Effects of Plant Volatiles on the EAG and Behavioral Responses of *Batocera horsfieldi* Hope (Coleoptera: Cerambycidae)[1]

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ABSTRACT This paper reports on the EAG and behavioral responses of unmated females, unmated males, and mated females of *Batocera horsfieldi* (Hope) (Coleoptera: Cerambycidae) to ten plant volatiles. Seven volatiles tested from the host species *Viburnum awabuki* K., *Betula luminifera* H. Winkl, *Juglans regia* L., and *Populus tomentosa* Carr. were β-myrcene, (*Z*)-3-hexen-1-ol, nonanal, dichloromethyl ether, salicyaldehyde, 3-methylbutyric acid, and trichloroethylene. Three volatiles tested from non-host conifers were (1*S*)-(−)-β-pinene, (1*R*)-(+)-α-pinene, and (1*S*)-(−)-α-pinene. The EAG responses of unmated *B. horsfieldi* females to these ten volatiles were tested at five concentrations (0.0001, 0.001, 0.01, 0.1, and 0.2 μL/μL). For eight of the volatiles, the EAG responses did not change significantly after their concentration was increased to 0.1 μL/μL. However, the maximum EAG response to 3-methylbutyric acid was at 0.0001 μL/μL and it decreased at higher concentrations. The EAG response to salicyaldehyde increased throughout the range of concentrations. The EAG response of mated females to salicyaldehyde at 0.1 μL/μL was significantly stronger than for unmated females or unmated males. EAG data showed that (1*S*)-(−)-β-pinene at 0.2 μL/μL had a strong repellent affect on unmated female and unmated male *B. horsfieldi* adults ($P < 0.01$). At 0.2 μL/μL, (*Z*)-3-hexen-1-ol had an attractive effect on unmated females ($P < 0.05$) and salicyaldehyde had an attractive effect on mated female *B. horsfieldi* adults ($P < 0.05$) in a Y-tube olfactometer. There was no significant difference in the repellent or attractive effects of dichloromethyl ether, 3-methylbutyric acid, or (1*R*)-(+)-α-pinene at five concentrations to unmated female or male *B. horsfieldi* adults.

KEY WORDS *Batocera horsfieldi* Hope, plant volatiles, EAG response, behavior response, Y-tube olfactometer

Batocera horsfieldi (Hope) (Coleoptera: Cerambycidae) is an important wood-boring insect that damages many hardwoods, such as *Populus* spp. (Salicaceae), *Juglans regia* (L.) (Juglandaceae), *Fagus engleriana* (Seem) (Fagaceae), *Rosa multiflora* (Thunb) (Rosaceae), *Viburnum awabuki* (K.) (Caprifoliaceae), *Betula*

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luminifera (H.Winkl) (Betuleae), and *Ligustrum lucidum* (Ait) (Oleaceae) (Sun & Zhao 1991). *Batocera horsfieldi* is mainly distributed in China, Vietnam, Japan, India, and Burma (Myanmar) (Chen et al. 1959). This species needs 2–3 years for each generation on *Populus* in China. The overwintering adults exit their holes at the end of April. The life span of the adults is about nine months, including the overwintering period (Xia et al. 2005). Since the 1990s, *Populus* spp. has been planted in large areas in the southern part of China, including Jiangsu, Zhejiang, Fujian, Anhui, Jiangxi, Hunan, Guangdong, Guangxi, and Sichuan Provinces. Consequently, the population of *B. horsfieldi* has increased rapidly, which has led to serious damage to *Populus* stands. Now *B. horsfieldi* is the main wood-boring insect on *Populus* spp. in these areas and negatively affects the construction of forestry eco-environment and the development of *Populus* spp. industry (Chen & Luo 2001). Due to the damage caused by *B. horsfieldi* larvae, *Populus* trees may become weak or even die. This species affects the growth of the trees, reduces wood quality, and causes significant losses in wood production (Mei et al. 1998).

Recently, production of *Juglans regia* in northern China, the major production area of this plant, has been severely damaged by *B. horsfieldi* with over 70% of the trees being infested. This is the main constraint for the development of the *J. regia* industry (Wang et al. 2004). Moreover, as the climate becomes warmer due to global warming, *B. horsfieldi* may migrate to more northerly areas and expand its range. Also, this pest can rapidly adapt to and damage new hosts (Li et al. 2009).

Pest Risk Analyses (PRA) are issued by the world trade organization to minimize the influence of quarantine inspection upon trade. Quarantine restrictions for all countries should be based on PRAs (Chen et al. 2002). In China, PRA values are classified into four levels (i.e., r = 2.50–3.00 means exceptional risk, r = 2.00–2.49 means high risk, r = 1.50–1.99 means moderate risk, and r = 1.00–1.49 means low risk) (Zheng & Zhao 2005). Li et al. (2009) determined that the PRA for *B. horsfieldi* in China was 2.04, making it the most dangerous pest to the forest industry. However, existing control techniques are not effective, and new and efficient methods for managing this pest are urgently needed.

Most plants have volatile "odors" that, as chemical signaling substances, affect insect behavior, and can play an important role in the interactions between plants and insects (Deng et al. 2004). Specific plant volatiles can help insects locate the plant, select an oviposition site, or serve as a repellent (Du 2001). Recently, the use of the non-host volatiles to regulate pest behavior has been given much attention in the mangement of pest populations and their damage (Xian et al. 2003, Yang et al. 2003, Zhang et al. 2003). Non-host volatiles could disturb normal physiological activities of the pest and cause malnutrition, teratogenesis, crippling, a reduction in reproduction, or death (Pang 1999). The responses of almost 100 kinds of longhorn beetles (Coleoptera: Cerambycidae) to host volatiles have been studied, including species in the genera *Monochamu*, *Xylotrechus*, and *Hylotrupes.* Moreover, the responses of *Phoracantha semipunctata* (F.), *Arhopalus tristis* (F.), *Monochamus alternates* (Hope), and other species to volatiles from non-host plants also have been studied (Allison et al. 2004). In this paper, we selected three common volatiles from non-host conifers, and seven main volatiles from four host plants (*Viburnum awabuki* K., *Betula luminifera* H.Winkl, *Juglans regia* L., and *Populus tomentosa* Carr.) (Liang 2007) to

determine the EAG responses of *B. horsfieldi* adults. Results of bioassays using a Y-tube olfactometer were used to confirm the biological significance of the EAG responses.

Materials and Methods

Insect source. Adult *B. horsfieldi* were collected from *Populus* in Luojiang County in Deyang, China in late April 2010. These adult longicorn beetles had just finished their emergence and had not mated. After sexing, the longicorns were put into a 60 cm × 60 cm × 60 cm breeding cage made of stainless steel mesh. Beetles were fed *V. awabuki* at 25 ± 2°C. The characteristics used to identify mated *B. horsfieldi* were the villi on the abdomen of mated males and the obvious mating plaques on the backside of the mated females (Ji et al. 1996). Female *B. horsfieldi* lay eggs in batches every 8–10 days. During this latent period, females need additional nutrition for the maturation of more eggs (Xia et al. 2005). Therefore, for this experiment, we selected adult females who had mated 10 days previously and were ready to lay eggs.

Preparation of volatiles. Seven volatiles from the host plants *V. awabuk*, *B. luminifera*, *J. regia,* and *P. tomentosa* were tested: trichloroethylene (100% pure), (*Z*)-3-hexen-1-ol (98% pure), nonanal (95% pure), dichloromethyl ether (98% pure), β-myrcene (≥80% pure), salicyaldehyde (>99.5% pure), and 3-methylbutyric acid (98% pure). Also, three volatile compounds from non-host conifers were tested: (1*S*)-(−)-β-pinene (98% pure), (1*R*)-(+)-α-pinene (95% pure), and (1*S*)-(−)-α-pinene (98% pure). The volatiles β-myrcene, (1*R*)-(+)-α-pinene, and (*Z*)-3-hexen-1-ol were obtained from Huancheng Industrial Joint-Stock Company (Tokyo, Japan), while the other seven compounds were obtained from the Jingchun Chemical Reagent Company, Ltd. (Shanghai, China). These compounds were dissolved separately in liquid paraffin (Atoleine) (Kelong Chemical Reagents Plant, Chengdu, China), mixed thoroughly, and prepared into solutions. In order to determine the EAG and behavioral responses *of B. horsfieldi* adults to different concentrations of the same volatile, solutions of 0.0001, 0.001, 0.01, 0.1 and 0.2 μL/μL were made for each of the ten volatiles. Atoleine was used as the standard check.

Determination of EAG. There are eleven segments of the antenna of *B. horsfieldi*, but the flagellum has the strongest EAG response (Yang 2008). Thus, the terminal section of the flagellum was cut off with a scalpel so that 1 mm on its end was removed. This piece of flagellum was glued onto a recording electrode using Spectra360® electrode gel (Parker Laboratories, Inc., Fairfield, NJ) so that the distance between the odorous pipe and the antenna was 1 cm. The EAG was determined according to Du's (1994) method. A 2 μL odor source sample was taken with a micro-sampler. It was uniformly dripped onto a 2 cm × 0.5 cm filter paper strip that was put into a 10-cm sample tube whose ending was connected to the odor stimulating control device. When the baseline was stable, the stimulation was begun. The stimulation time was 0.5 seconds and the interval between stimulations was 30 seconds, which guaranteed recovery of the antennal receptors. For each compound, six antennae (from 6 adults) were tested, and each antenna was stimulated five times. The mean of the observed value for each sample was divided by the mean of the two check values to give the relative value

of EAG response. We used a Syntech electroantennography (EAG) system (Syntech, Hilversum, The Netherlands), including their software.

The EAG experiments were done in two steps. The first step was to determine the EAG values for unmated female *B. horsfieldi* adults to ten volatiles at five concentrations. The second step was to use the optimal concentration (0.1 μL/μL) determined from the first stage to determine the EAG responses for unmated males and mated adults.

Laboratory bioassay of volatiles. A glass Y-tube olfactometer with an inside diameter of 15 cm was used to conduct the bioassays. The main arm of the device was 30 cm long, and the two side arms were 25 cm long. The angle between the two side arms was 75°, and the ends of the arms had ground-glass edges. The two side arms were connected to two 250-mL volumetric flasks by telfon tubes. The preparation method of the volatiles was the same as for the EAG test, and 10 μL was taken from different concentrations of the volatile for the bioassays. Atoleine was used as the check, and filter papers (1 × 1 cm) were used as the solvent carriers. Filter papers were put into the two volumetric flasks separately. The volumetric flasks were connected by Telfon tubes to a distilled water humidification bottle and an air purification bottle. The air flow speed was controlled at 0.5–0.6 L/min. Tests were run 08:00–12:00 am when the temperature of the laboratory was 25 ± 1°C. *B. horsfieldi* adults were introduced into the inlet of the main arm of the Y-tube, and after they had moved forward 2 cm from the inlet, we began timing. Adults had to make a choice at the junction of the Y-tube to one of the side arms. Tests were conducted for five minutes to observe each *B. horsfieldi*. If an insect went forward 5 cm into a side arm and stayed for at least one minute, it was recorded as having made a choice of this odor, otherwise it was recorded to have made no choice. After every five adults, the two arms of the Y-tube were interchanged to eliminate the possible influence of the different arms on the behavior of *B. horsfieldis*. When each treatment was finished, the Y-tube olfactometer, telfon tubes, and the volumetric flasks were washed with alcohol and allowed to air dry. There were three replications for each test, and 30 *B. horsfieldis* were used for each replication.

There is an optimal concentration range of the responses to plant volatile for each kind of insect. Usually, when it is lower than the value within the range, the response rate will be greater as the concentration increases, but when it is within the range, the rate will be smaller as the concentration increases (Fan et al. 2003). Therefore, the bioassay test also included two steps. The first step was to use the Y-tube olfactometer to determine the behavioral responses of unmated males, unmated females, and mated females to the ten volatiles at the maximum concentration (0.2 μL/μL). In the second step, three volatiles that caused non-significant behavioral responses in the first step, dichloromethyl ether, 3-methylbutyric acid, and (1*S*)-(−)-α-pinene, were selected for further testing. The behavioral responses of unmated females and males to different concentrations of these volatiles were determined.

Statistical analysis. Statistical analysis was performed using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL). A one-factor randomized complete block analysis of variance (ANOVA) was conducted on the EAG data and behavioral responses data. Fisher's protected least significant difference (LSD) multiple comparison procedure was used in the behavioral responses and EAG responses of unmated male, unmated female, and mated female adults to

the volatiles (Dong et al. 2000, Liu et al. 2005). A Chi-square test was used to compare the rate of repellency and the rate of attraction (Sokal & Rohlf 1995). The rate of repellency, rate of attraction, and response rate were calculated according to the following formulas (Ding et al. 1996, Yan et al. 2006):

Rate of repellency = (total number of *B. horsfieldis* in the control arm/total number of *B. horsfieldis* tested) × 100%;

Rate of attraction = (total number of *B. horsfieldis* in the treatment arm/ total number of *B. horsfieldis* tested) × 100%;

Response rate = ((total number of *B. horsfieldis* in the control arm + total number of *B. horsfieldis* in the treatment arm)/ total number of *B. horsfieldis* tested) × 100%;

Selection rate = (total number of *B. horsfieldis* in the treatment arm/ (total number of *B. horsfieldis* tested – no behavior response of number of *B. horsfieldis*)) × 100%.

Results

EAG response of unmated female *B. horsfieldi* adults to ten volatiles. The EAG responses of the unmated female *B. horsfieldi* adults to different concentrations of ten volatiles are shown in Figure 1. The EAG response values of unmated females to five volatiles ((1*S*)-(−)-β-pinene, (1*R*)-(+)-α-pinene, (1*S*)-(−)-α-pinene, β-myrcene, and dichloromethyl ether) increased with concentrations from 0.0001 to 0.01 μL/μL, before reaching a plateau. The EAG response values of the unmated females to (*Z*)-3-hexen-1-ol, nonanal, and trichloroethylene reached a maximum when their concentrations were 0.1 μL/μL and decreased at higher concentrations. For salicyaldehyde, the EAG value continued to increase throughout the range of concentrations. However, for 3-methylbutyric acid, the maximum EAG value was at 0.0001 μL/μL and the response flattened out at higher concentrations (Fig. 1).

EAG and behavioral responses of *B. horsfieldi* adults to ten plant volatiles. The EAG responses of *B. horsfieldi* adults to different plant volatiles are shown in Table 1. The EAG responses of unmated females to (1*S*)-(−)-β-pinene and to (*Z*)-3-hexen-1-ol were significantly higher than to the other volatiles ($F = 485.51$; $P < 0.01$). Their EAG responses to dichloromethyl ether and salicyaldehyde were low and not significantly different from each other ($F = 485.51$; $P > 0.05$).

Unmated males had the strongest EAG responses to (*Z*)-3-hexen-1-ol, (1*S*)-(−)-β-pinene, and β-myrcene (Table 1). LSD revealed that the EAG responses of the unmated males to (*Z*)-3-hexen-1-ol were significantly higher than that for eight of the other volatiles ($F = 621.39$; $P < 0.01$). Their responses to salicyaldehyde and trichloroethylene were not significantly different from each other ($F = 621.39$; $P > 0.05$).

Mated females had the strongest EAG responses to salicyaldehyde, (*Z*)-3-hexen-1-ol, and (1*S*)-(−)-β-pinene (Table 1). The EAG responses of mated females to salicyaldehyde and (*Z*)-3-hexen-1-ol were significantly higher than that for other volatiles ($F = 355.38$; $P < 0.01$). EAG response values to dichloromethyl ether and 3-methylbutyric acid were low and were not significantly different from each other ($F = 355.38$; $P > 0.05$).

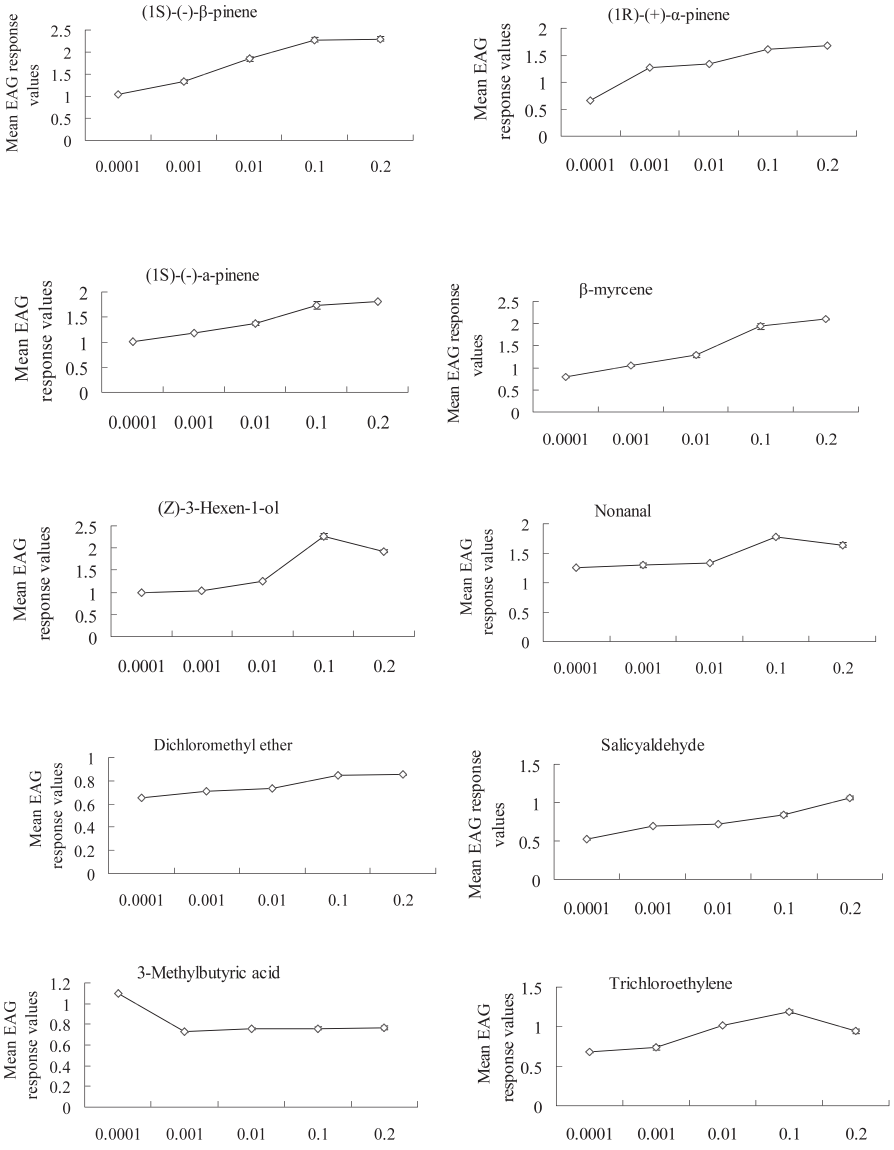

Fig. 1. EAG response of unmated female adults of *Batocera horsfieldi* to ten volatiles.

Table 1. EAG responses of *Batocera horsfieldi* adults to different volatiles (0.1 μL/μL).

Volatiles	EAG values[a]		
	Unmated female	Unmated male	Mated female
(1*S*)-(−)-β-pinene	2.269 ± 0.010 Aa	2.207 ± 0.036 ABab	2.079 ± 0.046 Bb
(1*R*)-(+)-α-pinene	1.620 ± 0.044 Da	1.276 ± 0.026 Eb	1.618 ± 0.034 Ca
(1*S*)-(−)-α-pinene	1.736 ± 0.001 Cb	1.896 ± 0.064 CDa	1.665 ± 0.048 Cb
β-myrcene	1.936 ± 0.001 Ba	2.019 ± 0.051 BCa	1.779 ± 0.001 Cb
(*Z*)-3-hexen-1-ol	2.276 ± 0.009 Aa	2.359 ± 0.005 Aa	2.310 ± 0.006 Aa
Nonanal	1.770 ± 0.001 Ca	1.744 ± 0.019 Da	1.586 ± 0.061 Cb
Dichloromethyl ether	0.853 ± 0.009 Fab	0.917 ± 0.005 Fa	0.821 ± 0.010 Eb
Salicyaldehyde	0.839 ± 0.001 Fc	1.139 ± 0.013 Eb	2.499 ± 0.060 Aa
3-methylbutyric acid	0.741 ± 0.011 Gb	0.633 ± 0.010 Gc	0.819 ± 0.001 Ea
Trichloroethylene	1.180 ± 0.008 Ea	1.149 ± 0.023 Ea	1.133 ± 0.009 Da

[a]Mean ± SE.
Means in the same column followed by the same capital letter are not significantly different ($P < 0.01$), ANOVA followed by LSD.
Means in the same row followed by the same lowercase letter are not significantly different ($P < 0.01$) according to Duncan's multiple range test.

EAG response values to salicyaldehyde for mated females were significantly higher than for unmated males or unmated females ($F = 118.24$; $P < 0.01$) (Table 2). However, responses to (*Z*)-3-hexen-1-ol ($F = 2.24$; $P > 0.05$) and trichloroethylene ($F = 7.35$; $P > 0.05$) were not significantly different among the three groups of *B. horsfieldi*.

Table 2. Behavioral responses of unmated female *Batocera horsfieldi* to different volatiles in a Y-tube bioassay.

Volatiles[a]	Repellent rate (%)	Attractive rate (%)	χ^{2} [b]	Response rate (%)
(1*S*)-(−)-β-pinene	72.2	21.1	7.40 **	93.3
(1*R*)-(+)-α-pinene	43.3	51.1	0.17 ns	94.4
(1*S*)-(−)-α-pinene	58.9	34.4	1.44 ns	93.3
β-myrcene	37.8	58.9	0.99 ns	96.7
(*Z*)-3-hexen-1-ol	27.8	64.4	4.06 *	92.2
Nonanal	31.1	65.6	3.13 ns	96.7
Dichloromethyl ether	55.6	38.9	0.59 ns	94.4
Salicyaldehyde	60.0	34.4	1.62 ns	94.4
3-methylbutyric acid	47.8	48.9	0.02 ns	96.7
Trichloroethylene	32.2	60.0	2.05 ns	92.2

[a]Volatiles tested at 0.2 μL/μL.
[b]Significance levels of χ^{2} test indicated by ns (non-significant; $P > 0.05$), * ($P < 0.05$), and ** ($P < 0.01$).

Table 3. Behavioral responses of unmated male *Batocera horsfieldi* to different volatiles in a Y-tube bioassay.

Volatiles[a]	Repellent rate (%)	Attractive rate (%)	χ^{2} [b]	Response rate (%)
(1*S*)-(−)-β-pinene	74.5	17.8	9.45 **	92.2
(1*R*)-(+)-α-pinene	48.9	43.3	0.05 ns	92.2
(1*S*)-(−)-α-pinene	58.9	36.7	1.18 ns	95.6
β-myrcene	35.6	62.2	1.69 ns	97.8
(*Z*)-3-hexen-1-ol	33.3	64.4	2.49 ns	97.8
Nonanal	38.9	60.0	1.00 ns	98.9
Dichloromethyl ether	61.1	35.6	1.58 ns	96.7
Salicyaldehyde	45.6	50.0	0.06 ns	95.6
3-methylbutyric acid	40.0	48.9	0.06 ns	93.3
Trichloroethylene	35.6	61.1	1.58 ns	96.7

[a]Volatiles tested at 0.2 μL/μL.
[b]Significance levels of χ^{2} test indicated by ns (nonsignificant; $P > 0.05$) and ** ($P < 0.01$).

The results of the Y-tube bioassays showed that *B. horsfieldi* adults had high overall response rates (over 92%) to the ten volatiles at the concentration of 0.2 μL/μL (Tables 2, 3, & 4). Unmated females showed no significant difference between repellent rate and attractive rate for eight of the volatiles (Table 2). However, there was a significant ($\chi^{2} = 7.40$; df = 1; $P < 0.01$) repellent effect toward (1*S*)-(−)-β-pinene, and a significant ($\chi^{2} = 4.06$; df = 1; $P < 0.05$) attractive effect toward (*Z*)-3-hexen-1-ol. Unmated males showed no significant difference between repellent rate and attractive rate for nine of the volatiles (Table 3). Only (1*S*)-(−)-β-pinene had a significant ($\chi^{2} = 9.45$; df = 1; $P < 0.01$) repellent effect.

Table 4. Behavioral responses of mated female *Batocera horsfieldi* to different volatiles in a Y-tube bioassay.

Volatiles[a]	Repellent rate (%)	Attractive rate (%)	χ^{2} [b]	Response rate (%)
(1*S*)-(−)-β-pinene	62.2	35.6	1.88 ns	97.8
(1*R*)-(+)-α-pinene	51.1	45.6	0.07 ns	96.7
(1*S*)-(−)-α-pinene	57.8	41.1	0.56 ns	98.9
β-myrcene	45.6	52.2	0.06 ns	97.8
(*Z*)-3-hexen-1-ol	38.9	58.9	0.87 ns	97.8
Nonanal	45.6	53.3	0.11 ns	98.9
Dichloromethyl ether	58.9	36.7	1.18 ns	95.6
Salicyaldehyde	26.7	66.7	4.40 *	93.3
3-methylbutyric acid	47.8	47.8	0.00 ns	95.6
Trichloroethylene	44.4	51.1	0.06 ns	95.6

[a]Volatiles tested at 0.2 μL/μL.
[b]Significance levels of χ^{2} test indicated by ns (nonsignificant; $P > 0.05$) and * ($P < 0.05$).

Table 5. Selection rate of *Batocera horsfieldi* adults to different volatiles in a Y-tube bioassay.

Volatiles	Selection rate (%)[a]		
	Unmated females	Unmated males	Mated females
(1*S*)-(−)-β-pinene	22.61 ± 1.49 Eab	19.05 ± 3.02 Eb	37.52 ± 3.27 Ea
(1*R*)-(+)-α-pinene	55.35 ± 2.14 ABCa	46.85 ± 1.26 CDa	47.56 ± 1.44 CDEa
(1*S*)-(−)-α-pinene	37.43 ± 0.41 DEa	38.65 ± 1.65 Da	42.25 ± 0.97 CDEa
β-myrcene	60.33 ± 0.23 ABa	63.64 ± 1.01 Aa	54.54 ± 1.04 BCb
(*Z*)-3-Hexen-1-ol	69.86 ± 3.82 Aa	65.86 ± 2.53 Aa	60.23 ± 1.11 Ba
Nonanal	68.03 ± 2.40 Aa	60.65 ± 1.40 ABab	52.81 ± 1.46 BCb
Dichloromethyl ether	42.01 ± 1.82 CDa	36.56 ± 1.60 Da	38.51 ± 2.38 DEa
Salicyaldehyde	35.81 ± 1.30 DEc	51.15 ± 2.47 BCb	73.42 ± 2.50 Aa
3-methylbutyric acid	50.54 ± 1.48 BCDa	46.45 ± 2.19 Da	50.82 ± 2.66 BCDa
Trichloroethylene	65.42 ± 2.21 ABa	64.26 ± 0.46 Aa	53.77 ± 1.55 BCb

[a]Percent of insects choosing the test compound versus the atoleine control, mean ± SE.
Means in the same column followed by the same capital letter are not significantly different ($P < 0.01$), ANOVA followed by LSD.
Means in the same row followed by the same lowercase letter are not significantly different ($P < 0.01$), ANOVA followed by LSD.

Mated females also showed no significant difference between repellent rate and attractive rate for nine of the volatiles (Table 4). Only salicyaldehyde had a significant ($\chi^2 = 4.40$; df = 1; $P < 0.05$) attractive effect to mated females in the Y-tube olfactometer experiments.

Unmated females had the highest selection rates for (*Z*)-3-hexen-1-ol (69.9%), nonanal (68.0%), trichloroethylene (65.4%), and β-myrcene (60.3%), and the lowest selection rate for (1*S*)-(−)-β-pinene (22.6%) (Table 5). Unmated males had the highest selection rates for (*Z*)-3-hexen-1-ol (65.9%), trichloroethylene (64.3%), β-myrcene (63.6%), and nonanal (60.7%), and the lowest selection rate for (1*S*)-(−)-β-pinene (19.1%). Mated females had the highest selection rate for salicyaldehyde (73.4%), and the lowest selection rate for (1S)-(−)-β-pinene (37.5%). Among the three groups (unmated females, unmated males, and mated females), the behavioral responses to (1*R*)-(+)-α-pinene ($F = 11.77$; $P > 0.05$), (1*S*)-(−)-α-pinene ($F = 18.84$; $P > 0.05$), (*Z*)-3-hexen-1-ol ($F = 12.26$; $P > 0.05$), dichloromethyl ether ($F = 16.84$; $P > 0.05$), and 3-methylbutyric acid ($F = 7.59$; $P > 0.05$) were not significantly different; however, there were significant differences among groups for (1*S*)-(−)-β-pinene ($F = 288.06$; $P < 0.01$), β-myrcene ($F = 34.47$; $P < 0.01$), nonanal ($F = 54.03$; $P < 0.01$), salicyaldehyde ($F = 386.31$; $P < 0.01$), and trichloroethylene ($F = 44.03$; $P < 0.01$) (Table 5).

Behavioral responses of unmated female and unmated male *B. horsfieldi* adults to different concentrations of three volatiles. As shown previously (Tables 2 & 3), there were no significant behavioral responses of unmated female and unmated male adults in Y-tube bioassays to dichloromethyl ether, 3-methylbutyric acid, and (1*R*)-(+)-α-pinene at 0.2 μL/μL. Results showed that for both unmated males and unmated females, there were no significant

behavioral responses over four lower concentrations (0.0001, 0.001, 0.01, and 0.1 μL/μL) of these volatiles (data not shown).

Discussion

Batocera horsfieldi adults had strong EAG responses to (*Z*)-3-hexen-1-ol, which is one of the main green leaf volatiles (Visser et al. 1979) from their host plant. The green leaf volatile concentrations are related to the growth status of the plant as well as whether the plant has been damaged by a pest (Lou & Cheng 2000). Several studies have shown that green leaf volatiles not only have attractive effects on pests, but they also influence the production and release of pheromones in some pests (Dickens et al. 1990, Landolt et al. 1994, Reddy & Guerrero 2004, Wang et al. 2005).

In the wild, *B. horsfieldi* uses multiple host plants. Typically, the host for adult feeding is different from the host required by the larvae (oviposition host) (Liang et al. 2008). After emergence, *B. horsfieldi* adults fly to adjacent *R. multiflora*, *V. awabuki*, *B. luminifera*, or other host plants to feed. After they feed and mate, females return to the oviposition host (e.g., *P. tomentosa*) to lay eggs (Jiang et al. 1999). Volatile signaling substances released by the host plant directly influence the adult insect's selection of a food source and choice of an oviposition site (Hanks 1999). Salicyaldehyde, which is released by *P. tomentosa*, is the main odorous substance used by *B. horsfieldi* for oviposition on this host. Salicyaldehyde has a repellent effect on unmated females, but it has an attractive effect on mated females. Therefore, after feeding, mated females use salicyaldehyde as an attractant and fly back to the *P. tomentosa* to lay eggs.

Non-host plants can disturb the behavior of pests while they are seeking a host for feeding or oviposition (Zhang et al. 2003). Suckling et al. (2001) showed that if burned pine, the favorite host of *A. tristis*, was treated with a mixture of the non-host compounds (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, and mineral oil at a ratio of 1:1:2, trap counts decreased to 20% of those with untreated pine, and the oviposition rate was reduced by 98.5%. Yan et al. (2006) showed that the non-host volatile turpentine oil (0.6 μmol/μL) had a strong repellent effect on female *Xylotrechus rusticus* (L.). The host plants of *B. horsfieldi* are hardwoods. Therefore, for this study, we selected the main volatiles of conifers (pinenes) as non-host volatiles. Our results revealed that the non-host volatiles (1*S*)-(−)-β-pinene and (1*S*)-(−)-α-pinene had significant repellent effects on *B. horsfieldi* adults. Future studies should include spraying these compounds on host plants to prevent recognition by *B. horsfieldi*. Borden et al. (2001) showed that the mixture of 1-hexanol and phenylcarbinol reduced trap counts of *Trypodeneron lineatum* (Olivier) by 80%. Aharoni et al. (2003) showed that linalool produced by transgenic *Arabidopsis* had a repellent effect on *Myzus persicae* (Sulzer). These studies showed that there is a bright future for pest control through chemical and ecological technology.

The EAG responses of *B. horsfieldi* for some of the compounds differed among unmated females, unmated males, and mated females. The gender and mating status of insects can play important roles in host seeking behavior. This might be reflected in differences in the number of the antenna receptors between the female and male adults or in the existence of qualitative physiological differences in olfaction (Raguso et al. 1996). Unmated females and males had different

behavioral responses to different concentrations of three volatiles. The phytophagous insect usually uses their olfactory receptors to recognize specific volatiles from the host plant (Barata et al. 2002). Different concentrations of the same volatile have various effects on the physiological activity of the phytophagous insect. The study performed by Leskey et al. (2001) showed the response values of *Conotrachelus nenuphar* (Herbst) to linalol and 3-hydroxy-2-butanone at the concentration of 1.0% were lower than the reference value, while the attraction effect at the concentration of 0.01% and 0.001% was higher than the reference.

During actual applications of plant volatiles for pest management, the controlled slow release of an optimum concentration of volatiles is an important factor in the development of this technology as well as the key point for research. The volatiles released by plants are a complex mixture of many kinds of secondary volatile substances. If these exogenous volatiles were sprayed on living plants, they might influence the insect (Yan et al. 2003). The repellent effects of the (1*S*)-(−)-β-pinene, and the attractive effects of (*Z*)-3-hexen-1-ol and salicyaldehyde should be tested in future forest experiments.

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