

D(+)-Camphor as the key phytochemical cue is promising for the trap monitoring in a new monophagous camphor tree borer *Pagiophloeus tsushmanus* (Coleoptera: Curculionidae)

Cong Chen

Nanjing Forestry University

Tian Xu

Nanjing Forestry University

Shouyin Li

Nanjing Forestry University

Mingyu Xue

Nanjing Forestry University

Yadi Deng

Nanjing Forestry University

Binqi Fan

Forest Station of Shanghai

Chufeng Yang

Forest Station of Shanghai

Dejun Hao (✉ djhao@njfu.edu.cn)

Nanjing Forestry University

Cong Chen

Zhaoqing University

Research Article

Keywords: *Cinnamomum camphora*, *Pagiophloeus tsushmanus*, monophagous weevil pest, D(+)-Camphor, host-plant volatiles, trapping device

Posted Date: April 7th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2777265/v1>

License: (cc) (i) This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The landscape plant, *Cinnamomum camphora*, is a broad-spectrum anti-insect tree species due to a diversity of terpenoids, such as D(+)-Camphor. However, *C. camphora* is selected as the unique host tree species by a monophagous weevil pest, *Pagiophloeus tsushmanus*. Deciphering the key olfactory signal components regulating its host preference could facilitate the monitoring and control strategies for this insect pest, but which is poorly understood. Herein, two host volatiles, D(+)-Camphor and Ocimene, can induce GC-EAD/EAG reactions in both male and female adults antennae. Correspondingly, Y-tube olfactometer assays showed that the two compounds were attractive to both male and female adults. In the field assays, self-made trap device baited with 5 mg dose D(+)-Camphor captured significantly more *P. tsushmanus* adults than isopropanol solvent control, but there was no sexual bias in adults captured. The trunk gluing trap device baited with bait can capture adults, but the number is significantly less than that of the self-made trap device and adults often fall after struggling. The cross baffle trap device never traps adults. Neither Ocimene nor isopropanol solvent control could capture adults. When used in combination, Ocimene cannot enhanced the attraction of D(+)-Camphor to both female and male adults. These results indicate that D(+)-camphor is a key active compound of *P. tsushmanus* adults for host location. Combination of the host-volatile lure based on D(+)-camphor and the self-made trapping device is promising to monitor and control this novel pest *P. tsushmanus* in *C. camphora* plantations.

Key Message

- *Cinnamomum camphora* is a broad-spectrum anti-insect tree species, mainly due to its rich in common insect repellent compound D(+)-Camphor
- *C. camphora* is the unique host tree species of the new wood-boring monophagous pest *Pagiophloeus tsushmanus*
- Electroantennographic and biological activity assays revealed that host volatiles D(+)-Camphor is the key olfactory signal of *P. tsushmanus* for host location
- Good trapping effect needs to be matched with appropriate device, self-made trapping device is the most suitable for capturing this pest, and our findings provide an eco-friendly control strategy for this pest
- D(+)-Camphor, a common insect repellent, has been selected as a key signal for host location by *P. tsushmanus*. The discovery of survival and evolution strategy of this pest using vacant niches has important ecological significance for understanding the mechanism of pest infestation or green prevention and control of pests

Introduction

Some phytophagous beetles, with a narrow host range, are highly specific to one or several host plant species (Xue et al, 2011; Li et al, 2017; Tang et al, 2022). For instance, both *Eucryptorrhynchus brandti* and *E. scrobiculatus* are host-specific pests of *Ailanthus altissima* (Wen et al, 2021). Host chemicals play an important role in the coevolution of plants and monophagous beetles (Landolt and Phillips, 1997; Rasmann and Agrawal, 2011). A very interesting phenomenon was found during previous indoor and field observation. The content of D(+)-Camphor in camphor tree *Cinnamomum camphora* Presl (Laurales: Lauraceae) is relatively high. However, this common insect repellent compound D(+)-Camphor is used by a monophagous camphor tree borer *Pagiophloeus tsushimanus* Morimoto (Chen et al. 2021; Li et al., 2021) for host location.

Camphor tree, *C. camphora*, is an important landscape tree species and widely distributed in south China (Wang et al., 2012). Some previous studies have shown that *C. camphora* has strong insect pests repellent activities due to that monoterpenoids are the main components of essential oil of *C. camphora*, which have larvicidal and ovicidal activities against some beetles of stored products (Nenaah et al., 2011; Guo et al., 2016; Jiang et al., 2016; Wu et al., 2020). Monoterpenoid volatile D(+)-camphor is the key component of camphor essential oil (Li et al., 2021; Li et al., 2022). Because of the insecticidal activity of these terpenoids, *C. camphora* plantations are less likely to be attacked or selected as a host by various herbivorous insects. However, *C. camphora* had been selected as the unique host plant by the new weevil pest *P. tsushimanus* (Chen et al. 2021; Li et al., 2021). *P. tsushimanus* was originally discovered in Tsushima Island of Kyushu in Japan and described by Morimoto (1982). Since the initial report of the monophagous camphor tree borer *P. tsushimanus* in China in 2014, *C. camphora* plantations have been widespread damaged by this destructive weevil pest in all administrative districts of Shanghai (Zhang et al., 2017; Chen et al. 2021; Li et al., 2021). *P. tsushimanus* has one generation a year in Shanghai. Adults usually overwinter in grooves on the underside of branches or branch nodes, and a handful of mature larvae overwinter in tunnels (Chen et al. 2021). Adults often aggregate for feeding on the tender bark of twigs or newly emerged buds, and larvae bore into the phloem, or sometimes cambium (Chen et al. 2021; Li et al., 2021). What's more, *C. camphora* plantations in adjacent areas outside Shanghai has been seriously threatened (Chen et al. 2021). Therefore, it is urgently needed to develop effective monitor or control strategies for this pest .

The larvae of wood-boring beetles live beneath the bark during the whole damage period with the characteristics of concealed activities and strong destructiveness, which lead to this pest difficult to control. Semiochemicals play a key role in mate, host, and oviposition location during the plant-insect interaction (Hanks, 1999; Roh et al., 2021). Plant volatiles are important olfactory cues in host selection of phytophagous beetles. Volatiles that mediate insect behavior have received a lot of attention. For example, *Cyrtotrachelus buqueti* is the main oligotrophic pest in bamboo forest, and the volatile substances of bamboo play a decisive role in its host location (Tang et al, 2022). Host plant-derived volatiles of *Ailanthus altissima* play an important role in the host oriented behavioral response of two weevils *Eucryptorrhynchus scrobiculatus* *E. brandti* (Wen et al, 2021). Traps baited with volatiles attractants are a economical and valuable measure for pest monitoring and control (Crook et al. 2014). In general, polyphagous insects distinguish host and non-host plants via the quantity of volatile

components, while monophagous insects identify host plants by the quality of volatiles (Rajapakse et al. 2010). For many specialized insects, the active components in their host plant volatiles have been identified. For instance, volatile (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) released from host plant *Alternanthera philoxeroides* (Mart.) Griseb showed significantly higher luring effect to adults of *Agasicles hygrophila* Selman & Vogt (Li et al., 2017). Isothiocyanates released by cruciferous plants is harmful to common herbivorous insects, but it can strongly attract adults to feed or stimulate the oviposition of specialized insect females *Plutella xylostella* Linnaeus (Liu et al., 2020). Therefore, it is more advantageous to develop attractants with strong attraction and simple components by using the strong tendency of monophagous insects to host specific odor compounds.

The biological characteristics of pests are the core basis developing effective control strategies (Shahid et al. 2017). In our previous study, an interesting phenomenon found during early indoor and field observation is that *P. tsushimanus* always has a special preference for *C. camphora* to other Lauraceae trees (Li et al., 2021). However, it is difficult to establish a stable population on close species (Li et al. 2019). At present, the mechanism of host identification and location of this pest and key phytochemicals in above process are still unclear. Therefore, the key active components of host selection preference of it were identified by chemical analysis combined with pest behavior determination in this study. This study aims to determine the effective compounds for developing the attractants, further promoting the eco-friendly control strategies for this pest. In addition, it can deepen our understanding of the interspecific chemical communication in *P. tsushimanus*, especially about the mechanisms of host identification and location.

Materials And Methods

Insects

Pagiophloeus tsushimanus pupae were collected from trunks of *C. camphora* trees at Maogang (30°56'6.15" N, 121°12'32.76" E), Songjiang District, Shanghai city, China in 2020 and 2021, which were placed in the wells of petri dish (six round wells with 2 cm in diameter) with wet cotton balls until adults' emergence. Then the juvenile adults were immediately collected, and their sex was distinguished by differences in their external genitalia. Ten males or females were reared in each plastic container (60 cm long, 30 cm wide, 20 cm high) with 10 small circles hole (diameter 1 mm) for ventilation (Chen et al., 2021). Adults were kept in a incubator (26 ± 1 °C, 60 ± 5% relative humidity, 14:10 light photoperiod), and fed with the fresh two-year-old twigs of camphor trees.

Headspace aeration of host volatiles

Two-year-old twigs of camphor tree (15 cm length) were utilized for analysis of headspace volatiles. The air outlet was fitted with volatiles trap, composed by a glass tube and Porapak Q adsorbent (200 mg; Sigma-Aldrich, St. Louis, MO, USA). Adsorbent secured in tube by glass wool plugs. Charcoal-filtered air was pulled (350 ml/min) through host-produced volatiles collection container (500 mL conical flask) for 24 h by atmosphere auto-sampling instrument (Beijing Municipal Institute of Labour Protection, Beijing,

China). The Porapak Q and traps were cleaned using Soxhlet extracted dichloromethane before used. Trapped volatiles were eluted from each adsorbent tube with 1 mL dichloromethane and loaded into chromatographic sample vials. Volatile extracts per 1 mL were concentrated to 200 μ L by a nitrogen blower to increase the concentration. Each sample vial was sealed until used in experiments. Six repetitions were set up in this experiment, and each repetition contains 20 twigs.

Identification of antennally-active compounds in host volatiles

Volatile extracts derived from *C. camphora* twigs were analyzed by coupled gas chromatography-electroantennogram detection (GC-EAD) using a 6890N chromatography-flame ionization detector fitted with a 30m capillary column HP-5 (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent Technologies Inc, USA, California). The parameters are as follows: 1) The temperature of injector and detector were 250°C, and the mode of injection was set in splitless. 2) The carrier gas is high purity nitrogen, and the flow rate is set to 45 mL per min. 3) The oven was programmed from 40°C, then 10°C per min to 80°C. 5°C per min to 100°C. 1°C per min to 105°C. 5°C per min to 120°C. 10°C per min to 250°C for 3 min. 4) A press-fit Y-type splitter (Agilent) was used to evenly distribute the nitrogen carrier gas flow between the GC's flame ionization detector (FID) and the EAG. The GC effluent was directed into the airflow tube (15 mm ID) of EAD and mixed with humidified air (flow rate: 300 mL/min, 20°C). and then passed through the antenna preparation. The mixed gas blows to the antennal preparation. The antennae of virgin female and male adults were cut from the base with scissors and forceps. And then the end of the antenna was quickly cut off about 0.5 mm with anatomical scissors. The two ends of the antenna were mounted on the two electrodes of EAG with a capillary glass tube (the tube was filled with normal saline, the silver wire was immersed in it and connected with the electrode, and the bubbles were removed before the test to avoid interference). After connection, we align the antenna with the center of the air outlet and 1 cm away from the air outlet, and wait until the baseline of the antenna potential is stable. An analogue-to-digital conversion board (IDAC-232, Syntech) was used to input the signals of electroantennographic (EAG) and FID into a computer, and the Syntech GC-EAD software was used to display and analyze signals. Antennae from ten females and ten males were respectively used for GC-EAD analysis, and the trial for each antenna was repeated three times.

Chemical analyses of host volatiles

Volatile extracts derived from *C. camphora* were identified using coupled gas chromatography-mass spectrometry (GC-MS) (Thermo Electron, USA). The related parameters of gas chromatography were the same as described in the GC-EAD experiment. Mass spectra were taken in EI mode (70 eV). The temperatures of ion source and transmission line were 250°C. The scanning range was 30 to 350 u. All compounds in the volatile extracts were preliminarily identified via the matching of mass spectra and database spectra, and then confirmed by the retention time and mass spectrum matching of key compounds and those of authentic standards. A set of linear alkanes from C8-C28 was used for GC-MS

analysis under the same conditions to calculate Kovat's index. The information of pure compounds used in this experiment was listed in Table 1.

Table 1

The information of pure compounds used for chemical identification, laboratory, and field assays

Maker	Compound	CAS no.	Purity (%)	Source
A	Ocimene	3779-61-1	90	Shanghai yuanye Bio-Technology Co., Ltd
B	D(+)-Camphor	464-49-3	96	Shanghai Aladdin Biochemical Technology Co., Ltd
C	Mineral oil	8042-47-5	96	Shanghai Aladdin Biochemical Technology Co., Ltd
D	Isopropanol	67-63-0	99	Shanghai Aladdin Biochemical Technology Co., Ltd

Electroantennographic (EAG) assays

Different doses (0.01, 0.1, 1, 10, 100 µg) of two main compounds (i.e., D(+)-Camphor and Ocimene) and mixed components [10 µg D(+)-Camphor : Ocimene = 1 : 1] were prepared with mineral oil.

Electrophysiological activities of female and male adults antennae in response to D(+)-Camphor and Ocimene at different dose or mixed components [D(+)-Camphor : Ocimene = 1 : 1] were examined using the EAG technique. EAG responses of *P. tsushimanus* adults antennae flagellomeres (seventh to ninth) to all treatments were tested. Before each test, 10 µL of each solution was dropped uniformly into a piece of filter paper strip (2 cm length × 0.5 cm width), which was inserted into a Pasteur pipette (15 cm length) and used as odor stimuli. Mineral oil was prepared as solvent control treatment.

Use a blade to cut off the antennae completely along the base of the antennae, and then cut off a bar at the end of the antennae. The base and end of each antennae were inserted into a glass pipette containing Kaiserin saline solution. The base was used as a neutral electrode and the end as a recording electrode (Sattelle et al., 1980). Electric continuity between antennae and DC mode with AC/DC un-6 amplifier connected to PC equipped with EAG 2.0 program (syntech laboratories, Hilversum, the Netherlands). Charcoal-filtered and humidified air (500 mL/min) continued to blow onto the antenna (seventh to ninth) passing through a stainless-steel delivery pipe (1cm inner diameter), and the outlet was about 1cm away from the antennae.

After the antenna connection was completed and the baseline was stable, the EAG test can be carried out. The filter paper strip with the given volatile compound was push into a new Pasteur pipette and connected with to the stimulussource tube, and start recording after gently pressing the pedal. The flow rate of each stimulation was 60 mL/s, the stimulation lasted for 0.2 s, and the interval between the two stimulation should be more than 60 s to ensure that the antennae recovered completely from the last

stimulation (Giacinto et al., 2019). In order to keep the activity of the antennae, the time to treat the antennae should be reduced as much as possible. At the beginning and end of each group of experiments, 10 μ L of mineral oil (control treatment) were tested respectively. In the dose-response experiment, it should be from low concentration to high concentration. Each treatment was tested on female antennae ($N=20$) and male antennae ($N=20$). Each antenna was tested with 3 repetitions respectively.

Olfactometer assays

Olfactory responses of male and female adults to two main compounds (i.e., D(+)-Camphor and Ocimene) and mixed components [D(+)-Camphor : Ocimene = 1 : 1] at different doses were investigated in Y-tube glass olfactometer assays. The dimensions of the Y-tube glass olfactometer were as follows, main arm: 40 cm length, two choice arms: 20 cm length at a 60° to each other, internal diameter of all arms: 4 cm. The olfactometer was placed in an environment with uniform light under a 500 W halogen lamp, and consistent background color at the bottom and around. Teflon® tubing was used to connect two 250 ml glass fasks to the ends of two choice arms. The glass fask contains a piece of filter paper strip (5 cm length $\times$ 0.5 cm width) loaded with odor stimuli or solvent control treatment.

A stream of air (500 mL/min/arm) filtered by carbon and humidified continuously blow into each choice arm through the fasks.

Each compound dose (1, 10, 100 μ g) and mixed components dose [D(+)-Camphor : Ocimene = 1 : 1, 10 μ g] were prepared as odor stimuli. Mineral oil was prepared as solvent control treatment. Before each test, 10 μ L of each solution was applied evenly to a piece of filter paper strip (5 cm length $\times$ 0.5 cm width), which was inserted into fask. Healthy male and female adults in the same physiological state and without mating were selected for this experiment, which were placed individually in the plastic container (60 $\times$ 30 $\times$ 20 cm) at $26 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ relative humidity for 24 h without food supply before the test. A single virgin male or female adult was placed into the opening at the starting end of the main arm and recorded as making a choice if it crawled and reached to the end of a choice arm within 20 min. Adults that did not crawl and reach the end of either choice arm within 20 min were recorded as no response. Each adult was only used once in each test. Before each assay, the components of Y-tube olfactometer were cleaned with acetone, and then rinsed thoroughly in distilled water, dried at room temperature. The treatment and control arms of the Y-tube olfactometer were physically alternated after every test. All tests were performed at the forest-entomology laboratory of Nanjing Forestry University from 9 am to 5 pm under the same environmental conditions, such as $26 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ relative humidity. Each treatment was tested female adults ($N=36$) and male adults ($N=36$). Three repetitions were set for each treatment, respectively.

Field assays

Field assays were conducted at the plantations cultivated with 15-year-old *C. camphora* at Maogang (30°56'6.15" N, 121°12'32.76" E), Songjiang District, Shanghai, where an outbreak of *P. tsushimanus*

infestation was first discovered in 2014 (Huang et al. 2014). Three 20 m×20 m sample plots were established and ten trees in each plot had been infested by *P. tsushimanus* were randomly selected. All larval tunnels were dissected on the surface of the trunk of each tree, which were located by a cue of frass. The density of larva below 2 m in the trunk of *C. camphora* plantations is 23.70 ± 10.33 larvae per tree (Tab.S1). The distance between trees was 3.0 m, mean height and the diameter at breast height of *C. camphora* trees were 5.0 ± 1.2 m and 21.1 ± 6.3 cm (Chen et al., 2021). In addition, a few other insects have occasionally been found to infest *C. camphora* plantations such as *Orthaga olivacea* Warren, *Thalassodes quadraria* Guenée, and *Diaphania perspectalis* Walker. (Chen et al., 2021). Lures were prepared by heat-sealing polyethylene tubing (~ 7 cm×4.9 cm, wall thickness 0.05 mm; Uline, Pleasant Prairie, WI, USA) containing 1 mL solution [D(+)-Camphor, Ocimene or mixed components diluted in isopropanol] or solvent control treatment (isopropanol).

Experiment 1– Effect of different kinds of traps on trapping efficiency

Three kinds of traps were selected in this experiment(Fig. 4), including cross baffle trapping device (Fig. 4-A), self-made trapping device (Fig. 4-B), and trunk gluing trapping device (Fig. 4-C). Trapping device B refitted from 5 L mineral water bottle. One third of the bottle were cut off and close to the trunk. Several openings larger than the insect body were arranged on the above and side of the bottle. The inner side of the bottle was coated with colorless and tasteless glue. The tasteless glue was replenished weekly. The bottles were fixed with thin iron wire and close to the trunk of *C. camphora*. Lures were respectively hung in the center of trapping device A and B. The lure was fixed in the middle of the trapping device C, and the back of lure was jacked up with branches to avoid being stuck by glue and affecting the release of volatiles. All trapping devices (including lures) were randomly placed on the trunk of *C. camphora* 1.5 m above the ground and ≥ 20 m apart. Collection cups of trapping device A and B contained mixed solution of automobile antifreeze and water (1 : 2) to kill and preserve trapped adults. This experiment was conducted from June 1 to July 1 in 2020. Trapped female and male adults were collected and lures were replaced weekly. Each trapping device and control group were set 6 repetitions respectively in the experiment. Each treatment contains 24 repetitions. Each trapping device was baited with: 1) 5 mg D(+)-Camphor in 1 mL isopropanol ($N=6$) and 2) 1 mL isopropanol ($N=6$).

Experiment 2– Effect of the height of trapping device on trapping efficiency

This experiment was also conducted from June 1 to July 1 in 2020 with D(+)-Camphor (5 mg/mL) combined trapping device B. Trapping device B were placed on the trunk of *C. camphora* at three heights: high (2 m above the ground), middle (1.5 m above the ground), and low (0.3 m above the ground) (Fig. 5). Each height included: (1) 5 mg D(+)-Camphor in 1 mL isopropanol ($N=6$) and (2) 1 mL isopropanol ($N=6$). The related methods were the same as experiment 1.

Experiment 3– Effect of dose of host volatile components on trapping efficiency

This experiment was tested from June 1 to July 1 in 2020 with trapping device B. The volume of each lure, the use of trapping device B, and the setting of the control group were the same as the Experiment 1.

Lures were replaced, trapped adults were, and tasteless glue was replenished collected weekly. All treatments included: (1) 1 mg D(+)-Camphor in 1 mL isopropanol ($N=6$), (2) 5 mg D(+)-Camphor in 1 mL isopropanol ($N=6$), (3) 10 mg D(+)-Camphor in 1 mL isopropanol ($N=6$), (4) 1 mg Ocimene in 1 mL isopropanol ($N=6$), (5) 5 mg Ocimene in 1 mL isopropanol ($N=6$), (6) 10 mg Ocimene in 1 mL isopropanol ($N=6$), and (7) 1 mL isopropanol ($N=6$).

Experiment 4– Effect of combination of host volatile components on trapping efficiency

According to the screening results of the above trapping conditions, the lures of D(+)-Camphor (5 mg) and Ocimene (5 mg), mixed components (D(+)-Camphor : Ocimene=1:1, 5 mg), trapping device B placed on the trunk of *C. camphora* at middle height (1.5 m above the ground) were selected to carry out the test of trapping effect in *C. camphora* plantations from July 1 to September 30 in 2020. Tasteless glue (apply to the inside of trap B) was replenished, lures were replaced, and trapped adults were collected weekly. All treatments included: (1) 5 mg D(+)-Camphor in 1 mL isopropanol ($N=6$), (2) 5 mg Ocimene in 1 mL isopropanol ($N=6$), (3) 5 mg D(+)-Camphor and 5 mg Ocimene in 1 mL isopropanol ($N=6$), and (4) 1 mL isopropanol ($N=6$). The related methods were the same as experiment 1.

Data Analysis

For laboratory assays, the negative polarity deflection (mV) of a stimulus was used to test the EAG response of each compound or mixed solutions (Light et al., 1992). The EAG responses (mV) of each stimulus was that the absolute EAG responses (mV) to each stimulus was subtracted by the mean response of two nearest mineral oil controls (Raguso et al., 1998). The mean EAG responses (mV) of each compound or mixed solutions tested were analysed by variance (ANOVA) and compared of mean value by the Tukey's HSD (Honestly Significant Difference) test ($P=0.05$). The mean EAG responses (mV) of female and male to each stimulus were compared using the Student's *t*-test ($P=0.05$). Binomial tests (expected value = 0.5) were applied to compare the numbers of adults selected between two odor stimuli in Y-tube olfactometer bioassays (Xu et al., 2020). Nonresponding adults were not included in the statistical analysis. In field bioassays, Kruskal-Wallis H test followed by pairwise contrast tests were applied to statistically analyze differences in the amount of trapping across treatments. Bonferroni correction was used to adjust the multiple comparisons to control the experiment-wise error rate. Significantly differences of sex ratio was analyzed by Chi-square test. The significance level of various treatments in this paper was $\alpha=0.05$. All data were analysed using SPSS 20.0 (IBM SPSS Statistics, Chicago, IL, USA) and plotted using Origin 2018 (OriginLab Inc., Northampton, UK).

Results

Identification of antenally-active compounds in host volatiles

Two components (1 and 2) of headspace volatiles collected from *C. camphora* volatiles consistently stimulated EAD responses in terms of the antennae of female (Fig. 1-A) and male adults (Fig. 1-B). Two components were identified 1 as Ocimene [retention time (RT) 9.71 min, Cas No. 13877-91-3] and 2 as

D(+)-Camphor [RT 13.23 min, Cas No. 464-49-3] by coupled GC-MS library match, and further confirmed with authentic standard by co-injection.

Electroantennographic (EAG) assay

The sensitivity of female and male *P. tsushimanus* adults to ascending dose of two single component and one mixed components were showed in Fig. 5. For all dose tested of Ocimene, there were significant differences among the mean EAG responses of the different dose both in females ($F = 1979.755$, $df = 4$, $P < 0.05$) and males ($F = 1648.967$, $df = 4$, $P < 0.05$) (Fig. 2-A). The mean EAG responses of female adults ($F = 1216.971$, $df = 4$, $P < 0.05$) and male ($F = 481.145$, $df = 4$, $P < 0.05$) adults antennae to D(+)-Camphor were also significant differences at all dose (Fig. 2-B). In the range of dose tested of Ocimene, the mean EAG responses amplitude varied from 0.078 ± 0.001 mV to 0.455 ± 0.002 mV in females and from 0.058 ± 0.003 mV to 0.423 ± 0.001 mV in males. Amplitude of mean EAG responses of female adults antennae to all dose of D(+)-Camphor is from 0.099 ± 0.003 to 0.636 ± 0.001 . For male ones, the mean EAG responses amplitude varied from 0.083 ± 0.006 to 0.526 ± 0.005 . The EAG responses of female adults antennae to Ocimene were significant higher than that of male adults at 0.01 ($t = 7.321$, $df = 4$, $P < 0.05$), 1 ($t = 6.061$, $df = 4$, $P < 0.05$) or 100 μ g ($t = 18.755$, $df = 4$, $P < 0.05$). The same difference existed in the EAG responses of adults to D(+)-Camphor at 0.1 μ g ($t = 6.259$, $df = 4$, $P < 0.05$), 10 μ g ($t = 6.375$, $df = 4$, $P < 0.05$) or 100 μ g ($t = 22.157$, $df = 4$, $P < 0.05$).

Strong EAG responses (0.488mV) were elicited by mixed components in both females and males (Fig. 2-C). The mean EAG responses of females was significantly higher than that of males for mixed components ($t = 4.311$, $df = 4$, $P < 0.05$).

Olfactometer assays

Y-tube olfactometer bioassays showed that the numbers of both females and males selecting D(+)-Camphor increased first and then decreased with the dose from 0.1 μ g to 100 μ g. D(+)-Camphor showed significantly stronger attraction to females than control at 100 μ g dose ($P = 0.035$), but there was no significant difference for males ($P = 0.189$). D(+)-Camphor at 10 μ g dose showed significantly stronger attraction to both females ($P < 0.001$) and males ($P = 0.001$) than control. Either females ($P = 0.690$) or males ($P = 0.678$) were not significantly attracted by D(+)-Camphor at 1 μ g dose (Fig. 3-A).

In Y-tube olfactometer bioassays, the response rate of both females and males selecting Ocimene also increased first and then decreased with increasing dose. The tendency of both females ($P = 0.006$) and males ($P = 0.008$) to Ocimene at 10 μ g dose showed significantly stronger than control. However, there was no significant difference in the response rate of females or males to 1 μ g ($P = 0.286$, $P = 0.230$) and 100 μ g ($P = 0.076$, $P = 0.061$) dose (Fig. 3-B).

Mixed components showed significantly stronger attraction to females ($P = 0.002$) and males ($P = 0.001$) than control at 10 μ g dose. The response rate of males to mixed components than that of females (Fig. 3-C).

Field assays

Experiment 1– Effect of different kinds of traps on trapping efficiency

There was a significant difference in the number of adults captured by different trapping devices baited with lures (D(+)-Camphor or isopropanol) ($H = 21.138$, $df = 5$, $P = 0.001 < 0.05$). The number of adults captured by trapping device B baited with D(+)-Camphor was significantly more than those of trapping device A and all isopropanol solvent control treatment (all of P was less than 0.0001, respectively, < 0.003 , Bonferroni corrected significance level) (Fig. 4).

Trapping device B baited with D(+)-Camphor captured significantly more female adults than those of other treatments except the trapping device C ($P = 0.001$ in both comparisons, respectively, < 0.0033), while there was no significant difference in the number of male adults among all treatments ($P > 0.0033$, in all pairwise contrasts).

There were four females and three males were captured at trapping device B, and 1 females and 2 males were captured at trapping device C, but the differences were not statistically significant between the sexes of adults captured at trapping device B ($\chi^2 = 0.032$, $P = 0.858$) or trapping device C ($\chi^2 = 0.139$, $P = 0.709$) baited with D(+)-Camphor respectively. However, no adults were captured at trapping device A.

Experiment 2– Effect of the height of trapping device on trapping efficiency

The results showed that there was a significant difference in the number of adults captured at different heights of trapping device B baited with lures (D(+)-Camphor or isopropanol) ($H = 22.744$, $df = 5$, $P < 0.0001$). Significantly more adults captured at high height of trapping device B baited with 5 mg D(+)-Camphor than those of all isopropanol solvent control treatment ($P = 0.001$ in both comparisons, respectively, < 0.003 , Bonferroni corrected significance level) (Fig. 5).

Number of males captured by trapping device B baited with D(+)-Camphor at high height was significantly more than those of other treatments except for middle height ($P = 0.001 < 0.003$, in all pairwise contrasts), while there was no significant difference in the number of female adults among all treatments ($P > 0.003$).

A total of 9 females and 6 males were captured in experiment 2. Specifically, there were 3 females and 4 males were captured at high height, 4 females and 2 males were captured at middle height, and 2 females were captured at low height, but there was no sexual bias in adults captured at high height ($\chi^2 = 0.032$, $P = 0.858$), middle height ($\chi^2 = 0.178$, $P = 0.673$), or low height ($\chi^2 = 1.333$, $P = 0.248$).

Experiment 3– Effect of dose of host volatile components on trapping efficiency

Significant difference was shown in the number of adults caught by different dose of all treatments ($H = 34.458$, $df = 6$, $P < 0.0001$). Trapping device B baited with 5 mg D(+)-Camphor captured significantly more adults than other treatments except 10 mg D(+)-Camphor ($P = 0.001$ in all pairwise contrasts, $< 0.05/21 =$

0.0023). No significant difference was detected between the number of adults caught by 10 mg and other treatments ($P > 0.0023$, in all pairwise contrasts) (Fig. 6).

There were 7 females and 6 males in total caught in this experiment, of which 5 females and 4 males were caught by 5 mg D(+)-Camphor, 2 females and 2 males were caught by 10 mg D(+)-Camphor. There was no sexual bias in the number of adults caught by 5 mg D(+)-Camphor ($\chi^2 = 0.020$, $P = 0.887$) or 10 mg D(+)-Camphor ($\chi^2 = 0$, $P = 1$). However, no adults were captured at different dose of Ocimene and solvent control treatment.

Experiment 4– Effect of combination of host volatile components on trapping efficiency

The results of trapping for three months were analysed by Kruskal Wallis H test, which showed that significant difference existed in the number of adults caught by major and minor host volatile components ($H = 20.320$, $df = 3$, $P < 0.0001$). Trapping device B baited with 5 mg D(+)-Camphor caught significantly more females and the total of both sexes than those with the isopropanol solvent control treatment or 5 mg Ocimene ($P = 0.001$ in both comparisons, respectively, $< 0.05/6 = 0.0083$, Bonferroni adjusted significant level). When combined with Ocimene, fewer adults were caught by trapping device B baited with 5 mg D(+)-Camphor + Ocimene than that of 5 mg D(+)-Camphor only, but the difference was not significant ($P = 0.724 > 0.0083$). Although the mixed component did not increase the number of captured adults, it was still significantly higher than those of Ocimene ($P = 0.003 < 0.0083$) or control group ($P = 0.003 < 0.0083$). No adults were captured by trapping device B baited with the single component of Ocimene, which was the same as the result of Experiment 3 (Fig. 7).

A total of 16 females and 10 males were caught in experiment 4. Trapping device B baited with 5 mg D(+)-Camphor attracted 9 females and 5 males, and trapping device B baited with 5 mg D(+)-Camphor + Ocimene attracted 7 females and 5 males. The number of females caught in both groups was more than that of males, but no significant difference between the sexes ($\chi^2 = 0.152$, $P = 0.696$, in 5 mg D(+)-Camphor; $\chi^2 = 0.049$, $P = 0.825$, in 5 mg D(+)-Camphor + Ocimene).

Discussion

The wood-boring monophagous pest *P. tsushimanus* prefers to damage the camphor tree *C. camphora*. Based on the above phenomena, the key olfactory signal components of this preference behavior was identified in this study. The compounds D(+)-Camphor and Ocimene in the volatiles of the host plant *C. camphora* can both cause the antennal electroantennographic response and olfactory responses in the Y-tube, but the field activity test results show that only D(+)-Camphor has the attractant activity, and there is no gender bias in the quantity. The attractant needs to be matched with a suitable trapping device to obtain a good trapping effect. The trapping device B (self-made trap device) is suitable for capturing adults. However, although trapping device C (trunk gluing trap device) can capture adults, the number is significantly less than that of the self-made trap device, and adults often struggle and fall down. In addition, the trapping device A (cross baffle trap device) never traps adult animals.

Antennal electroantennographic response of adults caused by host plant volatiles

With the rapid development of chemical analysis technology, a large number of key components involving in chemical communication of wood-boring beetles have been identified (Mitsuyoshi et al., 2002; Xing et al., 2017; Xu et al., 2017; Xu et al., 2020). GC-EAD method is one of the optimal methods for screening active components, which has been applied to the identification of pheromone components in various insects (Xu et al., 2017; Xu et al., 2020; Guo et al., 2020). In this study, we found that compounds D(+)-Camphor and Ocimene in the volatiles of the host plant *C. camphora* that have GC-EAD response to both female and male adults. At the same time, both male and female adults had greater EAG response to the single or mixed components of D(+)-Camphor and Ocimene. The results of GC-EAD and EAG responses showed that D(+)-Camphor and Ocimene had antennal electrophysiological activities for both female and male adults. The components with electrophysiological activities may be luring components or repellent components, which needs to be comprehensively judged in combination with the results of laboratory and field behavior assays (Kong et al., 2001).

Bioactivity of host plant volatiles

After the preliminary identification of key active components of host plant volatiles, bioactivity of these components were investigated and three kinds of field trapping devices were developed for this pest in this study, and the effects of different factors on the capture effect were measured at the same time.

The results of olfactometer assays showed that male and female adults had a strong response rate to 10 µg D(+)-Camphor or Ocimene, and the response rate of D(+)-Camphor was higher than that of Ocimene, respectively. Additionally, the results of field assays showed that D(+)-Camphor also had attractive activity to both female and male adults. Previous surveys have shown that D(+)-Camphor is considered as pest repellent agent (Guo et al., 2016; Jiang et al., 2016; Nenaah et al., 2011; Singh et al., 2012). Interestingly, our results indicate that D(+)-Camphor is used by *P. tsushimanus* adults as a key olfactory signals for host location. Similar result has been reported that the isothiocyanates released by cruciferous plants can strongly attract *P. xylostella* Linnaeus adults, but it is harmful to many other insects (Liu et al., 2020). Toxic substances isothiocyanates were used by *P. xylostella* for host location, which can effectively repel natural enemies or other food competitors. This survival strategy may be also used by *P. tsushimanus* to occupy this vacant niche and cause outbreaks. However, Ocimene or isopropanol solvent control treatment had never attracted adults, and Ocimene had no attractant activity and had no synergistic enhance when combined with D(+)-Camphor. This may be due to the fact that monophagous insects are more sensitive to the types and quality of host plant volatiles than polyphagous insects (Rajapakse et al. 2010), although Ocimene exhibits certain attractive effects in indoor short-distance behavioral measurements. In addition, it also may be due to the repellent effect of Ocimene to insects, and Ocimene is one of the important volatile components released by plants always induced by insect feeding damage. For example, Ocimene would released by tea plants after being fed by

insects such as *Empoasca vitis* Gothe, *M. aurolineatus*, or *Ectropis oblique* Prout (Sun et al., 2010; Sun et al., 2012; Cai et al., 2014). The mixed components contained Ocimene has a strong repellent effect on *M. aurolineatus* (Han et al., 2017). The content of Ocimene was high in the extract of *Chloroxylon swietenia* Apiaceae, which not only has a strong repellent effect, but also poisons or inhibits food on *Spodoptera litura* Fabricius (Kiran et al., 2017).

No adult was ever trapped by trapping device A (cross baffle trap device) with lures. We speculated that since *P. tsushimanus* adults always fly less and mainly move by crawling (Chen et al., 2021), they may be difficult to be captured after flying and hitting the baffles of trapping device A. The number of adults captured by trapping device C (trunk gluing trap device) with lures was less than that of trapping device B (self-made trap device). It is possible that the trapped adults can fall off after struggling, and consequently cannot be accurately counted. From this, the most suitable trapping device for the attraction of *P. tsushimanus* adults in the field is the trapping device B (self-made). The number of adults captured by self-made trap device with lures hung at high and medium height were more than that of the low height, which may be related to the oviposition position of *P. tsushimanus* adults mainly distributed in the high and medium height of the trunk. Therefore, they often come into contact with the high height trapping device first. However, there is no significant difference in the number of adults captured by the trapping device B with lures hung at high and medium height. Therefore, in order to facilitate operation, the medium height of the trapping device should be a priority in the field assays. Dose-response test showed that the number of adults captured by 5 mg was more than that of 10 mg D(+)-Camphor, which was similar with the field assay result of *A. glabripennis* (Xu et al., 2020). These results indicated that the dose of lures in insect chemical communication needs to be moderate, not the greater the better. Therefore, the suitable conditions for field monitoring and control this pest are as follows: trapping device B (self-made), lure with 5 mg D(+)-Camphor, hung at medium height of the trunk.

The volatiles from host plants in nature exist in the manner of mixed components, and consequently multi-component mixtures is highly effective in luring the target insects (Binder et al., 1995; Xing et al., 2017). In this study, we preliminarily discovered a single component D(+)-Camphor that possessed a certain attractive activity. while other assistant or synergistic components remain to be identified in the future. In addition, field trapping is commonly affected by various environmental factors, such as temperature, humidity, and rainfall (Ssini et al., 1996; de Abreu et al., 1997; Hallett et al., 1999). Therefore, these factors affecting the trapping efficiency need to be further considered. Through the above related research, the purpose is to improve or optimize the trapping effect of lures with D(+)-Camphor as key active component.

Relationship among host plant, pest and their parasitic natural enemy insect

After being attacked by herbivorous insects, plants will also release volatiles to recruit some natural enemies, so-called indirect defense (Vet et al., 1992; Havill et al., 2010). For example, after being attacked by *Spodoptera exigua* Hübner, *Zea mays* Linn releases volatiles to attract the natural enemies *Cotesia*

marginiventris Cresson of *S. exigua* (Tunings et al., 1992). *Brassica oleracea* releases volatiles after being damaged by *Pieris rapae* to attract parasitoid *Cotesia glomerata* of *P. rapae* (Blaakmeer et al., 1992). In the camphor trees plantations, a natural enemy parasitoid *Pseudocyanopterus pagiophloeusis* Samartsev & Li parasitizing the larvae of *P. tsushmanus* can be found every year, which is a new species and described from Shanghai, China (Samartsev et al., 2021). However, this new species parasitoid *P. pagiophloeusis* has never been lured in the trapping devices at field assays. Based on the above results, it is speculated that *P. tsushmanus* can avoid eavesdropping on the information in interspecific chemical communication by the natural enemy parasitoid *P. pagiophloeusis* when using D(+)-Camphor as an important olfactory signal in host recognition. Similarly, the defense substance isothiocyanates of Cruciferae plants is used by specialized insect females *Plutella xylostella* Linnaeus as olfactory signal compounds to locate the host plant for oviposition (Liu et al., 2020). Toxic compounds can be used by pests to repel other competitors or natural enemies, which are often selected as the key olfactory signal for locating hosts. It is a good survival and evolution strategy for pests to occupy vacant ecological niche (toxic compounds), which may be the main reason for pests invasion, population reproduction, and rapid expansion in host plants.

Conclusion and outlook

Previous results have shown that *P. tsushmanus* can adapt to and thrive in *C. camphora* plantations. Further more, *C. camphora*, a broad-spectrum insect repellent tree species, was selected as the unique host tree species of *P. tsushmanus*. This study has found that D(+)-Camphor is the key olfactory signal of *P. tsushmanus* preference for *C. camphora*. D(+)-Camphor is used by *P. tsushmanus* for host location. Although the decoys currently used need to be further optimized, for example, the mining of synergistic components, component distribution ratio, etc., it can be clearly that D(+)-Camphor is an indispensable key component in the development and utilization of decoys. In addition to interspecific chemical communication, the reproduction of insect population also includes the intraspecific chemical communication of courtship between female and male adults. Therefore, future studies should focus on the identification of pheromone components of intraspecific chemical communication between female and male adults, in order to comprehensively explore the adaptive mechanism of *P. tsushmanus* to its host plant and continuously control this pest below the economic threshold.

Declarations

Funding: The research was funded by National Natural Science Foundation of China (Grant number 32271884) and the Science and Technology Commission of Shanghai Municipality (Grant number 18391903200).

Conflicts of interest: The authors declare no competing financial interest.

Authors' contributions: Cong Chen and Dejun Hao conceived and designed the experiments and wrote the manuscript. Tian Xu, Shouyin Li, Mingyu Xue, and Yadi Deng performed the experiments and analysed

the data. Yadi Deng Binqi Fan, and Chufeng Yang collected insects. All authors read and approved the manuscript.

Acknowledgments

We acknowledge Peng Su, Liang Chen, and Yangyang Han for their assistance. Thanks for the financial support of the above funds.

References

1. Binder B F, Robbins J C, Wilson R L (2019) Chemically mediated ovipositional behaviors of the european corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J Chem Ecol* 21: 1315-1327.
2. Blaakmeer A, Geervliet J B F, Loon J J A (1994) Comparative headspace analysis of cabbage plants damaged by two species of *Pieris caterpillars*: consequences for in-flight host location by *Cotesia parasitoids*[J]. *Entomol Exp Appl* 73: 175-182.
3. Cai X M, Sun X L, Dong W X, et al (2014) Herbivore species, infestation time, and herbivore density affect induced volatiles in tea plants. *Chemoecology* 24: 1-14.
4. Chen C, Zhang C C, Li S Y, et al (2021) Biological traits and life history of *Pagiophloeus tsushmanus* (Coleoptera: Curculionidae), a pest on camphor trees in China. *J For Res* 32: 1979-1988.
5. Crook D J, David R L, Mastro V C (2014) Identification of a potential third component of the male-produced pheromone of *Anoplophora glabripennis* and its effect on behavior. *J Chem Ecol* 40: 1241-1250.
6. de Abreu J M (1997) Attraction of *carpophilus dimidiatus* and *C. obsoletus* (Coleoptera: Nitidulidae) to aggregation pheromones in stored cocoa. *Agrotropicap* 9: 41-48.
7. Guo S S, Geng Z F, Zhang W J, et al (2016) The chemical composition of essential oils from *Cinnamomum camphora* and their insecticidal activity against the stored product pests. *Int J Mol Sci* 17: 1836.
8. Guo X J, Yu Q Q, Chen D F, et al (2020) 4-Vinylanisole is an aggregation pheromone in locusts. *Nature* 584: 584-588.
9. Hallett R H, Oehlschlager A C, Borden J H (1999) Pheromone trapping protocols for the asian palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)[J]. *Int J Pest Manag* 45: 231-237.
10. Han J J, Li X W, Liu F J, et al (2017) Feeding Selection of Tea Cultivars by the Tea Weevil and the Four Induced Terpenoids. *Journal of Tea Science* 37: 220-227 (In Chinese).
11. Hanks L M (1999) Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annu Rev Entomol* 44: 483-505.
12. Havill N P, Raffa K F (2010) Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. *Ecol Entomol* 25: 171-179.

13. Jiang H, Wang J, Song L, et al (2016) GC×GC-TOFMS analysis of essential oils composition from leaves, twigs and seeds of *Cinnamomum camphora* L. Presl and their insecticidal and repellent activities. *Molecules* 21: 423-434.
14. Kiran S R, Reddy A S, Devi P S, et al (2006) Insecticidal, antifeedant and oviposition deterrent effects of the essential oil and individual compounds from leaves of *Chloroxylon swietenia* DC. *Pest Manag Sci* 62: 1116-1121.
15. Kong X B, Wang R, Gao W, et al (2001) Technique and applications of coupling gas chromatography with electroantennographic detector. *Entomological Knowledge* 38: 304-309 (In Chinese).
16. Landolt P J, Phillips T W (1997) Host plant influences on sex pheromone behavior of phytophagous insects. *Annu Rev Entomol* 42: 371-391.
17. Li N, Li S, Ge J (2017) Manipulating two olfactory cues causes a biological control beetle to shift to non-target plant species. *J Ecol* 105: 1534-1546.
18. Li S Y, Chen C, Jia Z Y, et al (2021) Offspring performance and female preference of *Pagiophloeus tsushmanus* (Coleoptera: Curculionidae) on three Lauraceae tree species. *J Appl Entomol* 145: 530-542.
19. Li S Y, Chen C, Li H, et al (2019) Effects of feeding on diets containing components of different plants on the development and detoxifying enzyme activities in *Pagiophloeus tsushmanus* (Coleoptera: Curculionidae) larvae. *Acta Entomologica Sinica* 62: 1286-1296 (In Chinese).
20. Li S Y, Wang J T, Chen C, et al (2022) Tolerance, biochemistry and related gene expression in *Pagiophloeus tsushmanus* (Coleoptera: Curculionidae) exposed to chemical stress from headspace host-plant volatiles. *Agric For Entomol* 24: 189-203.
21. Light, M D, Kamm J A, Buttery R G (1992) Electroantennogram response of alfalfa seed chalcid, *Bruchophagus roddi* (Hymenoptera: Eurytomidae) to host- and non host-plant volatiles. *J Chem Ecol* 18: 333-352.
22. Liu X L, Zhang J, Yan Q, et al (2020) The molecular basis of host selection in a crucifer-specialized moth-science direct. *Curr Biol* 30: 4476-4482.
23. Mitsuyoshi Y H, Hiroshi M, Kihachiro O (2002) Volatile components of Japanese cedar cultivars as repellents related to resistance to *Cryptomeria* bark borer. *J Wood Sci* 48: 51-55.
24. Morimoto K (1982) The family Curculionidae of Japan I Subfamily Hylobxinae. *Esakia* 19: 51-121.
25. Nenaah G E, Ibrahim S I A (2011) Chemical composition and the insecticidal activity of certain plants applied as powders and essential oils against two stored-products coleopteran beetles. *J Pest Sci* 84: 393-402.
26. Raguso R A, Light D M (1998) Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and 'green leaf volatiles'. *Entomol Exp Appl* 86: 287-293.
27. Rajapakse C N K, Walter G H, Moore C J, et al (2010) Host recognition by a *Polyphagous lepidopteran* (*Helicoverpa armigera*): primary host plants, host produced volatiles and neurosensory stimulation. *Physiol Entomol* 31: 270-277.

28. Rasmann S, Agrawal A A (2011) Evolution of specialization: a phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophthalmus*). *Am Nat* 177: 728-737.
29. Roh G H, Cha D H, Park C G (2021) Olfactory attraction to aggregation pheromone is mediated by disti-flagellum of antennal segments in *Riptortus pedestris*. *J Asia Pac Entomol* 24: 415-420.
30. Saini R K, Hassanali A, Andoke J, et al (1996) Identification of major components of larviposition pheromone from larvae of tsetse flies *Glossina morsitans morsitans* Westwood and *Glossina morsitans centralis* Machado. *J Chem Ecol* 22: 1211-1220.
31. Samartsev K, Hao D J, Li T (2021) A new species of the genus *Pseudocyanopterus* van Achterberg, Cao & Yang (Hymenoptera, Braconidae, Braconinae) from China. *J Hymenopt Res* 86: 79-92.
32. Shahid A A, Mudasir A D, Mahendiran G, et al (2017) The first record of pear psylla *Cacopsylla bidens* (Hemiptera: Psyllidae) from India along with notes on seasonal occurrence and some elements of its biology. *Orient Insects* 52: 101-111.
33. Singh R, Jawaid T (2012) *Cinnamomum camphora* (Kapur): review[J]. **Phcog J** 4: 1-5.
34. Sun X L, Wang G C, Cai X M, et al (2010) The tea weevil, *Myloccerinus aurolineatus*, is attracted to volatiles induced by conspecifics. *J Chem Ecol* 36: 388-395.
35. Sun X L, Wang G C, GAO Y, et al (2012) Screening and field evaluation of synthetic volatile blends attractive to adults of the tea weevil, *Myloccerinus aurolineatus*. *Chemoecology* 22: 229-237.
36. Tang H, Li Y Q, Luo C B (2022) Integrated Analysis of metabolome and transcriptome of *Bambusa emeiensis* shoots in response to bamboo snout beetle *Cyrtotrachelus buqueti* (Coleoptera: Curculionidae). *Phyton-Int J Exp Bot* 91: 1227-1244.
37. Tunings T C, Tumlinson J H (1992) Systemic release of chemical signals by herbivore-injured corn. *P NATL ACAD SCI USA* 89: 8399-8402.
38. Vet L E M, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annu Rev Entomol* 37: 141-172.
39. Wang Z C, Jiang X X, Jiang H Y, et al (2012) Application of major tree species for the CAST stereoscopic landscape demonstration project. *Journal of landscape research*, 4: 65-68, 71.
40. Wen X J, Yang K L, Piñero J C, et al (2021) Contrasting behavioral and electrophysiological responses of *Eucryptorrhynchus scrobiculatus* and *E. brandti* (Coleoptera: Curculionidae) to volatiles emitted from the tree of heaven, *Ailanthus altissima*. *Insects*, 12: 68-68.
41. Wu M T, Ni L, Lu H X, et al (2020) Terpenoids and their biological activities from *Cinnamomum*: a review. *J chem* 5097542. <https://doi.org/10.1155/2020/5097542>.
42. Xing Y, Chi D F, Jia Y, et al (2017) EAG and Behavioral responses of *Cryptorrhynchus lapathi* (Coleoptera: Curculionidae) to twelve plant volatiles. *Scientia Silvae Sinicae* 53: 159-167.
43. Xu T, Hansen L, Dong H C, et al (2020) Identification of a female-produced pheromone in a destructive invasive species: Asian longhorn beetle, *Anoplophora glabripennis*. *J Pest Sci* 93: 1321-1332.

44. Xu T, Yasui H, Teale S A, et al (2017) Identification of a male-produced sex-aggregation pheromone for a highly invasive cerambycid beetle, *Aromia bungii*. Sci Rep 7: 7330.
45. Xue H, Li W, Nie R, et al (2011) Recent speciation in three closely related sympatric specialists: inferences using multi-locus sequence, post-mating isolation and endosymbiont data. PLoS One 6: e27834.
46. Zhang C C, Wang M, Su P, et al (2017) Mating and oviposition behaviors of *Pagiophloeus tsushimanus* (Coleoptera: Curculionidae). Acta Entomologica Sinica 60: 1208-1215 (In Chinese).

Figures

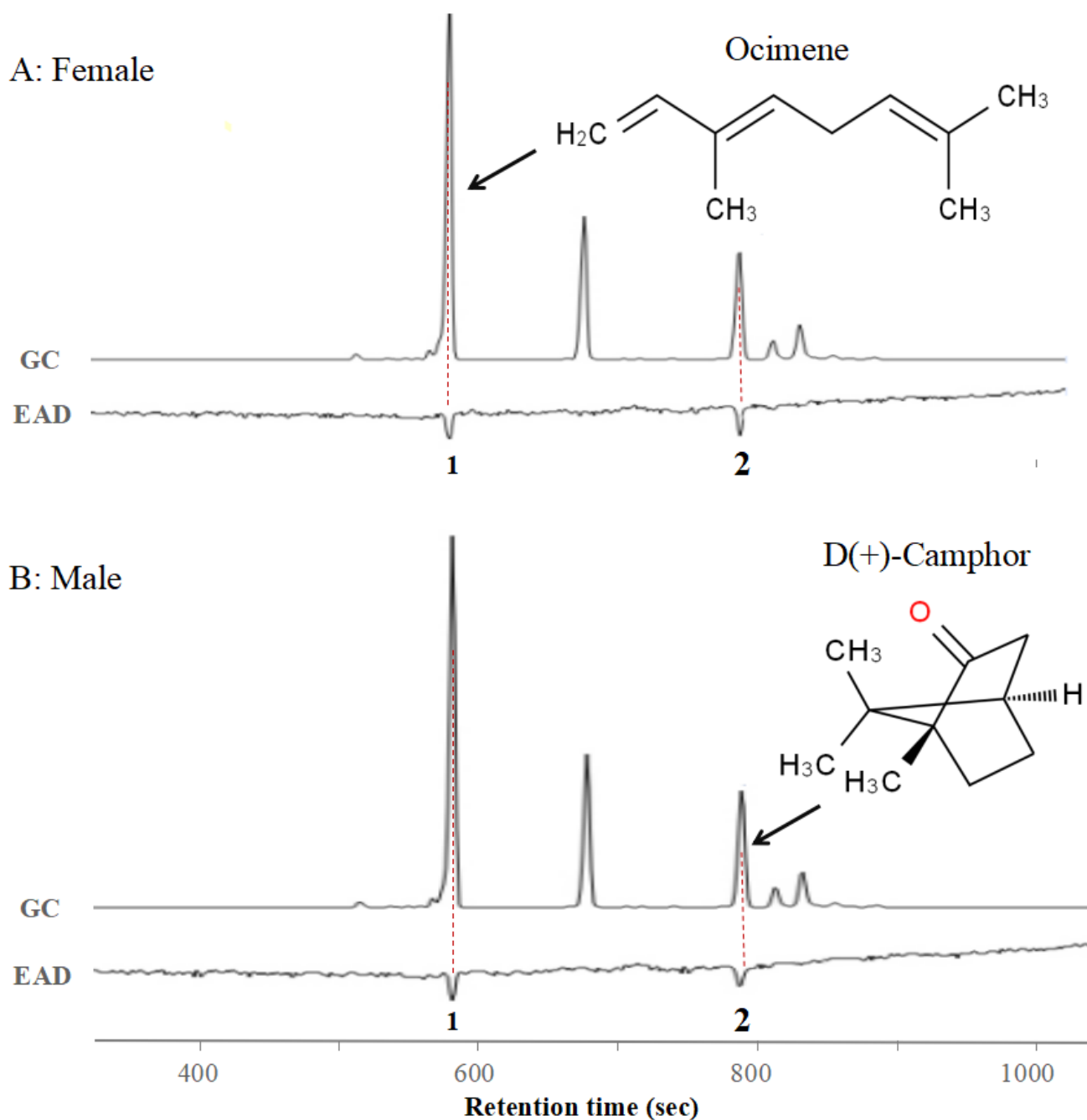


Figure 1

GC-EAD response of female (A) and male (B) adults antennae to volatiles of *C. camphora*. Number "1" refers to Ocimene and "2" refers to D(+)-Camphor.

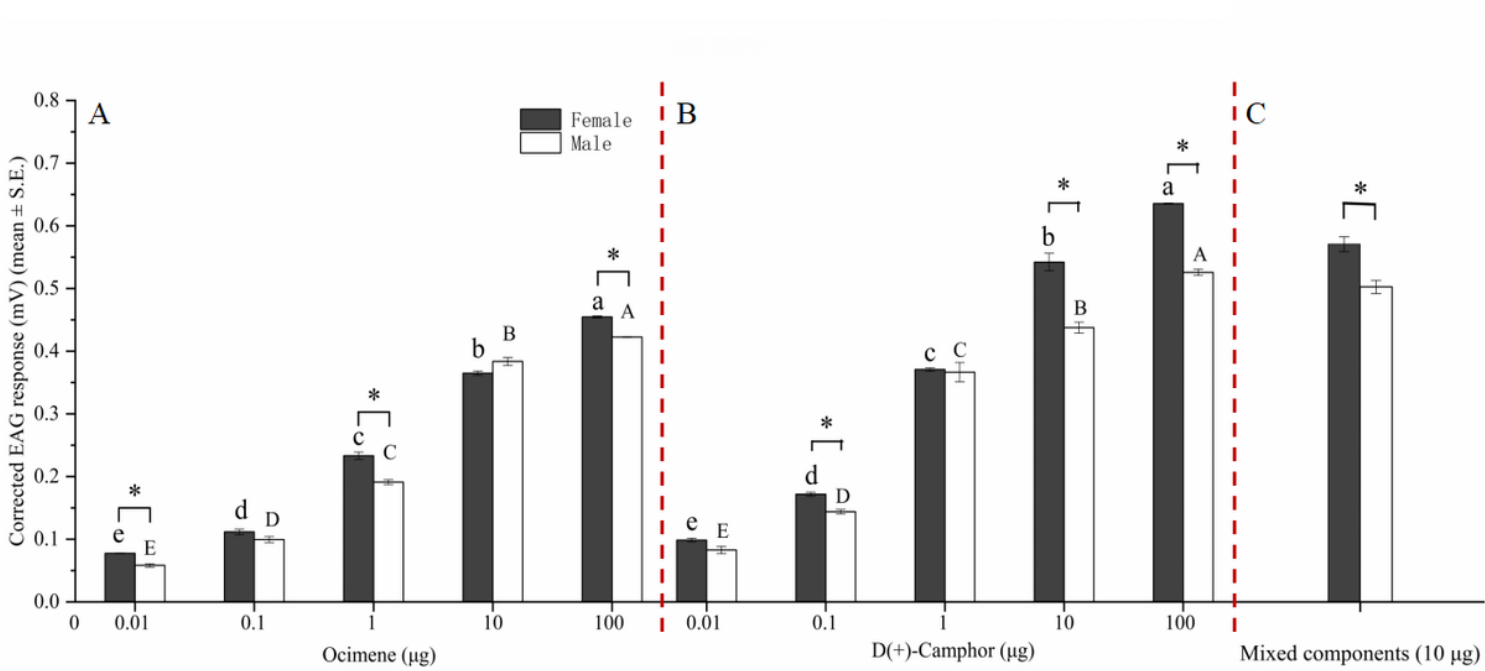


Figure 2

EAG response of females and males antennae to two single component and one mixed components. Single component Ocimene (A), single component D(+)-Camphor (B), and mixed components [D(+)-Camphor : Ocimene = 1 : 1] (C). Different capital letters indicate that the EAG response values of male adults to different concentration of the compound are significantly different ($P < 0.05$), different small letters indicate significantly different of female adults ($P < 0.05$), and asterisk "*" indicate significantly different between female and male adults ($P < 0.05$).

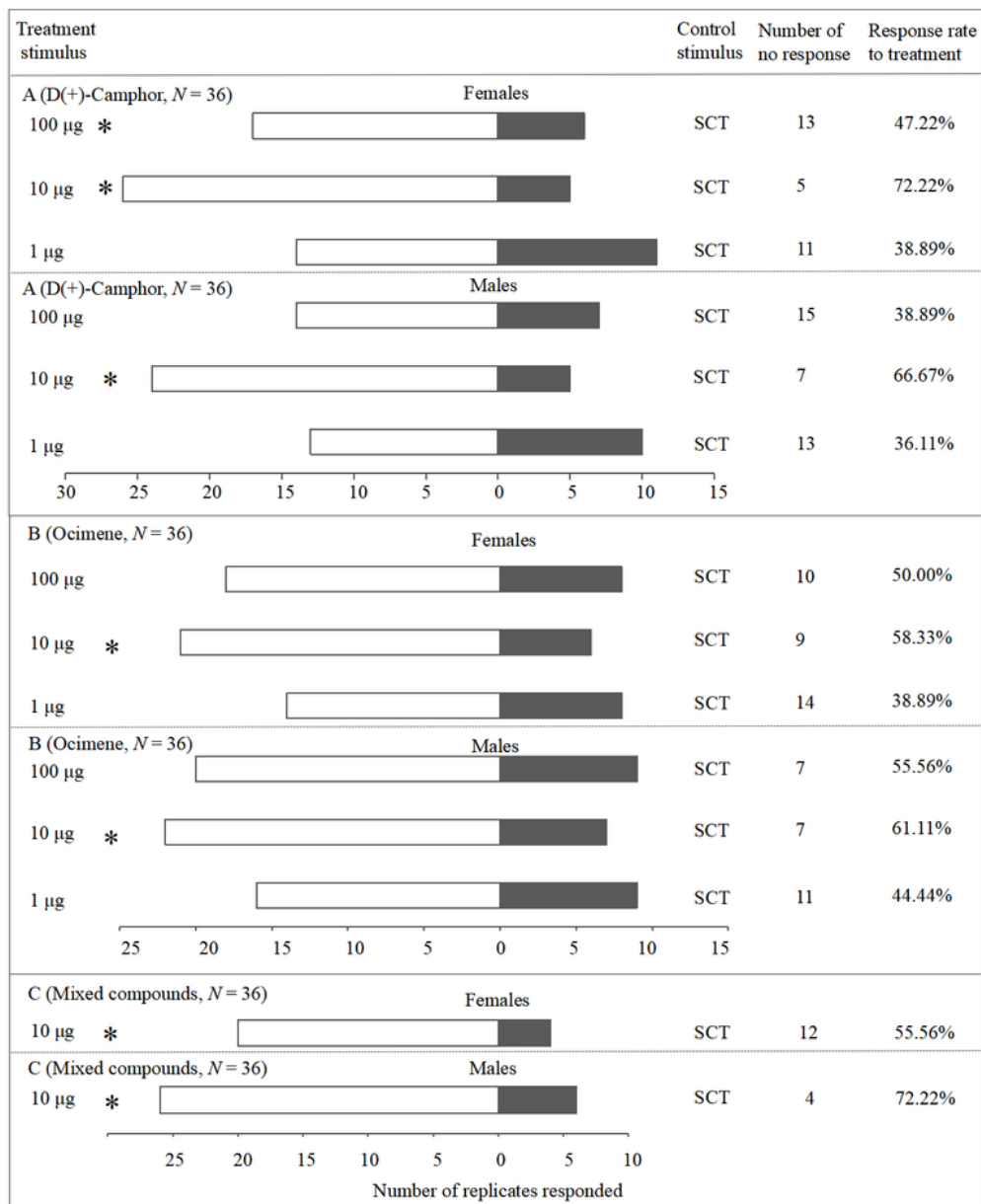


Figure 3

The responses of females and males to different dose of D(+)-Camphor (A), Ocimene (B), and mixed components [D(+)-Camphor : Ocimene = 1 : 1] (C) vs. solvent control treatment (SCT). Bars with an asterisk (*) indicate significantly different between treatment stimulus and solvent control treatment ($P < 0.05$).

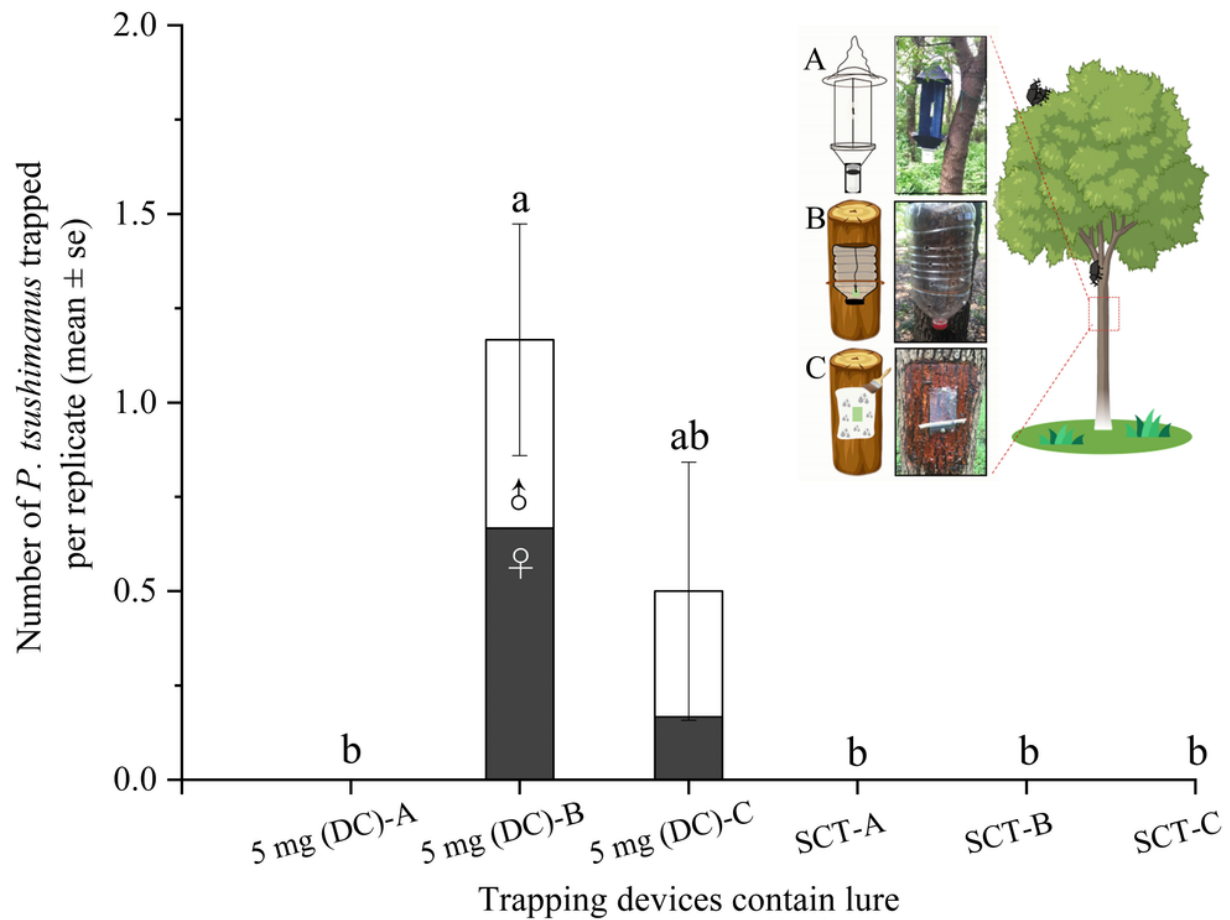


Figure 4

Number of *P. tsushimanus* adults captured at different trapping devices baited with 5 mg D(+)-Camphor (DC) or isopropanol solvent control treatment (SCT). Cross baffle trap device (A), self-made trap device (B), and trunk gluing trap device (C). Kruskal-Wallis H test followed by pairwise contrast tests were applied to compare each pair of treatments. Bars with the different letter are significant difference ($P < 0.003$, Bonferroni adjusted significant level).

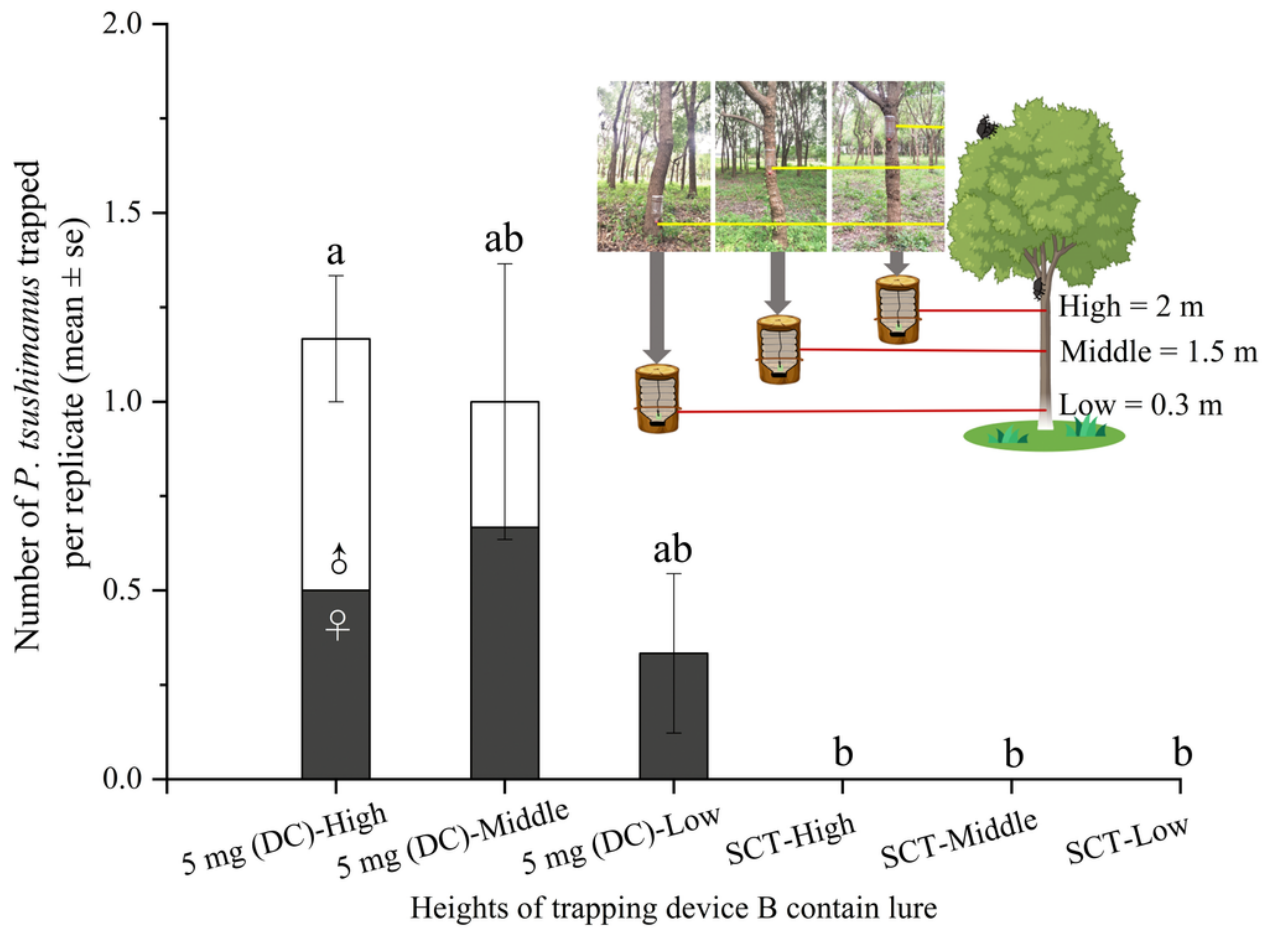


Figure 5

Number of *P. tsushimanus* adults captured at different heights of trapping device B baited with 5 mg D(+)-Camphor (DC) and isopropanol solvent control treatment (SCT). High (2 m above the ground), middle (1.5 m above the ground), and low (0.3 m above the ground). Kruskal-Wallis H test followed by pairwise contrast tests were applied to compare each pair of treatments. Bars with the different letter are significant difference ($P < 0.05/15 = 0.0033$, Bonferroni adjusted significant level).

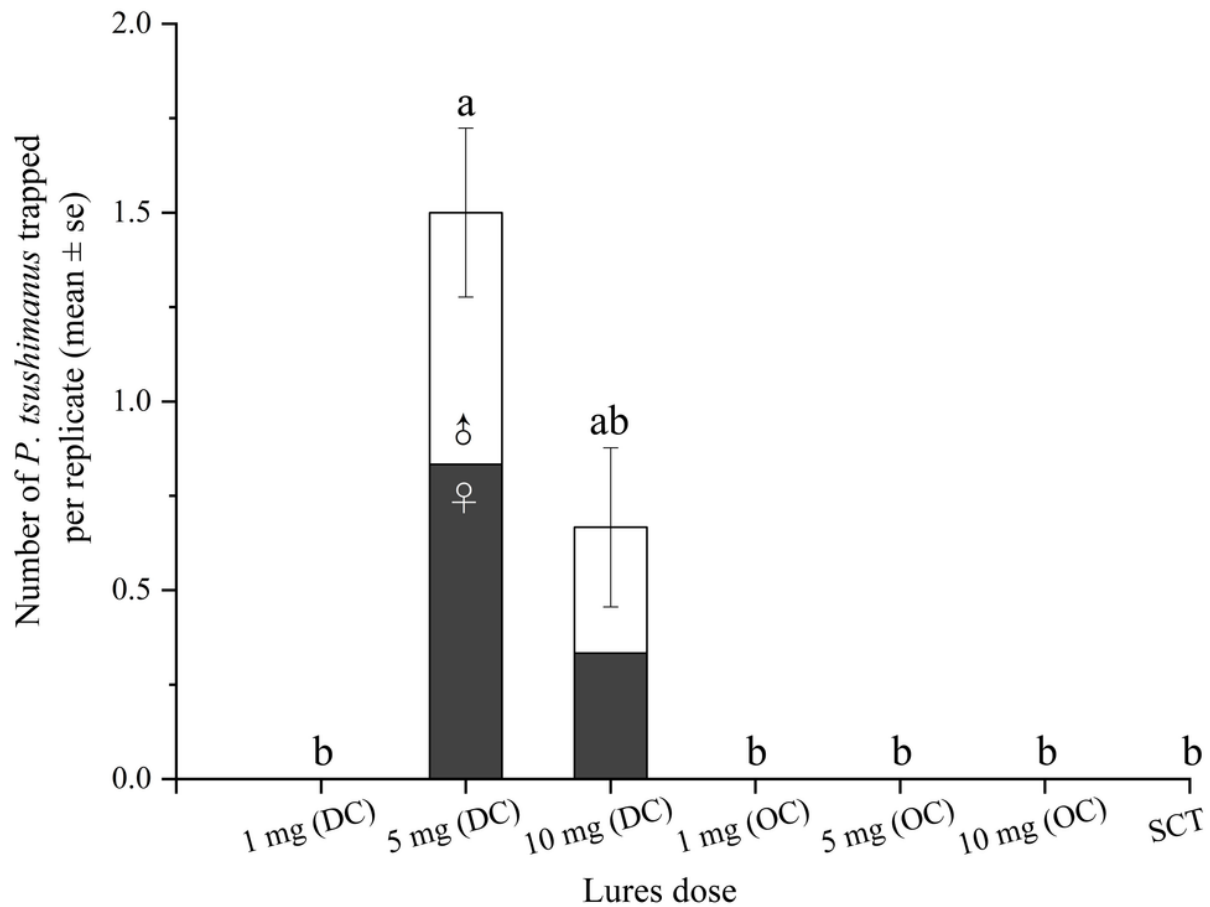


Figure 6

Number of *P. tsushimanus* adults captured at trapping device B baited with different lures of D(+)-Camphor (DC), Ocimene (OC), and isopropanol solvent control treatment (SCT). Kruskal-Wallis H test followed by pairwise contrast tests were applied to compare each pair of treatments. Bars with the different letter are significant difference ($P < 0.05/21 = 0.0023$, Bonferroni adjusted significant level).

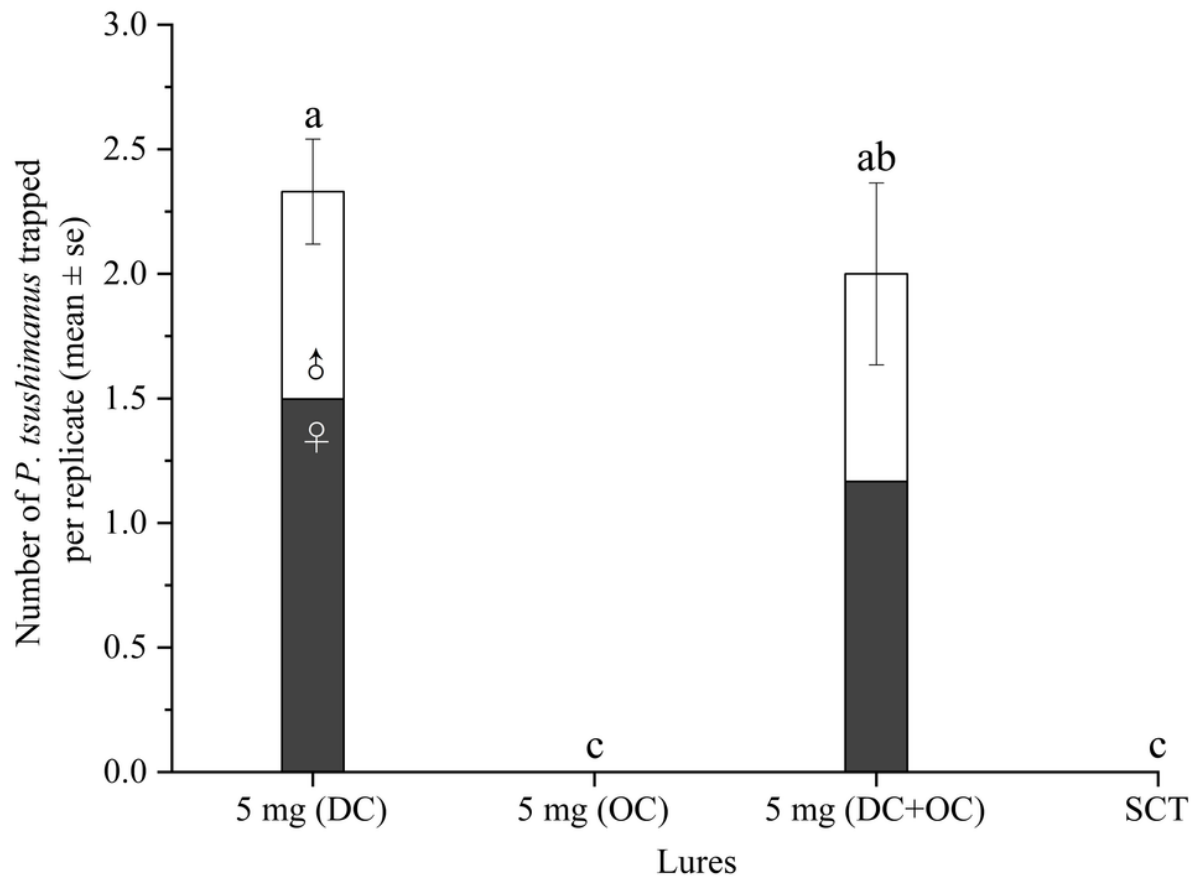


Figure 7

Number of *P. tsushimanus* adults captured at trapping device B baited with different lures of D(+)-Camphor (DC), Ocimene (OC), D(+)-Camphor+Ocimene (DC+OC), and isopropanol solvent control treatment (SCT). Kruskal-Wallis H test followed by pairwise contrast tests were applied to compare each pair of treatments. Bars with the different letter are significant difference ($P < 0.05/6 = 0.0083$, Bonferroni adjusted significant level).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)