Screening and Identification of two repellent active volatiles to Hyphantria cunea

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Abstract

The repellent tree species or their volatiles of *Hyphantria cunea* can be used for "push and pull strategy" integrated control to improve the capture to *H. cunea* in the wild. The response of *H. cunea* to repellent tree species and volatiles was determined using insect electrophysiological technique and Y-tube olfactometer test. *H. cunea* 5th, 6th instar larvae and virgin females were significantly more repulsive to the branches of *Larix gmelinii* and *Syringa oblata* than to blank control (P<0.05). Two mutual antennal active volatile compounds (α-pinene and (+)-limonene) from the branches of two tree species were identified by gas chromatography-electroantennographic detection/mass spectrometry (GC-EAD/MS) and electroantennography (EAG) responses of *H. cunea* females and males existed significant dose-dependent relationship (P<0.05). The highest EAG response value was induced at the highest concentration (100 μL/mL). In addition, 2 volatile compounds (100 μL/mL) had significant repellent effects on *H. cunea* 4-6th instar larvae and adults (P<0.05), and could notably inhibit the host (*Salix matsudana*) selection of 5th and 6th instar larvae and both sex adults (P<0.05). In conclusion, α-pinene and (+)-limonene as potential candidates can repellent *H. cunea* 4-6th larvae or adults, and play an important role in effective green prevention and control.

Introduction

Hyphantria cunea (Drury) (Lepidoptera: Erebidae), as a worldwide quarantine pest insect, has a wide range of distribution and reproductive solid ability, which has more than 630 host plants in the world, including mulberry, elm, poplar and other broad-leaved tree species, along with the serious impact on social and economic development every year (Sllivan and Ozman-Sullivan 2012). According to the difference of photoperiod and temperature, one or two generations occur yearly in China with the overlap of generations seriously (Tadashi 2007). The adults of *H. cunea* were nocturnal, often laying eggs on the back of the host leaves, and flying on the wind thousands of meters away (Luo et al. 2018). In the larval stage, there are 6–7 stages, and the younger larvae (1–4 stages) cluster in the net to feed on the leaves of host plants, and disperse to harm different hosts after the 4th stage, which the natural crawling distance is about 500 m owning the strong diffusion ability. Then, the old larvae habitually pupate under trees (Chen 2018). *H. cunea* is widely distributed in the N19°-55° area, which has strong adaptability (Qiu et al. 2022). Due to the high risk of large-scale occurrence in China, effective prediction and management of *H. cunea* has become an important forestry control work.

In the last decade of studies, chemical pesticides are often sprayed for pest control in the pest outbreak period, but pest resistance undermines the development of chemical pest control. Therefore, efficient natural alternatives and environmentally friendly pest control methods are desperately needed to reduce the risk of chemical pesticides poisoning the environment and non-target species (Mohammed et al. 2021). Insect repellents based on plant volatiles have become a good choice and play an important role, which are widely used to interrupt the behavior of some insects seeking hosts in recent years (Jia et al. 2022). Repellents are mostly used to repel mosquitoes or control stored grain pests and other agricultural insects (Zeng et al. 2022). For example, DEET can interfere with an insect's olfactory system,

thereby preventing it from recognizing the host's scent (Feng et al. 2014). Repellent compounds often stem from insect repellent plants, such as *Juglans regia*, *Cyperus articulates* and *Caulerpa scalpelliformi* serve as repellent plants to *Aedes albopictus* (Wang et al. 2022), *Tribolium casteneum* (Abubakar et al. 2000) and *Spodoptera litura* (Sahayaraj et al. 2022), respectively, which are considered to be an essential source for screening repellent active volatiles. In addition, exploiting insect oviposition inhibitors based on repellent compounds (Li et al. 2023), the combination of repellent active substances and selected pesticide auxiliaries (Li 2021), all provide new ideas for effective pest management.

Studying pests' olfactory and behavioral responses and assessing plant-insect interactions are critical to developing management strategies. Up to now, many *H. cunea* attractant ingredients can be screened and developed, namely *trans*-2-hexenyl acetate, nonanal, hexanal, *trans*-2-hexenal, hexanol, isoamyl acetate, acetic acid cis-3-hexenyl ester, acetophenone and *trans*-2-hexen-1-ol are all potential attractant ingredients (Tang et al. 2012a). At the same time, dibutyl phthalate and phytol can stimulate the mating and oviposition of female *H. cunea* (Peng et al. 2020). While sex pheromone for male moths have been identified and used, which (3Z,6Z)9,10-epoxyheneicosadiene, (9Z,12Z,15Z)-octadecatrienal, and (3Z,6Z)9,10-epoxyheneicosa-triene in a 1:10:1 ratio has remarkable trapping activity for *H. cunea* at a higher cost (Zhang and Schlyter 1996), thus, the practical application of attractant compounds still faces many environmental challenges. Also, applying repellent tree species or repellent compounds can further improve the control effect of *H. cunea* in forests, which is of great significance for the rational use of "push and pull strategy" to control *H. cunea*.

Building on the preliminary findings, the feeding rate of *H. cunea* 4th instar larvae to *Larix gmelinii* and *Syringa oblata* was significantly lower than that of *Salix matsudana*, *Tilia amurensis* and other preferred tree species (Lv et al. 2023), and virgin female adults had obvious rejection behavior to *S. oblata*. According to the habit of *H. cunea* to break the net and spread after 4th instar larvae, the behavioral responses of the 4th to 6th instar larvae and virgin adults were further measured to the fresh branches and leaves of the two tree species. Meanwhile, the volatile odors from *L. gmelinii* and *S. oblata* were identified by gas chromatography-electroantennographic detection (GC-EAD) and Gas chromatography-mass spectrometry (GC-MS), which caused the antennae electrophysiological response of *H. cunea* adults. The sensitivity of *H. cunea* to two common active volatiles was tested, including electroantennographic (EAG) of adults, behavioral selection of 4-6th instar larvae and virgin adults, to determine the repellent ability of these two compounds to *H. cunea*, which laid a foundation for the development and practical application of repellents for *H. cunea*.

Materials and methods

Insect rearing, plant sampling and standard compounds

H. cunea eggs were purchased from the Insect Virus Research and Development Center, Institute of Forest Ecology, Environment and Protection. They were reared in an incubator with a constant temperature of 25 ± 1℃, relative humidity of 70%-80%, and photoperiod of 16L: 8D. They were fed with

the artificial diet until 4-6th instar larvae or pupae, then the newly feathered adults were separated by sexes.

Fresh branches with leaves of *L. gmelinii* and *S. oblata* were cut from the mature trees grown on the Northeast Forestry University campus for olfactometer bioassays and volatile collections from June to September, 2022.

α-Pinene (CAS: 80-56-8, ≥ 98%), (+)-Limonene (CAS: 5989-27-5, ≥ 95%) were all purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Liquid paraffin (solution) was purchased from Tianjin Yongda Chemical Reagent Company Limited (Tianjin, China).

Olfactometer bioassays

The behavioral responses of *H. cunea* 4-6th instar larvae (main arm: 15 cm, side arms: 10 cm, inner diameter: 2.5 cm) and virgin female and male adults (main arm: 30 cm, side arms: 25 cm, inner diameter: 5 cm) to different odor sources were measured with Y-type olfactometer. Teflon hoses were used to connect a vacuum pump, an activated carbon tube, a flowmeter (with a flow rate of 200 mL/min), and the odor source to each side arm of the Y-type olfactometer in sequence.

Odor source settings in different experiments: Fresh branches and leaves were put into polyethylene (PE) bags and inserted into the water bottle. At the same time, the control group was empty PE bags and then respectively connected to the two side arms of the Y-type olfactometer. In the compound experiment, 10 μL of samples or solvent control were added to filter paper (0.5 cm × 2 cm) in source bottles as a group to determine the response of *H. cunea*. In the combination experiment, the prefer host plant of *H. cunea* (according to existing research) was selected for the background odor. Clean air was connected to the PE bag containing the fresh branches and leaves of *S. matsudana*, and then to the source bottle with 10 μL samples on filter paper, compared to the combination of *S. matsudana* and liquid paraffin.

The experiments were conducted in a climate-controlled room (25°C and 70%-80% relative humidity). Furthermore, newly feathered adults were under dark conditions and 4-6th instar larvae were starved for 24 hours before the experiment. For each testing run, 5 *H. cunea* larvae or adults were introduced from the end of the main arm of the Y-tube. While 30 insects were divided into a group and repeated 3 times. When the tested insect passed 1/2 of a side arm and stayed for more than 5min, it was recorded as a positive response. The number of insects that responded in each side arm of the Y-tube was recorded for each test. We calculated the treatment response rate and control response rate:

Treatment response rate (%) = Total number of insects in the treatment arm/ (total number of insects - the total number of unselected insects) × 100%

Control response rate (%) = Total number of insects in the control arm / (total number of insects - the total number of unselected insects) × 100%

Collection of volatiles from fresh tree branches with leaves

Dynamic headspace sampling adsorption method (refer to Birhanu et al. 2023) was used to collect volatiles from fresh branches of the two tree species (with three aeration samples for each tree species). The vacuum sampler was connected to the PE bag and pumped for 5–10 min until the bag was vacuumed, then pumped until it was full of air. After 20 minutes of balance, the air inlet end of the vacuum sampler (with the flow rate of 200 mL/min), activated carbon tube, PE bag, adsorbent tube (containing 100 mg PoraPak Q adsorbent) and the air outlet end were connected successively. Each aeration lasted 4 hours from 16:00 to 20:00 when *H. cunea* adults were most active. After aeration, the adsorbent column was removed and rinsed with 1mL n-hexane 2 times, followed by 1mL dichloromethane 2 times. The eluents for each aeration run were combined, concentrated, and collected in a 2mL sample bottle, sealed, and stored at -20℃ refrigerator before use.

Gas chromatography-electroantennographic detection (GC-EAD) and Gas chromatography-mass spectrometry (GC–MS)

Plant volatile samples (2μL) were injected into an Agilent 7890A GC-FID System equipped with a non-polar capillary column (HP-5MS; 30 m × 0.25 mm × 0.25 μm; Agilent Technologies Inc, the United States) that allowed simultaneous flame ionization detection (FID) and coupled to an EAG detector (IDAC-4) (Ockenfels Syntech GmbH, Kirchzarten, Germany) of the separated volatile compounds. Nitrogen was used as the carrier gas at 2 mL/min flow and the injector temperature was 250°C. The heating program was as follows: 40°C for 2 min, linear ramp at a rate of 5°C /min rising to 120°C, and then ramp to 280°C at 15°C /min with the 250°C of detector temperature. The outlet for the EAD was held in a humidified air stream flowing at 300 mL/min via Stimulus Controller CS-55 over the antennal preparation. EAD recordings were made using Ag-AgCl wire-glass capillary electrodes filled with saline solution on cut antennae of newly feathered male and female *H. cunea* adults. The antennal signals were stored and analyzed by the Spike software. The antennae of 5 females and 5 males were tested for each volatile sample. FID peaks that elicited EAD responses for at least three runs were considered EAD-active and marked for identification by coupled GC-MS.

GC-MS analyzed EAD active volatile samples on an HP 7890A GC coupled with an HP 5973 mass selective detector (Agilent Technologies, USA) using the same GC column type and similar conditions described above. The specific MS method included the ionization mode of EI 70eV, helium as the carrier gas (at 2 mL/min), transfer line temperature at 250°C, the scanned area being 30–500 amu, and scanning speed at 1000amu/s. Compounds were identified by comparing retention times with those of authentic standards and with mass spectra of standards in the NIST5.0 database.

Electroantennographic (EAG) recordings

All electroantennographic (EAG) recordings were made with a Syntech EAG setup. The moth antenna with the head was fixed on the forked electrode to the probe with conductive adhesive. Five concentrations (0.01, 0.1, 1.0, 10, and 100 μL/mL) of two compounds with liquid paraffin solutions plus the solvent control were tested on *H. cunea* antennae of both males and females. Stimuli were prepared

by applying the chemical solutions or control (10 μL each) on a piece of filter paper (0.5 cm × 2 cm) in a Pasteur pipette. The stimuli were tested in order of sample concentration from low to high, and “control” stimuli were applied before and after the chemical treatments were tested. Each stimulation lasted 0.1 sec. It was followed by a minimum 30-s purge period of filtered air to ensure recovery of antennal receptors. The same sample was tested for 5 times, and each compound/concentration was tested on 3 antennae of each sex. The EAG response signals to different doses of the 3 tested compounds were stored and analyzed and the calculation formula is shown below:

Relative EAG response value (%) = 2 *□sd/(□sk_1 + □sk_2)

Where,□sd is the average of 5 EAG response values (mV) to the same compound at the same concentration within the same testing round; □sk_1 is the average of 5 EAG response values (mV) to the control liquid paraffin (control) before the chemical sample testing, whereas the□sk_2 is the average of 5 EAG response values (mV) to the control liquid paraffin (control) after test of the sample to be tested (mV).

Data analysis

A matched sample *T*-test in SPSS24.0 was used to analyze the olfactory selection behavior of the 4-6th instar larvae and adults of *H. cunea*. One-way analysis of variance (ANOVA) was performed for EAG data, followed by the Duncan multiple range test in SPSS24.0 to separate means.

Results

Olfactory behavioral responses of H. cunea to fresh branches of L. gmelinii and S. oblata

In the Y-tube dual-choice assays, *H. cunea* 5th and 6th instar larvae were significantly attracted to the blank control compared to the fresh branches (bearing leaves) of *L. gmelinii* and *S. oblata* (*P*<0.05) (Fig. 1A/B) with the average responsive rates being 57.97% (5th instar) and 64.86% (6th instar) (*L. gmelinii*), 67.60% (5th instar) and 69.73% (6th instar) (*S. oblata*). The average responsive rate of 4th instar larvae to the blank control (58.93%) was higher than the branches of *L. gmelinii*, which to the branches of *S. oblata* (80.88%) were higher than the control, but 4th instar larvae showed no significant attractions to the two tree species (*P*□0.05).

Compared to the clean air, the fresh branches of *L. gmelinii* and *S. oblata* had a repellent effect on *H. cunea* virgin females (*P*<0.05) (Fig. 2A/B), which the average response rates to the blank control were 60.17% and 62.75%, respectively. In contrast, the virgin males didn’t elicit any significant behavioral response to the two tree species (*P*□0.05), with the higher average response rates to the control (50.98%) than *L. gmelinii* and *S. oblata* (55.9%) than the control.

Identification of EAD-active volatile compounds

GC-EAD/MS analyses indicated that *H. cunea* antennae of both sexes consistently responded to two volatile components, α-pinene and (+)-limonene from the fresh branches of *L. gmelinii* and *S. oblata* (Fig. 3A/B). Moreover, in the volatiles from branches of *L. gmelinii*, the average relative contents of α-pinene and (+)-limonene were 13.64% and 1.81%, respectively. From *S. oblata* volatiles, the average relative contents of two components were 1.86% (α-pinene) and 1.22% [(+)-limonene] (Table 1).

Table 1
Retention time (min) and relative content (%) of the two EAD-active volatile compounds identified from fresh branches of two tree species by GC-MS.

	CAS no.	Retention time (min)	*L. gmelinii*	*S. oblata*
			Relative content (%) (mean ± SE)	
α-Pinene	80-56-8	7.227	13.64 ± 0.58	1.86 ± 0.21
(+)-Limonene	5989-27-5	9.979	1.81 ± 0.21	1.22 ± 0.08

EAG dose-responses of H. cunea adults to two co-active compounds

According to the results of EAG, different dose-dependent response curves of *H. cunea* virgin males' and females' antennae were observed for the five concentrations of α-pinene and (+)-limonene (Fig. 4A/B). Among them, the EAG responses of both sexes to α-pinene at 100 μL/mL dosages were the topmost, and the responses of virgin females at 100 μL/mL (1.50%) and 0.1 μL/mL (1.39%) were notably higher than to those of the three other concentrations (10 μL/mL: 1.36%; 1 μL/mL: 1.15%; 0.01 μL/mL: 0.77%) (P<0.05) (Fig. 4A). While the EAG responses of males at 100 μL/mL of α-pinene (1.45%) were significantly higher than that at the other four concentrations (P<0.05), with a positive correlation trend, and from low to high the relative response values were 0.88%, 1.08%, 1.07%, and 1.22%. Similar to α-pinene, (+)-limonene at 100 μL/mL induced the highest average EAG response of adult antennae, namely 1.73% (females) and 1.62% (males), and the effect on antennae of female moths was higher than that at two low concentrations (0.01 μL/mL: 1.07%; 0.1 μL/mL: 1.24%) (P<0.05) (Fig. 4B). The response of male moths to (+)-limonene at 100 μL/mL, 10 μL/mL (1.52%) and 1 μL/mL (1.47%) was notably higher than that at 0.1 μL/mL (1.19%) and 0.01 μL/mL (1.00%) (P<0.05).

Behavioral responses of H. cunea to two co-active compounds in Y-Tube bioassay

In the Y-tube dual-choice bioassays of larvae, 100 μL/mL of α-pinene had extremely significant repellent effects on the 4th, 5th and 6th instars larvae (P<0.01) (Fig. 5A), and the average response rates were 89.32%, 94.97% and 86.36% in order. In addition, (+)-limonene at 100 μL/mL showed extremely significant repellent effects on 5th instar larvae of *H. cunea* (P<0.01) (Fig. 5B), with the average response rate being 95.08% for the control (liquid paraffin), while its repellent effect was notable to 4th and 6th instar larvae (P<0.05), which the average response rates of the control were 81.11% and 76.07%.

The results of adults' Y-tube bioassays showed that α-pinene and (+)-limonene at 100 μL/mL had repellent effects on both virgin sexes of *H. cunea* (P<0.05) (Fig. 6A/B). Thereinto, the average response

rates of females in control were 63.23% (α-pinene) and 62.96% [(+)-limonene], and for males were 61.42% (α-pinene) and 64.86% [(+)-limonene].

Effects of two EAD active volatiles on host selection of H. cunea larvae and adults

In order to investigate the effect of two repellent volatiles on the host selection of *H. cunea*, we measured the responses of 4-6th instar larvae and virgin adults to the combination of *S. matsudana* fresh branches with compounds [*S. matsudana* combined with α-pinene at 100 μL/mL; *S. matsudana* combined with (+)-limonene at 100 μL/mL; *S. matsudana* with liquid paraffin (control)], which the dual-choice results showed in Fig. 7A/B. Then compared with 100 μL/mL of α-pinene and *S. matsudana* fresh branches, the 5th, 6th instar larvae and virgin male and female adults significantly preferred the control group ($P<0.05$) (Fig. 7A), with the average response rates of 78.76% (5th instar larvae), 78.24% (6th instar larvae), 59.12% (females), 65.97% (males), however, 4th instar larvae (59.03%) showed no significant response ($P>0.05$). Similar to α-pinene, the average response rate of 5th instar larvae (66.26%), 6th instar larvae (66.91%), female (65.24%) and male (79.60%) to the control were notably higher than that of *S. matsudana* plus (+)-limonene (100 μL/mL) ($P<0.05$), but the response of 4th instar larvae (52.38%) was still not obvious ($P>0.05$).

Discussion

This study was conducted using two tree species, *L. gmelinii* and *S. oblata*, which were repellent to *H. cunea*, to search for key chemosensory clues between plants and insects. *H. cunea* 5th, 6th instar larvae and virgin female adults significantly rejected the odor of *L. gmelinii* and *S. oblata*, but males and 4th instar larvae were inapparent. In line with the living habits of *H. cunea*, male moths often recognize the sex pheromones released by females, and may not be sensitive to the recognition of plant volatiles, which is consistent with our studies (Tang et al.2012a). The 4th instar larvae just began to break the net with the weak activity selection, distinguishing between hosts and non-hosts inferiorly, while the 5th instar larvae entered the binge stage, and the gathered larvae dispersed and fed alone (Cheng 2022). With repellent plants as the research object, searching for possible candidates of plant-derived repellents that meet the criteria of sustainable biology (Nina et al. 2022), or rational use of repellent plants to protect the target hosts (Wu et al. 2019), all contributed to future efforts and new research directions for the pest green control.

The study found that two active volatiles, α-pinene and (+)-limonene were important terpenes compounds, which commonly existed in the volatiles of Pinaceae, such as *Larix principis-rupprechtii* (Li et al. 2022), *Larix olgensis* (Chen et al. 2013), *Pinus koraiensis* (Liu et al. 2021), *Pinus tabuliformis* (Wang et al. 2006) and so on. Previously, α-pinene and limonene were identified in the volatiles of *L. gmelinii* and *S. oblata* (Qiang et al. 2019, Ju et al. 2021), consistent with our results. The volatiles of *S. oblata* leaves were extracted by hydrodistillation, and found to contain α-pinene, which varied greatly with different seasons (Hui et al. 2008), but the content and proportion of compounds were discrepant with those in our experiment. The possible reason was that the volatile collection method was different. Xu et al.

(2020) determined that the content of α-pinene released by the green tree *S. oblata* was high without (+)-limonene, unlike in our study since the adsorbents Tenax and PoraPak Q used in the two above-mentioned the former only picked leaves, while fresh branches and leaves were collected in this study.

α-Pinene and (+)-limonene were the common volatiles of *L. gmelinii* and *S. oblata*, and their relative contents and proportions were diverse. In subsequent EAG experiments, male and female *H. cunea* adults showed significant dose-dependent responses. At the highest concentration of 100 μL/mL, 4-6th instar larvae and adults all showed highly consistent rejection of the two compounds, suggesting that they may be the important chemical clues to distinguish hosts and non-hosts of *H. cunea*. In other studies, α-pinene and (+)-limonene also showed significant repellent activity against *Sitophilus oryzae* (Fouad et al. 2021), *Hylastinus obscurus* (Tania et al. 2007) and *Pinus koraiensis* cone pests (Luo et al. 2022). The female and male adults of *Plutella xylostella* showed significant EAG and repelling response to the *Polygonum* essential oil. The GC-MS results showed that (+)-α-pinene, one of the main components, could be developed as a target repellent for the moth (Song et al. 2022). α-Pinene and (+)-limonene were attractive to female adults of *Spodoptera frugiperda*, while (+)-limonene was repellent to males (Wang 2022). Furthermore, repellent compounds may have different effects on the growth and reproduction of insects. For example, limonene from pine volatiles can interfere with *Thaumetopoea pityocampa* laying of eggs (Panzavolta et al. 2015). α-Pinene, *trans*-anethole, and other compounds showed great potential as larvicides or hatching inhibitors in controlling *Pseudaletia unipuncta* (Rose et al. 2015). In the natural environment, plumes with different odor concentrations and ratios are recognized by insects (Aarti et al. 2019). But pests adapted more slowly to a mixture of different compounds than to a single component (Shu et al. 2022). According to the olfactory contrast hypothesis, qualitative and quantitative patterns with their contrast of background odors were equally critical (Diego et al. 2018). Hence, follow-up tests could try the effect of different proportions of mixtures on *H. cunea*, and test out more effective volatiles' ratio and concentration for further application research.

In this study, we tested whether two repellent compounds reduce the attraction of the host plant (*S. matsudana*) to *H. cunea* in an indoor simulation. The results showed that α-pinene and (+)-limonene at 100 μL/mL could significantly inhibit host selection of 5th, 6th instar larvae and virgin adults, which laid a foundation for developing repellents to protect the target forest or tree species in the field. For example, limonene applied to the host (*Solanum lycopersicum*) could significantly repel *Bemisia tabaci* (Nicholas et al. 2022). There may be differences in insect behavioral responses at various ages, such as the ethanol extracts of *Ageratina Adenophora* and *Acorus calamus* repelled *Protaetia brevitarsis* larvae gradually increased with the growth of age (Wu et al. 2021). While the larvae of the *H. cunea* grow to the 4th instar in the net screen, and then gradually break out, the population spreads to different host plants (Tadashi et al. 2007).

Consequently, suitable repellents can be used for different instars of *H. cunea* larvae in practical application, and scientific and reasonable application can improve the control effectiveness of *H. cunea*. Presently, it has been found that more plant volatiles with attractant activity for *H. cunea*, such as β-ocimene and 6-methyl-5-hepten-2-one, could not only attract female moths, but also enhance the

attractant effect of sex pheromones (Tang et al. 2012b, Wang 2020), nevertheless, few repellent compounds have been studied and applied in practice. In brief, the combination of repellent and attractant by push-pull pattern may increase the trapping effect of pheromone and improve the efficiency of green control of *H. cunea*. However, the study of insect behavior in the field is more complicated due to the limitation of laboratory conditions and the interference of various abiotic factors. This study provided basic data for the "push-pull strategy" and its comprehensive management of *H. cunea*, which had important practical application value.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution Statement

Jin-Yan Lv, Zhao-Jun Meng and Shan-Chun Yan conceived and designed research. Jin-Yan Lv conducted experiments, analyzed data and wrote the manuscript. Yan-Yan Li and Xin-Su Li assisted in experiments. All authors read and approved the manuscript.

Data Availability

The datasets generated during or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

This statement is not applicable.

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Figures

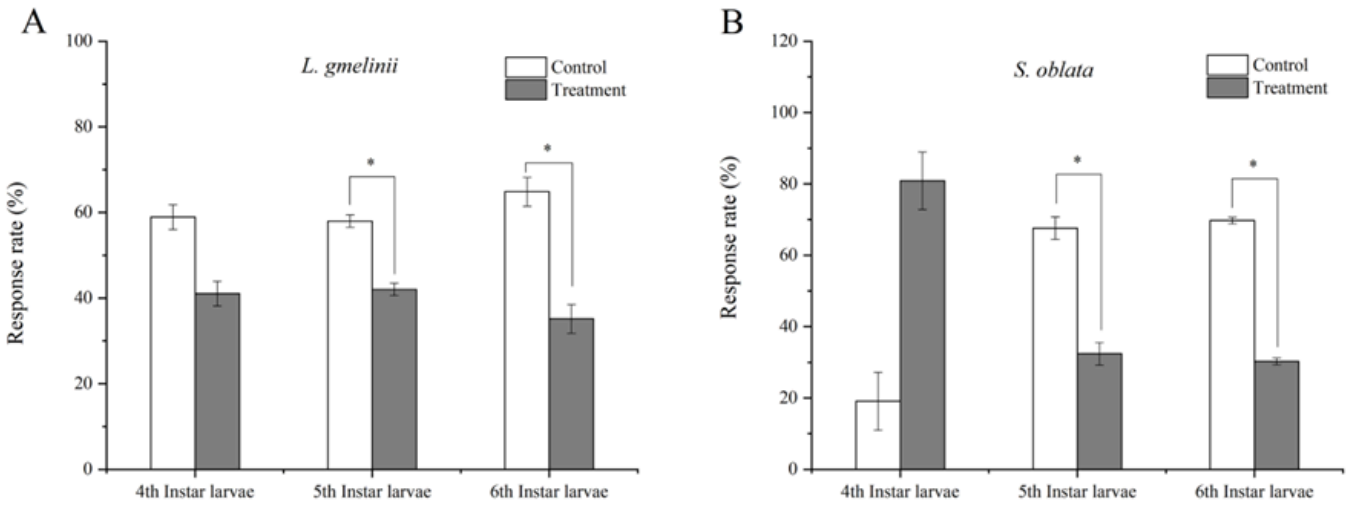

Figure 1

Behavioral responses of *H. cunea* 4-6th instar larvae to fresh branches of two tree species. The asterisk (*) indicates significant difference between the treatment and the control at P 0.05; the double asterisks

(**) indicate an extremely significant difference at $P \leq 0.01$; NS means not significant ($P \leq 0.05$) (matched sample *t*-test). The same as Fig 2, 5-7.

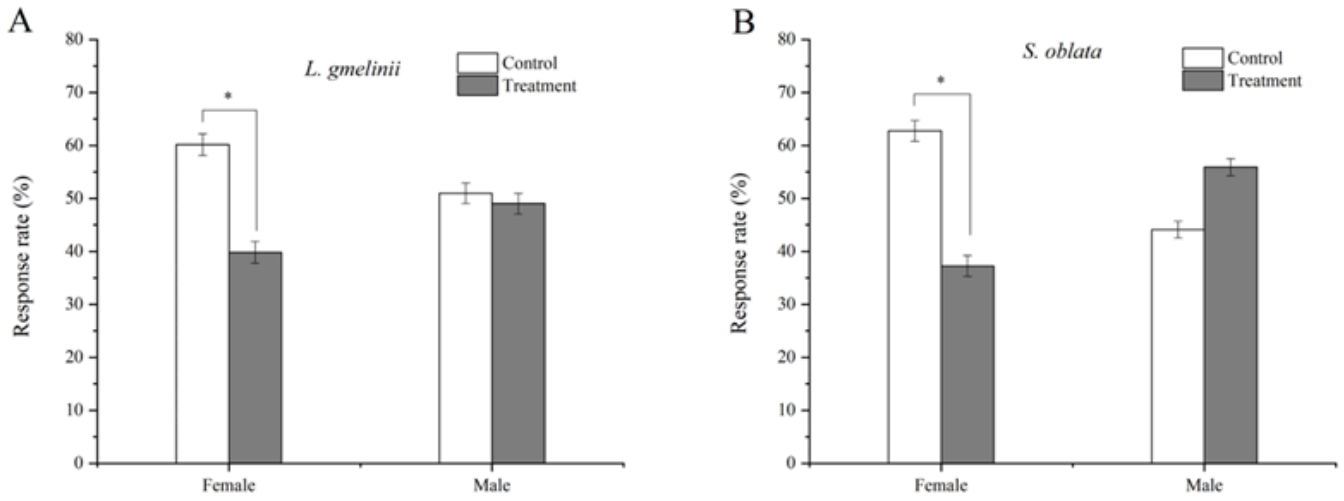

Figure 2

Behavioral responses of *H. cunea* virgin females and males to fresh branches of two tree species. (A) *L.gmelinii*, (B) *S. oblata*.

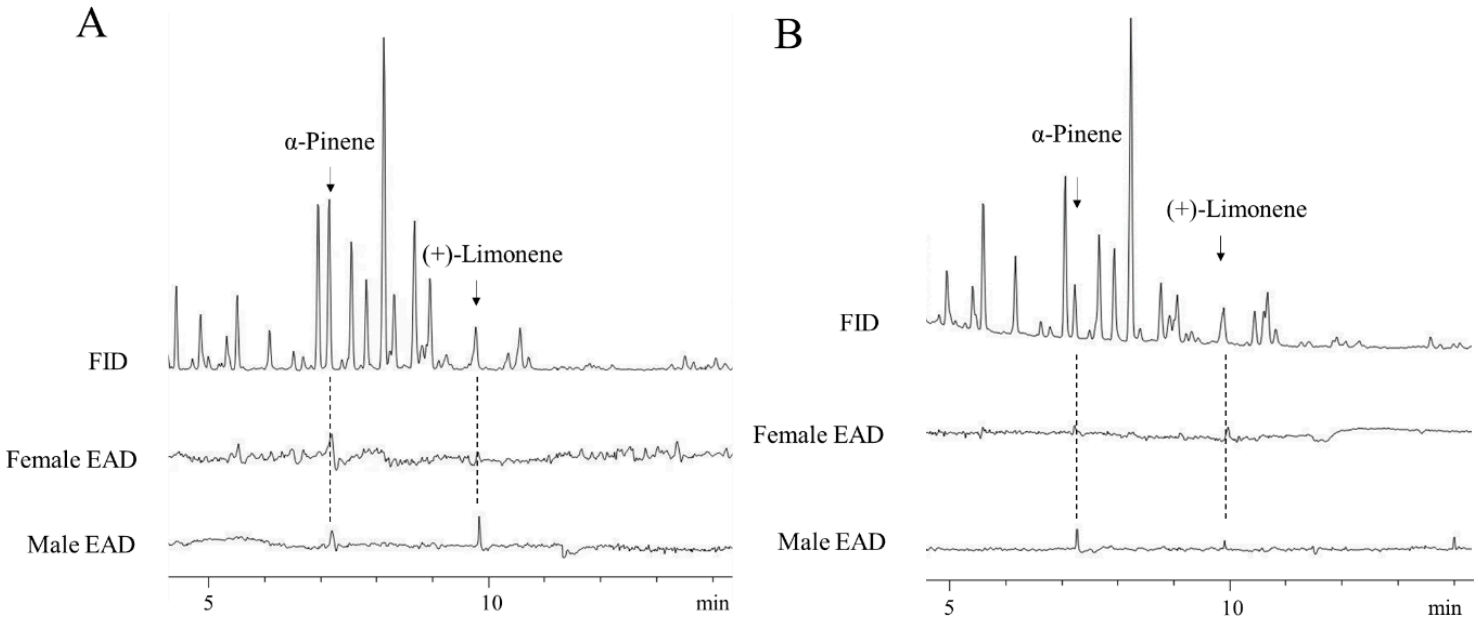

Figure 3

Simultaneously recorded GC-flame ionization detector (FID) and electroantennographic detector (EAD) (GC-EAD) responses of *H. cunea* antennae (female and male adults) to the volatile compounds collected from fresh branches of (A) *L.gmelinii*, (B) *S. oblata*.

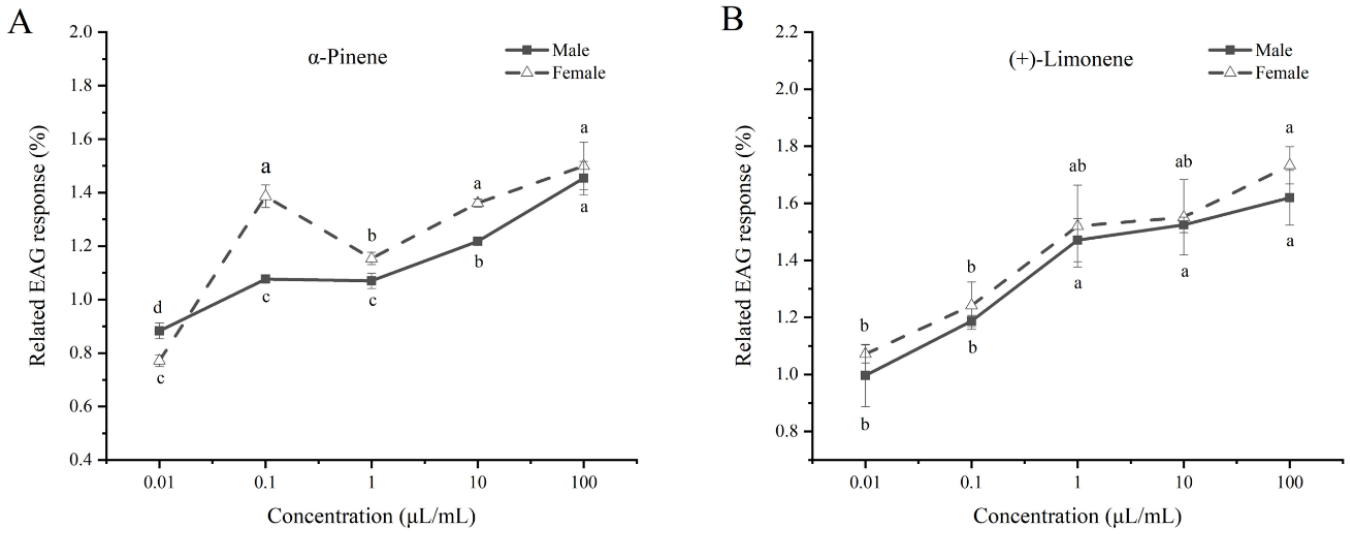

Figure 4

EAG responses of *H. cunea* virgin females and males to different concentrations of 2 EAD-active volatile compounds. The data in the figures are mean ± SE and means with different letters within the same sex were significantly different (One-way analysis of variance and Duncan's test, *P*□0.05). (A) α-Pinene, (B)(+)-Limonene.

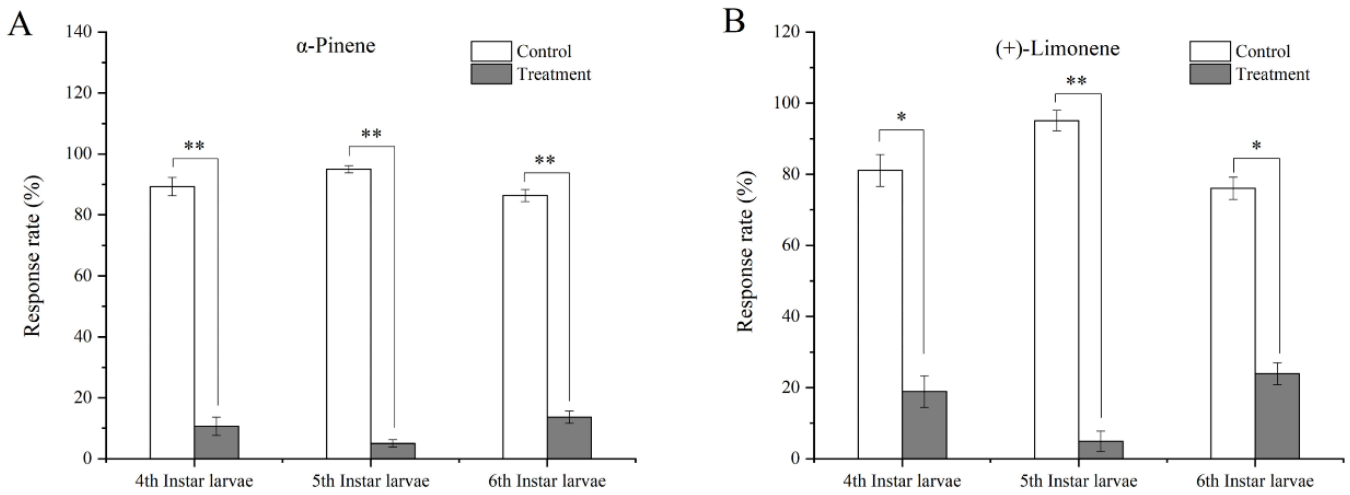

Figure 5

Behavioral responses of *H. cunea* 4-6th instar larvae to two EAD-active volatile compounds at 100 μL/mL (A) α-Pinene and (B) (+)-Limonene in the Y-Tube bioassay.

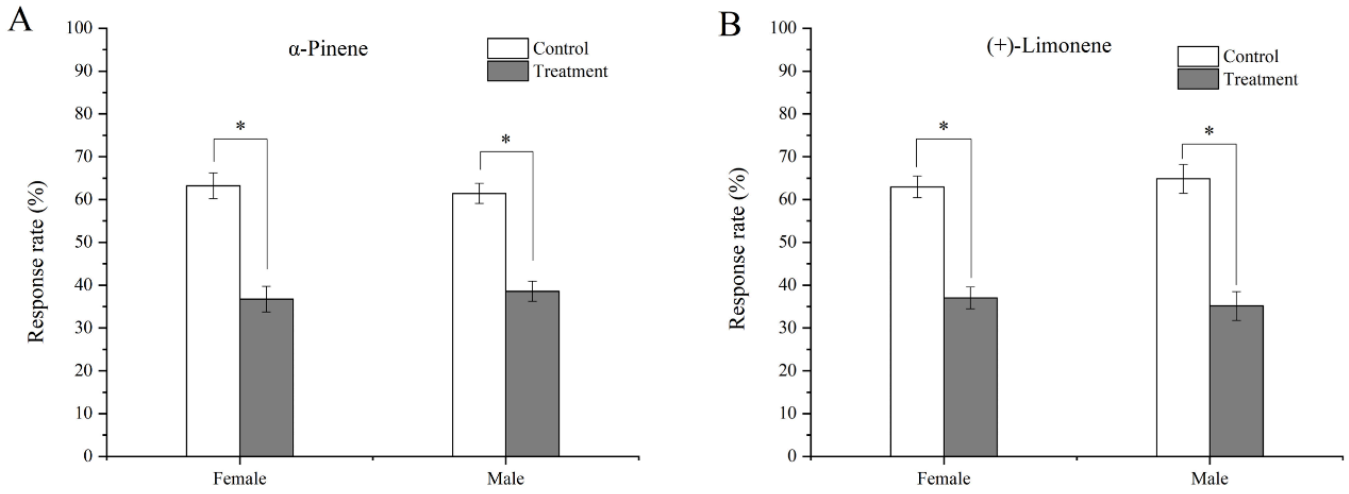

Figure 6

Behavioral responses of *H. cunea* virgin females and males to two EAD-active volatile compounds at 100 μL/mL (A) α-Pinene and (B) (+)-Limonene.

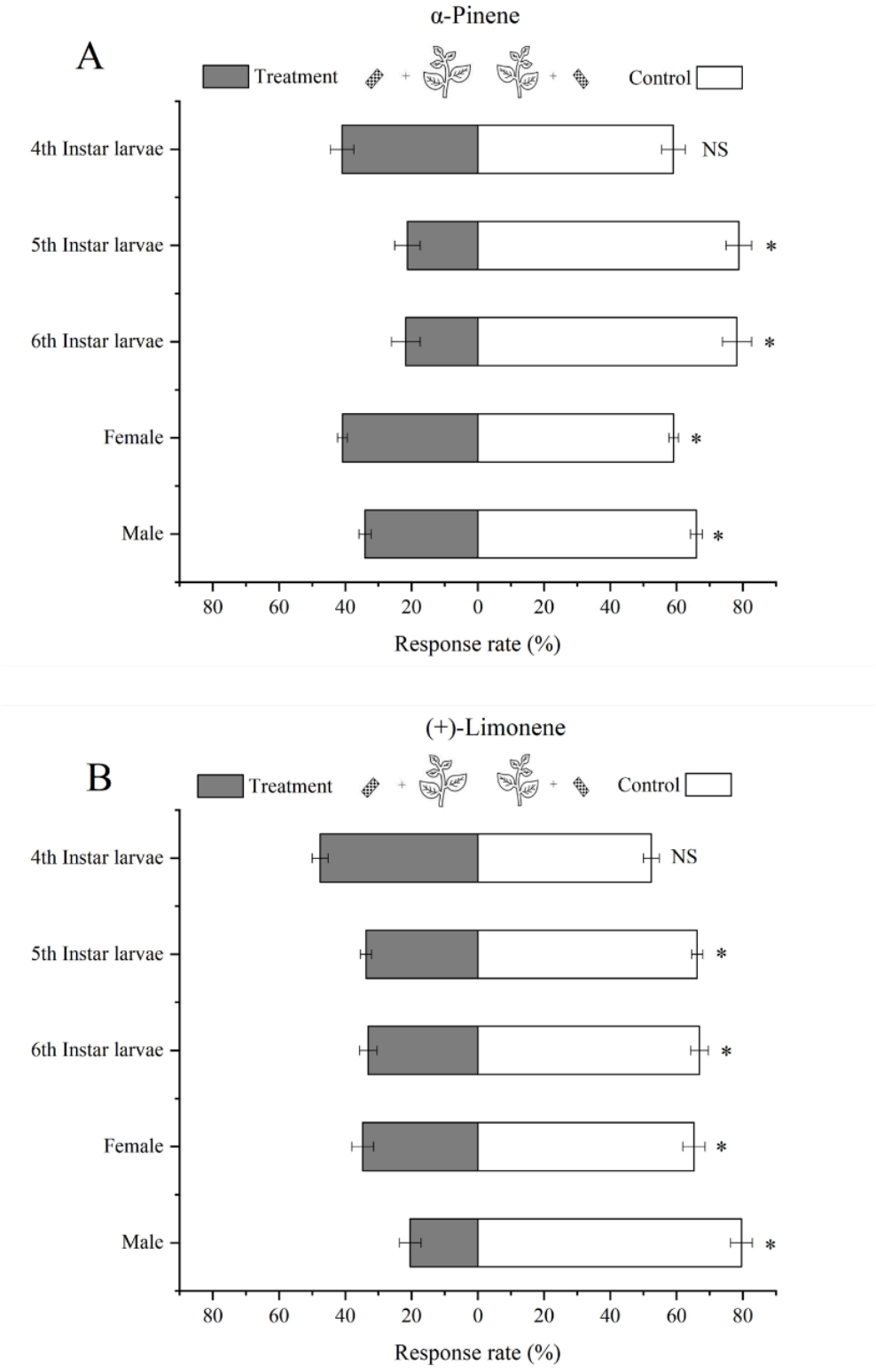

Figure 7

Behavioral responses of *H. cunea* 4-6th instar larvae and virgin females and males to two EAD-active volatile compounds at 100 μL/mL with fresh branches of *S. matsudana* (A) α-Pinene, (B) (+)-Limonene.