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# Male-Produced Sex Pheromone of the Cerambycid Beetle *Hedypathes betulinus*: Chemical Identification and Biological Activity

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**Abstract** We identified, synthesized, determined the diel periodicity of release, and tested the bioactivity of components of the male-produced sex pheromone of *Hedypathes betulinus* (Coleoptera: Cerambycidae: Lamiinae). Gas chromatographic-mass spectrometric analysis of headspace volatiles from adult beetles showed three male-specific compounds, which were identified as (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (major component), (*E*)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone), and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol. Release of these chemicals was dependent on time of the photoperiod and presence of the host plant. Pheromone release took place primarily during the photophase, with maximum release occurring between 4 and 6 hr after the onset of photophase. The amount of pheromone released by males was much greater when they were in the presence of their host plant than when they were not. In Y-tube olfactometer tests, a ternary mixture of the compounds was attractive to female beetles, although the individual compounds were not attractive by themselves. Addition of volatiles from the host plant greatly increased the attractiveness of the ternary pheromone mixture and of the major pheromone component alone.

**Key Words** Cerambycidae · Sex pheromone · Identification · Y-olfactometer · Host-plant volatile chemicals

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## Introduction

In the southern region of Brazil, *Hedypathes betulinus* (Coleoptera: Cerambycidae: Lamiinae) (Klug 1825) is the most important pest of green maté (*Ilex paracguariensis*), a popular tea in the region. Larvae bore into and feed on the branches and trunks of green mate, causing disruption of translocation and death of plants. As larvae bore they produce sawdust, which accumulates at the base of the stalk (Cassanello, 1993). Eradication of *H. betulinus* is labor-intensive and expensive because suitable pesticides are not available, and infestations are controlled by manually collecting adult beetles or removing infested branches or trees.

Semiochemical-based monitoring and mass trapping of *H. betulinus* adults are potential tactics for the control of this pest. Recently, responses of *H. betulinus* to volatile chemicals released by conspecifics were demonstrated in olfactometer bioassays (Fonseca and Zarbin, 2009). Extracts of males were attractive to females but not to males, while extracts of females were unattractive to either sex, suggesting that males produce a sex pheromone.

In this study, we identified and synthesized male-specific compounds released by *H. betulinus*, and tested their attractiveness alone and in combination with host plant volatiles. Additionally, we determined the influence of host plant and photoperiod on pheromone release.

## Methods and Materials

*Insects* Adult insects were used in each experiment, and were collected from a green maté plantation located in São Mateus do Sul, Paraná, Brazil. Adults were sexed according to the method of Cassanello (1993); males have a thicker

antennal scape and foreleg femur than do females. After sexing, males and females were placed in separate plastic boxes (7.5×8.5 cm) and fed green maté branches. Insects were maintained at 25±2°C at 60±5% RH, under a 12:12 hrL/D photoperiod. The mating history of field-captured beetles was unknown.

*Pheromone Collection and Analysis* Groups of either 4 males or 4 females were placed in an all-glass aeration chamber, and the released volatiles were trapped on Super Q columns (200 mg; Alltech, Deerfield, IL, USA) as previously reported (Zarbin et al., 2003). Samples were collected continuously over 15 day by using a humidified and charcoal-filtered airstream (1 $l.min^{-1}$). Volatiles from each aeration were eluted from the Super Q columns with distilled hexane, with the adsorbent traps changed after 10 collections. Extracts were concentrated to 400 µl (one insect equivalent per 100 µl) using argon (Zarbin et al., 1999; Zarbin, 2001).

In order to investigate the effect of host plant on release of pheromone, beetle volatiles were collected as described above for 24 h over 3 day, either in the presence or absence of a small branch of host plant ($N$=3 per treatment). The same procedure was performed to determine the diel rhythm of volatile release from males during the photophase and scotophase and, subsequently, every 2 hr of the photophase (over 3 day; $N$=3 males per treatment). Plant volatiles were also collected from 67 g of green maté branches. Branches were placed in all-glass aeration chambers, and volatiles were collected over 24 hr. After elution from traps, the collections were concentrated (10 g of green maté branches/100 µl) under argon (Zarbin et al., 1999; Zarbin, 2001). Data were analyzed by ANOVA, followed by a Tukey *post-hoc* test using BioEstat 3.0 software (Ayres et al., 2003).

Extracts were analyzed by gas chromatography-mass spectrometry (GC–MS) using a Shimadzu gas chromatograph (model 17A) coupled to a Shimadzu QP5050A electron ionization mass detector. The GC was operated in the splitless mode, and was equipped with a DB-5 (30 m× 0.25 m×0.25 µm) or a DB-Wax (30 m×0.25 m×0.25 µm) capillary column (both Agilent Technologies, Santa Clara, CA, USA). The column oven was maintained at 50°C for 3 min, and then increased to 250°C at 7°C.$min^{-1}$ to 250°C. The concentration of the major chemical component from the sex-specific volatiles was determined by using an external standard curve (1, 10, 100, 500, 1,000, 1,500, and 2,000 ppm) based on tridecane.

*Synthesis* $^{1}$H- and $^{13}$C-NMR spectra were recorded using a Bruker ARX-200 spectrometer (200 and 50 MHz, respectively) in a $CDCl_3$ solution. Chemical shifts are expressed in ppm relative to $CDCl_3$ (7.27 and 77.23 ppm for $^{1}$H and $^{13}$C NMR, respectively). The infrared (IR) spectra were measured as films using a Bomem B100 spectrometer. Crude products were purified by flash or vacuum flash chromatography on silica gel (230–400 mesh). (*E*)-6,10-Dimethyl-5,9-undecadien-2-one (geranylacetone) **(A)** is commercially available (Fluka® 99.5% *E* isomer; Sigma-Aldrich, Milwaukee, WI, USA, 60/40 *E*/*Z* isomers). (*E*)-6,10-Dimethyl-5,9-undecadien-2-ol **(B)** and (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate **(C)** were synthesized as described in Fig. 1.

*Synthesis of (E)-6,10-dimethyl-5,9-undecadien-2-ol (B)* A dry THF solution (5 ml) of ketone **A** (Fluka®, 99.5%) (1 g, 5.1 mmol) was added slowly to a suspension of $LiAlH_4$ (0.290 g, 7.7 mmol) in THF (15 ml) at 0°C. The mixture was stirred for 5 hr at room temperature, then cooled to 0°C, hydrolyzed with $NaOH_{(aq)}$ (15%; 5 ml), and extracted several times with diethyl ether. The combined ether extracts were washed with water, dried over $Na_2SO_4$, and concentrated. The crude product was purified by flash chromatography (hexane/ethyl acetate, 8/2), yielding alcohol **B** (0.909 g, 90% yield). $^{1}$H NMR (200 MHz, $CDCl_3$): δ 1.19 (d, 3H, $J$=6.16 Hz), 1.44–1.52 (m, 3H), 1.60 (s, 3H), 1.62 (s, 3H), 1.68 (s, 1H), 1.92–2.14 (m, 6H), 3.73–3.87 (m, 1H), 5.02–5.21 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 16.0, 17.7, 23.5, 24.4, 25.7, 26.6, 39.2, 39.7, 68.0, 123.9, 124.3, 131.4, 135.7. IR (ν Max, $cm^{-1}$): 824, 1082, 1127, 1377, 1447, 2853, 2915, 2966, 3349.

*Synthesis of (E)-6,10-dimethyl-5,9-undecadien-2-yl acetate (C)* An excess of acetic anhydride (0.5 ml, 5 mmol) and pyridine (0.5 ml) was added to alcohol **B** (0.800 g, 4.0 mmol) in dichloromethane (15 ml). The solution was stirred for 12 hr, diluted with dichloromethane (20 ml), and then washed with $HCl_{(aq)}$ (10%). The organic layer was

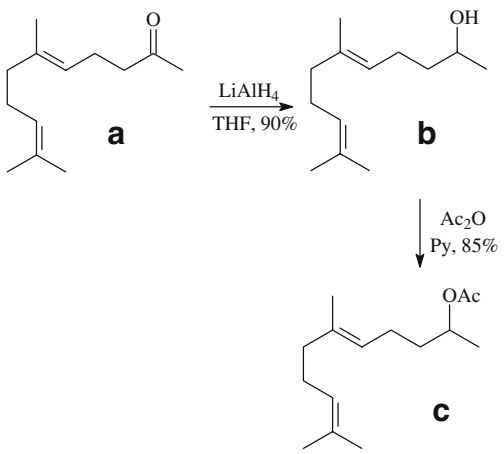


**Fig. 1** Racemic synthesis of (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (**b**) and (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (**c**) from (*E*)-6,10-dimethyl-5,9-undecadien-2-one (**a**)

washed with saturated $NaHCO_3$ solution before the dichloromethane solution was separated, dried over $Na_2SO_4$, and concentrated. After flash chromatography (hexane/ethyl acetate, 9/1), the acetate **C** was obtained at 85% yield (0.784 g). $^1$H NMR (200 MHz, $CDCl_3$): δ 1.22 (d, 3H, *J*=6.26 Hz), 1.50–1.65 (m, 8H), 1.68 (s, 3H), 1.94–2.10 (m, 9H), 4.79–4.98 (m, 1H), 5.03–5.17 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 15.9, 17.7, 20.0, 21.4, 23.9, 25.7, 26.7, 36.0, 39.7, 70.7, 123.4, 124.3, 131.4, 135.7, 170.8. IR (ν Max, $cm^{-1}$): 1018, 1058, 1127, 1240, 1371, 1439, 1736, 2853, 2921, 2971.

*Laboratory Bioassay of Synthetic Pheromone* Female *H. betulinus* responses to synthetic pheromone racemic mixtures were tested in a binary choice Y-tube olfactometer, using humidified, charcoal-filtered air at 4 $l.min^{-1}$. The olfactometer consisted of a Y-shaped glass tube (4×40 cm) with two 20-cm arms. Hydrochloric acid and ammonium hydroxide were mixed to confirm the plume distribution of odor sources throughout the system (Baker and Linn, 1984). Odor sources were placed at the base of one arm of the olfactometer, and consisted of a 2×2 cm piece of filter paper loaded with synthetic pheromone, plant volatiles, or hexane (control). A single female beetle was placed at the base of the main olfactometer tube, and its behavior was observed for 20 min. A female that walked upwind and made contact, within 20 min., with a filter paper containing either the treatment or control odor source was recorded as a positive or negative response, respectively. Females that did not contact either the treatment or control odor source were excluded from the statistical analysis. The Y-tube was cleaned with alcohol and left to dry for 5 min after testing 4 females. The positions of the olfactometer arms were reversed upon changing the odor sources. A preliminary study showed that there was no difference in the responses of males or females in the olfactometer when both of the arms were blank, indicating a lack of positional effect of the experimental setup. Nine experiments testing female responses were performed: 1) to 10 μl of the plant volatiles extract; 2) to 5 μg (5 μl of a solution of 1 μg/μl hexane) of synthetic major pheromone component; 3) to 8 μg of synthetic major component; 4) to 5 μg of synthetic major component plus 10 μl of the plant volatiles collection; 5) to 8 μg of synthetic major component plus 10 μl of the plant volatiles collection; 6) to ternary mixture **I** (5 μg **C**: 0.43 μg **A**: 0.038 μg **B**); 7 to ternary mixture **II** (8 μg **C**: 0.69 μg **A**: 0.06 μg **B**); 8) to ternary mixture **I** plus 10 μl of the plant volatiles collection; and 9) to ternary mixture **II** plus 10 μl of the plant volatiles collection. The ternary mixture ratio [91.4:7.9:0.70 of racemic (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate, (*E*)-6,10-dimethyl-5,9-undecadien-2-one and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol, respectively] that was used in the experiment was similar to that found in male volatile collections We tested at least 30 individuals in each experiment, with the odor sources replaced after each female tested. Each individual was tested only once. Experiments were performed during the fourth to ninth hour of the photophase, when adult beetles were active (see Results).

Data were analyzed by the Chi-squared test using BioEstat 3.0 software (Ayres et al., 2003).

## Results

The chromatographic profile of volatiles from male and female *H. betulinus* showed three male-specific compounds, **A**, **B**, and **C**, with the following retention times (Rts) and Kovat's Indices (KIs) on the DB-5 column: **A**: Rt=16.65 min., KI=1,448; **B**: Rt=16.72 min, KI=1,454; **C**: Rt=18.84 min, KI=1,573. The ratio between these components was calculated as 7.9:0.7:91.4, respectively, based on the area of the GC peaks (Fig. 2). The mass spectrum of compound **A** was simple, with a base peak at *m/z* 43, a fragment at *m/z* 69, and a molecular ion of 194 Da (Fig. 3a). When this spectrum was compared to the NIST library, it was evident that the compound might be (*E*)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) or its 5*Z* isomer (nerylacetone). Compound (**A**) was identified as geranylacetone by co-injection of the crude extract with geranyl- and nerylacetone standards on the two GC columns. Identification was based on the similarity of retention times and fragmentation pattern.

Compound **B** had a similar retention time as component **A**. The mass spectrum (Fig. 3b) showed a molecular ion of *m/z* 196, a base peak at *m/z* 41, and other fragments at *m/z* 69 (90%) and 153 (34%), suggesting that **B** may be related to geranylacetone with hydrogenation at the carbon 5, or the corresponding alcohol, geranylacetol. Reduction of compound **A** with $LiAlH_4$ (Zarbin et al., 1998) yielded an alcohol with the same retention time and mass spectrum as compound **B**, confirming its chemical structure as (*E*)-6,10-dimethyl-5,9-undecadien-2-ol. In a previous study, this alcohol was identified as the primary component of the male-produced sex pheromone of the longhorn beetles, *Tetropium fuscum* and *Tetropium cinnamopterum*, members of the Spondylidinae subfamily, and was named fuscumol (Silk et al., 2007).

The mass spectrum of component **C** showed a base peak at *m/z* 109, fragments at *m/z* 43 (60%), *m/z* 69 (51%), and had a molecular weight of 238 Da (Fig. 3c). The molecular weight of component **C** was 42 Da greater than component **B**, and the retention time more than 2 min longer. These differences, in addition to the appearance of a fragment at *m/z* 43, suggested the presence of an acetyl group. This was

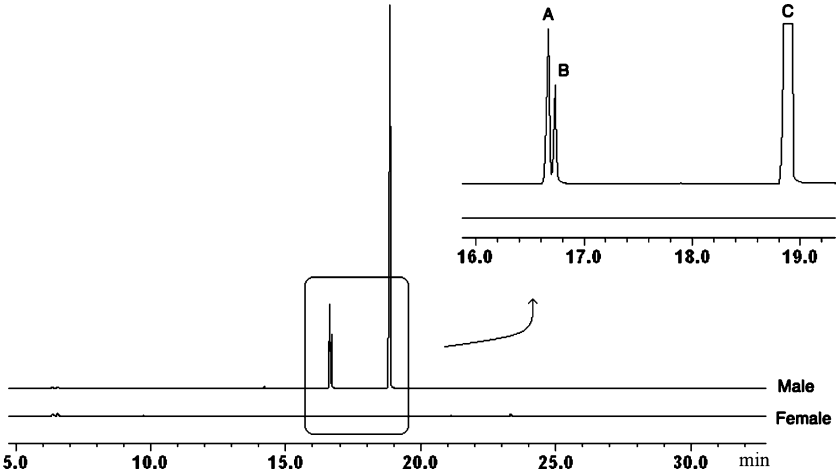


**Fig. 2** Gas chromatographic analyses of volatiles obtained from male and female *Hedypathes betulinus*. The three male-specific compounds are (*E*)-6,10-dimethyl-5,9-undecadien-2-one (**a**), (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (**b**), and (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (**c**)

also supported by the appearance of a fragment at *m/z* 178, resulting from the loss of acetic acid ($M^+$ - 60). Acetylation of alcohol **B** by acetic anhydride and pyridine (Leal et al., 1999) resulted in an ester with a fragmentation pattern and retention time that perfectly matched that of compound **C**. Thus, the major compound was identified as (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (fuscumol acetate).

The production of (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate **C** was dependent on the presence of host plant. Beetles that fed on green maté released more total volatiles (344.2±86.5 μg/insect/day) than beetles that did not feed on green maté (0.53±0.35 μg/insect/day) ($F_{1,4}$=157.02, $P$< 0.05) (Fig. 4a). The release of pheromone components occurred during the photophase rather than the scotophase ($F_{1,4}$=14.93, $P$<0.05) (Fig. 4b), with maximum release occurring between 4 and 6 hr after the onset of the photophase (Fig. 4c). Six hours after the onset of the photophase, the amount of pheromone released decreased ($F_{5,12}$=94.08, $P$<0.001), from that of peak release, to an amount similar to that at the start of the photophase (Fig. 4c).

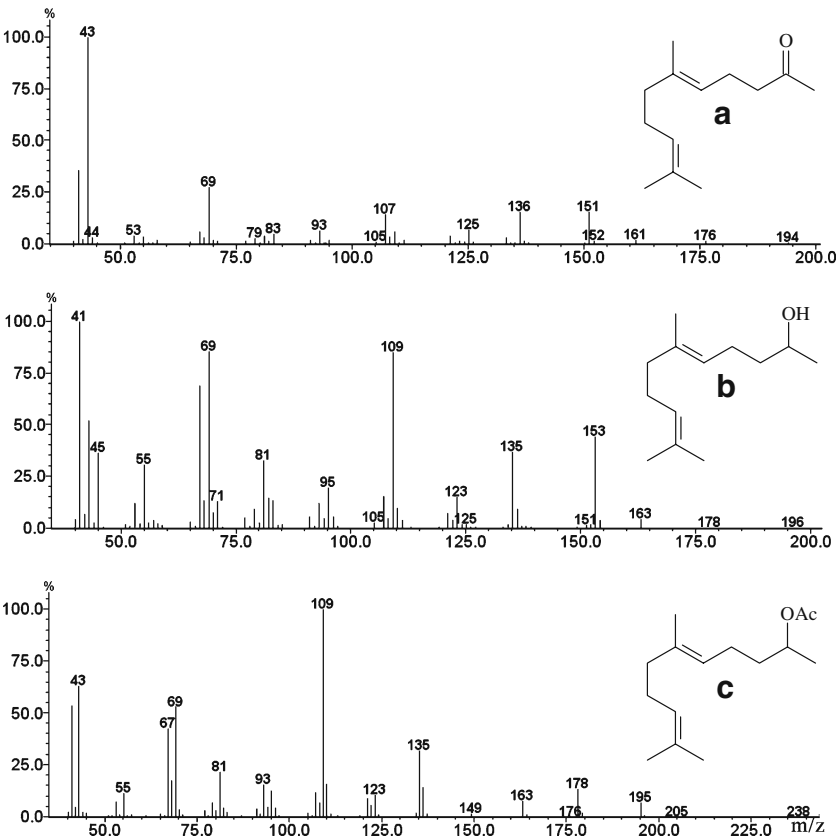


**Fig. 3** Mass spectra of compounds **a** [(*E*)-6,10-dimethyl-5,9-undecadien-2-one], **b** [(*E*)-6,10-dimethyl-5,9-undecadien-2-ol], and **c** [(*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate]

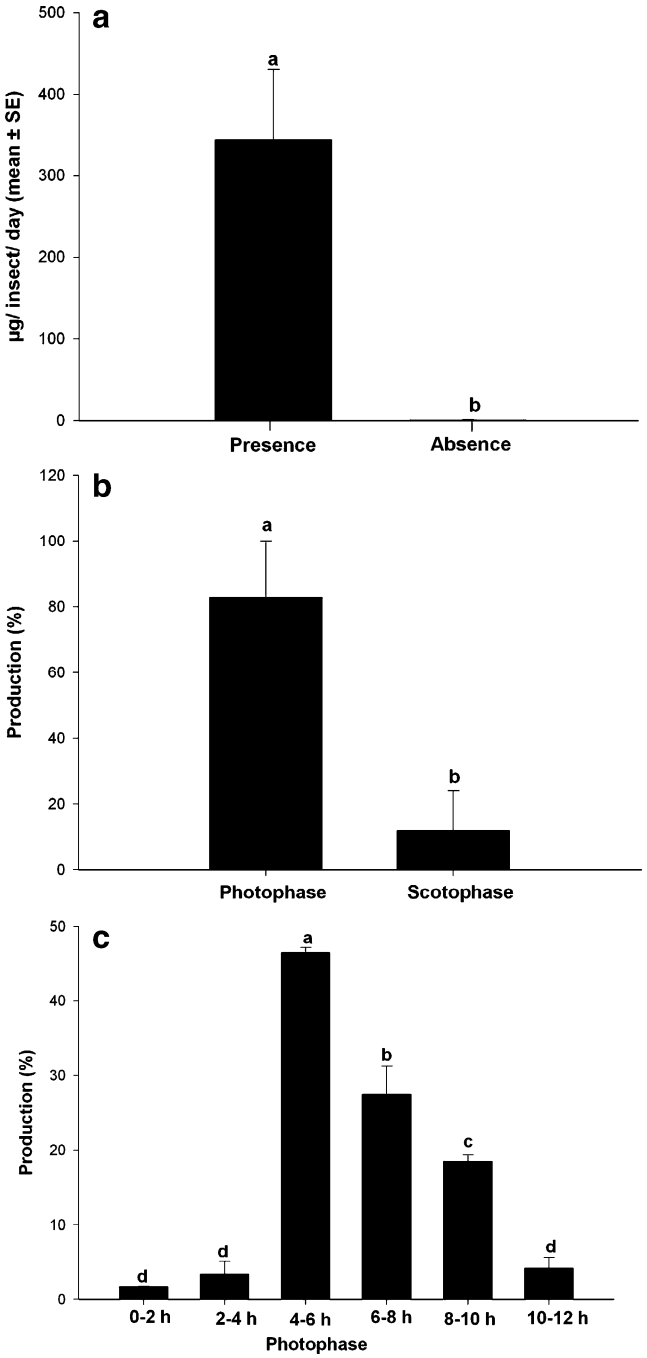


**Fig. 4** Comparison of the amounts of the major component **C** [(*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate] collected from aeration of *Hedypathes betulinus* males: **a** in the presence and absence of green maté branches; **b** between the photophase and scotophase; **c** among different times of the 12 hr of the photophase. Mean values followed by the same letter are not significantly different (ANOVA followed by a Tukey post-hoc test; $N=3$)

Female *H. betulinus* responses to the various treatments are shown in Tables 1 and 2. The major component alone at 5 µg ($\chi^2=0.034$; $df=1$; $P>0.05$) and 8 µg ($\chi^2=0.143$; $df=1$; $P>0.05$), and the ternary mixture **I** alone ($\chi^2=0.29$; $df=1$; $P>0.05$) were not attractive to females (Table 1). However, ternary mixture **II** was attractive when compared with the control ($\chi^2=5.452$; $df=1$; $P<0.05$).

Although plant volatiles alone were not attractive to females ($X^2=0.133$; $df=1$; $P=0.715$), addition of these volatiles to the various pheromone component mixtures increased attraction. A greater number of females were attracted to the combination of host plant volatiles and component **C** at both doses tested, 5 µg ($\chi^2=7.53$; $df=1$; $P=0.006$) and 8 µg ($\chi^2=11.64$; $df=1$; $P<0.001$). Similarly, both doses of ternary mixture **I** ($\chi^2=5.828$; $df=1$; $P=0.015$) and **II** ($\chi^2=9.783$; $df=1$; $P=0.001$), as well as major component **C,** were attractive when combined with host-plant volatiles (Table 2).

## Discussion

We identified the male-produced pheromone of *H. betulinus* as a mixture of three compounds: (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (major), (*E*)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone), and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol. Within the sub-family Lamiinae, a male-produced volatile pheromone that attracts both sexes (i.e., an aggregation pheromone) has been identified previously for *Anoplophora glabripennis* (Zhang et al., 2002; Nehme et al., 2009). In field tests, addition of host-plant volatiles to this aggregation pheromone increased attraction of *A. glabripennis*, particularly for virgin females (Nehme et al., 2010). Evidence for a male-produced contact sex pheromone has been reported for *Steirastoma breve* (Liendo et al., 2005) and *Monochamus galloprovincialis* (Ibeas et al., 2008), while *A. glabripennis* females produce a contact recognition pheromone (Zhang et al., 2003). Our bioassay data indicated that the male-produced pheromone of *H. betulinus* is a sex pheromone, as conspecific males were not attracted to the pheromone. Thus, this is probably the first volatile sex pheromone identified from this subfamily. However, this needs to be confirmed in rigorous field tests by testing different ratios and concentrations of the compounds.

The male-produced compounds we identified in *H. betulinus* are different from male-produced sex or aggregation pheromones of other cerambycid species in the subfamily Cerambycinae (Allison et al., 2004; reviewed by Ray et al., 2006; Hanks et al., 2007; Lacey et al., 2008). The pheromones of most of these species typically are compounds with short chain (6–10 carbon) α-hydroxyl ketones or (α, β)-diols (Hanks et al., 2007; Lacey et al., 2008), although two exceptions are the male-produced aggregation pheromone of *Phymatodes lecontei* [(*R*)-2-methylbutan-1-ol] (Hanks et al., 2007), and a component of the sex pheromone of *Hylotrupes bajulus* (1-butanol) (Reddy et al., 2005). The pheromone components of *H. betulinus* also are different

**Table 1** Responses of *Hedypathes betulinus* females to various synthetic racemate treatments in a Y-tube olfactometer

| Odor sources | | Responses (%) ♀ |
|---|---|---|
| Main compound **C** (5 μg) vs. Control | Main compound **C** | 41.7 |
| | Control | 38.9 |
| | Not decided | 19.4 |
| | *N*=36 | |
| Main compound **C** (8 μg) vs. Control | Main compound **C** | 41.7 |
| | Control | 36.1 |
| | Not decided | 22.2 |
| | *N*=36 | |
| Ternary Mixture **I** vs. Control | Ternary mixture **I** | 44.8 |
| | Control | 36.8 |
| | Not decided | 18.4 |
| | *N*=38 | |
| Ternary Mixture **II** vs. Control | Ternary mixture **II** | 59.5* |
| | Control | 24.3 |
| | Not decided | 16.2 |
| | *N*=37 | |

*Statistically significant, Chi-square test, $P<0.05$

Hexane = solvent control; Ternary Mixture **I**=(5 μg **C**: 0.43 μg **A**: 0.038 μg **B**); Ternary Mixture **II**=(8 μg **C**: 0.69 μg **A**: 0.06 μg **B**); Ternary mixture ratio was calculated from male extract (91.4: 7.9: 0.70 of racemic (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate **C**, (*E*)-6,10-dimethyl-5,9-undecadien-2-one **A** and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol **B**, respectively)

from the known female-produced pheromones of other cerambycids: N-(2′*S*)-methylbutanoyl-2-methylbutylamine is a pheromone component of *Migdolus fryanus* (Anoploderматinae) (Leal et al., 1994), 3,5-dimethyldodecanoic acid is a pheromone component of *Prionus californicus* (Prioninae) (Rodstein et al., 2009), and (*S*)-10-oxoisopiperitenone has been identified as a pheromone of *Vesperus xatari* (Vesperinae) (Boyer et al., 1997).

Multi-component pheromones previously have been reported for some species of Cerambycidae. For example, Lacey et al. (2008) identified eight male-specific compounds from *Megacyllene caryae*, with initial field tests

**Table 2** Responses of *Hedypathes betulinus* females to various synthetic racemate treatments and/or host plant volatiles (HPV) in a Y-tube olfactometer

| Odor sources | | Responses (%) ♀ |
|---|---|---|
| HPV vs. Control | HPV | 32.6 |
| | Control | 37.2 |
| | Not decided | 30.2 |
| | *N*=43 | |
| Main compound **C** (5 μg) plus HPV vs. Control | Main compound **C** plus HPV | 56.8* |
| | Control | 20.5 |
| | Not decided | 22.7 |
| | *N*=44 | |
| Main compound **C** (8 μg) plus HPV vs. Control | Main compound **C** plus HPV | 69.4* |
| | Control | 16.7 |
| | Not decided | 13.9 |
| | *N*=36 | |
| Ternary Mixture **I** plus HPV vs. Control | Ternary mixture **I** plus HPV | 72.2* |
| | Control | 13.9 |
| | Not decided | 13.9 |
| | *N*=36 | |
| Ternary Mixture **II** plus HPV vs. Control | Ternary mixture **II** plus HPV | 66.7* |
| | Control | 13.3 |
| | Not decided | 20 |
| | *N*=30 | |

*Statistically significant, Chi-square test, $P<0.05$

Hexane = solvent control; Ternary Mixture **I**=(5 μg **C**: 0.43 μg **A**: 0.038 μg **B**); Ternary Mixture **II**=(8 μg **C**: 0.69 μg **A**: 0.06 μg **B**); Ternary mixture ratio was calculated from male extract (91.4: 7.9: 0.70 of racemic (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate **C**, (*E*)-6,10-dimethyl-5,9-undecadien-2-one **A** and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol **B**, respectively)

determining that none of these compounds was attractive individually. Volatiles collected from male *Rosalia funebris* contained a major male-specific compound, (*Z*)-3-decenyl (*E*)-2-hexenoate, and several minor compounds (Ray et al., 2009). Despite the attraction, in the field, of both sexes of *R. funebris* to lures baited with synthetic (*Z*)-3-decenyl (*E*)-2-hexenoate, the authors suggested that the minor components may form part of the natural pheromone, and that traps baited with a more complete blend could attract more beetles.

Our results demonstrated that addition of host-plant volatiles to the pheromone enhanced attraction to females (Table 2). Host plant volatiles often enhance an insect's response to sex pheromones, and this effect may result in true synergism; a response to the pheromone and plant volatile mixture that is greater than the sum of responses to the individual components (Reddy and Guerrero, 2004). Increased pheromone attraction due to the presence of host odors occurs in several insect groups, such as moths (Dickens et al., 1990, 1993; Reddy and Guerrero, 2000; Deng et al., 2004; Yang et al., 2004), beetles (Phillips et al., 1984; Byers et al., 1990; Nakamuta et al., 1997; Zhang and Schlyter, 2003), and flies (Landolt et al., 1992). In the Cerambycidae, host odor and pheromone synergism has been documented in *Anaglyptus subfasciatus* (Nakamuta et al., 1997), *Tetropium fuscum* and *T. cinnamopterum* (Silk et al., 2007), and *A. glabripennis* (Nehme et al., 2010). Only in *A. glabripennis* are adults attracted to plant volatiles alone, and in that case, this effect is more apparent for males than females (Nehme et al., 2010).

Studies of numerous cerambycid species suggest that conspecific location in these species is mediated first by attraction to host-plant volatiles and subsequently to pheromones (Hanks, 1999; Allison et al., 2004). Ginzel and Hanks (2005) hypothesized that cerambycine mate location and recognition results from three sequential behaviors: (1) both sexes are independently attracted to larval hosts by plant volatiles; (2) males attract females at short-range with pheromones; and (3) males recognize females by contact pheromones. In our study, female *H. betulinus* were not attracted to host-plant volatiles alone, indicating that mate location involves only stages 2 and 3, consistent with previous observations of the mating behavior of this species (Fonseca and Zarbin, 2009).

In summary, we have identified the sex pheromone of *H. betulinus* and described the temporal pattern of pheromone release. Future studies will determine the stereochemistry of components **B** and **C**, the biosynthesis and site of production of the components, as well as the activity of the pheromone and host-plant volatiles in the field.

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