Response of the Woodborers *Monochamus carolinensis* and *Monochamus titillator* (Coleoptera: Cerambycidae) to Known Cerambycid Pheromones in the Presence and Absence of the Host Plant Volatile α-Pinene

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ABSTRACT In recent years, several attractant pheromones have been identified for cerambycid beetles, including 2-(undecyloxy)-ethanol (hereafter monochamol) for *Monochamus galloprovincialis* (Olivier), *M. alternatus* Hope, and *M. scutellatus* (Say). This study screened eight known cerambycid pheromones or their analogues (including monochamol) as potential attractants for *M. carolinensis* Olivier and *M. titillator* (F.), in the presence and absence of the host volatile α-pinene. Monochamol attracted *M. carolinensis* in the presence and absence of α-pinene, whereas *M. titillator* was only attracted to the combination of monochamol and α-pinene. (2*R**,3*R**)-2,3-Hexanediol also attracted both *M. carolinensis* and *M. titillator*, but only in the presence of α-pinene. Subsequent coupled gas chromatography–mass spectrometry and gas chromatography–electroantennogram detection analyses of extracts of volatiles collected from both sexes demonstrated that male *M. carolinensis* and *M. titillator* release monochamol, and that antennae of males and females of both species detect it. These results indicate that monochamol is a male-produced pheromone for both *M. carolinensis* and *M. titillator*.

KEY WORDS Cerambycidae, pheromone, host volatile, *Monochamus*, 2-(undecyloxy)-ethanol

There are an estimated 35,000 species of cerambycid beetles worldwide (Lawrence 1982), with an estimated North American fauna of 956–1,400 species (USDA 1985, Downie and Arnett 1996, Yanega 1996, Arnett 2000). Adults often have long antennae and commonly are called longhorned or longicorn beetles. The larvae of most species bore in the phloem and xylem tissues of stressed, dying, or recently-dead woody plants (Linsley 1959), and many species are primary agents for the degradation of woody biomass in forests (Edmonds and Eglitis 1989). Larval feeding initiates the breakdown of woody tissues while simultaneously creating access routes for wood-rotting fungi and other wood-boring agents. As a result, longhorned beetles play critical roles in nutrient cycling in forests. However, larval feeding can result in significant economic losses to standing and fallen timber, and some species are considered primary [e.g., *Anoplophora glabripennis* (Motschulsky)] and secondary pests [e.g., *Monochamus scutellatus* (Say)]. Economic losses result from the attack and associated mortality of healthy trees, and lumber damaged by larval tunnelling also is degraded, resulting in further economic losses.

Chemical cues (e.g., floral, trunk, leaf and smoke volatiles, bark beetle pheromones) and signals (pheromones) play important roles in host and mate location in cerambycid beetles (Allison et al. 2004). Behavioral and chemical analyses have identified pheromones or attractants for several species from the subfamilies Cerambycinae (e.g., Lacey et al. 2004, 2009; Hanks et al. 2007; Ray et al. 2009); Prioninae (e.g., Rodstein et al. 2011, Barbour et al. 2011); Lamiinae (e.g., Pajares et al. 2010, Teale et al. 2011, Mitchell et al. 2011); Aseminae or Spondylidinae (e.g., Silk et al. 2007, Sweeney et al. 2010); and Lepturinae (Ray et al. 2011, 2012). An increasing body of literature suggests that pheromone motifs often are conserved within genera, tribes, and subfamilies (see Mitchell et al. 2011).

Cerambycid beetles of the genus *Monochamus* Dejean (commonly referred to as sawyer beetles) can be significant secondary pests. Adult sawyer beetles are known to use host volatiles and bark beetle pheromones to locate potential hosts (Allison et al. 2001, 2003, 2012a; Pajares et al. 2004; Miller and Asaro 2005; but see Fan et al. 2010). Females deposit eggs into the

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phloem through oviposition pits or cracks in the bark and larvae develop in the phloem for 3–4 wk before entering the sapwood to construct U-shaped galleries where pupation will occur. Larval tunnelling results in degradation of the wood for lumber and occasionally in tree death (Gandhi et al. 2007). Equally or more important is the role of adults of *Monochamus* species as vectors of the pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, the causal agent of pine wilt disease that can decimate forests of susceptible pines (Wingfield et al. 1982, Zhao 2008).

Recently, the compound 2-(undecyloxy)-ethanol (hereafter monochamol) was identified as a pheromone for *M. galloprovincialis* (Olivier) (Pajares et al. 2010), *M. alternatus* Hope (Teale et al. 2011), and *M. scutellatus* (Fierke et al. 2012), and serves as an attractant and probable pheromone for *M. carolinensis* Olivier (Hanks and Millar 2012), *M. clamator* (LeConte) (Macias-Samano et al. 2012), *M. notatus* (Drury) (Fierke et al. 2012), and *M. obtusus* Casey (Macias-Samano et al. 2012). The objectives of this study were to 1) screen known cerambycid pheromones and their analogues for attraction of *M. carolinensis* and *M. titillator* (F.); 2) measure the role of the host volatile α-pinene in the responses of both species; and 3) use coupled gas chromatography–mass spectrometry (GC–MS) and gas chromatography–electroantennogram detection (GC–EAD) analyses to locate monochamol in extracts of volatiles from the beetles, to verify that it is indeed a pheromone of these two species.

Materials and Methods

Semiochemicals. Racemic 3-hydroxy-2-hexanone was prepared from 3-hydroxy-1-hexyne as described by Imrei et al. (2012). The homologs 3-hydroxy-2-octanone and 3-hydroxy-2-decanone were synthesized in analogous fashion, substituting 3-hydroxy-1-octyne and 3-hydroxy-1-decyne as the starting materials, respectively. Racemic (2*R**,3*R**)- and (2*R**,3*S**)-2,3-hexanediols were prepared by OsO_4-catalyzed oxidation of (*E*)- and (*Z*)-2-hexenes (GFS Chemicals, Powell, OH), respectively, as described in Lacey et al. (2004). (2*R**,3*R**)- and (2*R**,3*S**)-2,3-octanediols were synthesized in similar fashion from (*E*)- and (*Z*)-2-octenes (GFS Chemicals, Powell, OH). 2-(Undecyloxy)-ethanol was synthesized as described by Pajares et al. (2010).

Experiments 1, 3, 5, and 6 used ultra-high release pouches containing α-pinene (172 ml; chemical purity ≥95%, enantiomeric purity 95% [-]; release rate ≈ 2 g/d at 20°C [Contech Enterprises Inc., Victoria, BC, Canada]) as a representative host plant volatile (Allison et al. 2004). The pheromone compounds were released from polyethylene sachets (Fisherbrand zipper seal sample bags, 51-micron wall thickness, 5 cm by 7 cm, Fisher, Scientific, Pittsburgh, PA) loaded with 50 mg (release rate ≈ 100 μg/h) of each synthetic pheromone diluted in 1 ml of 95% ethanol (experiments 1–4). Ethanol is an efficient carrier for these pheromone compounds, but is attractive to some cerambycids at high doses (Allison et al. 2004, Miller 2006). At the doses used in this study, ethanol was unlikely to be attractive to cerambycid beetles (Hanks et al. 2007), but to be sure, isopropanol was used as a carrier (and control) in experiments 5 and 6.

Field Experiments. Four preliminary field experiments were conducted to test for attraction of beetles to known cerambycid pheromones in central Louisiana. Experiments 1 and 3 used 48 multiple-funnel traps deployed in a linear array of eight replicate blocks of six traps per block, using black, eight-unit multiple-funnel traps (Contech Enterprises Inc.). Experiments 2 and 4 deployed 40 eight-unit black multiple-funnel traps in a linear array of eight replicate blocks of five traps per block. Experiments 1 and 2 tested the same four cerambycid pheromones in the presence (experiment 1) or absence (experiment 2) of the host volatile α-pinene. The following treatments were assigned randomly within each replicate block: experiment 1: 1) solvent control; 2) solvent control plus α-pinene; 3) 3-hydroxy-2-decanone plus α-pinene; 4) 3-hydroxy-2-octanone plus α-pinene; 5) 3-hydroxy-2-hexanone plus α-pinene; and 6) (2*R**,3*R**)-2,3-hexanediol plus α-pinene; experiment 2: 1) solvent control; 2) 3-hydroxy-2-decanone; 3) 3-hydroxy-2-octanone; 4) 3-hydroxy-2-hexanone; and 5) (2*R**,3*R**)-2,3-hexanediol. Experiments 3 and 4 tested four additional cerambycid pheromone compounds in the presence (experiment 3) and absence (experiment 4) of the host volatile α-pinene. The following treatments were assigned randomly within each replicate block: experiment 3: 1) solvent control; 2) solvent control plus α-pinene; 3) (2*R**,3*S**)-2,3-octanediol plus α-pinene; 4) (2*R**,3*R**)-2,3-octanediol plus α-pinene; 5) (2*R**,3*S**)-2,3-hexanediol plus α-pinene; and 6) monochamol plus α-pinene; experiment 4: 1) solvent control 2); (2*R**,3*S**)-2,3-octanediol; 3) (2*R**,3*R**)-2,3-octanediol; 4) (2*R**,3*S**)-2,3-hexanediol; and 5) monochamol.

In 2011, two additional field experiments (experiments 5 and 6) were conducted to confirm the activity of (2*R**,3*R**)-2,3-hexanediol and monochamol, and to verify the role of the host volatile α-pinene in the responses of *M. carolinensis* and *M. titillator* (see Results). Both experiments had 88 funnel traps deployed in a linear array of eight replicate blocks of 11 traps per block using black eight-unit multiple-funnel traps. The following treatments were assigned randomly within each replicate block: experiment 5: 1) 3-hydroxy-2-decanone; 2) 3-hydroxy-2-octanone; 3) 3-hydroxy-2-hexanone; 4) (2*R**,3*R**)-2,3-hexanediol; 5) 3-hydroxy-2-decanone plus α-pinene; 6) 3-hydroxy-2-octanone plus α-pinene; 7) 3-hydroxy-2-hexanone plus α-pinene; 8) (2*R**,3*R**)-2,3-hexanediol plus α-pinene; 9) solvent control; 10) α-pinene; and 11) solvent control plus α-pinene; experiment 6: 1) (2*R**,3*S**)-2,3-octanediol; 2) (2*R**,3*R**)-2,3-octanediol; 3) (2*R**,3*S**)-2,3-hexanediol; 4) monochamol; 5) (2*R**,3*S**)-2,3-octanediol and α-pinene; 6) (2*R**,3*R**)-2,3-octanediol and α-pinene; 7) (2*R**,3*S**)-2,3-hexanediol and α-pinene; 8) monochamol and α-pinene; 9) isopropanol; 10) α-pinene; and 11) isopropanol and α-pinene. Treatments 1–3 and 5–7 were in-

cluded as part of a larger bioassay of potential cerambycid attractants.

Experiment 1 was run during 28 July–4 August, 8–21 September, and 19–28 October 2010. Traps were baited with fresh lures on 28 July, 8 September, and 19 October 2010, and captured beetles collected on 4 August, 21 September, and 28 October 2010. Experiment 2 was run during 28 July–4 August, 2–15 September, and 19–28 October 2010. All traps were baited with fresh lures on 28 July, 2 September, and 19 October and all captured beetles collected on 4 August, 15 September, and 28 October. Experiments 3 and 4 were run 11–18 August, 6–14 October, and 3–11 November 2010. All traps were baited with fresh lures on 11 August, 6 October, and 3 November and all captured beetles collected on 18 August, 14 October, and 12 November. Experiment 5 was run 4–18 April, 9–23 May, and 13–27 June 2011. All traps were baited with fresh lures on 4 April, 9 May, and 13 June and all captured beetles collected on 18 April, 23 May, and 27 June. Experiment 6 was run 18 April–2 May, 23 May–6 June, and 27 June–11 July. All traps were baited with fresh lures on 18 April, 23 May, and 27 June and all captured beetles collected on 2 May, 6 June, and 11 July.

All six experiments were run in the Kisatchie National Forest, Catahoula Ranger District, in stands of predominately *Pinus taeda* L. and mixed hardwoods that had experienced a prescribed burn preceding trap deployment in 2010. Traps were suspended individually from a rope strung between two trees so that the collection cup of each trap was 0.5–1.5 m above ground level and each trap was ≥2 m from any tree. All traps were treated with Fluon PTFE to enhance capture efficiency (Graham et al. 2010, Allison et al. 2011), equipped with a wet collection cup with 150–200 ml of a solution of propylene glycol and water, and were spaced ≈20 m apart within and between blocks. Species of *Monochamus* were identified following Yanega (1996) and Lingafelter (2007). Voucher specimens of *M. carolinensis* and *M. titillator* have been deposited in the Louisiana State Arthropod Museum.

Preparation and Analysis of Beetle-Produced Volatiles. Live *M. titillator* and *M. carolinensis* were shipped to the UC Riverside quarantine facility (USDA–APHIS permit P526P-09-01886) from the LSU AgCenter. Volatiles were collected from 10 *M. titillator* males and 8 females. Individual beetles were held in 500-ml glass canning jars fitted with inlet and outlet tubes, and each insect was provided sprigs of conifer foliage as food. Humidified, charcoal-filtered air was drawn through each chamber at 1 liter/min, trapping the headspace volatiles on collectors fashioned from a 1-cm-long by 3.2-mm-diameter bed of thermally desorbed activated charcoal (50–200 mesh; Fisher) held between glass wool plugs in a glass tube. Collections of headspace odors were conducted in a glasshouse under natural light at temperatures of 22–26°C. A first group of four female and male *M. titillator* were aerated for 3 d, then the collectors were changed and aerations were continued for an additional 5 d. A second group of four females and six males were aerated twice for a single day with half being provided twigs with the cut ends covered with aluminum foil, and the others provided only 10% sugar water. Trapped volatiles were eluted with 3- by 250-μl CH_2Cl_2.

Headspace extracts were prepared in similar fashion in Illinois from two male and one female *M. carolinensis.* When not being used for headspace collections, beetles were held individually under ambient laboratory light and temperature conditions, and fed on fresh-cut sprigs of *Pinus strobus* L. The first male used for headspace collections emerged from a log in the laboratory on 21 April 2010 and was aerated with pine sprigs for 24-h periods by using the methods and apparatus described above, with the exception that volatiles were collected on 150-mg HaySep Q polymer (Sigma-Aldrich, St. Louis, MO) rather than activated charcoal. Beetles were aerated five times between 23 April and 2 July 2010. The second male and the female were caught in a trap baited with monochamol on 4 June, and each was aerated separately on 30 June and 2 July. Controls consisting of pine sprigs alone were aerated in parallel with each beetle aeration so that host plant compounds could be identified in the extracts from the insects and excluded from further consideration as possible pheromones. Collectors were eluted with three by 500-μl CH_2Cl_2, and extracts were stored in a freezer until needed for analyses. The single extract from the field-collected male that was found to contain monochamol by GC analysis was shipped to UC Riverside for further analyses by GC–MS and GC–EAD.

Headspace extracts were analyzed in splitless mode by coupled gas chromatography-electroantennography (GC–EAD) on a DB-Wax column (30 m by 0.25 mm in diameter, 0.25-μm film; J&W Scientific, Folsom CA) programmed from 50°C/1 min, 15°C/min to 250°C, hold 30 min. The GC–EAD apparatus and analysis conditions recently have been described in detail (Ray et al. 2012). The antennal preparation consisted of the terminal 2–4 segments, and antennae from both sexes were used in the analyses. Extracts were reanalyzed with an Agilent 6890N gas chromatograph (Wilmington, DE) fitted with a DB-5 column (30 m by 0.25 mm in diameter, 0.25-μm film; J&W Scientific, Folsom CA), operated in splitless mode. The GC was interfaced to an Agilent 5975C mass selective detector. The GC was programmed from 40°C/1 min, then 10°C/min to 280°C for 20 min. Monochamol in samples (see Results) was identified conclusively by matching its retention time and mass spectrum to those of an authentic standard.

Statistical Analyses. The experimental designs were similar for all six experiments (eight randomized complete blocks and three collection dates) so the data were analyzed similarly. Total catches per trap of *M. carolinensis* and *M. titillator* were analyzed using a blocked multiresponse permutation procedure (MRBP: McCune et al. 2002). To look for an interaction between treatment and collection date, MRBP was used to determine if collection date affected the treatments within an experiment similarly (i.e., if the pattern of

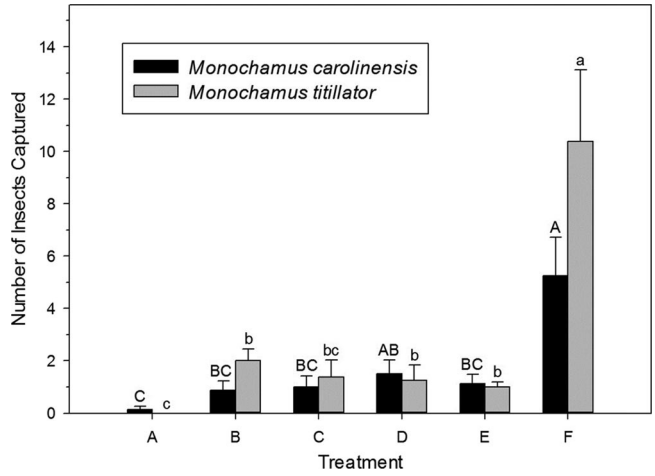

Fig. 1. Mean total captures (+SE) of *M. carolinensis* (black bars) and *M. titillator* (gray bars) in traps baited with the solvent (ethanol) control (A); solvent control plus α-pinene (B); 3-hydroxy-2-decanone plus α-pinene (C); 3-hydroxy-2-octanone plus α-pinene (D); 3-hydroxy-2-hexanone plus α-pinene (E); and (2*R**,3*R**)-2,3-hexanediol plus α-pinene (F) (experiment 1). Eight replicates per treatment. Means (±SE) with the same letter (uppercase: *M. carolinensis*, lowercase: *M. titillator*) are not significantly different at $P = 0.05$.

treatment effects was similar among collection dates). Comparison of the patterns of treatment effects among collection dates within experiments strongly suggested that there were no collection period by treatment interactions for any of the experiments. As a result, catches from each collection period were summed by treatment within blocks for each species. In experiments 5 and 6, the total number of *M. carolinensis* and *M. titillator* first was subjected to separate MRBP analysis for each species, including all treatments. Because experiments 1–4 failed to detect attraction of either species to the trap treatments 1–3 and 5–7 (see Results), and the primary goal of this study was to measure attractants for *M. carolinensis* and *M. titillator*, the data subsequently were analyzed without treatments 1–3 and 5–7.

The advantage of MRBP is that the assumptions regarding the distribution of dependent variables are relaxed (Mielke and Berry 2001). All analyses were conducted with PC-ORD 6.0 (MjM Software Design, Gleneden Beach, OR) by using Euclidean distances to construct the distance matrix with blocks aligned before analysis (McCune et al. 2002). The multiplicity effect was controlled using step-up FDR (see Benjamini and Hochberg 1995 and Garcia 2004 for a discussion of the benefits of this approach).

Results

Field Experiments. In total, 2,318 *M. carolinensis* and 887 *M. titillator* were captured in experiments 1–6, with ≈86 and 72% of the *M. carolinensis* and *M. titillator* being captured in experiments 5 and 6 . Too few beetles were captured in experiments 2 and 4 for analyses (experiment 2 captured 12 *M. carolinensis* and three *M. titillator;* experiment 4 captured 19 *M. carolinensis* and two *M. titillator*).

In total, 79 *M. carolinensis* and 128 *M. titillator* were captured in experiment 1. There was a significant treatment effect for both species (*M. carolinensis:* $T = -5.99, P < 0.0001$; *M. titillator:* $T = -8.88, P < 0.0001$). The overall pattern of trap capture was similar for the two species (Fig. 1): traps baited with (2*R**,3*R**)-2,3-hexanediol plus α-pinene captured the most beetles, and traps baited with the solvent control alone, the fewest (Fig. 1). For both species, the (2*R**,3*R**)-2,3-hexanediol plus α-pinene treatment captured significantly more beetles than all other treatments except the 3-hydroxy-2-octanone plus α-pinene treatment for *M. carolinensis.* The solvent control plus α-pinene treatment captured more *M. titillator* than did the solvent control alone treatment.

In total, 211 *M. carolinensis* and 117 *M. titillator* were captured in experiment 3, and treatment effects were significant for both *M. carolinensis* ($T = -10.95, P < 0.0001$) and *M. titillator* ($T = -11.21, P < 0.0001$). For both species, monochamol plus α-pinene was the only trap treatment to capture significantly more beetles than the solvent control plus α-pinene treatment (Fig. 2).

In experiment 5, 193 male and 283 female *M. carolinensis* and 144 male and 129 female *M. titillator* were captured, respectively, and in the complete analysis treatment effects were significant for male ($T = -8.66, P < 0.0001$) and female ($T = -13.73, P < 0.0001$) *M. carolinensis* and for male ($T = -8.77, P < 0.0001$) and female ($T = -8.23, P < 0.0001$) *M. titillator.* Subsequent analyses with treatments 1–3 and 5–7 excluded also indicated that there was a significant treatment effect for male ($T = -4.81, P = 0.0008$) and female ($T = -8.60, P < 0.0001$) *M. carolinensis* and for male ($T = -7.04, P < 0.0001$) and female ($T = -6.14, P = 0.0001$) *M. titillator.* For both males and females of both species, (2*R**,3*R**)-2,3-hexanediol alone was

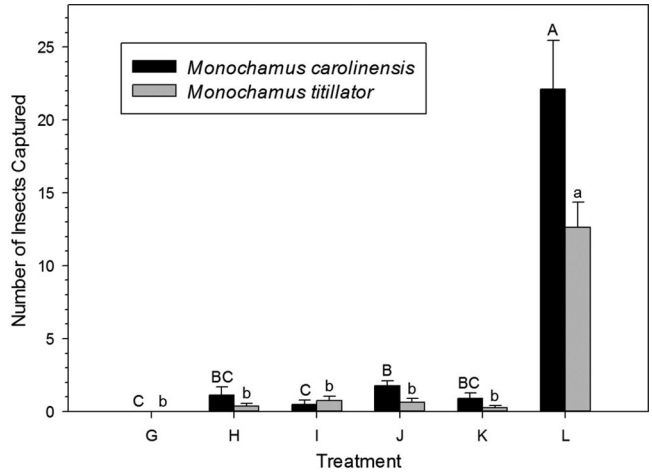

Fig. 2. Mean total captures (+SE) of *M. carolinensis* (black bars) and *M. titillator* (gray bars) in traps baited with the solvent (ethanol) control (G); solvent control plus α-pinene (H); (2*R**,3*S**)-2,3-octanediol plus α-pinene (I); (2*R**,3*R**)-2,3-octanediol plus α-pinene (J); (2*R**,3*S**)-2,3-hexanediol plus α-pinene (K); and monochamol plus α-pinene (L) (experiment 3). Eight replicates per treatment. Means (+SE) followed by the same letter (uppercase: *M. carolinensis*, lowercase: *M. titillator*) are not significantly different at $P = 0.05$.

not attractive, whereas the blend of (2*R**,3*R**)-2,3-hexanediol plus α-pinene was significantly attractive (Fig. 3).

In total, 614 male and 907 female *M. carolinensis* and 189 male and 175 female *M. titillator* were captured in experiment 6 and in the complete analysis treatment effects were significant for male ($T = -15.99$, $P < 0.0001$) and female ($T = -16.29$, $P < 0.0001$) *M. carolinensis*, and for male ($T = -12.37$, $P < 0.0001$) and female ($T = -12.88$, $P < 0.0001$) *M. titillator*. Subsequent analyses with treatments 1–3 and 5–7 excluded also indicated that treatment effects were significant for male ($T = -9.84$, $P < 0.0001$) and female ($T = -9.70$, $P < 0.0001$) *M. carolinensis* and for male ($T = -8.44$, $P < 0.0001$) and female ($T = -8.60$, $P < 0.0001$) *M. titillator*. Pairwise comparisons of total catch (with treatments 1–3 and 5–7 excluded) indicated that for male and female *M. carolinensis*, monochamol alone captured more beetles than the solvent control, and that monochamol plus α-pinene captured more beetles than all other treatments (Fig. 4). In contrast, pairwise comparisons of the *M. titillator* trap catches indicated that monochamol alone was not attractive, but that monochamol plus α-pinene captured more beetles than all other treatments (Fig. 4).

Analysis of Beetle-Produced Volatiles. Extracts of headspace volatiles collected from male *M. titillator* held on conifer sprigs contained large amounts of host

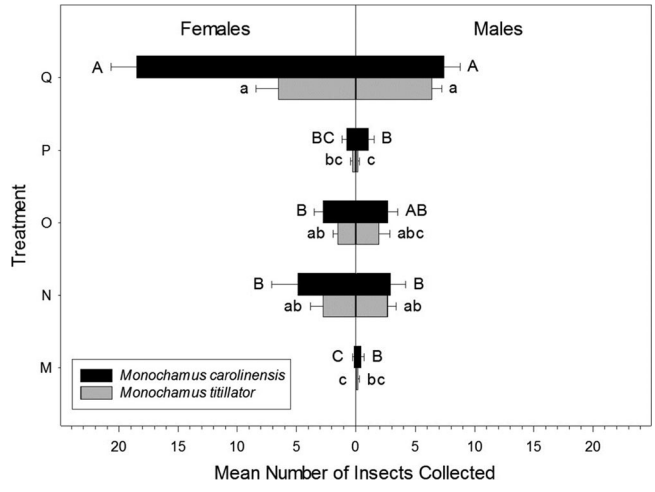

Fig. 3. Mean total captures (+SE) of *M. carolinensis* (black bars) and *M. titillator* (gray bars) in traps baited with the solvent (isopropanol) control (M); α-pinene (N); solvent control plus α-pinene (O); (2*R**,3*R**)-2,3-hexanediol (P); and (2*R**,3*R**)-2,3-hexanediol plus α-pinene (Q) (experiment 5). Eight replicates per treatment. Means (+SE) followed by the same letter (uppercase: *M. carolinensis*, lowercase: *M. titillator*) are not significantly different at $P = 0.05$.

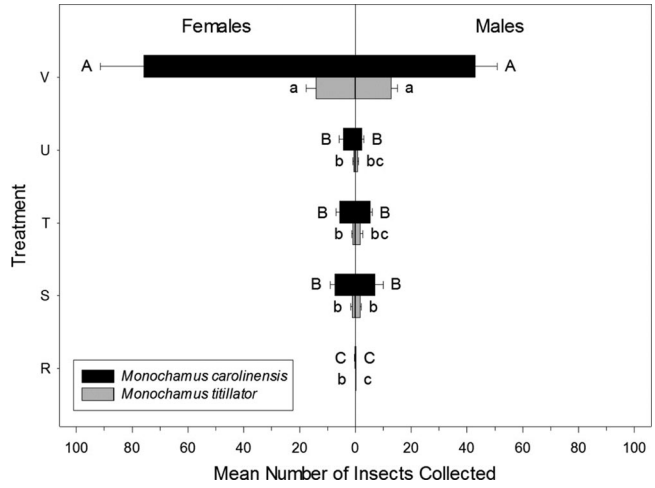

Fig. 4. Mean total captures (+SE) of *M. carolinensis* (black bars) and *M. titillator* (gray bars) in traps baited with the solvent (isopropanol) control (R), α-pinene (S), solvent controls plus α-pinene (T), monochamol (U) and monochamol plus α-pinene (V) (experiment 6). Eight replicates per treatment. Means (+SE) followed by the same letter (uppercase: *M. carolinensis*, lowercase: *M. titillator*) are not significantly different at $P = 0.05$.

plant volatiles, and smaller and more variable amounts of monochamol. Monochamol was detected in extracts from males by coupled GC–EAD analyses, using antennae from both male (Fig. 5) and female *M. titillator* (Fig. 6). The presence of monochamol in the extracts from males was confirmed by retention time matches on polar DB-Wax and nonpolar DB-5 columns, and by matching its mass spectrum with that of an authentic standard. Monochamol was not detected in GC–MS analyses of extracts from female *M. titillator*.

The presence of monochamol also was confirmed by GC–MS and GC–EAD analyses of an extract of headspace volatiles from a male *M. carolinensis*, and was not detected from simultaneous aerations of a female specimen. As with *M. titillator*, antennae from both males (Fig. 7) and females (Fig. 8) responded to monochamol when the extract was analyzed by GC–EAD.

Discussion

Interest in the chemical ecology of cerambycid beetles has increased in recent years, primarily because of a heightened awareness of the potential for introduc-

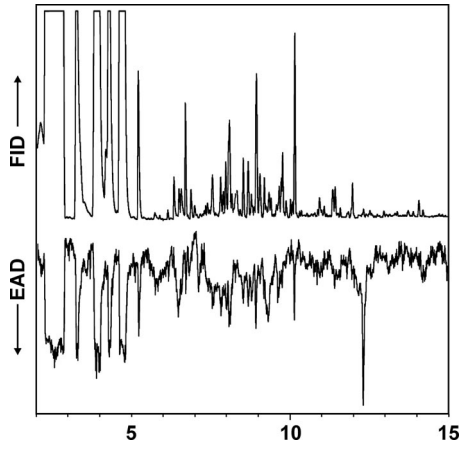

Fig. 5. Representative coupled gas chromatography-electroantennogram analysis of an extract of headspace odors from a male *M. titillator* aerated on pine sprigs. Upper trace is the chromatogram, lower inverted trace is the electroantennogram signal from the antenna of a male *M. titillator*. The response at ≈12.2 min was elicited by monochamol. DB-Wax column, 50°C for 1 min, then 15°C per min to 250°C, hold for 30 min.

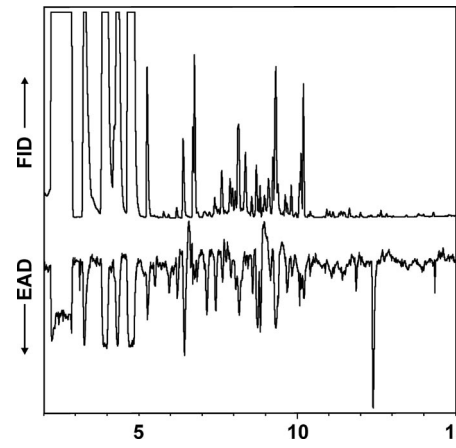

Fig. 6. Representative coupled gas chromatography-electroantennogram analysis of an extract of headspace odors from a male *M. titillator* aerated on pine sprigs. Upper trace is the chromatogram, lower inverted trace is the electroantennogram signal from the antenna of a female *M. titillator*. The response at ≈12.25 min was elicited by monochamol. DB-Wax column, 50°C for 1 min, then 15°C per min to 250°C, hold for 30 min.

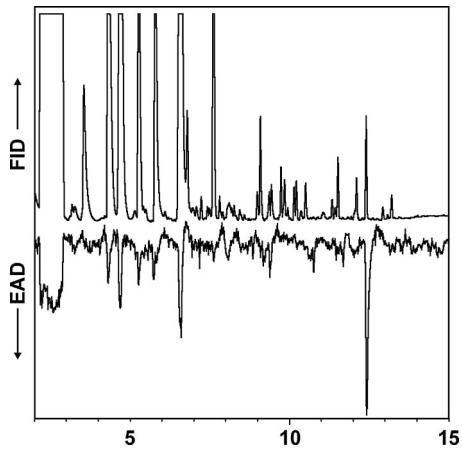

Fig. 7. Representative coupled gas chromatography-electroantennogram analysis of an extract of headspace odors from a male *M. carolinensis* aerated on pine sprigs. Upper trace is the chromatogram, lower inverted trace is the electroantennogram signal from the antenna of a male *M. carolinensis*. The response at ≈12.3 min was elicited by monochamol. DB-Wax column, 50°C for 1 min, then 15°C per min to 250°C, hold for 30 min.

tion of exotic cerambycids in wood products, dunnage, and nursery stock (Aukema et al. 2010). As a result, the literature of known cerambycid pheromones and attractants has expanded dramatically in the last decade (see Hanks 1999, Allison et al. 2004, Millar et al. 2009, Barbour et al. 2011, Mitchell et al. 2011). An emergent trend from these studies is that cerambycid pheromone motifs appear to be phylogenetically conserved (e.g., [*R*]-3-hydroxyhexan-2-one within the subfamily Cerambycinae; 3,5-dimethyldodecanoic acid within the genus *Prionus* Casey in the subfamily Prioninae; [*E*]-6,10-dimethyl-5,9-undecadien-2-ol within the genus *Tetropium* Kirby in Richardson [but see Mitchell et al. 2011]; monochamol within the genus *Monochamus*) (see Mitchell et al. 2011). This study reports the results of field tests that demonstrated that male and female *M. carolinensis* and *M. titillator* were attracted to monochamol, and chemical analyses confirmed that this compound is a male-produced pheromone for both species. These results are consistent with the hypothesis that the pheromone monochamol is conserved within the genus *Monochamus.* Furthermore, the evidence to date suggests that monochamol may be specific to the genus because it has not been reported as a pheromone or attractant for species in any other cerambycid genus.

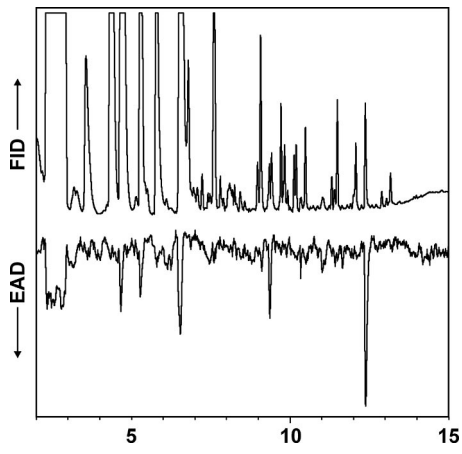

Fig. 8. Representative coupled gas chromatography-electroantennogram analysis of an extract of headspace odors from a male *M. carolinensis* aerated on pine sprigs. Upper trace is the chromatogram, lower inverted trace is the electroantennogram signal from the antenna of a female *M. carolinensis*. The response at ≈12.25 min was elicited by monochamol. DB-Wax column, 50°C for 1 min, then 15°C per min to 250°C, hold for 30 min.

The conservation of the pheromone monochamol within the genus raises questions about how sympatric *Monochamus* spp. such as *M. carolinensis* and *M. titillator* maintain reproductive isolation. Among many insects, the behavioral mechanisms that facilitate pair formation are hypothesized to be the same as those that prevent hybridization. For example, specificity of pheromone response has been hypothesized to mediate mate location and reproductive isolation in bark beetles (Lanier and Wood 1975; but see Allison et al. 2012b). The observation that the sympatric species *M. carolinensis* and *M. titillator* rely on the same mechanism for mate location (monochamol) suggests that in these species, the mechanisms involved in mate location and reproductive isolation may differ.

Monochamus alternatus, M. carolinensis, M. gallo-provincialis, M. titillator, and *M. scutellatus* all attack stressed hosts. Hanks (1999) summarized mate location in "stressed host" species as depending on mutual attraction to the larval host followed by male reliance on antennal contact to recognize potential mates. Using this model, monochamol may mediate the attraction of the sexes to the larval host and once on the host, contact pheromones on the female cuticle may mediate reproductive isolation. Although contact pheromones have not been identified from females of any *Monochamus* spp., behavioral studies suggest their existence in *M. alternatus* (Kim et al. 1992), *M. gallo-provincialis* (Ibeas et al. 2008, 2009), and *M. saltuarius* (F.) (Kim et al. 2006).

Costs associated with attraction to heterospecifics would be expected to generate selection that would favor divergence of mate location mechanisms. However, both of our study species rely on the location of an ephemeral resource for larval development, and mating occurs on the larval host. These factors may generate directional selection that both opposes and is stronger than selection associated with the costs of heterospecific attraction. In addition, the relative population densities and frequencies of occurrence of both species on host material may significantly reduce the potential for heterospecific attraction and ultimately the need for divergence of mate location mechanisms. Alternatively, one or both species may possess additional mechanisms to prevent attraction to

heterospecifics (e.g., additional pheromone components).

Unexpectedly, both male and female *M. carolinensis* and *M. titillator* were attracted to traps baited with (2*R**,3*R**)-2,3-hexanediol plus α-pinene (but not to traps baited with (2*R**,3*R**)-2,3-hexanediol alone). Although it is possible that (2*R**,3*R**)-2,3-hexanediol is an additional pheromone component for either or both species, to date this compound has only been found as a pheromone component for species in the subfamilies Cerambycinae and Prioninae (Hanks et al. 2012). Its possible presence in extracts of volatiles was impossible to determine by GC–MS or GC–EAD because of the large amounts of host volatiles that coeluted in the region of the chromatogram where this compound would have eluted. Alternatively, *M. carolinensis* and *M. titillator* may eavesdrop on the communication systems of sympatric species that also use stressed, dying, or recently-dead conifers as larval hosts (see Lacey et al. 2004, Hanks et al. 2007, Barbour et al. 2011). Further behavioral and chemical analyses are required to test these hypotheses.

Ginzel and Hanks (2005) proposed a three-stage sequence for mate location in some species from the subfamily Cerambycinae that exploit stressed hosts. In stage 1, both sexes locate larval hosts by using plant volatiles; in stage 2, males attract females over shorter distances with pheromones; and in stage three males recognize females by contact pheromones. Fan et al. (2007) suggested that this hypothesis might apply to all cerambycid species that exploit stressed hosts. The synergy between pheromone and host volatiles observed in this study and others [*M. alternatus* (Teale et al. 2011); *M. galloprovincialis* (Ibeas et al. 2008, Pajares et al. 2010); *M. carolinensis* (Hanks et al. 2012); *Tetropium fuscum* (F.), *T. cinnamopterum* Kirby in Richardson, and *T. castaneum* (L.) (Silk et al. 2007, Sweeney et al. 2010); and *A. glabripennis* (Nehme et al. 2009, 2010)] does not support this model, suggesting instead that pheromones play a much larger role in attraction of conspecifics. Furthermore, the observation of synergy between pheromones and host volatiles suggests that both the insect-produced signal and the host-produced cue are required for attraction and that the combination of the two stimuli together may have a larger active space than either stimulus alone (Linn et al. 1987).

Although our knowledge of the chemical ecology of cerambycids has increased rapidly in recent years, our understanding of the full panoply of signals and cues that mediate host location and reproductive behaviors in this insect family is still limited. Unlike communication systems in which the female sex pheromone has the single function of attracting a conspecific male for mating (e.g., Lepidoptera), the chemical communication systems of cerambycids appear to be more complex. First, both insect-produced and host plant compounds may be required to obtain significant levels of response. Second, the signal may be multifunctional, indicating both the presence of potential mates and host resources. Third, an increasing body of evidence suggests that exploitation of the pheromonal signals of heterospecific guild members as kairomones to locate high quality hosts may be commonplace among cerambycids. These findings suggest that, in addition to studying the roles of semiochemicals as signals within a species, the possible effects of those chemicals on other members of its feeding guild and community also should be determined.

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