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Attraction of adult *Harmonia axyridis* to volatiles of the insectary plant *Cnidium monnieri*

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ABSTRACT

Plant volatiles mediate the interactions within plant-insect communities and influence the distribution and behavior of insects. Natural enemies often utilize the volatiles that released from plants to locate the profitable patches for foraging. The lady beetle *Harmonia axyridis* (Pallas) is a dominant generalist predator of various pests in agriculture fields, yet little is known about the chemical cues which the predator employs to locate its prey. In this study, we tested the attractiveness of volatiles emanating from healthy and aphid-infested insectary plant *Cnidium monnieri* (L.) Cuss. to *H. axyridis* adults in a Y-tube olfactometer. Next, using coupled gas chromatography – electroantennogram detection (GC-EAD) and gas chromatography – mass spectrometry (GC–MS) identified the volatile components which elicited electrophysiological activity in *H. axyridis* adults. Lastly, Y-tube olfactometer tests in laboratory and field traps were used to verify the effectiveness of the volatile components. In olfactometer trials, *H. axyridis* adults preferred healthy and aphid-infested plants versus control. In GC–MS and GC-EAD trials, two antenna-active components (1,2-diethylbenzene and p-diethylbenzene) were detected among the volatile blends emanating from healthy and aphid-infested plants; and in ensuing behavioral assays, *H. axyridis* adults were attracted to two synthetic volatile compounds. Under field conditions, the number of *H. axyridis* adults as recorded in traps for two synthetic volatile components at three different concentrations (100 mg/ml, 10 mg/ml, and 1 mg/ml) were significantly higher than those for mineral oil (control). These findings could provide an effective means for the cultivation of insectary plants to develop natural enemy attractants and support the biological control in the field.

1. Introduction

Plants provide food and shelter to many insects and play a key role in the orchestration of multi-trophic interactions (Xu and Turlings, 2018). The interactions between plants and insects frequently resulted from the detection of a diversity of plant volatiles by the well-developed olfactory system of insects (Xu and Turlings, 2018). Plants usually emit a bouquet of volatile organic compounds (VOCs) of high volatility, low molecular weight, and lipophilic character, and these compounds easily meander a few meters to dozens of meters away from the source in natural environments (Pichersky et al., 2006; Pinto et al., 2008; Dicke et al., 2009; Loreto and Schnitzler, 2010). VOCs released from different structures of plant such as leaves, flowers, roots, and fruits (Kigathi et al., 2019). These act as a language that plants used for their communication and interaction with the surrounding environment (Dudareva et al., 2006). VOCs can also mediate many interactions within a plant-insect community and influence the behavior and distribution of insects (Dicke, 2015; Song et al., 2017). The host searching ability of natural enemies mainly depends on the chemical cues in field conditions (Dicke, 2016; Thiel et al., 2017). For instance, the volatiles from sunflower could attract the natural enemies and pollinators broadly (Jones and Gillett, 2005; Adedipe and Park, 2010).

When a plant was damaged by herbivorous insects, additional plant volatiles known as herbivore-induced plant volatiles (HIPVs) released

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from it (Paré and Tumlinson, 1999; Xiu et al., 2019b). HIPVs are complex blends consisting of phenolics, terpenoids, and green leaf volatiles (Howe and Jander, 2008), and play a central role in the recruitment of various insects (Dicke and Van Loon, 2000; Aartsma et al., 2017). The predator lady beetle *Coccinella septempunctata* was observed to aggregate in the soybean plants that infested with the soybean aphid *Aphis glycines* Matsumura in fields, while this response was thought to be mediated by aphid-induced plant volatiles (Zhu and Park, 2005). In previous investigations, the HIPVs of methyl salicylate (MeSA) proved to be a broad-spectrum attractant and bring into being considerable responses from key beneficial insect groups including the families Coccinellidae, Syrphidae, Anthocoridae, lacewings, parasitic Hymenoptera, and predaceous Heteroptera (James and Price, 2004; Rodriguez-Saona et al., 2011). Therefore, plant volatiles (e.g. VOCs and HIPVs) were used not only by herbivorous insects to find their hosts, but also by the natural enemies of the herbivores to find their prey (Aartsma et al., 2017; Xu and Turlings, 2018).

The lady beetle, *Harmonia axyridis* (Pallas) is the generalist predator of aphids and other soft-bodied insect pests in various cultivated lands (Spellman et al., 2006; Qiu et al., 2010; Brown, 2011; Song et al., 2017). In recent years, the artificial breeding and field release of *H. axyridis* for biological control have received increasing attention (Xiu et al., 2019a). Chemicals mediated (VOCs, HIPVs, aphids, and honeydew) played various roles of fundamental functions in the ecology of lady beetles, including the finding and predation of prey, detection of mates and competitors, aggregation and defense mechanisms against natural enemies (Hemptinne and Dixon, 2000; Provost et al., 2006; Pasteels, 2007; Leroy et al., 2010; Sloggett et al., 2011; Wang et al., 2015). Volatiles from the nettle *Urticadioica* L. and Chinese pagoda *Sophora japonica* L. flowers attracted the adults of *H. axyridis* under laboratory and field conditions (Leroy et al., 2012; Xiu et al., 2019a). Moreover, the volatiles from twigs and leaves of *S. japonica* possessed the most attractive activity to *H. axyridis* by using the Y-olfactory instrument (Xue et al., 2008). Some healthy plants have not effectively attracted the adults of *H. axyridis*, but the plants damaged by herbivorous arthropods showed high level of attraction. Likewise, the volatiles of prickly ash *Zanthoxylum bungeanum* damaged by mealybug *Phenacoccus azalea* and tea shoots attacked by tea aphids *Toxoptera aurantii* (Boyer de Fonscolombe) attracted to the adults of *H. axyridis* (Han and Chen, 2002; Xie et al., 2004). The volatiles from four different aphid × plant combinations attracted the adults of *H. axyridis* (Xiu et al., 2019b). A number of synthetic compounds have been identified as attractants, such as (*E*)-β-farnesene, (−)-β-caryophyllene, (*Z*)-3-hexenol, and (*E*)-2-hexenal, equally attract *H. axyridis* (Verheggen et al., 2007; Leroy et al., 2012). However, there is limited research to use plant volatiles to attracted *H. axyridis* for large-scale biological control in the fields.

Cnidium monnieri (L.) Cuss., is an annual herb of the umbelliferae family, which has important medicinal and economic value and widely distributed in most parts of China. It was initially discovered to maintain a number of natural enemies of insect pests including lady beetles, notably *H. axyridis* adults and larvae, and has the basic feature of an insectary plant (Yang et al., 2018). In previous field survey, it was found that many adults and larvae of *H. axyridis* gathered on these plants. In addition, it was also found a few of celery aphid, *Semiaphis heracleid* (Takahashi) on some plants (approximately 30 aphids per plant). Moreover, there were no significant differences in the population of *H. axyridis* in plants with and without celery aphids (unpublished results). However, there is still lack of study regarding the mechanism of chemical ecology of *C. monnieri* plants to attract the natural enemies. The main objectives of this study were to: (1) Whether the volatiles from *C. monnieri* are effective in attracting *H. axyridis*; (2) Whether a few of celery aphids on *C. monnieri* stimulated more *H. axyridis* to gather on the plants; (3) Whether active compounds effectively trapping *H. axyridis* in the field. In this study, we identify plant volatiles that attract *H. axyridis* adults from healthy and aphid-infested plants of *C. monnieri,* examining their behavioral and electrophysiological responses in adults of *H. axyridis,* and evaluation of their potential in the field application as attractants. Our studies are useful for further planting insectary plants and developing the synthetic attractant of *H. axyridis* to biological control.

2. Materials and methods

2.1. Experiment materials

Insects: The eggs, larvae and adults of *H. axyridis* were collected from farmland and orchard near the Yantai Experimental Station of the Institute of Zoology, Chinese Academy of Sciences (37°43′34.5″ N, 120°55′16.7″ E) (Shandong Province, China). After collection, the eggs were placed in Petri dishes (diameter: 9 cm), while the larvae and adults were placed in mesh cages (25 × 25 × 30 cm) an indoor and held at approximately 25 °C, 60% relative humidity (RH) and 14:10 h L:D photoperiod. After hatching of the eggs, the larvae were moved to the above cages and raised in the same environment. Beetle colonies were fed with the pea aphid *Acyrthosiphon pisum* Harris, reared on broad bean *Vicia faba* L. seeding in the laboratory. For all the behavioral response and electrophysiological trials 4 to 10-day unmated female and male adults of *H. axyridis* were used. The celery aphids were collected from the insectary plant *C. monnieri* and the colonies were fed with *C. monnieri* in the laboratory.

Plants: The insectary plant *C. monnieri* was planted in pots (25 × 16.5 cm) in the laboratory and held at approximately 25 °C, 60% relative humidity (RH) and 14:10 h L:D photoperiod. Upon emergence, plants were covered with zippered gauze cages (65 × 65 × 120 cm) to prevent entry of insects. Once plants had reached the flowering stage, half of the plants were infested with celery aphids, 30 third- or fourth-instar juveniles of *S. heracleid* were placed on each plant for 24 h. Both healthy and aphid-infested plants were used in behavioral response trials and volatiles collection.

Reagents: The synthetic chemicals were used in both the laboratory and the field trials (Table S1), while the mineral oil (Aladdin, Shanghai, China) was used as blank and solvent in the experiment.

2.2. Behavioral responses to plant volatiles

Insect behavioral responses to plant volatiles were conducted using Y-tube olfactometer. The olfactometer have the following specifications: a 3 cm inner diameter clear glass tube with a 20 cm long central tube and two 20 cm long lateral arms with a 60° angle at the Y-junction. The apparatus was placed in a 100 × 100 × 60 cm chamber that was illuminated with two 40 W fluorescent lamps (light intensity 2000 lx) maintained at 25 ± 1 °C, 60 ± 5% RH. An atmospheric sampler (QC-1S, Beijing Institute of Labor Instrument, Beijing, China) pushed air through a drying tower (250 ml) filled with activated charcoal. An Erlenmeyer flask filled with distilled water and an oven bag (30 × 40 cm), the airflow was maintained at 400 ml/min and entered the olfactometer via silicone tube. During study, two oven bags were used while in each of the bags connected to either of two arms of the olfactometer. Three different arrangements were used for assessment of behavioral responses of *H. axyridis* females and males in the olfactometer: (1) Healthy plants Vs. blank; (2) Aphid-infested plants Vs. blank; (3) Healthy plants Vs. aphid-infested plants.

Celery aphids of the plants were cleaned before olfactory trials, and the plants were rinsed with water as aphid-infested plants. The top of each plant was sealed approximately at 25 cm (including leaves and flowers) with an oven bag as a source of odor. Before the behavioral responses, unmated *H. axyridis* adults were starved for 24 h, each individual was used only once. Plants were replaced after every hour and the Y-tube olfactometer was replaced with a clean one after four individuals had been tested. For each treatment, 60 female and 60 male adults of *H. axyridis* were tested. All bioassays were conducted between

08:00 am and 18:00 pm. The choice of each *H. axyridis* adults after set period of time was recorded. To begin the test, an individual was released at the bottom of the Y-tube and given 5 min reaction time. Once the adult of *H. axyridis* crossed the right or left arm of the apparatus and stayed there for at least 5 s, a choice was recorded. If an individual has not made a choice for either arm within the allotted 5 min., it was removed and recorded as "no choice".

2.3. Identification of the active volatiles

***Volatiles collection*:** Headspace sampling was used to collect healthy and aphid-infested plant volatiles in the laboratory. Once plants reached the flowering stage, healthy and aphid-infested plants were moved to the laboratory for volatiles collection. The selected plant parts (including leaves and flowers) were sealed in an oven bag (40 × 50 cm). Clean air was introduced from the lower part of the bag and allowed out of the top through a volatile collection trap, a glass tube (inner diameter: 5 mm) containing 200 mg of 80–100 mesh PoraPak^{TM} Q adsorbent (Bulk Packing Material, Altech. Assoc., USA). Before collection, the tube containing PoraPak™ Q adsorbent at 180 °C, and nitrogen flow rate (10 ± 2) ml/min to activate for 8 h (In the preliminary test, we eluted the activated adsorbent with n-hexane and determined its composition by GC–MS. The results see Fig. S2). The collection of volatiles lasted for 6 h (06:00–12:00) and replicated six times. It was added a total of 1 ml HPLC-grade n-hexane (C_6H_{14}) (Aladdin, Shanghai, China) solution to eluted the volatile compounds. Extracts were stored at −20 °C in 4 ml glass vials (Agilent, USA) and later used for coupled gas chromatography-electro antennogram detection (GC-EAD) and gas chromatography-mass spectrometry (GC–MS) trials.

GC-EAD recordings: GC-EAD studies were conducted with samples of volatiles from healthy and aphid-infested plants against either female or male of *H. axyridis* adults to detect antenna-active compounds. The GC (6890N, Agilent Technologies, Palo Alto, CA, USA) procedures were as follows: the initial column temperature was 50 °C (1 min hold) with 5 °C/min increasing until 240 °C. The techniques of EAD (Syntech, Hilversum, Netherlands) used in these trials were similar to those described in Xiu et al. (2019b). Initially, the head of *H. axyridis* adult female or male was excised from its body with a scalpel, so that all the segments and basal nerves were still connected, and the tip of the antenna was cut slightly to facilitate electrical contact. Secondly, the tip of the antenna was connected to a reference electrode (diameter: 1 mm), and the basal antenna was immersed in Ringer's saline solution (Table S2). The signal pass through the amplifier (UN-06, Syntech), then connect to the IDAC converter (Auto Spike, IDAC2/3, Syntech), finally connect to the date acquisition card of the computer, collect and analyze the data through the computer software (EAD 2.3, Syntech). Ultimately, if one component emerged at least 6 times in every 10 successful recordings, it was considered as a physiologically active compound.

***GC–MS analysis*:** We analyzed 2 μl of the volatile samples that elicited GC-EAD active peaks using GC–MS detection (6890N-5975N, Agilent Technologies, Palo Alto, CA, USA). The GC method followed the above-mentioned procedures. The volatiles were separated by GC method successively enter quadrupole mass spectrum, the ionization mode was EI 70 eV, the flow rate of helium as carrier gas was 1 ml/min., the temperature of source and transfer line was 250 °C, the scanned range was 35–450 amu and the scanning speed was 1000 amu/s. Active volatiles were tentatively identified by comparing mass spectrum with those of authenticated samples in the NIST 2008 database and were further confirmed by co-injecting the collected volatiles with commercially available synthetic chemical standards on both non-polar HP-5MS and polar DB-WAX columns (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Palo Alto, CA, USA) with peak enhancement indicating co-elution.

2.4. Behavioral response to antenna-active components

Behavioral responses of the adults of *H. axyridis* to each antenna-active component were evaluated in the Y-tube olfactometer following protocols as above, 2 μl identified chemical stimuli (antenna-active compound diluents, 10 μl/ml) and a control (mineral oil, the solvent) were applied separately onto 20 × 50 mm filter paper and placed into a plastic tube that was randomly connected to either olfactometer arm. New filter papers were used after every individual *H. axyridis* adults with a total of 60 females and 60 males subject to be tested.

2.5. Field trapping

A field trap experiment was also conducted in the apple orchard to determine the role of 1,2-diethylbenzene (Adamas, Shanghai, China) and p-diethylbenzene (Aladdin, Shanghai, China) volatiles to attract the adults of *H. axyridis* under field conditions at Yantai Experimental Station during 20 June − 5 July 2019. The active compounds lure was consisted of 1,2-diethylbenzene or p-diethylbenzene, macro-porous adsorbent resin (Haoju, Tianjin, China), non-woven bag (6.5 × 8.5 cm), and slow-release ball (diameter: 4.5 cm, many holes in the surface). We dissolved 1,2-diethylbenzene and p-diethylbenzene in mineral oil at three concentrations (100 mg/ml, 10 mg/ml, and 1 mg/ml) and extracted 1 ml of the liquid with each concentration and set aside into a non-woven bag with 10 g macro-porous adsorption resin, then kept the non-woven bag into the slow-release ball. Each concentration of an active compound was used in a total of three traps and the mineral oil was used in controls. A slow-release ball was placed into a pot trap (length: 20 cm; upper diameter: 30 cm; lower diameter: 25 cm). Traps were hung from branches of apple trees and with 1–1.5 m distance from the ground. Each trap was placed more than 30 m apart and traps with different compounds and concentrations were randomly arranged in the apple orchard. Every three days, the number of *H. axyridis* adults in each pot was counted, all adults were removed from the trap and lures were replaced.

2.6. Data analysis

For the behavioral responses, the null hypothesis was that lady beetles showed no preference for either olfactometer arm (i.e., 50:50 responses). This assumption was analyzed using χ^2 goodness-of-fit tests, with no response individuals excluded from the analysis. A one-way ANOVA was performed to assess the effect of each active compound concentration on lady beetle trap capture rates. For all analysis, the number of trapped *H. axyridis* adults was transformed by $\log_{10}(x + 1)$ to meet normality assumptions. All statistical analysis was conducted using SPSS 20.0.

3. Results

3.1. Behavioral responses of the adults of H. axyridis to plant odors

In Y-tube olfactometer assays, *H. axyridis* adults preferred odors of healthy plants versus blank (Fig. 1 and Table 1). Also, the beetles preferred odors of aphid-infested plants versus blank (Fig. 1 and Table 1). The odors of aphid-infested plants in comparison with the healthy plants, *H. axyridis* adults had no significant differences (Fig. 1 and Table 1). In the three groups above, there were no significant differences between female and male of *H. axyridis* adults (Table 2).

3.2. GC-EAD and GC–MS analysis

GC-EAD recordings with volatiles were taken from healthy plants and aphid-infested plants. A total of two antenna-active components were detected among the volatile blends emanating from healthy and aphid-infested plants. The two antenna-active components elicited the GC-EAD response in females and males of *H. axyridis* adults (Fig. 2). The

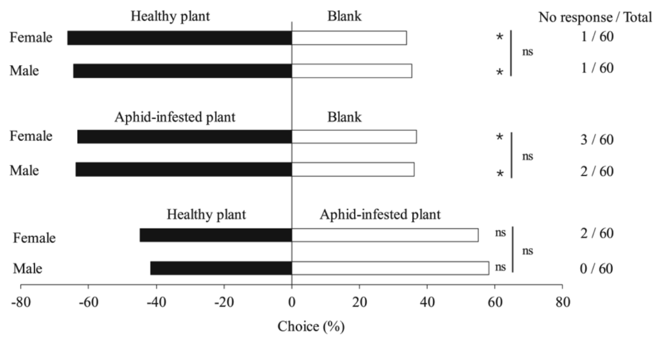


Fig. 1. Behavioral responses of female and male of *Harmonia axyridis* adults to different combinations in the Y-tube olfactometer assays. "*" denotes a significant difference at the P < 0.05 level; "ns" indicates no significant difference.

Table 1
Results for the chi-square tests regarding the effects of female and male of the *H. axyridis* adults to different combinations in the Y-tube olfactometer assays.

Combinations	Gender	χ^2	*df*	*P*
Healthy plant Vs. Blank	Female	6.119	1	0.013
	Male	4.898	1	0.027
Aphid-infested plant Vs. Blank	Female	3.947	1	0.047
	Male	4.414	1	0.036
Healthy plant Vs. Aphid-infested plant	Female	0.621	1	0.431
	Male	1.667	1	0.197
1,2-diethylbenzene Vs. Mineral oil	Female	8.067	1	0.005
	Male	4.898	1	0.027
p-diethylbenzene Vs. Mineral oil	Female	6.119	1	0.013
	Male	4.267	1	0.039

Table 2
Results for the chi-square tests regarding the effects between females and males of the *H. axyridis* adults to different combinations in the Y-tube olfactometer assays.

Combinations	χ^2	*df*	*P*
Healthy plant Vs. Blank	0.037	1	0.847
Aphid-infested plant Vs. Blank	0.005	1	0.944
Healthy plant Vs. Aphid-infested plant	0.12	1	0.729
1,2-diethylbenzene Vs. Mineral oil	0.206	1	0.65
p-diethylbenzene Vs. Mineral oil	0.1	1	0.752

antenna-active peaks, as identified by GC–MS, included 1,2-diethylbenzene and p-diethylbenzene (Fig. 2 and Fig. S1).

3.3. Behavioral responses to antenna-active compounds

Olfactometer trials showed that females and males of *H. axyridis* adults significantly preferred 1,2-diethylbenzene and p-diethylbenzene, as compared to the control (Fig. 3 and Table 1). Comparing the above two groups, there were no significant differences between females and males of *H. axyridis* adults (Table 2).

3.4. Evaluation of the field level trapping

The number of *H. axyridis* adults were trapped in 1,2-diethylbenzene attractant at three concentrations were significantly higher than control ($F_{3,8}$ = 5.983, P = 0.019; Fig. 4a). Also, the number of *H. axyridis* adults were trapped in p-diethylbenzene attractant at three concentrations were significantly higher than control ($F_{3,8}$ = 7.136, P = 0.012; Fig. 4b). In addition, the trapping effect of the two active compounds decreased with the decrease of concentration. The highest *H. axyridis* adults capture rate was recorded for 100 mg/ml p-diethylbenzene, reaching 4.33 ± 0.67 individuals per trap.

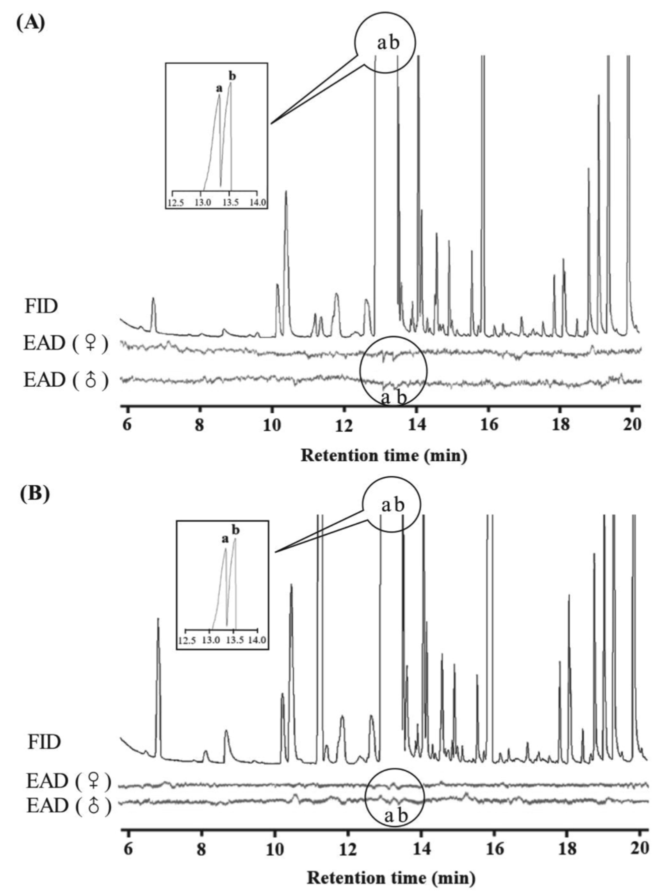


Fig. 2. Coupled GC-EADs of female and male of *Harmonia axyridis* adults to healthy plant volatiles (A) and aphid-infested plant volatiles (B). "a" means the antenna-active component 1,2-diethylbenzene; "b" means the antenna-active component p-diethylbenzene; "FID" means the volatile blends export by GC; "EAD" means the electrophysiological response of *H. axyridis* antenna.

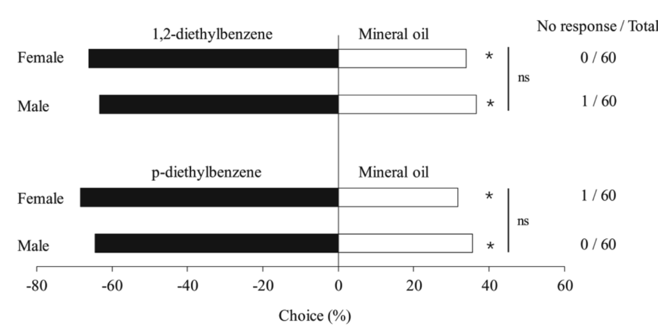
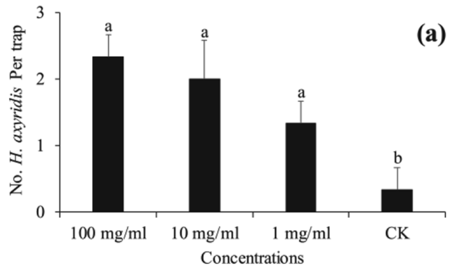


Fig. 3. Behavioral responses of female and male of *Harmonia axyridis* adults to antenna-active component in the Y-tube olfactometer assays. "*" denotes a significant difference at the P < 0.05 level; "ns" indicates no significant difference.

4. Discussion

Plants are known to emit volatile organic compounds (VOCs) in response to various stresses, include plant species, leaf growth stage, herbivore species, herbivore age and identity, and phytopathogen (Takabayashi et al., 1994; Rostás et al., 2006; Abel et al, 2009; De Moraes et al., 2001; Toome et al., 2010; Cai et al., 2014; McCormick et al., 2014; Ponzio et al., 2014). Natural enemies of herbivorous insects often utilize plant volatiles to locate advantageous patches for foraging (Hare, 2011; Oliveira and Pareja, 2014). In this study, we used olfactory behavior analysis and electrophysiological responses of insect antennae



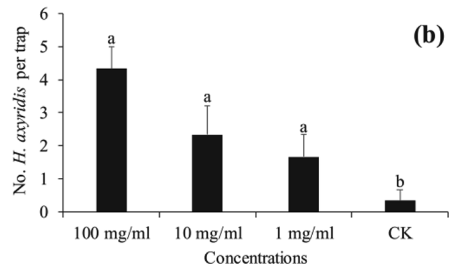


Fig. 4. Field trapping effect of three different concentrations of 1,2-diethylbenzene (a) and p-diethylbenzene (b) on the *Harmonia axyridis* adults. Data are presented as the means ± SEMs. Different lowercase indicates a significant difference at the $P < 0.05$ level.

in the laboratory and trapping test in the field to elucidate the role of plant volatiles in attracting natural enemies and possibly enhancing the pest's biological control.

Plant volatiles play an important role in recruiting the natural enemies (Snoeren et al., 2010; Lucchi et al., 2017; Uefune et al., 2017). When the plants were damaged by herbivorous insects, the quantity of volatile released from damaged plants was more than that released form the normal plants. Then the plants damaged by herbivorous insects enhanced the attraction of natural enemies (Steidle et al., 2001; Dudareva et al., 2004; Holopainen and Blande, 2012; Oliveira and Pareja, 2014). However, some studies showed that the aphids appeared not to induce a reaction at all in the plant, despite a very heavy infestation (Turlings et al., 1998). In our previous investigation, a few of celery aphids were found in some of the insectary plants *C. monnieri*, and there was no significant difference in the population of *H. axyridis* in plants with and without celery aphids (unpublished results). Our olfactometer trials showed that *H. axyridis* adults to be attracted to volatiles emitted by healthy and aphid-infested plants, and there was no significant difference in volatile components between healthy and aphid-infested plants, it likely that too few aphids on plants or absence of emissions of volatiles in response to aphid infestation (Turlings et al., 1998). Additionally, the volatiles from aphids, aphid honeydew, and aphid-emitted alarm pheromones also attract natural enemies (Verheggen et al., 2007; Leroy et al., 2012). In this study, the aphids were cleaned from plants before olfactometer trials and volatile collection, and washed plants with water. Thus, the aphids and honeydews likely affected *H. axyridis* behavioral assessments to minor extent.

Plants such as woody and herbaceous could attract predators during flowering period. In a similar way as in earlier trials with Chinese pagoda *Sophora japonica* (Xiu et al., 2019a), *Eleutherococcus senticosus* and *E. sessiliflorus* (Liu et al., 2002), perennial herb *Scabiosa tschiliensis* Grünning (Liu et al., 2004), bugle weed *Ajuga reptans*, dill *Anethum graveolens*, marigold *Tagetes tenuifolia*, sunflower *Helianthus annuus*, and golden rod *Solidago* sp. (Adedipe and Park, 2010). As an insectary plant, *C. monnieri* could maintain a number of predatory natural enemies (Yang et al., 2018; Cai et al., 2019). The study showed that during the flowering period of *C. monnieri*, a large number of predatory natural enemies were gathered on the plants, such as *H. axyridis*, *Propylaea japonica*, *Hippodamia variegata*, *Chrysoperla sinica*, and *Episyrphus balteata* (unpublished results). The olfactometer trials showed that *H. axyridis* adults attracted to the volatiles emitted by flowering stage of *C. monnieri* plants.

Plant volatiles were composed of many components, but for certain natural enemies, single or more volatile compounds could prove highly striking (Yu et al., 2010). Likewise, for the predators *H. axyridis*, a single synthetic compound of nonanal from *S. japonica* that elicited a significant electrophysiological response (Xiu et al., 2019a). In this study, the antenna-active components were 1,2-diethylbenzene and p-diethylbenzene. Previous studies have shown that 1,2-diethylbenzene and p-diethylbenzene could induce electrophysiological responses in antennae of *H. axyridis* adults (Xiu et al., 2019b). In this field trials, antenna-active compounds 1,2-diethylbenzene and p-diethylbenzene independently engrossed the *H. axyridis* adults. Field attraction of single active compounds to important natural enemies has also been demonstrated (Lee, 2010; Rodriguez-Saona et al., 2011; Silva et al., 2017). Previous study showed that the number of natural enemies' response to the mixture of antenna-active components were more than those to individual compounds. For example, the parasitoid *Opius dissitus*, a blend of active volatiles was significantly more attractive than any one single component (Wei and Kang, 2006). Therefore, it is necessary to research the attraction effect of different combinations of active compounds on natural enemies in the future works.

Conservation biological control (CBC), which aimed to control pests by promoting the population of natural enemies, had received increasingly attention in recent decades (Tschumi et al., 2016; Gurr et al., 2017; Michaud, 2018). However, practically the lower population of natural enemies and an overall temporal asynchrony with target pests limited their wide uses (Kean et al., 2003). Our investigations provide a basis for the production and utilization of natural enemy attractants and possible improvement of biological control. At the same time, *C. monnieri* in our study could also be used as an insectary plant to improve biological control. The insectary plant *C. monnier* could provide various conveniences to natural enemies including shelter, nectar, alternative prey/hosts and pollen (SNAP) (Naranjo et al., 2015; Gurr et al., 2017). Especially flowering plants could provide natural enemies with pollen, nectar and other nutrients, which played an important role in the longevity, activity and fertility of natural enemies (Venzon et al., 2006; Kopta et al., 2012; Ramsden et al., 2015). The insectary plant *C. monnieri* not only emits volatile organic compounds that attract natural enemies, and also provide pollen, nectar, prey, and shelter to natural enemies. Therefore, we should strengthen the study of combined application of active substances and insectary plants in the future research.

CRediT authorship contribution statement

Zhiping Cai: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Fang Ouyang:** Data curation, Formal analysis, Supervision, Writing - original draft. **Jianwei Su:** Data curation, Formal analysis, Methodology, Software. **Xingrui Zhang:** Investigation. **Chenglin Liu:** Investigation. **Yunli Xiao:** Conceptualization, Project administration, Supervision, Writing - review & editing. **Jianping Zhang:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Feng Ge:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2020.104189.

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