate	106-36-5	7.98	—	0.35±0.05 ²	—	—	—	0.42±0.02	—
7	<i>n</i> -Butyl acetate	123-86-4	8.20	2.63±0.31 ³	3.18±0.47 ³	1.01±0.24 ⁵	—	5.08±1.05 ^b	3.02±0.25 ^b	1.47±0
8	Ethyl-2-methylbutyrate	7452-79-1	10.27	—	21.44±2.45 ³	13.86±0.55 ⁵	—	—	21.87±5.08	21.28±
9	2-Methylbutyl acetate	624-41-9	12.21	9.34±0.65 ³	11.53±1.75 ³	5.17±0.64 ⁵	—	17.74±2.14 ^b	11.01±1.19 ^b	7.78±1
10	Styrene	100-42-5	12.58	—	—	2.08±0.55 ⁵	—	—	—	3.32±1
11	<i>n</i> -Propyl butyrate	105-66-8	13.37	0.66±0.11 ³	0.32±0.01 ²	—	—	1.29±0.32	0.39±0.03	—
12	Butyl propionate	590-01-2	14.12	1.07±0.27 ³	0.36±0.01 ²	—	—	2.11±0.66	0.43±0.05	—
13	Amyl acetate	628-63-7	14.48	0.47±0.03 ²	0.65±0.01 ²	0.35 ¹	—	0.95±0.14	0.79±0.08	0.64
14	Methyl caproate	106-70-7	15.16	—	—	0.44±0.03 ²	—	—	—	0.81±0
15	Ethyl tiglate	5837-78-5	16.18	—	0.42 ¹	—	—	—	0.46	—
16	Propyl 2-methylbutyrate	37064-20-3	16.72	0.68±0.07 ³	1.23±0.36 ³	0.96±0.13 ⁵	—	1.30±0.21 ^a	1.11±0.09 ^a	1.49±0
17	<i>n</i> -Butyl butyrate	109-21-7	20.62	2.89±0.43 ³	—	—	—	5.59±1.21	—	—
18	Ethyl caproate	123-66-0	20.79	0.35±0.06 ²	4.32±0.94 ³	5.74±0.70 ⁵	—	0.60±0.10 ^a	4.75±2.90 ^b	8.94±1
19	Hexyl acetate	142-92-7	21.91	4.21±0.61 ³	3.71±0.26 ³	2.88±0.19 ⁵	—	8.15±1.82 ^a	3.85±1.00 ^a	4.28±0
20	Butyl 2-methylbutyrate	15706-73-7	24.09	2.75±0.45 ³	1.16±0.34 ³	0.97±0.11 ⁵	—	5.30±1.11 ^b	1.05±0.12 ^a	1.41±0
21	2-Methylbutyl butyrate	51115-64-1	25.60	0.53±0.02 ²	—	—	—	1.07±0.13	—	—
22	Propyl caproate	626-77-7	29.22	1.58±0.04 ³	—	0.48±0.04 ⁴	—	2.99±0.25	—	0.81±0
23	2-Methylbutyl 2-Methylbutyrate	2445-75-8	29.81	0.75±0.09 ³	—	0.55±0.04 ²	—	1.44±0.24	—	0.97±0
24	Hexyl propionate	2445-76-3	30.06	0.80±0.20 ³	—	—	—	1.58±0.50	—	—
25	Isoamyl 2-methylbutyrate	27625-35-0	32.14	0.37±0.05 ³	—	—	—	0.71±0.13	—	—
26	Hexyl isobutyrate	2349-07-7	32.73	0.27±0.03 ²	—	—	—	0.53±0.00	—	—
27	Butyl hexanoate	626-82-4	35.09	11.57±0.34 ³	0.96±0.12 ³	1.42±0.22 ⁵	—	22.00±2.51 ^b	0.94±0.20 ^a	1.99±0

28	Ethyl octanoate	106-32-1	35.16	—	0.82±0.17 ³	1.63±0.36 ⁵	—	0.88±0.33	2.86±0	
29	Hexyl 2-methylbutyrate	10032-15-2	36.88	11.91±0.95 ³	4.34±0.81 ³	6.09±1.10 ⁵	—	22.74±3.34 ^b	4.12±0.55 ^a	9.00±2
30	2-Methylbutyl hexanoate	2601-13-0	37.40	1.50±0.06 ³	—	0.39±0.04 ³	—	2.83±0.21	—	0.57±0
31	Amyl hexanoate	540-07-8	38.68	1.07±0.05 ³	—	—	—	2.03±0.26	—	—
32	Propyl octanoate	624-13-5	38.79	0.28±0.03 ³	—	—	—	0.52±0.03	—	—
33	Ethyl trans-4-decenoate	76649-16-6	41.51	—	0.32 ¹	0.74±0.06 ⁵	—	—	0.42	0.97±0
34	Hexyl hexanoate	6378-65-0	41.78	6.35±0.34 ³	0.38±0.07 ²	0.79±0.20 ⁵	—	12.12±1.67 ^b	0.47±0.13 ^a	1.31±0
35	Butyl caprylate	589-75-3	41.78	—	—	0.27±0.03 ²	—	—	—	0.42±0
36	<i>n</i> -Tetradecane	629-59-4	42.16	—	—	0.24±0.04 ³	—	—	—	0.40±0
37	Isoamyl caprylate	2035-99-6	43.49	0.36±0.01 ³	—	—	—	0.69±0.07	—	—
38	α -Farnesene	502-61-4	45.20	36.55±5.01 ³	30.57±2.52 ³	49.24±1.02 ⁵	—	67.88±5.40 ^a	30.57±6.36 ^a	74.25±

^a Identity of compounds was confirmed by comparison of mass spectrum and retention time with those of the respective synthetic standards.

^b NIA: fungi non-infected apples; MDA: mechanically damaged apples; PDA: *P. digitatum*-infected apples; PPD: *P. digitatum* in potato dextrose agar medium.

^c Values are mean ± SE ($n = 2\sim 5$). The superscript numbers of mean values indicated the times that the compound was identified in five repetitions of the same treatment.

^d Values are mean ± SE ($n = 2\sim 5$). Different letters within the same row indicated the significant difference among four treatments ($P < 0.05$).

Figures

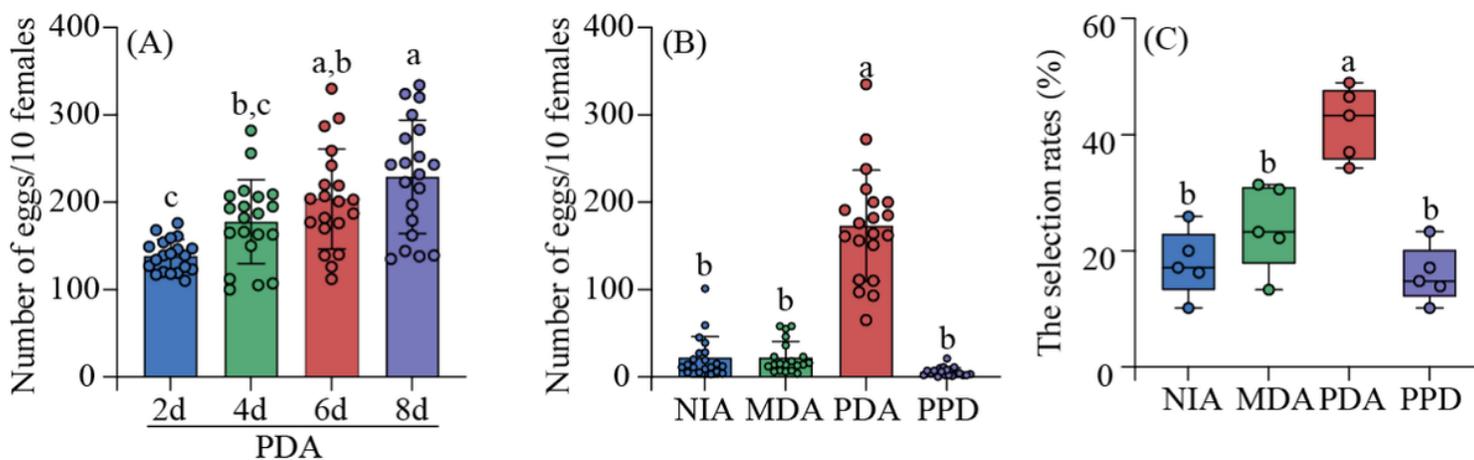


Figure 1

The selection behavior of mated YPM females. (A) the average egg numbers of mated YPM females on *P. digitatum*-infected apples among different infection time (2, 4, 6, and 8 d, respectively). (B) the average egg numbers of mated YPM females among PPD, NIA, MDA, and PDA. (C) the selection rates of four-arm olfactometer experiments among the odors from PPD, NIA, MDA, and PDA. Different letters indicated significant difference among different treatments (Tukey-HSD test after ANOVA, $P < 0.05$). Fungi non-infected apples (NIA), Mechanically damaged apples (MDA), *P. digitatum*-infected apples (PDA), and *P. digitatum* in potato dextrose agar medium (PPD).

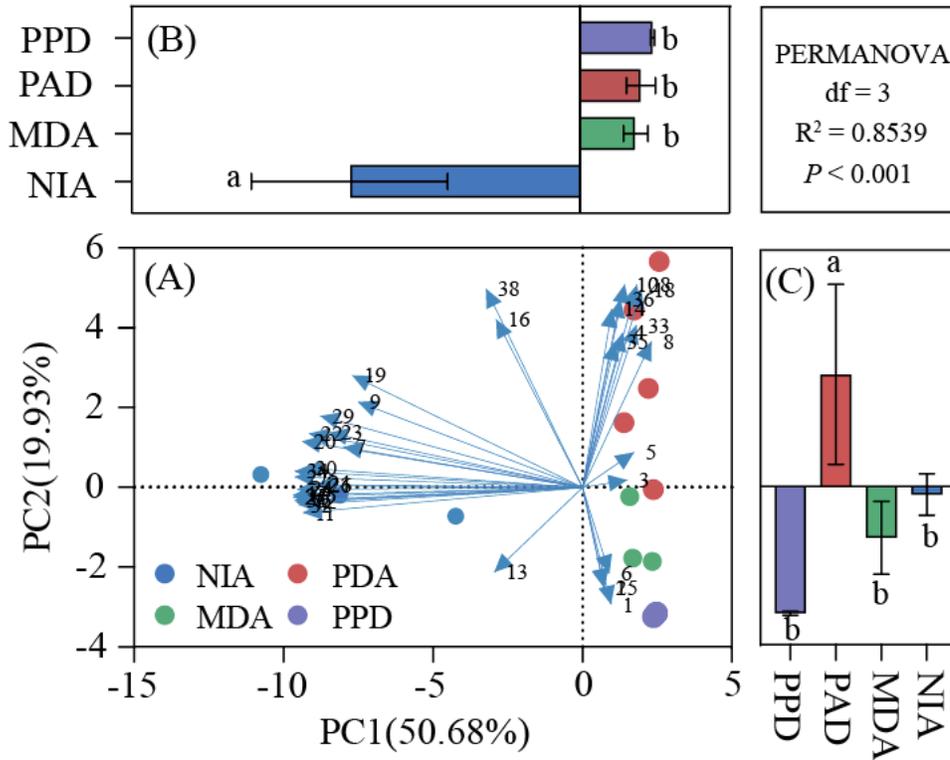


Figure 2
Principal component analysis based on 38 compounds which were obtained from NIA, MDA, PDA, and PPD. (A) The percentage of total variance explained by the first two principal components (PCs). (B) The overall distribution of PC1 scores within four treatments. (C) The overall distribution of PC2 scores within four treatments. Different letters indicated significant difference among four treatments (Tukey-HSD test after ANOVA, $P < 0.05$). The numbers in the graph were the same as those in Table 1. Fungi non-infected apples (NIA), Mechanically damaged apples (MDA), *P. digitatum*-infected apples (PDA), and *P. digitatum* in potato dextrose agar medium (PPD).

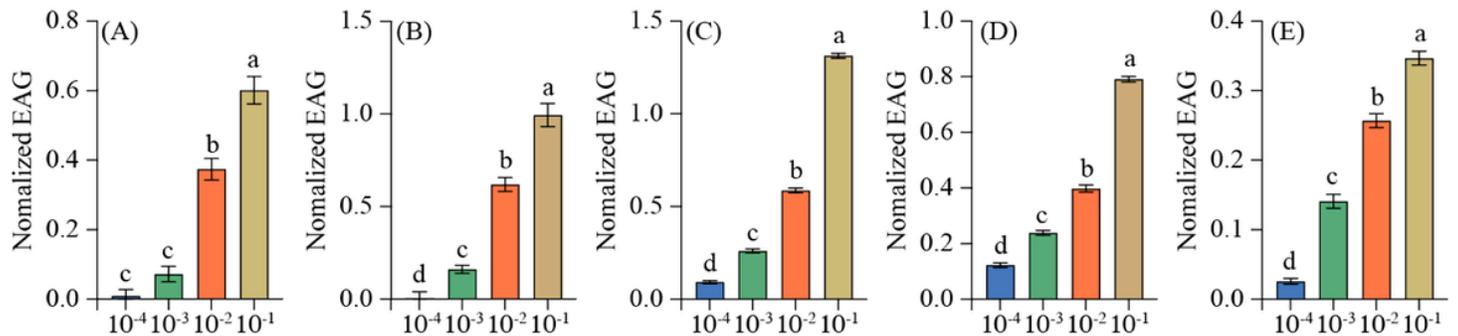


Figure 3
EAG responses of mated YPM females to five individual compounds. Bars represented mean \pm SE ($n = 15$). Different letters on the bars indicated significant difference among the four concentrations of the same compounds (Tukey-HSD test after ANOVA, $P < 0.05$). (A) styrene, (B) methyl 2-methylbutyrate, (C) methyl caproate, (D) butyl caprylate, (E) *n*-tetradecane.

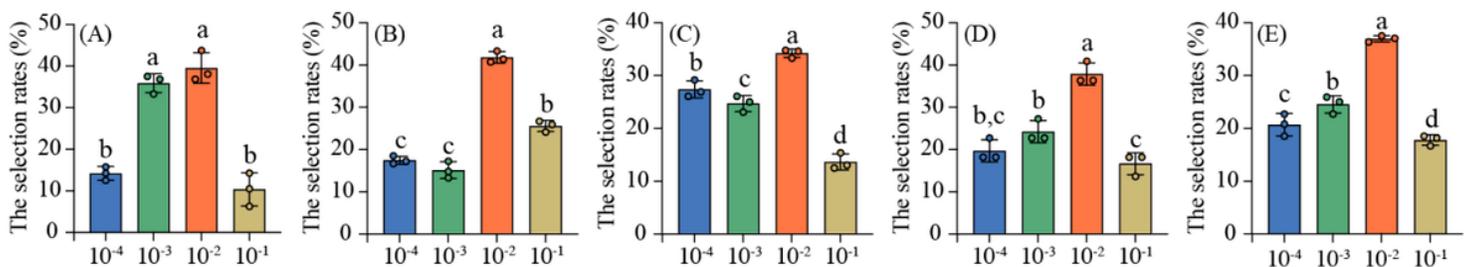


Figure 4

The selection rates of mated YPM females among the four concentrations (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} (v/v)) of the same compounds in four-arm olfactometer. Different letters on the bars indicated significant difference among the four concentrations of the same compounds (Tukey-HSD test after ANOVA, $P < 0.05$). (A) styrene, (B) methyl 2-methylbutyrate, (C) methyl caproate, (D) butyl caprylate, (E) *n*-tetradecane.

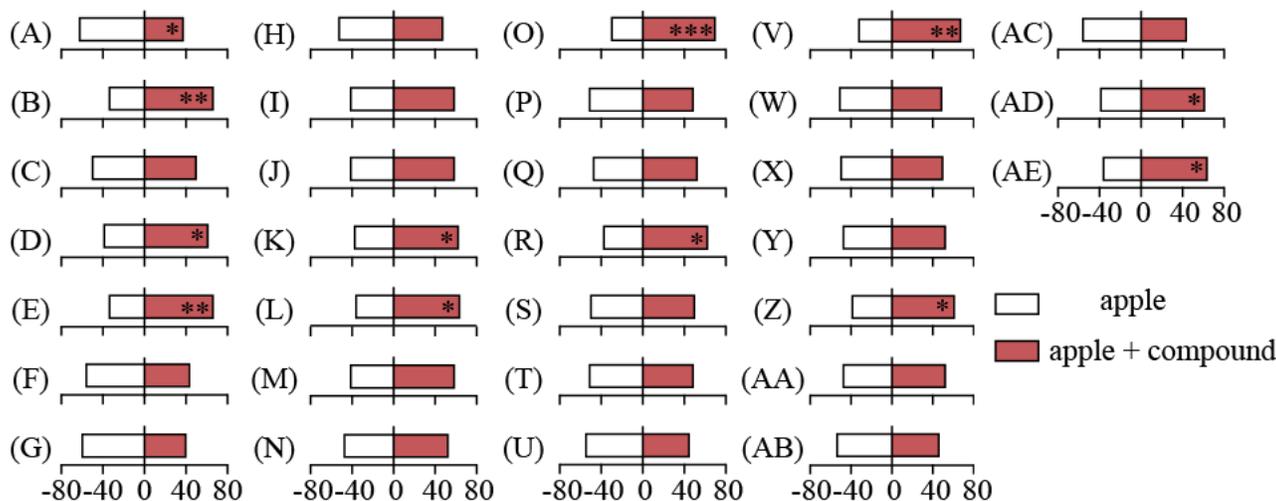


Figure 5

Behavioral responses of mated YPM females to apples with and without compounds in Y-tube olfactometer. The bars represented the percentage of the responding mated YPM females to apples with or without compounds. Stars indicated significant difference in Y-tube olfactometer assays using χ^2 test ($*P < 0.05$, $**P < 0.01$). (A) individual compound (1), (B) individual compound (2), (C) individual compound (3), (D) individual compound (4); (E) individual compound (5); (F) mixed compounds (1+5); (G) mixed compounds (1+2); (H) mixed compounds (1+4); (J) mixed compounds (1+3); (K) mixed compounds (2+3); (L) mixed compounds (2+4); (M) mixed compounds (2+5); (N) mixed compounds (3+4); (O) mixed compounds (3+5); (P) mixed compounds (4+5); (Q) mixed compounds (1+2+3); (R) mixed compounds (1+2+4); (S) mixed compounds (1+2+5); (T) mixed compounds (1+3+4); (U) mixed compounds (1+3+5); (V) mixed compounds (1+4+5); (W) mixed compounds (2+3+4); (X) mixed compounds (2+3+5); (Y) mixed compounds (2+4+5); (Z) mixed compounds (3+4+5); (AA) mixed compounds (1+2+3+4); (AB) mixed compounds (1+2+3+5); (AC) mixed compounds (1+2+4+5); (AD) mixed compounds (1+3+4+5); (AE) mixed compounds (2+3+4+5); (AE) mixed compounds (1+2+3+4+5). Compound (1): styrene; Compound (2): methyl 2-methylbutyrate; Compound (3): methyl caproate; Compound (4): butyl caprylate; Compound (5): *n*-tetradecane.

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