

The pheromone verbenone and its function in *Dendroctonus armandi* (Coleoptera: Curculionidae: Scolytinae)

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Abstract. The Chinese white pine beetle, *Dendroctonus armandi* Tsai and Li is a native species of bark beetle and one of the most destructive in much of western China. Little is known about the characterization of the pheromones *trans*-verbenol and verbenone, and their functions in *D. armandi* are unknown. Electroantennogram tests (EAG) and olfactory assays (Y-tube assays) in the laboratory revealed that (1) *trans*-verbenol may be an anti-aggregation pheromone for male and an aggregation pheromone for female *D. armandi* and (2) female beetles are more attracted to controls (hexane) than low concentrations of verbenone and male beetles more attracted to controls than high concentrations of verbenone. Field trials indicated that the addition of verbenone to bait used to trap *D. armandi* remarkably decreased the efficiency of field trapping. These results indicate that verbenone is an anti-aggregation pheromone for male *D. armandi*. This research provides evidence of the role of verbenone among the different types of pheromones. The pheromone verbenone clearly could be used to protect healthy Chinese white pines.

INTRODUCTION

The Chinese white pine beetle, *Dendroctonus armandi* Tsai and Li, is considered to be a serious pest in the Qinling and Bashan Mountains in China as it kills mature Chinese white pines (*Pinus armandi* Franch), resulting in huge economic losses. In China, over 3×10^8 m³ of Chinese white pines have been killed by *D. armandi* since the 1970s (Xie & Lv, 2012). As *D. armandi* is a major threat to the health of *P. armandi*, it may pose a significant threat to the development of Qinling and Bashan Mountain forest ecosystems. Management measures in recent years include forest tending, chemical control and semiochemical-based trapping. *D. armandi* mainly attacks healthy *P. armandi* trees that are over 30 years old (Chen & Tang, 2007). In recent years, *D. armandi* begun to attack much younger *P. armandi* (Chen et al., 2015). *D. armandi* completes its life cycle in the phloem of *P. armandi* and the adult stage leave this host and attacks new host trees. *D. armandi* females attack a tree and then use some kind of semiochemical to attract both males and females, which is similar to what is recorded for *Dendroctonus valens* (Liu et al., 2006; Pureswaran et al., 2008a). Semiochemical communication in bark beetles “enables host and mate location, aggregation and resource partitioning” (Borden et al., 1986; Liu et al., 2013), and aggregation pheromones ensure successful

colonization and reproduction of bark beetles. Therefore, semiochemicals can be used to protect pines by reducing the initial attack rate of the beetle (Stephen, 2001; Faccoli & Stergulc, 2008; Blazenec & Jakus, 2009). EAGs of the different sexes of *D. armandi* recorded in response to some terpenes of different concentrations from its host *P. armandi* differ significantly (Wang et al., 2011a, b; Zhang et al., 2010, 2011). GC-MS analyses of extracts of the hindgut of *D. armandi* (Xie & Lv, 2012; Chen et al., 2015) have provided compounds that used in the field trapping experiments. The use of β -caryophyllene in a blend of monoterpenes increases the numbers trapped in the field (Xie & Lv, 2012). Aggregation pheromone and host volatiles from Chinese white pine should be used together in field trapping experiments (Chen et al., 2015). Non-host volatiles and frass of *D. armandi* have been tested on *D. armandi* in laboratory olfactometer trials (Wu et al., 2012; Zhao et al., 2014; Zhang et al., 2015).

Some of the components of the pheromone produced by different species of *Dendroctonus* are the same, although the gender of the individual producing them and the functions of the components may vary (Pitman et al., 1969). *trans*-Verbenol is a component of the pheromones produced by the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Renwickj, 1969), the western pine beetle,

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Dendroctonus brevicomis LeConte (John et al., 1984) and the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Greis et al., 1990; Pureswaran et al., 2000). Verbenone is a component of the pheromones produced by *D. brevicomis* and *D. frontalis* (Fettig et al., 2009; Sullivan et al., 2007). Furthermore, the use of verbenone to protect *Pinus contorta* trees from attack by *D. ponderosae* and *D. valens* has been extensively studied (Gillette et al., 2006, 2009, 2012).

Although *trans*-verbenol and verbenone has been detected using gas chromatographic and mass spectral (GC-MS) analyses of the hindguts of female beetles and the fumes emanating from *P. armandi* logs naturally attacked by *D. armandi* (Xie & Lv, 2012; Chen et al., 2015), the characterization and function of *trans*-verbenol and verbenone in *D. armandi* are unknown. Moreover, there is little information on the release and function of semiochemicals. We identified the characteristics of *trans*-verbenol and verbenone produced by *D. armandi* based on electrophysiological responses and laboratory olfactometer trials. Verbenone was further studied in a field trial. The results of these studies could provide a basis for future studies and might be used in the biocontrol of these beetles.

MATERIALS AND METHODS

Insects

Chinese white pines infested with *D. armandi*, were collected from the Qinling Mountains, Shaanxi, China (33°26'53.0"N, 108°28'48.3"E, at a mean altitude of 1500 m) in November 2015. Logs cut from these Chinese white pines were placed in a nylon plastic net (of mesh size ≤ 0.8 mm) in the laboratory and watered twice every day to keep them moist. The beetles that flew from the logs were collected everyday, sorted by sex and stored at 4°C in a refrigerator. Only active beetles were used in the EAG experiment and laboratory olfactometer trials (Wood, 1982; Light, 1983).

Chemicals

The chemicals used in EAG assays, laboratory bioassays and field trials were (R)-(+)- α -pinene (98% c.p., 97% e.e.), (-)- β -pinene (99% c.p.), (+)-3-carene (>98% c.p.), (S)-(-)- α -pinene (>98% c.p., $\geq 81\%$ e.e.), trichloroethylene (>99.5% c.p.), (1S)-(-)-verbenone (>93% c.p., 82% e.e.) and hexane (HPLC certified) obtained from Sigma-Aldrich Co., and (-)-*trans*-verbenol (>95% c.p.) obtained from Bedoukian Research INC, 21 Finance Drive, Danbury, CT06810-4192, USA.

EAG assays

The test method used was that described by Zhang et al. (2010). The antennae of *D. armandi* were dissected under a microscope and were connected between two electrodes. The recording electrode was connected to the distal edge of the club and the indifferent electrode to the scape of the antenna using a conductive adhesive. Filter paper (5 by 50 mm) was used to carry 20 μ L of a test solution of the odour, the solvent was then allowed to evaporate for 30 s and then the filter paper was inserted into a Pasteur pipette (10 mm diameter by 15 cm long).

Filter paper treated with 20 μ L hexane alone were used as controls. One end of the Pasteur pipette was inserted into a hole in a metal tube (15 mm diameter by 15 cm long), the other end of which was connected to an air controller (model CS-05b, Syntech, the Netherlands) that delivered humidified air at a constant

rate of 40 mL/min. The open end of the metal tube was positioned 1.2 cm away from the antenna. In the experiment, the air controller delivered 0.2 s flows of air to the antenna. To ensure that the test antenna fully recovered from a stimulus, there was a 60 s interval between tests. The test of each pheromone was from a low concentration to a high concentration (Zhang et al., 2010). A hexane-only control and standard solution (1-hexanol at 1 μ g/ μ L in hexane) was presented before and after each test pheromone. Each pheromone was tested on five male beetles and five female beetles, and the trials were repeated at least five times for each EAG preparation.

Laboratory olfactometer trials

Olfactometer bioassays were conducted in a glass Y-tube (15 cm main arm, 10 cm side arm, 15 mm diameter, with 75° side arm inside angle) with 400 mL/min airflow through main arm. The method of testing is described by Liu et al. (2013). Four tests were carried out. In the first, one chamber contained filter paper treated with hexane and the other filter paper treated with *trans*-verbenol diluted in hexane (0.001, 0.1, 1, or 10 μ g/ μ L). In the second test, one chamber contained filter paper treated with hexane and the other filter paper treated with (1S)-(-)-verbenone diluted in hexane (0.001, 0.1, 1, or 10 μ g/ μ L). In the third test, the attraction of the beetles to *trans*-verbenol in (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene (1 : 1 : 1) at a concentration of 1/10 μ g/ μ L versus (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene (1 : 1 : 1) was determined. In the fourth test, the attraction of beetles to (1S)-(-)-verbenone in (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene (1 : 1 : 1) at a concentration of 1/10 μ g/ μ L versus (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene (1 : 1 : 1) was determined. A mixture of (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene (1 : 1 : 1) was used as a solvent in the third and fourth test. The (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene (1 : 1 : 1) mentioned above was used without dilution. Approximately 30 min before each trial, the adults were introduced into a separate holding tube and not exposed to any test odour before their release. Ten μ L of each test solution was placed on each filter paper strip (5 by 50 mm) and allowed to evaporate for 30 s before being placed in the main arm of the Y-tube. In the experiment, a beetle was released into the main arm of the Y-tube and given 10 min to make a choice. The choice of *D. armandi* for a particular side arm of the Y-tube was recorded when the beetle was 5 cm inside one of the arms of the Y-tube. To eliminate any directional bias, the side arms of the Y-tube were reversed after every test of each treatment. The Y-tube was cleaned with 100% ethanol before use. The experiment with the Y-tube was done at 25°C and a relative humidity of 70%. For the test of each different concentration, at least 30 females and 30 males were used.

Field trials

Field trapping with (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene, (S)-(-)- α -pinene, trichloroethylene and (1S)-(-)-verbenone was done at Huoditang (33°18'–33°28' N, 108°21'–108°39' E, altitude 1400–1500 m) and Pingheliang (33°34'–33°22' N, 108°24'–108°36' E, altitude 1500–1800 m) Forestry Stations, Shaanxi Province, China (May 20 to August 31, 2013–2014). Huoditang and Pingheliang were chosen to determine whether the beetles responded to compounds tested similarly at different geographical locations. The trees at Huoditang and Pingheliang were approximately 20–50 years old and 12–20 m tall and were infested with *D. armandi*. An outbreak of *D. armandi* beetles occurred at Huoditang in 2014, which resulted in a substantial increase in the numbers of males and females trapped. A systemic thinning of the forest was carried out in September 2014. To ensure that this did not influence the results of our field

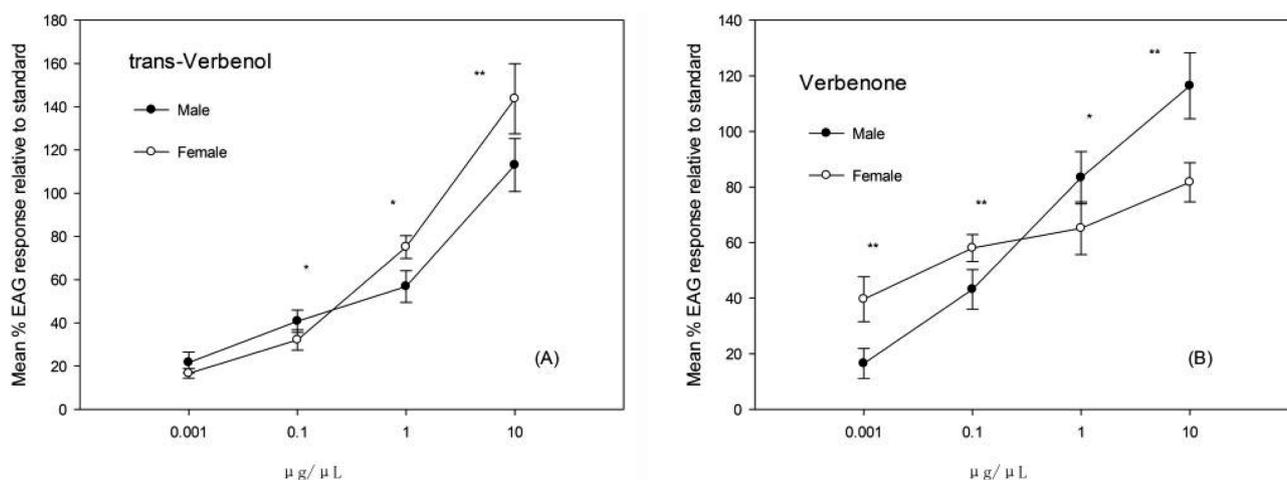


Fig. 1. EAG response (mean % ± SE) of *D. armandi* to *trans*-verbenol and verbenone measured relative to the standard solution (1-hexanol at 1 µg/µL in hexane). Error bars are standard errors based on five replicates. Asterisks denote a significant difference between males and females in their response to the same concentration (t test; * P ≤ 0.05; ** P ≤ 0.01).

trials, this experiment ended on August 31, 2014. Multiple funnel traps were used in all the field trials, which were obtained from Sino-Czech Trading Co. Ltd., Beijing, China.

In 2013, the field trapping experiment was done at Huoditang and Pingheliang Forestry Stations from May 20 to August 31. Four treatments (A, B, C, D) were compared (Table 5). Treatment A was the control. In 2014, the field trapping experiment was done at Huoditang and Pingheliang Forestry Stations from May 20 to August 31. Four treatments (E, F, G, H) were tested (Table 5). Treatment E was the control. The method used is that described by Xie & Lv (2012). The mixed reagent treatments were released from a 15 mL slow-release plastic vial at a release speed of 200 mg a day. Eight multiple-funnel traps were set up at random with 10 replicates per treatment. This field equipment was installed in early May 2013 and 2014 before the flight period of *D. armandi*. The traps were spaced at least 30 m apart and were checked every three days. Live *D. armandi* were sexed using the fact that males stridulate (McGhehey, 1968).

Statistical analysis

The results of EAG assays were corrected for solvent and systematic bias by subtracting the mean response to the solvent-only controls before and after exposure to each sample from the response to the test compound. In order to compensate for the effect of the decline in activity of the test antennae and individual differences between experiments, the EAG assays were standardized by calculating the EAGs as percentages relative to the response to the standard solution (1-hexanol at 1 µg/µL in hexane). Mann-Whitney tests using SPSS (1999) were used to determine significant differences between sexes in the relative EAG responses. In the olfactometer bioassays, chi-square tests using SPSS (1999) were used to compare the responses to the different compounds. For the field trapping experiments, statistical analysis was conducted using one-way ANOVA to detect if the numbers of female

and male *D. armandi* trapped were significantly affected by the treatments and LSD multiple comparison used to determine significant differences among treatments.

RESULTS

EAG assays

The EAGs revealed the olfactory responses of *D. armandi* to different concentrations of *trans*-verbenol and verbenone (Fig. 1). Analyses of the same compound at four different concentrations revealed significant differences between the sexes. Mann-Whitney tests revealed significant differences between males and females in their antennal responses to *trans*-verbenol at 0.1, 1, 10 µg/µL (P_{0.001µg/µL} = 0.117, P_{0.1µg/µL} = 0.028, P_{1µg/µL} = 0.016, P_{10µg/µL} = 0.009). At the 0.001 and 0.1 µg/µL concentrations, the antennae of male adult *D. armandi* were more sensitive to *trans*-verbenol. At the 1 and 10 µg/µL levels, females were more sensitive to *trans*-verbenol. The EAGs revealed that female and male beetles differed significantly in their responses to verbenone at concentrations of 0.001, 0.1, 1 and 10 µg/µL (P_{0.001µg/µL} = 0.009, P_{0.1µg/µL} = 0.009, P_{1µg/µL} = 0.028, P_{10µg/µL} = 0.009). At the 0.001 and 0.1 µg/µL levels, the antennae of adult female *D. armandi* were more sensitive to verbenone. At the 1 and 10 µg/µL levels, males were more sensitive to verbenone.

Laboratory olfactometer trials

The Y-tube data for four different concentrations of *trans*-verbenol revealed that males were more repelled by certain concentrations of *trans*-verbenol (0.001 µg/µL and

Table 1. Attraction of male and female *D. armandi* to various concentrations of *trans*-verbenol (T-V) in Y-tube assays^a.

	Hexane × T-V _{0.001 µg/µL}			Hexane × T-V _{0.1 µg/µL}			Hexane × T-V _{1 µg/µL}			Hexane × T-V _{10 µg/µL}		
	Hexane	T-V _{0.001 µg/µL}	χ ²	Hexane	T-V _{0.1 µg/µL}	χ ²	Hexane	T-V _{1 µg/µL}	χ ²	Hexane	T-V _{10 µg/µL}	χ ²
Male	25	8	8.758**	20	13	1.485	18	15	0.273	23	10	5.121*
Female	16	16	0.000	16	16	0.000	17	15	0.125	5	27	15.125**
χ ²	4.628*			0.740			0.013			19.371**		

^a Table entries are the numbers of *D. armandi* of each sex that responded to either the control or treatment stimulus. Asterisks mean significant differences (* P ≤ 0.05, ** P ≤ 0.01, chi-squared test).

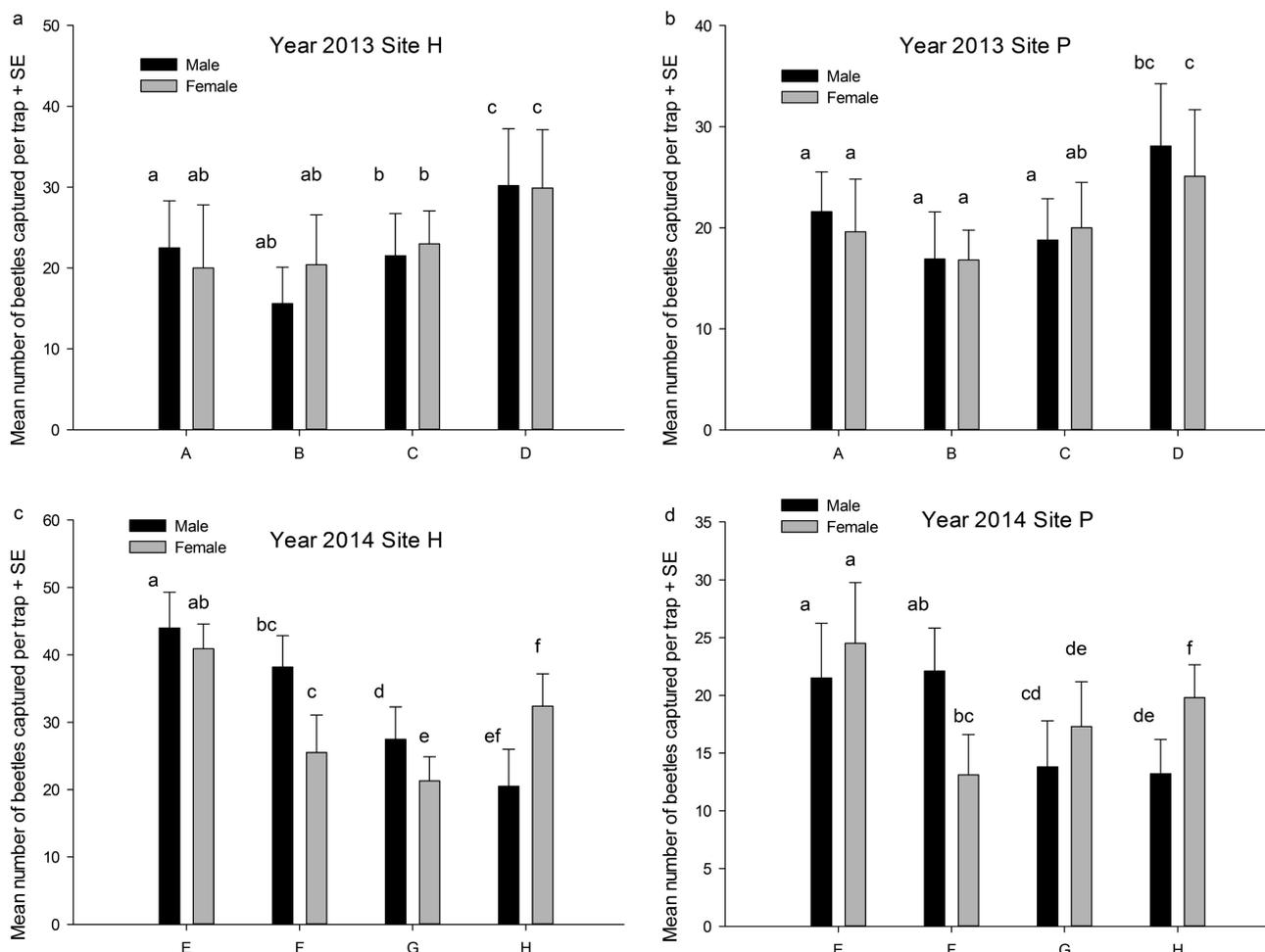


Fig. 2. Annual mean captures of adult *D. armandi* by eight kinds of reagent. Bars indicate mean response with standard errors and different letters above bars indicate significant differences at $P \leq 0.05$ based on LSD multiple comparison (ANOVA). The reagent ratios in groups A–H are in table 5. Reagents were released as a mixture at 250 mg/day.

10 $\mu\text{g}/\mu\text{L}$) than by the hexane control (Table 1). Females were more attracted to the 10 $\mu\text{g}/\mu\text{L}$ concentration of *trans*-verbenol than the hexane-alone control. The responses of the female and male beetles to *trans*-verbenol were significantly different ($P \leq 0.05$) at concentrations of 0.001 $\mu\text{g}/\mu\text{L}$ and 10 $\mu\text{g}/\mu\text{L}$. When (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene and *trans*-verbenol were compared as stimuli in the Y-tube assays, the responses of female and male beetles were not significantly different (Table 3).

The Y-tube data for four different concentrations of verbenone revealed that males were more repelled by certain concentrations of verbenone (1 $\mu\text{g}/\mu\text{L}$ and 10 $\mu\text{g}/\mu\text{L}$) (Table 2). Females were more repelled by the 0.001 $\mu\text{g}/\mu\text{L}$ concentration of verbenone. The responses of female and male beetles to the 1 $\mu\text{g}/\mu\text{L}$ concentration of ver-

benone differed significantly ($P \leq 0.01$). When (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene and verbenone were compared as stimuli in the Y-tube assays, females were significantly more repelled by verbenone (1 $\mu\text{g}/\mu\text{L}$) in X [(R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene = 1 : 1 : 1] than males (Table 3), while males were significantly more repelled by verbenone (10 $\mu\text{g}/\mu\text{L}$) in X ((R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene = 1 : 1 : 1) than females.

Field trials

The numbers of males and females trapped differed significantly between the different treatments (Table 4). In 2013, the responses of *D. armandi* to the four different reagent mixtures (A, B, C, D) were similar at Huoditang (site H) and Pingheliang (site P) (Fig. 2a, b). When the propor-

Table 2. Attraction of male and female *D. armandi* to various concentrations of verbenone (V) in Y-tube assays.

	Hexane \times V _{0.001 $\mu\text{g}/\mu\text{L}$}			Hexane \times V _{0.1 $\mu\text{g}/\mu\text{L}$}			Hexane \times V _{1 $\mu\text{g}/\mu\text{L}$}			Hexane \times V _{10 $\mu\text{g}/\mu\text{L}$}		
	Hexane	V _{0.001 $\mu\text{g}/\mu\text{L}$}	χ^2	Hexane	V _{0.1 $\mu\text{g}/\mu\text{L}$}	χ^2	Hexane	V _{1 $\mu\text{g}/\mu\text{L}$}	χ^2	Hexane	V _{10 $\mu\text{g}/\mu\text{L}$}	χ^2
Male	17	17	0.000	13	21	1.882	27	13	4.900*	23	9	6.125 *
Female	24	11	4.829*	19	14	0.758	12	21	2.455	16	16	0.000
χ^2	2.467			2.511			7.045**			3.216		

^a Table entries are the numbers of *D. armandi* of each sex that responded to either the control or treatment stimulus. Asterisks mean significant differences (* $P \leq 0.05$, ** $P \leq 0.01$, chi-squared test).

Table 3. Attraction of male and female *D. armandi* to *trans*-verbenol-X and verbenone-X blend in Y-tube assays^a.

		Male	Female	χ^2
T-V/ $X_{1 \mu\text{g}/\mu\text{L}} \times X$	T-V/ $X_{1 \mu\text{g}/\mu\text{L}}$	12	19	2.765
	X	18	12	
	χ^2	1.200	1.581	
T-V/ $X_{10 \mu\text{g}/\mu\text{L}} \times X$	T-V/ $X_{10 \mu\text{g}/\mu\text{L}}$	11	14	0.603
	X	20	17	
	χ^2	2.613	0.290	
V/ $X_{1 \mu\text{g}/\mu\text{L}} \times X$	V/ $X_{1 \mu\text{g}/\mu\text{L}}$	16	12	1.873
	X	17	25	
	χ^2	0.030	4.568*	
V/ $X_{10 \mu\text{g}/\mu\text{L}} \times X$	V/ $X_{10 \mu\text{g}/\mu\text{L}}$	11	19	6.137*
	X	26	13	
	χ^2	6.081*	1.125	

^a Table entries are the numbers of *D. armandi* of both sexes that responded positively to either the positive control (X) or treatment stimulus. The results are for bioassays with *trans*-verbenol and verbenone in X at concentrations of 1 and 10 $\mu\text{g}/\mu\text{L}$, respectively. X present (R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene = 1: 1: 1. T-V/ $X_{1 \mu\text{g}/\mu\text{L}}$ and T-V/ $X_{10 \mu\text{g}/\mu\text{L}}$ mean *trans*-verbenol in X at concentrations of 1 and 10 $\mu\text{g}/\mu\text{L}$, respectively; V/ $X_{1 \mu\text{g}/\mu\text{L}}$ and V/ $X_{10 \mu\text{g}/\mu\text{L}}$ mean verbenone in X at concentrations of 1 and 10 $\mu\text{g}/\mu\text{L}$, respectively. Asterisks indicate significant differences (* $P \leq 0.05$, ** $P \leq 0.01$, chi-squared test).

tion of (+)-3-carene was increased or (S)-(-)- α -pinene was added, there was no significant difference in the numbers of either male or female *D. armandi* captured at H and P. However, both male and female *D. armandi* at sites P and H were attracted in significantly higher numbers to the complete blend of (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene and trichloroethylene (1 : 1 : 1) than to (R)-(+)- α -pinene, (-)- β -pinene and (+)-3-carene (1 : 1 : 1).

The field experiment in 2014 revealed the same pattern in the captures of beetles at P and H. At the two locations, significantly more beetles were trapped by funnel traps with the mixture (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene than when verbenone was added (Fig. 2c, d), except for males caught by traps baited with treatment F at site P. When a low relative proportion of verbenone was added to (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene (1 : 1 : 1), the number of females caught significantly declined. When a higher relative proportion of verbenone was added to (R)-(+)- α -pinene, (-)- β -pinene, (+)-3-carene (1 : 1 : 1), the number of males caught significantly declined. Overall, field trapping in 2014 revealed that the addition of ver-

benone reduced the number of male *D. armandi* captured in traps by 20%–38%, and of females by 19%–48%.

DISCUSSION

This is the first behavioral test of the response of *D. armandi* to *trans*-verbenol and verbenone. We identified an electrophysiological response to *trans*-verbenol and verbenone by the antennae of both sexes as well as a dose-dependent behavioral response to *trans*-verbenol and verbenone in both sexes in olfactometer bioassays. Furthermore, the characterization and function of verbenone was verified in field trials at Huoditang and Pingheliang Forestry Stations in 2013 and 2014. *Dendroctonus* spp. are known to use semiochemicals and host volatiles when attacking host trees and attracting partners (Schlyter & Birgersson, 1999; Byers & Zhang, 2012). Semiochemicals might be a key factor determining the successful mass colonization by *Dendroctonus* spp., which can overcome the defense system of host trees (Chen et al., 2015). *trans*-Verbenol [4,7,7-trimethylbicyclo(3.1.1)hept-3-en-2-ol], is the important component of the aggregation pheromone pro-

Table 4. ANOVA of the numbers of males and females of *Dendroctonus armandi* captured by multiple funnel traps during field-trapping at Huoditang and Pingheliang Forestry Stations, Shaanxi Province, China (20 May to 31 August 2013–2014) (n = 10 traps per treatment).

	Sources of variation	Male				Female			
		Sum of squares	df	F	Sig.	Sum of squares	df	F	Sig.
Year 2013	Between groups	1078.900	3	10.981	0.000	629.475	3	5.011	0.005
Site A	Within groups	1179.000	36			1507.300	36		
	Total	2257.900	39			2136.775	39		
Year 2013	Between groups	719.300	3	10.452	0.000	358.475	3	4.807	0.006
Site B	Within groups	825.800	36			894.900	36		
	Total	1545.100	39			1253.375	39		
Year 2014	Between groups	3337.300	3	43.313	0.000	2205.075	3	36.655	0.000
Site A	Within groups	924.600	36			721.900	36		
	Total	4261.900	39			2926.975	39		
Year 2014	Between groups	692.500	3	15.093	0.000	681.675	3	14.374	0.000
Site B	Within groups	550.600	36			569.100	36		
	Total	1243.100	39			1250.775	39		

Table 5. The regent ratios used in the field trapping experiment at Huoditang and Pingheliang Forestry Stations in 2013 (group A, B, C, D) and 2014 (group E, F, G, H).

Group	Chemical regent					
	(R)-(+)- α -pinene	(-)- β -pinene	(+)-3-carene	(S)-(-)- α -pinene	trichloroethylene	verbenone
A	1	1	1			
B	1	1	3			
C	1	1	1	1		
D	1	1	1		1	
E	1	1	1			
F	1	1	1			0.001
G	1	1	1			0.01
H	1	1	1			0.1

duced by most *Dendroctonus* spp. (Blomquist et al., 2010). Its production most likely begins at the onset of feeding on a new host tree and ceases upon mating (Pureswaran et al., 2000). Verbenone [4,6,6-trimethylbicyclo(3.1)hept-3-en-2-one] is a pheromone stimulating very similar behavioral responses in *Dendroctonus* spp. (Borden, 1997). Verbenone is the auto-oxidation product of verbenol and has an anti-aggregation role in the majority of *Dendroctonus* spp. (Lindgren & Miller, 2002). Our results not only demonstrate that *trans*-verbenol may be an aggregation pheromone for females and an anti-aggregation pheromone for males, but also demonstrate that verbenone is an anti-aggregation pheromone in *D. armandi*. Based on gas chromatographic and mass spectrometry (GC-MS) analyses of volatiles collected from live invading unmated females, paired females and paired males of *D. armandi* (relevant data has not been published), the level of *trans*-verbenol produced by paired females was significantly higher than by unmated females and paired males. The effect of *trans*-verbenol was clearer. After a female mates, the concentration of *trans*-verbenol increases to that of paired females. High concentrations of *trans*-verbenol were released by paired females in order to attract other females to attack the same pine and inhibit males from releasing the signal “I have mated”. *trans*-verbenol played its role after the mating of female and male. This accords with the GC-MS analyses, the behavioral experiment and life history of *D. armandi*. Verbenone is produced when the beetles attacking a pine tree start competing for space and nutrition. Furthermore, the amount of verbenone produced by paired males was significantly higher in the GC-MS analysis (relevant data not presented). So verbenone also acts as anti-aggregation pheromone after mating. The aim of this study regarding verbenone was to determine whether it was an anti-aggregation pheromone and a further study is planned to determine how and when. The electroantennographic and behavioral responses of *Dendroctonus armandi* to trichloroethylene are reported by Wang et al. (2011b). Trichloroethylene is significantly more attractive for males than females. This was taken into consideration in the field experiment, but the reason for the lure effect is still not clear, but it may be acting as a synergist.

The EAG dose-response curve of *D. armandi* to *trans*-verbenol revealed that male beetles showed a significantly greater response to the control (hexane) than *trans*-verbenol and that female beetles a significantly greater response to *trans*-verbenol (10 $\mu\text{g}/\mu\text{L}$) than the control (Fig. 1). The

laboratory olfactometer trials showed that male beetles were more attracted to the controls (hexane) than to *trans*-verbenol and female beetles more attracted to *trans*-verbenol (10 $\mu\text{g}/\mu\text{L}$) than the control (Table 1). *trans*-Verbenol may act as an anti-aggregation pheromone for male and an aggregation pheromone for female *D. armandi*. The characterization and function of *trans*-verbenol in female *D. armandi* are similar to that of some other tree-killing *Dendroctonus* spp., such as *D. ponderosae* (Borden et al., 1987), *D. pseudotsugae* (Rudinsky et al., 1972) and *D. frontalis* (Payne et al., 1978; Pureswaran et al., 2008b). However, the characterization and function of *trans*-verbenol were not clear when X [(R)-(+)- α -pinene: (-)- β -pinene: (+)-3-carene = 1 : 1 : 1] was used as a control (Table 3). More work is needed on the characterization and function of *trans*-verbenol, especially its role as an anti-aggregation or aggregation pheromone.

The EAG dose-response curve of *D. armandi* to verbenone revealed that the response of female beetles was significantly greater to the control (hexane) than to low concentrations of verbenone and of male beetles significantly greater to the control (hexane) than high concentrations of verbenone. The laboratory olfactometer trials revealed that female beetles were more attracted to controls (hexane) than low concentrations of verbenone and male beetles more attracted to controls (hexane) than high concentrations of verbenone. Both the trapping at Huoditang and Pingheliang in 2014 revealed the same consistent trend of a decreasing female and male response to added verbenone, although the result for treatment F at Pingheliang was not significant (Fig. 2). At high concentrations of verbenone, the decreased response was more significant for males than for females. At low concentrations of verbenone, the decreased response was more significant for females than for males. The addition verbenone to bait greatly decreased the efficiency of field trapping of *D. armandi*. Verbenone has the potential to be used to control beetle populations and so protect healthy Chinese white pine. Collectively, this evidence supports our conclusion that for female and male *D. armandi* verbenone is an anti-aggregation pheromone. In bark beetles, the pheromone verbenone is produced from dietary α -pinene in the fat body (Blomquist et al., 2010) and is an anti-aggregation pheromone in most *Dendroctonus* spp. *D. armandi* infestations appear to be increasing throughout the Qinling Mountains, and it is often difficult to use physicochemical methods and semiochemical traps to protect healthy Chinese white pines against attack by *D.*

armandi. It might be possible to use the anti-aggregation pheromone verbenone to reduce the mortality of Chinese white pines and thereby protect healthy pines. There are field trapping and tree protection studies on *D. brevicomis*, *D. ponderosae* and *D. valens* (Gillette et al., 2006, 2012; Fettig et al., 2009). Although, a GC-MS analysis of hindgut extracts from female *D. armandi* detected verbenone (Chen et al., 2015; Xie & Lv, 2012), little is known about the function of verbenone as an anti-aggregation pheromone in *D. armandi*. In this study, verbenone significantly reduced the number of *D. armandi* trapped compared with the control at two sites by approximately 30%. This indicates that verbenone might be used to protect healthy Chinese white pines. The efficiency of verbenone pouch release devices is especially high when some *Dendroctonus* spp. beetles are abundant (Progar, 2003, 2005; Borden et al., 2006). We plan to use verbenone pouch release devices in future studies.

In summary, this is the first biological and behavioral analysis of *trans*-verbenol and verbenone as possible semiochemicals of *D. armandi* that has demonstrated the biological activities and characteristics of *trans*-verbenol and verbenone in electrophysiological, laboratory olfactometer experiments and field trials. Furthermore, studies are needed to confirm the attraction of both sexes of *D. armandi* to *trans*-verbenol and evaluate the use of verbenone for protecting trees.

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