Screening of Essential Oil Antifeedants in the Elm Pest Ambrostoma quadriimpressum (Coleoptera: Chrysomelidae)

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Screening of essential oil antifeedants in the elm pest *Ambrostoma quadriimpressum* (Coleoptera: Chrysomelidae)

*Yinliang Wang, Xue Xing, Hanbo Zhao, Qi Chen, Wenqi Luo, and Bingzhong Ren**

Abstract

The leaf beetle *Ambrostoma quadriimpressum* Motschulsky (Coleoptera: Chrysomelidae) is a major pest of elm (*Ulmus*; Rosales: Ulmaceae) in eastern Asia, and there is currently no effective, environmentally friendly, chemical method for its control. In this study, we measured *A. quadriimpressum* adults' electrophysiological and behavioral responses to 13 compounds, including 6 plant volatiles (linalool, α-pinene, methyl salicylate, indole, di-n-octyl phthalate, dimethyl naphthalene), 4 semiochemicals (benzyl alcohol, cinnamaldehyde, 1-undecene, anethol) known to elicit responses in closely related species, 2 pungent odorants (methanol, phenol), and 1 analogue (ethyl salicylate) of an elm volatile. Female leaf beetles were highly responsive to phenol and methanol, whereas male beetles responded to di-n-octyl phthalate and dimethyl naphthalene at a concentration of 10 μg/μL. Cinnamaldehyde elicited the highest electroantennogram responses in both male and female beetles. In screenings of semiochemicals, beetles of both sexes were significantly repelled by cinnamaldehyde at 1 μg/μL. In Y-tube olfactometer tests, beetles of both sexes were significantly repelled by cinnamaldehyde, at 1 μg/μL for females and 10 μg/μL for males. In choice tests, 90% of starved beetles chose control leaves over leaves treated with cinnamaldehyde at 10 μg/μL. These results suggest that cinnamaldehyde has potential value for control of this elm pest, acting as an antifeedant compound.

Key Words: insect antifeedant; electroantennogram; olfactometer

Resumen

El escarabajo de la hoja *Ambrostoma quadriimpressum* Motschulsky (Coleoptera: Chrysomelidae) es una plaga importante del olmo (Ulmus; Rosales: Ulmaceae) en Asia oriental, y actualmente no existe ningún método químicos para su control que es eficaz y respetuoso del medio ambiente. En este estudio, se midió la respuesta electrofisiológica y de comportamiento de adultos de A. *quadriimpressum* a 13 compuestos, incluyendo 6 volátiles de plantas (linalool, α-pineno, salicilato de metilo, indol, ftalato de di-n-octilo, dimetil naftaleno), 4 semioquímicos (bencilo alcohol, cinamaldehído, 1-undeceno, anetol) conocido para provocar respuestas en especies estrechamente relacionadas, 2 olores acres (metanol, fenol), y 1 analógica (salicilato de etilo) de un volátil de olmo. Las hembras de los escarabajos de las hojas fueron muy sensibles a fenol y metanol, mientras que los escarabajos machos respondieron a ftalato de di-n-octilo y naftaleno de dimetilo a una concentración de 10 g/l. El cinamaldehído provocó la mayor respuesta EAG en los escarabajos machos y hembras. En pruebas de detección de semioquímicos, escarabajos de ambos sexos fueron repelados de manera significativa por cinamaldehído a 1 mg/l. En las pruebas olfactometer Y-tubo, los escarabajos de ambos sexos fueron repelados de manera significativa por cinamaldehído, a 1 mg/l para las hembras y 10 mg/l para los machos. En las pruebas de escoger, el 90% de los escarabajos hambrientos escogieron las hojas de control sobre los tratados con cinamaldehído a 10 mg/l. Estos resultados sugieren que cinamaldehído tiene un valor potencial para el control de esta plaga del olmo, que actúa como un compuesto contra la alimentación.

Palabras Clave: contra la alimentación de insectos; electroantenograma; olfatómetro

The leaf beetle *Ambrostoma quadriimpressum* Motschulsky (Coleoptera: Chrysomelidae) is the dominant pest of elms (Rosales: Ulmaceae) in East Asia, especially northeastern China, where it feeds on shoots and leaves of several elm species (*Ulmus pumila* L.*, Ulmus macrocarpa* Hance, and *Ulmus davidiana* Planch.) (An et al. 2005). Adults and larvae feed on elm foliage from Apr to Oct, and adults overwinter in the soil. During outbreaks, elms may be completely defoliated, resulting in some tree mortality (Meng et al. 2009; Zhang et al. 2009) in both forests and city green spaces. In the past, prevention of damage from defoliation relied on use of pesticides such as phosphamidon, carbofuran, and omethoate—products with high mammalian toxicity (Liang et al. 1990; Liu 2010). These chemicals cause serious environmental pollution and are hazardous to humans, pollinators, and livestock. Hence, development of an effective, low-toxicity chemical to control this pest would be helpful.

Plant volatiles can influence insect behavior and may have potential as natural pesticides, lures, or antifeedants. For example, the addition of phenylacetaldehyde (a general floral odor compound) to traps significantly increased catch of *Lygus rugulipennis* Poppius and *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae) compared with unbaited traps (Koczor et al. 2012). For *Holotrichia oblita* (Faldermann) (Coleoptera: Melolonthidae), a pest of castor bean (*Ricinus communis* L.; Malpighiales: Euphorbiaceae), the leaf volatiles dibutyl phthalate and cinnamaldehyde were highly attractive, suggesting potential for

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use as lures (Li et al. 2013). Many authors have studied the toxicity and antifeedant activity of plant volatiles in pests (Huang & Ho 1998; Hernández-Lambraño et al. 2014; Wang et al. 2015). Most effective environmentally friendly antifeedants extracted from plants can be applied in the field against pests (Koczor et al. 2012). For instance, fruit and seed extracts from *Cabralea canjerana* Mart. (Sapindales: Meliaceae) had high larvicidal and toxic activity to *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae) at 1000 mg/kg (Magrini et al. 2015).

Herbivore attack is known to increase the emission of plant volatiles (Cossé et al. 2006). Such herbivore-induced plant volatiles (HIPVs) can be effective, safe, and environmentally friendly chemicals for managing pests. Whereas some HIPVs deter insects, others attract them (Dicke & Baldwin 2010). *Ambrostoma quadriimpressum* adults have been found to be attracted by some elm HIPVs (Cheng et al. 2010). For this study, 6 HIPVs were chosen (linalool, α-pinene, methyl salicylate, indole, di-n-octyl phthalate, dimethyl naphthalene). In addition, 4 bio-functional odorants were selected, all of which elicited significant electroantennogram (EAG) responses in other Coleoptera species but had not been tested in *A. quadriimpressum.* These included benzyl alcohol, predicted to be responsible for the host switch from Cucurbitaceae to Poaceae in *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae) (Hibbard et al. 1997), 1-undecene, suggested to be a male sex pheromone component, and cinnamaldehyde, which attracted both male and female adults of *Bruchus rufimanus* Boheman (Coleoptera: Chrysomelidae) (Bruce et al. 2011). Anethol, the 4th odorant, showed clear olfactory neural activation and attraction in some scarab beetles (Hansson et al. 1999). In this study, we investigated the EAG and behavioral responses of *A. quadriimpressum* to these HIVPs and bio-functional odorants, along with the possible repellent mechanisms of these odorants.

Materials and Methods

INSECTS

Adult beetles (*A. quadriimpressum*) were collected from field elm (*U. pumila*) trees on a single day in Jun 2014, during the peak of adult emergence (An et al. 2005). All beetles were collected in Jilin Province (44.5100000°N, 124.2300000°E), China, and the beetles were maintained in ventilated net cages (55 × 53 × 50 cm) at 26 ± 2 °C,75 to 85% relative humidity, and a 12:12 h L:D photoperiod. Beetles were fed fresh elm leaves (*U. pumila*), with mature leaves supplied every 2 to 3 d. One hundred adult beetles were held per cage. The sex ratio was approximately 1:1 (female to male) per cage.

TEST ODORANTS

Nine HIPVs are released in markedly increased amounts in elm trees attacked by *A. quadriimpressum* compared with undamaged trees (Cheng et al. 2010); of these, we chose 3: linalool (odorant 1), α-pinene (odorant 2), and methyl salicylate (odorant 3). Three HIPVs of other plant species were also selected: indole (odorant 4), di-n-octyl phthalate (odorant 5), and dimethyl naphthalene (odorant 6). In addition, 4 bio-functional odorants were selected, namely, benzyl alcohol (odorant 7) (Hibbard et al. 1997), cinnamaldehyde (odorant 8), 1-undecene (odorant 9) (Bruce et al. 2011), and anethol (odorant 10) (Hansson et al. 1999). Furthermore, methanol (odorant 11) and phenol (odorant 12) were tested because of their pungent odors, and odorant 3's analogue ethyl salicylate (odorant 13) was likewise tested.

The above mentioned 13 odorants were purchased from Sigma Aldrich (St. Louis, Missouri) and Aladdin (Shanghai, China) (Table 1). The odorants were dissolved in commercial paraffin oil (which by itself was the negative control) (molecular biology grade, Shanghai, China) to make stock solutions of 10 µg/µL from which 10-fold dilutions were made.

EXPERIMENT 1: ELECTROANTENNOGRAM RESPONSES TO ODOR SOURCES

To measure adult beetles' ability to perceive the test odors, we used an EAG assay, following procedures of Syed & Leal (2011) with a few modifications. Glass electrodes were filled with a solution of 1 M potassium chloride and 1% polyvinylpyrrolidone. The reference electrode was inserted into the eye of the beetle, and the recording electrode was placed in contact with the cut tip of the antenna by using a micromanipulator MP-12 and a high impedance AC/DC pre-amplifier (Syntech, Kirchzarten, Germany). The tip of a disposable syringe was oriented towards the antenna from a distance of 1 cm, and then 20 µL of stimulus dissolved in paraffin oil at the desired dose (from the lowest to the highest) was loaded on a filter paper strip. The strip was placed in the syringe, which delivered a continuous stream (500 mL/min) of humidified (60–70%) air, and compensatory flow was added. The stimulus duration was 0.1 s, and the signal from the antenna was recorded for 10 s. Two min periods were allowed between stimuli for recovery of the EAG sensitivity (Raguso et al. 1996). At least 3 individuals were tested (1 antenna preparation from each beetle) for each replicate, and

Table 1. Tested odorants, their properties, source, and dose.

Test odorant	Property	Purity	Source	Code	CAS
linalool	liquid	≥97%	Aladdin	odorant 1	78-70-6
α-pinene	liquid	98%	Aladdin	odorant 2	80-56-8
methyl salicylate	liquid	≥95.5%	Aladdin	odorant 3	119-36-8
indole	solid	≥99%	Aladdin	odorant 4	120-72-9
di-n-octyl phthalate	liquid	98%	Aladdin	odorant 5	117-84-0
dimethyl naphthalene	solid	96%	Aladdin	odorant 6	28804-88-8
benzyl alcohol	liquid	99.8%	Aladdin	odorant 7	100-51-6
cinnamaldehyde	liquid	≥95%	Aladdin	odorant 8	104-55-2
1-undecene	liquid	97%	Sigma Aldrich	odorant 9	821-95-4
anethol	liquid	99%	Sigma Aldrich	odorant 10	104-46-1
methanol	liquid	100%	Aladdin	odorant 11	67-56-1
phenol	solid	100%	Aladdin	odorant 12	108-95-2
ethyl salicylate	liquid	≥99%	Aladdin	odorant 13	118-61-6
paraffin oil	liquid	molecular biology grade	Vita	negative control	8042-47-5

each odorant was tested 3 times for each concentration (0.01, 0.1, 1, and 10 µg/µL) (total of 3 female and 3 male beetles for each odorant concentration). Negative controls (paraffin oil) were performed first for each preparation, and the EAG peaks of each individual were normalized to the negative control.

EXPERIMENT 2: SCREENING FOR BEHAVIORALLY ACTIVE ODORANTS

To determine if physiological perception of an odor in the EAG test translated into a behavioral response by the test beetle, the 5 odorants (5, 6, 8, 11, and 12) that induced the strongest EAG responses in male or female beetles were tested to measure their attractant or repellent effects on *A. quadriimpressum*. The experiments were performed in a 12 cm diameter glass Petri dish, placed under the midline of a parallel fluorescent light source (28 W, bar form). The distance between light bulb and Petri dish was 100 cm. Two 2.5 cm diameter filter papers were placed on opposite sides of the dish, and 20 µL of odorant at 1 µg/µL was added to one filter paper, and the same amount of paraffin oil was added to the other as a control. Then a circle 5 cm in diameter was marked around each filter paper. The test adult beetle was placed in the center of the Petri dish, and the lid replaced. The amount of time the beetle spent in either circle was recorded, and when the insect was out of either marked circle, it was recorded as "neutral." The positions of the odorant and control were exchanged, and the Petri dish was cleaned with dehydrated alcohol after each replicate. Each treatment lasted 15 min, and the data are presented as the average of at least 10 adult (5 male and 5 female) beetles. Beetles were used only once to avoid effects of attenuation of antennal sensitivity (Fig. 1A).

EXPERIMENT 3: Y-TUBE OLFACTOMETER TEST OF REPELLENCY OF CINNAMALDEHYDE

Based on the results from Exp. 2, odorant 8 appeared to be a repellent for the test beetle. To confirm its repellency, the response of the beetle to cinnamaldehyde was assessed in a Y-tube olfactometer. The compound was tested at 0.1, 1, and 10 µg/µL in paraffin oil. In the assays, 20 µL of the odorant at the test concentration was applied to a piece of filter paper (25 × 10 mm), which was then placed in one of the olfactometer arms. In the other arm, 20 µL paraffin oil was used as a negative control.

Thirty beetles of each sex were individually tested for each of the 3 concentrations. A parallel fluorescent light source was used to avoid light interference. The distance between the light bulb (28 W, bar form, placed parallel directly above the Y-tube) and the Y-tube (9 mm diameter, base length 55 mm, arm length 55 mm) was about 65 cm. Air that passed through the Y-tube was sourced from a pressurized tank of pure air; it was first filtered through active carbon and then humidified (60–70% humidity) by bubbling the air through a bottle filled with distilled water before entering the Y-tube. The air flow moved through both arms of the Y-tube olfactometer at a speed of 300 mL/min. Each individual beetle was placed at the entrance of the olfactometer (Fig. 2). A "choice" was recorded when the beetle entered an arm and stayed for 1 min. If an insect made no choice within 3 min, it was discarded. The Y-tube olfactometer was cleaned with dehydrated alcohol and allowed to air dry between trials with different sexes or odorant dilutions. The positions of the odor sources were exchanged after 15 beetles were tested.

EXPERIMENT 4: CHOICE TESTS FOR BEETLE FORAGING

Following confirmation of repellency of odorant 8 in Exp. 3, here we sought to measure reduction in feeding induced by repellent odor. Elm leaf discs (area 283.5 mm^2) were placed in a ring of filter paper (inside diameter 19 mm, outside diameter 30 mm) in a 90 mm diameter Petri dish, and beetles that had been starved for 4 d were introduced. The elm leaves and filter paper disks were cut using a cork punch. In each separate dish, the filter paper on the left side of the plate was impregnated with the negative control (20 µL paraffin oil) while the right side of the filter paper was impregnated with odorant 8 at concentrations of 0.1, 1, and 10 µg/µL. The position of the control and odorant 8 was exchanged after each experiment, and the food on both sides was checked after 1 h of foraging. Only 1 beetle at a time was placed in each Petri dish and 10 adults (5 females and 5 males) were tested for each concentration. To avoid any possible effect of light, trials were run in the dark, covering Petri dishes with aluminum foil. Beetle starting position was at the edge of the Petri dish. The leaf section on which the beetle was feeding at the 1 h mark was recorded, and the Petri dish was cleaned with dehydrated alcohol and air dried. A new leaf was added before the start of each replicate (Fig. 1B and 1C).

STATISTICAL ANALYSES

EAG responses (Exp. 1) were processed with the EAG software (EAG Pro version 2.0 software; Syntech Company, German) and analyzed by 1-way ANOVA. Treatment means relative to standard were

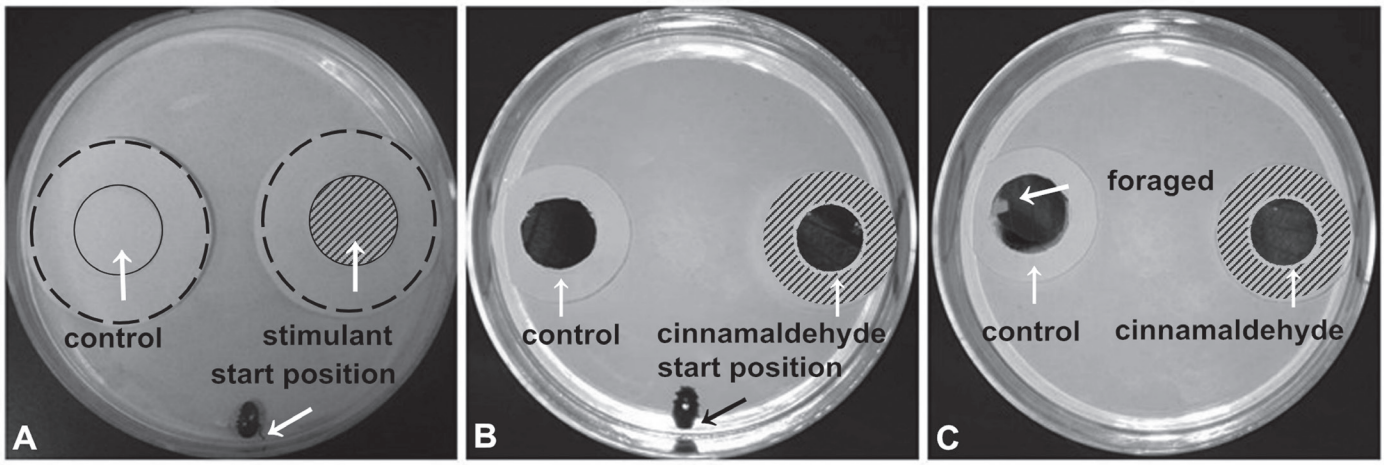

Fig. 1. Arena design for use in (A) Exp. 2 (screening for behaviorally active odorants) and (B & C) for Exp. 4 (choice test for beetle foraging).

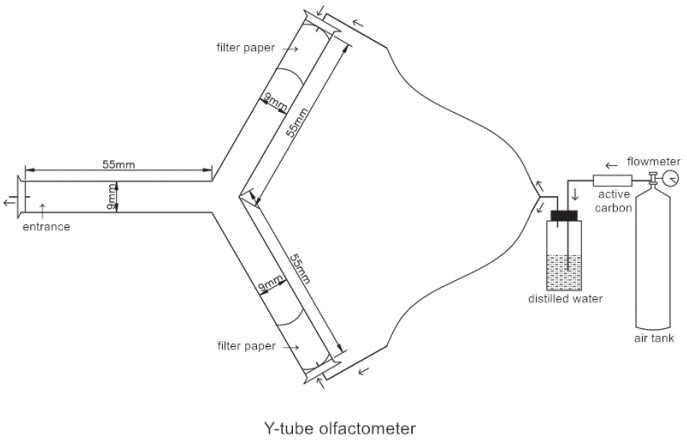

Fig. 2. Schematic of Y-tube olfactometer used in Exp. 3 for assaying repellency of cinnamaldehyde.

separated by Duncan's multiple range test at $P = 0.01$. Results from behavioral response assays (Exp. 2) were assessed using multiple *t*-tests to determine significant difference between odorants and the negative control at $P = 0.001$. Data from the Y-tube olfactometer assay (Exp. 3) and choice tests on beetle foraging (Exp. 4) were analyzed by χ^2-tests. Level of significance was Asymp. Sig. = 0.05. Statistical procedures for all tests were conducted using SPSS Statistical 19.0 (IBM, New York, New York), and the final results were plotted by Prism 6.0 (GraphPad Software, La Jolla, California).

Results

EXPERIMENT 1: ELECTROANTENNOGRAM RESPONSES TO ODOR SOURCES

For male beetles, odorants 5, 6, 8, and 12 each elicited a significant EAG response ($P < 0.01$) compared with the control, whereas odorants 1, 2, 3, 4, 7, 9, 10, 11, and 13 did not. For female beetles, odorants 6, 8, 9, 11, and 12 elicited significant EAG responses ($P < 0.01$) compared with the control, whereas odorants 1, 2, 3, 4, 5, 7, 10, and 13 did not (Table 2).

The dose dependence of the stimuli that elicited significant EAG responses was also tested. In female beetles, the EAG response to odorant 11 increased with increasing concentration and then started to decrease when the concentration reached 10 µg/µL (Fig. 3A), whereas the EAG response to odorant 12 was steady when the concentration was less than 1 µg/µL and increased until the concentration reached 10 µg/µL (Fig. 3B). In male beetles, the EAG response to odorant 6 peaked at 0.01 µg/µL (Fig. 3C), whereas the EAG response to odorant 5 increased with increasing concentration and peaked at 1 µg/µL (Fig. 3D). Both female and male beetles' EAG responses to odorant 8 rose with increasing stimulus concentration (Fig. 3E).

EXPERIMENT 2: SCREENING FOR BEHAVIORALLY ACTIVE ODORANTS

Of the 5 odorants that elicited significant EAG response in male or female beetles, odorant 11 had no significant attractant or repellent effect on either male ($P = 0.059$) or female ($P = 0.895$) beetles; odorant 6 had a significant attractant effect on male ($P < 0.001$) but not female beetles ($P= 0.0029$); odorant 5 showed repellent effects on female ($P < 0.001$) but not male beetles ($P = 0.010$); and likewise, odorant 12 showed repellent effects on female ($P < 0.001$) but not on male beetles ($P = 0.635$). However, most noteworthy was that odorant 8 had substantial repellent effects on both male and female beetles at 1 µg/µL ($P < 0.001$) (Table 3).

EXPERIMENT 3: Y-TUBE OLFACTOMETER TEST OF REPELLENCY OF CINNAMALDEHYDE

Y-tube olfactometer analysis indicated that odorant 8 had a significant repellent effect on female and male beetles at 1 and 10 µg/µL, respectively (Asymp. Sig. < 0.05) (Figs. 4A and 4B). At concentrations below 1 µg/µL, odorant 8 had no significant repellent effect on either sex (Asymp. Sig. > 0.05) (Table 4).

EXPERIMENT 4: CHOICE TESTS FOR BEETLE FORAGING

In the choice tests for beetle foraging, all starved beetles selected only 1 of the offered leaves in each trial. Starved beetles were significantly (Asymp. Sig. < 0.05) more attracted to the control leaves, as 9 of the 10 starved adults foraged on the control leaves when the concen-

Table 2. Mean (± SD) EAG relative responses of female and male *Ambrostoma quadriimpressum* beetles at 10 µg/µL in Exp. 1.

Female		Male	
Odorant	EAG response	Odorant	EAG response
odorant 8	10.00 ± 0.92A	odorant 8	7.12 ± 0.20A
odorant 12	6.07 ± 0.58B	odorant 5	6.58 ± 0.84AB
odorant 11	5.81 ± 0.48BC	odorant 6	6.41 ± 1.11AB
odorant 6	5.60 ± 0.46BC	odorant 12	6.11 ± 0.35ABC
odorant 9	5.52 ± 0.44BCD	odorant 11	4.99 ± 1.17BCD
odorant 7	4.52 ± 1.28BCDE	odorant 2	4.57 ± 0.71CDE
odorant 13	4.50 ± 0.19BCDE	odorant 1	4.36 ± 0.30CDE
odorant 4	4.33 ± 0.43CDE	odorant 9	4.23 ± 0.39DE
odorant 3	4.30 ± 0.08CDE	negative control	3.81 ± 1.01DEF
odorant 5	4.19 ± 0.39CDE	odorant 7	3.81 ± 0.23DEF
odorant 10	3.93 ± 0.22DE	odorant 3	3.19± 0.51DEF
negative control	3.17 ± 0.62E	odorant 10	3.17 ± 0.62DEF
odorant 2	3.15 ± 0.76E	odorant 4	3.07 ± 1.14EF
odorant 1	3.09 ± 1.03E	odorant 13	2.28 ± 0.64F

Means in a column followed by different letters are significantly different between the stimuli ($P \leq 0.01$; ANOVA and Duncan test).

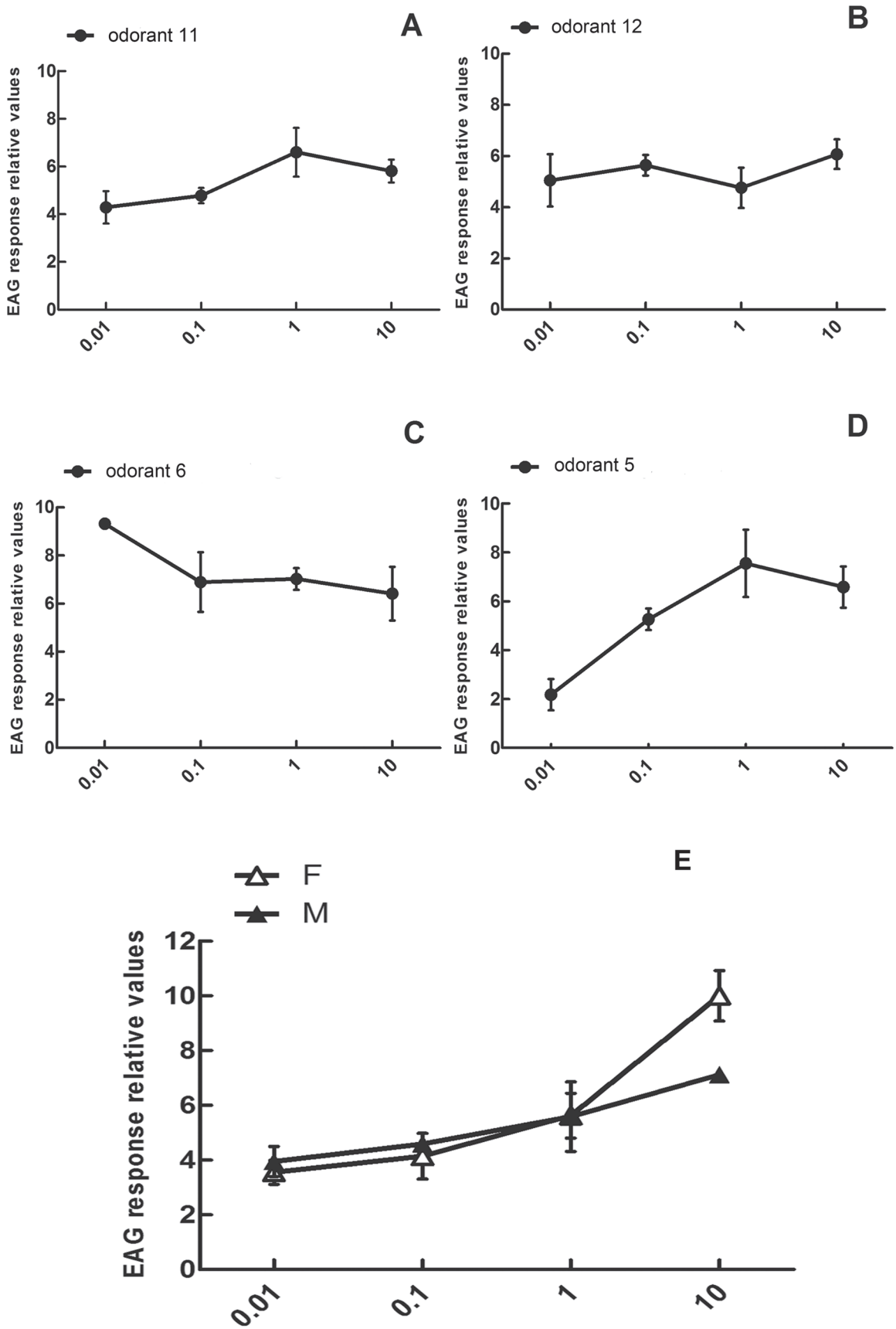

Fig. 3. Dose-response curves of stimuli. The x-axis represents stimulus concentration and the y-axis represents EAG response relative values. (A) Female dose-response to odorant 11. (B) Female dose-response to odorant 12. (C) Male dose-response to odorant 6. (D) Male dose-response to odorant 5. (E) Male and female dose-responses to odorant 8.

Table 3. Results of multiple *t*-tests of Exp. 2 at 1 µg/µl. F indicates female, M indicates male.

Odorant	*P* value	Staying time (odorant)	Staying time (control)	Difference	SE of difference	*t* ratio	df
F odorant 6	0.0030	330.6	544.2	−213.6	50.7853	4.20594	8
M odorant 6	<0.001*	551.4	223.2	328.2	16.1168	20.3639	8
F odorant 5	<0.001*	279.6	597.0	−317.4	36.4439	8.70927	8
M odorant 5	0.0103	342.0	493.2	−151.2	45.3671	3.33281	8
F odorant 11	0.8952	421.2	430.8	−9.6	70.6177	0.13594	8
M odorant 11	0.0594	464.4	404.4	60.0	27.3317	2.19526	8
F odorant 12	<0.001*	331.8	496.8	−165.0	20.1388	8.19315	8
M odorant 12	0.6356	423.0	411.6	11.4	23.1476	0.49249	8
F odorant 8	<0.001*	222.6	627.0	−404.4	20.8921	19.3566	8
M odorant 8	<0.001*	265.8	589.8	−324.0	20.2537	15.9971	8

*indicates significant differences between control and odorants ($P < 0.001$).

tration of odorant 8 was 10 µg/µL in the filter paper around the tested leaves (Fig. 4C and Table 4).

Discussion

Although chemical defenses and sex pheromones have been well understood in several species of Chrysomelidae (Michalski et al. 2008; Jimenez-Aleman et al. 2012), there have been no reports until now on the effect of antifeedants on chrysomelid beetles. Here, we not only identified odorant 8 as the most promising antifeedant against *A. quadriimpressum* adults but also investigated a possible repellent mechanism.

Cheng (2010) found that odorant 1 elicited a relatively high EAG response in female *A. quadriimpressum* beetles at 1 µg/µL, but in our case, odorant 1 did not elicit a significant EAG response in either male or female beetles even at 10 µg/µL. To confirm this observation, we rechecked the dose dependence of odorant 1 and determined that differences in the results may be caused by the concentration of the stimulus. In our study, the dose dependence of odorants 5, 6, 8, 11, and 12 were investigated on *A. quadriimpressum*. Responses of both female and male beetles were evaluated separately, providing a reference of the most suitable concentration for actual applications.

EAG response analysis showed that odorant 8 elicited the highest response in both sexes, so we performed further behavioral assays to test the effects of this compound on beetle behaviors. The Y-tube olfactometer results revealed that odorant 8 has repellent effects on both female and male beetles, and exhibited its optimal effect at 1 µg/µL (Asymp. Sig. < 0.05) and a significant but less pronounced effect at 10 µg/µL (Asymp. Sig. < 0.05). Interestingly, odorant 8 exhibited a re-

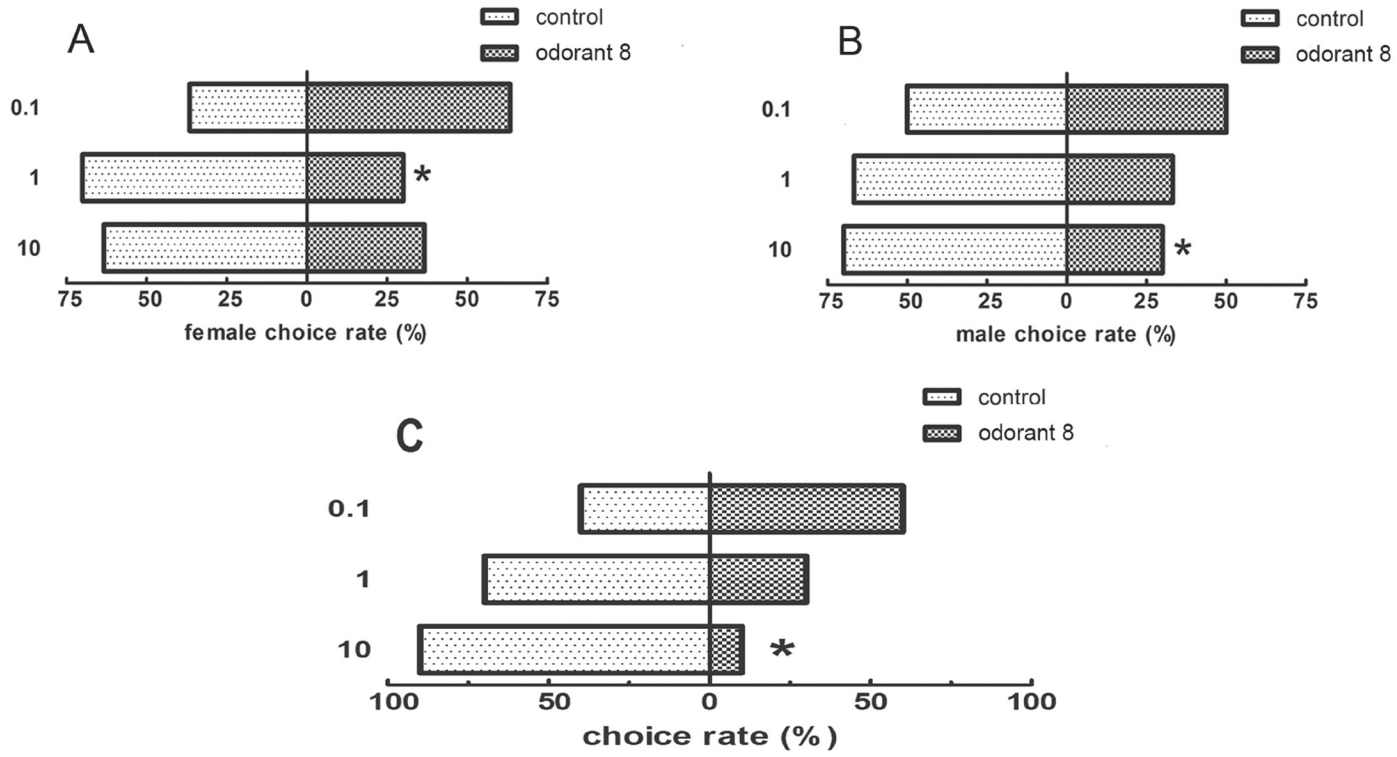

Fig. 4. (A & B) Response of female and male *Ambrostoma quadriimpressum* beetles to 3 concentrations of odorant 8 in the Y-tube olfactometer (A: female, B: male, $n = 30$). (C) The results of the choice foraging test ($n = 10$). * indicates significant difference by χ^2-analysis (Asymp. Sig. < 0.05).

Table 4. Chi-squared test results of Exp. 3 (Y-tube olfactometer test of repellency of cinnamaldehyde) and Exp. 4 (choice test for beetle foraging).

Experiment	Beetle sex and odorant concentration	No. of beetles tested	χ^2-value	df	Asymp. Sig.
Y-tube olfactometer test of repellency of cinnamaldehyde	female at 10 μg/μL	30	2.133	1	0.144
	male at 10 μg/μL	30	4.800	1	0.028*
	female at 1 μg/μL	30	4.800	1	0.028*
	male at 1 μg/μL	30	3.333	1	0.068
	female at 0.1 μg/μL	30	2.133	1	0.144
	male at 0.1 μg/μL	30	0.000	1	1.000
Choice test for beetle foraging	10 μg/μL	10	6.400	1	0.011*
	1 μg/μL	10	1.600	1	0.206
	0.1 μg/μL	10	0.400	1	0.527

*indicates asymptotic significance < 0.05 in Exp. 3 and Exp. 4.

pellent effect against both sexes of the beetle. Previous studies on the curculionid *B. rufimanus* showed that cone traps baited with odorant 8 collected from *Vicia faba* L. (Fabales: Fabaceae) flowers and blends of floral volatiles caught significantly more insects of both sexes than unbaited control traps (Bruce et al. 2011). However, it is interesting that food combined with odorant 8 significantly reduced food consumption in *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) larvae and had obvious antifeedant activity at concentrations of 27.2 and 54.4 mg/g of food (Huang & Ho 1998), similar to our results. Odorant 8 is the volatile of the host plant of *B. rufimanus*, which is not a host plant of *A. quadriimpressum* or *T. castaneum,* suggesting that odorant 8 would have a repellent effect on these 2 species rather than the attractant effect it has on other Coleoptera such as *B. rufimanus*.

To further explore the mechanism of repellency of odorant 8 to *A. quadriimpressum*, we designed and performed a choice test on foraging behavior, whose results were consistent with those of the Y-tube olfactometer analysis in as much as the starved beetles foraged on leaves from the control side (Asymp. Sig. < 0.05) when the concentration of odorant 8 was 10 μg/μL. Odorant 8 is low in mammalian toxicity, and its well-known properties make it ideal for agricultural use. Odorant 8 was first isolated in 1834 by Dumas and Péligot from cinnamon essential oil, and its insecticidal and antifeedant activities have been reported against a wide range of pests (Ma et al. 2014). Odorant 8 has recently been recognized as a very effective insecticide for controlling mosquito larvae. As little as 29 ppm of odorant 8 was able to kill 50% of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquito larvae in 24 h (Cheng et al. 2004).

Our EAG response and Y-tube olfactometer results showed the repellent effects of odorant 8 against *A. quadriimpressum*. This beetle is a monophagous species feeding on elm, but odorant 8 is not a volatile associated with elm foliage. It is the main compound of the essential oil extracted from *Laurus nobilis* L. (Laurales: Lauraceae), suggesting that the observed repellent effect is related to the monophagous nature of *A. quadriimpressum*. Although odorant 8 was not tested in the field in this study, our results suggest it to be a promising alternative to the highly toxic chemicals currently used to control *A. quadriimpressum* on urban elm trees.

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