The Influence of Host Plant Volatiles on the Attraction of Longhorn Beetles to Pheromones

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Abstract Host plant volatiles have been shown to strongly synergize the attraction of some longhorn beetle species (Coleoptera: Cerambycidae) to their pheromones. This synergism is well documented among species that infest conifers, but less so for angiosperm-infesting species. To explore the extent of this phenomenon in the Cerambycidae, we first tested the responses of a cerambycid community to a generic pheromone blend in the presence or absence of chipped material from host plants as a source of host volatiles. In the second phase, blends of oak and conifer volatiles were reconstructed, and tested at low, medium, and high release rates with the pheromone blend. For conifer-infesting species in the subfamilies Spondylidinae and Lamiinae, conifer volatiles released at the high rate synergized attraction of some species to the pheromone blend. When comparing high-release rate conifer blend with high-release rate α-pinene as a single component, species responses varied, with *Asemum nitidum* LeConte being most attracted to pheromones plus α-pinene, whereas *Neospondylis upiformis* (Mannerheim) were most attracted to pheromones plus conifer blend and ethanol. For oak-infesting species in the subfamily Cerambycinae, with the exception of *Phymatodes grandis* Casey, which were most attracted to pheromones plus ethanol, neither synthetic oak blend nor ethanol increased attraction to pheromones. The results indicate that the responses to combinations of pheromones with host plant volatiles varied from synergistic to antagonistic, depending on beetle species. Release rates of host plant volatiles also were important, with some high release rates being antagonistic for oak-infesting species, but acting synergistically for conifer-infesting species.

Keywords Coleoptera · Cerambycidae · Pheromone · Host plant volatiles · Synergist · Antagonist

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Introduction

Cerambycid beetles are ecologically important in forest ecosystems as primary decomposers of woody material, allowing carbon and other nutrients to cycle back into the ecosystem (Grove 2002; Ulyshen 2016). In addition, they cull weak, diseased, and stressed trees within forests, maintaining baseline mortality and turnover of trees, an important factor for healthy forest systems (Haack and Byler 1993; Teale and Castello 2011). In counterpoint to their beneficial role in coevolved systems, longhorn beetles have the potential to be ecologically and economically damaging when they are introduced into new habitats as invasive species (Haack et al. 2010; Parry and Teale 2011). Preventing introductions of wood-boring insects is particularly difficult because larvae can persist cryptically within lumber, wooden materials such as shipping crates and dunnage, and finished wooden products such as furniture, being shipped internationally (Haack 2006).

Prior to 2004, almost nothing was known about pheromones of cerambycids (Allison et al. 2004; Hanks 1999), but within the last decade a large number (>100) of

pheromones and pheromone candidates have been identified (Millar and Hanks 2016). Within the five subfamilies for which at least some pheromones have been identified, patterns in pheromone use are emerging. For example, all identified pheromones from the subfamilies Prioninae (Barbour et al. 2011; Ray et al. 2012a; Wickham et al. 2014, 2016) and Lepturinae (Ray et al. 2011, 2012b, 2014) are female-produced sex pheromones, whereas all known examples of volatile pheromones from the subfamilies Cerambycinae, Lamiinae, and Spondylidinae are male-produced aggregation pheromones that attract both sexes. Common pheromone motifs for cerambycine species include 2,3-hexanediols, 3-hydroxyhexan-2-ones (Hanks et al. 2007; Lacey et al. 2004, 2008, 2009; Ray et al. 2009, 2015; Schröder et al. 1994) and their C_8 analogs (Leal et al. 1995; Narai et al. 2015), whereas fuscumol [(E)-6,10-dimethyl-5,9-undecadien-2-ol] and its ester, fuscumol acetate [(E)-6,10-dimethyl-5,9-undecadien-2-yl acetate], are common pheromone components for the subfamilies Spondylidinae (Silk et al. 2007; Sweeney et al. 2010) and Lamiinae (Fonseca et al. 2010; Hughes et al. 2013; Mitchell et al. 2011). In addition, for the lamiine genus Monochamus and some related genera, monochamol [2-(undecyloxy)ethanol] is a proven or likely pheromone component for ~15 species (Allison et al. 2012; Fierke et al. 2012; Hanks and Millar 2013; Macias-Samano et al. 2012; Pajares et al. 2010, 2013; Ryall et al. 2014; Teale et al. 2011; Wickham et al. 2014). There also are several examples of attraction of cerambycids to pheromones of other cerambycid species that they themselves do not produce, possibly exploiting these signals as kairomones to locate suitable habitats (Hanks and Millar 2013; Hanks et al. 2007, 2012; Mitchell et al. 2011; Wickham et al. 2014; Wong et al. 2012).

Before pheromones were identified for cerambycid beetles, mimics of naturally produced suites of host plant volatiles (HPVs), such as α-pinene, turpentine, and related materials, were used as generic attractants for cerambycids (reviewed in Allison et al. 2004; Millar and Hanks 2016). Fermenting sugar-based baits also have been used as lures (Linsley 1959), with early work establishing ethanol as a general attractant for cerambycids and other saproxylic wood-boring insects that infest stressed, dying, and dead trees (Gara et al. 1993; Kelsey 1994; Kimmerer and Kozlowski 1982; Klimetzek et al. 1986; Montgomery and Wargo 1983). Thus, blends utilizing α-pinene and ethanol have been widely used as general attractants for cerambycids (e.g., Miller 2006), with conifer-infesting species typically being most strongly attracted to such blends. However, there are nuances, because in comparisons of α-pinene with more complex blends of monoterpenes, α-pinene was found to be more attractive than the monoterpene blends for some cerambycid species (Chénier and Philogène 1989; Morewood et al. 2002; Sweeney et al. 2004).

More recent studies have shown that HPVs may strongly synergize the attraction of cerambycid species to their pheromones. Among species in the subfamily Lamiinae, a number of Monochamus species are more strongly attracted to combinations of monochamol with α-pinene and ethanol than to monochamol alone (Allison et al. 2012; Fierke et al. 2012; Ryall et al. 2014; Teale et al. 2011). Similarly, Tetropium fuscum (F.) and Tetropium castaneum (L.) (subfamily Spondylidinae) respond only weakly to their pheromones alone, with attraction being significantly increased by a blend of five monoterpenes from their hosts (spruce; Picea species) with ethanol (Silk et al. 2007).

In contrast, less is known about the possible role of angiosperm HPVs as attractants or co-attractants (with pheromones) for cerambycids. In one of the few reported examples where angiosperm HPVs were tested, volatiles from green maté (Ilex paraguariensis A. St. Hill; Aquifoliales: Aquifoliaceae), the host plant of the lamiine species Hedypathes betulinus (Klug), were not attractive to the beetles in Y-tube assays, but they did increase attraction of female beetles to the male-produced pheromone (Fonseca et al. 2010). HPVs also have been intensively studied as possible attractants or co-attractants for the invasive Asian longhorned beetle, Anoplophora glabripennis Motschulsky, but the results have been mixed, with lures being relatively weakly attractive overall (Meng et al. 2014; Nehme et al. 2014; Wickham 2009). For the congeneric species Anoplophora malasiaca (Thomson), in which the reproductive behaviors have been studied intensively (reviewed in Yasui 2009), the evidence suggests that this species relies heavily on HPVs for mate-/host-location, eschewing the need for long-range pheromones. Although A malasiaca is highly polyphagous, populations reared from mandarin orange (Citrus unshiu Marc.; Sapindales: Rutaceae) are most strongly attracted to citrus HPVs, whereas willow-infesting (Salix schwerinii E. Wolf; Malphigiales: Salicaceae) populations orient preferentially towards willow HPVs (Yasui et al. 2011). Within the subfamily Cerambycinae, both sexes of Xylotrechus colonus (F.), Megacyllene caryae (Gahan), and Neoclytus mucronatus mucronatus (F.) were attracted to odors from logs of their host, shagbark hickory (Carya glabra Miller; Fagales: Juglandaceae) in Y-tube assays (Ginzel and Hanks 2005). In sum, these studies suggest that host volatiles also may be involved in attraction of angiosperm-infesting cerambycids.

Overall, these studies indicate that our knowledge of the interactions among HPVs and pheromones in the reproductive behaviors of cerambycids is limited. Thus, the goal of the work reported here was to obtain an overview of the influence of HPVs on attraction of cerambycids to male-produced pheromones, for species infesting both conifers and angiosperm trees (specifically oaks). Our main objectives were:

1) To determine whether volatiles from fresh host plant material affected the attraction of cerambycids to their pheromones, for species attacking either conifers or oaks;

2) To analyze the volatiles from fresh host materials, with the goal of reconstructing blends of host volatiles from synthetic chemicals;

3) To test those reconstructed blends of HPVs at various release rates with and without pheromones.

We also conducted follow-up experiments to test the importance of blend complexity for conifer-infesting species, and the role of ethanol alone as a possible host tree cue for oak-infesting species.

Methods and Materials

Field Sites and General Experimental Design Field bioassays were conducted at two sites in the San Bernardino National Forest (SBNF) in San Bernardino Co., CA, USA. The first site was near Jenks Lake (34°09′45.8″N 116°54′08.6″W) and was dominated by Ponderosa pine (*Pinus ponderosa* Douglas) and white fir (*Abies concolor* [Gordon]) (Pinales: Pinaceae), with some western black oak (*Quercus kelloggii* Newbury), canyon live oak (*Quercus chrysolepis* Liebm.) (Fagales: Fagaceae), bigcone Douglas-fir (*Pseudotsuga macrocarpa* [Vasey]) (Pinales: Pinaceae), and incense cedar (*Calocedrus decurrens* Torr.) (Pinales: Cupressaceae). This site was used for all three experiments. The second site was near the community of Seven Oaks (34°11′08.0″N 116°51′57.4″W) and was dominated by canyon live oak and interior live oak (*Quercus wislizeni* A. DC.), with some single-leaf pinyon pine (*Pinus monophylla* Torr. & Frém.) and Jeffrey pine (*Pinus jeffreyi* Balfour); this site was used for the first two experiments. Black cross-vane traps (Alpha Scents, Portland OR, USA) coated with Fluon® (Graham et al. 2010) were hung on tree branches at a height of ~1.5–2 m (first experiment) or on 1.5 m tall, L-shaped stands made from PVC pipe (second and third experiments). For all experiments, traps were placed 10–15 m apart in transects, with treatments initially assigned randomly to traps. Traps were checked twice weekly, with lures changed once weekly, at which time the trap order was rerandomized.

To attract a broad range of species, a blend of known cerambycid pheromones was used, as done in previous studies (Hanks and Millar 2013; Wong et al. 2012). The blend was formulated in isopropanol, using 25 mg/ml for pure compounds (i.e., monochamol) or 50 mg/ml for racemic compounds (all others). The blend contained the following compounds: racemic 3-hydroxyhexan-2-one (Bedoukian Research, Danville CT, USA); (2*R**,3*R**)-2,3-hexanediol (synthesized as described in Lacey et al. 2004); racemic fuscumol (Bedoukian Research); racemic fuscumol acetate (Bedoukian Research); monochamol (Bedoukian Research); and racemic 2-methylbutanol (Aldrich Chemical Co., Milwaukee WI, USA). The blend used in the first two experiments contained all six compounds, whereas (2*R**,3*R**)-2,3-hexanediol was omitted from the blend in the final experiment, because none of the cerambycine species that we had trapped in the first two experiments use 2,3-hexanediols as their pheromones. Lures consisted of 1 ml of the blend deployed in 2 mil wall thickness, low-density polyethylene resealable baggies (~5 × 7.6 cm; Fisher Scientific, Pittsburgh PA, USA).

Beetles were live trapped so that they could be used for pheromone collection and electrophysiological assays in the laboratory. Excess beetles were released at least 100 m from the field sites. Voucher specimens of all species captured in statistically significant numbers have been deposited in the Entomology Museum at UC Riverside.

Bioassay of Pheromones with Crude Host Plant Material The first experiment was designed to test the effects of crude tree volatiles in combination with the pheromone blend. The experiment ran from 4 May to 11 September 2012 at both study sites, with six spatial replicates (i.e., six trap transects). Host tree species included four conifer species (Jeffrey and Ponderosa pines, white fir, bigcone Douglas-fir) and three oak species (western black, interior live, and canyon live oak), all of which occur in the SBNF and are known hosts to a diversity of cerambycid species (Linsley and Chemsak 1997). Branches were harvested from 2 to 4 healthy trees of each of the species from April through June, and were chipped with an industrial chipper to increase surface area and enhance the release of volatiles. Chipped material was stored in sealed jars in a −20 °C freezer until deployed in the field. To make HPV lures, one liter of chipped material was bundled in aluminum window screen and tied to the top of the trap. Chipped material was replaced weekly, with a different species of conifer and oak being tested each week. The treatments were: 1) pheromone blend, 2) pheromone blend + chipped conifer material, 3) pheromone blend + chipped oak material, 4) chipped conifer material, 5) chipped oak material, 6) blank control.

Identification of Host Plant Volatiles and Reconstruction of HPV Blends Compounds were identified from the seven species of trees used in the first experiment, i.e., Jeffrey pine, Ponderosa pine, white fir, bigcone Douglas-fir, western black oak, interior live oak, and canyon live oak. Approximately 250 ml of chipped tree branches of a given species were placed in a 250 ml mason jar, with the lid fitted with air inlets and outlets. Charcoal-filtered air was pulled through the container at 250 ml per min, collecting the headspace volatiles on ~100 mg of thermally-desorbed activated charcoal (50–200 mesh; Fisher Scientific) held between glass wool plugs in a short piece of glass tubing. Volatiles were collected for 24–48 h at a time, with consecutive samples being taken to follow temporal changes in the emitted blend of volatiles. Volatiles were collected from 2 to 4 trees of each species. Adsorbed volatiles were eluted from the collector with 1 ml

of dichloromethane, and samples were analyzed with an HP 6890 gas chromatograph (Hewlett-Packard, now Agilent, Santa Clara CA, USA) fitted with a DB-17 column (30 m × 0.25 mm ID × 0.25 μm film; J&W Scientific, Folsom CA, USA), coupled to an HP 5973 mass selective detector. The temperature program used was 40 °C/1 min, then increased 5 °C/min to 280 °C, hold 5 min. To improve the chromatography of early eluting compounds, the injector temperature was set to 125 °C and injections were made in split mode. Spectra were taken in full scan mode with electron impact ionization (70 eV).

Compounds were identified tentatively by matches with database spectra (NIST 98, Agilent), and then confirmed by matching retention times and mass spectra with those of authentic standards. The absolute configurations of chiral compounds were determined by GC analysis using Cyclodex-B and Beta-Dex columns (both 30 m × 0.25 mm ID × 0.25 μm film; J&W Scientific). All samples were run on both columns, matching retention times with those of standards of known absolute configuration. Analyses were conducted using a temperature program of 50 °C/1 min, then 5 °C/min to 220 °C, hold 20 min, with an injector temperature of 100 °C. Samples were run in split mode.

Authentic standards of the host plant compounds were obtained from the following sources: Sigma-Aldrich (Milwaukee, WI, USA): (+)- and (-)-α-pinene, (+)-β-pinene, racemic 3-carene, (+)-limonene, racemic camphene, (Z)-3-hexenol; Alfa-Aesar (Ward Hill, MA, USA): (-)-β-pinene, (-)-limonene, 1,8-cineole (=eucalyptol), (-)-borneol, (+)- and (-)-camphor, 4-allylanisole (=estragole), benzaldehyde, hexanol, hexanal; Acros Organics (Geel, Belgium): myrcene, (E)-2-hexenal, (E)-2-hexenol; TCI Americas (Portland, OR, USA): (−)-trans-β-caryophyllene, methyl salicylate.

For the two synthetic blends of HPVs, the primary criteria used to decide which compounds to use in the blends were: 1) commonality of the compound among all conifer or all oak species sampled, 2) commercial availability of the compound (e.g., β-phellandrene was excluded because it was not readily available), 3) high abundance compounds, 4) compounds known to act as host plant cues in other insect-plant systems [e.g., (−)-trans-β-caryophyllene; Yasui et al. 2008], and 5) compounds that elicited strong antennal responses during coupled gas chromatography-electroantennogram detection (GC-EAD) screening tests (e.g., 4-allylanisole, see below). The ratios of compounds for the two blends were estimated from the ratios found in GC-MS chromatograms of the sampled volatiles, as determined by integrated peak areas (Tables 1 and 2). To correct for the relative volatilities of each compound in the blend, the compounds were blended initially in equal amounts, placed in the medium release rate device (7.5 ml glass vial, see below for details), and the headspace volatiles were sampled for 24 hr from a 250 ml mason jar in the same manner as the chipped material. These data were used to adjust the amounts of each compound added to the blends so that the resulting profile approximately matched that obtained from the sampled chipped material. Because the percent abundance among the

Table 1 Mean (±1 SE) relative abundances of volatile chemicals in headspace aerations of conifer species and enantiomeric composition for chiral compounds. Relative abundance of chemicals in the reconstructed synthetic blend is also included

Compound	Ponderosa pine (N=2)		Jeffrey pine (N=2)		Bigcone Douglas-fir (N=1)		White fir (N=1)		All conifers (N=6)	Synthetic conifer blend
	Mean ± SE	Chirality	Mean ± SE	Chirality	Rel. abundance	Chirality	Rel. abundance	Chirality	Mean ± SE	Rel. abundance
α-pinene	44.9 ± 5.7	(−)[1]	35.9 ± 6.2	(−)	8.59	racemic	12.0	2:1 (−):(+)	30.4 ± 6.9	21.4
camphene	5.0 ± 0.6	(−)	3.6 ± 2.1	(−)	0.35	racemic	0.57	(−)	3.0 ± 1.0	3.6
β-pinene	13.3 ± 1.0	2:1 (−):(+)	17.3 ± 1.9	(−)	37.4	(−)	45.3	(−)	24.0 ± 5.7	19.9
myrcene	4.4 ± 0.4	–	5.6 ± 0.1	–	2.84	–	2.1	–	4.1 ± 0.6	13.1
3-carene	6.9 ± 1.1	–	7.5 ± 5.5	–	4.36	–	3.1	–	6.1 ± 1.6	19.0
limonene	3.6 ± 0.03	(+)	12.6 ± 4.6	(+)	7.69	(+)	5.1	(+)	7.5 ± 2.1	14.4
β-phellandrene	6.4 ± 0.4	–	3.7 ± 0.1	–	19.0	–	20.9	–	10.0 ± 3.2	–
1,8-cineole	2.9 ± 0.1	–	0	–	0	–	0	–	0.97 ± 0.61	4.5
borneol	0.13 ± 0.04	(−)	0.16 ± 0.09	(−)	0.29	(−)	0.26	(−)	0.19 ± 0.04	0.45
camphor	0.08 ± 0	2:1 (−):(+)	0.08 ± 0.03	(−)	0.21	2:1 (−):(+)	0.10	(−)	0.10 ± 0.02	1.1
4-allylanisole	3.8 ± 1.0	–	2.4 ± 1.8	–	4.29	–	–	–	3.3 ± 0.7	1.1
(−)-trans-β-caryophyllene	1.6 ± 0.5	–	0.20 ± 0.20	–	1.85	–	3.4	–	1.8 ± 0.5	–
unidentified	7.2 ± 3.8	–	11.1 ± 3.2	–	13.1	–	7.3	–	9.5 ± 1.7	–

[1] Unless otherwise specified, indicates dominant isomer

Table 2 Mean (±1 SE) relative abundances of volatile chemicals in headspace aerations of oak species and enantiomeric composition for chiral compounds. Relative abundance of chemicals in the reconstructed synthetic blend is also included

Compound	Canyon live oak (N = 2)		Interior live oak (N = 3)		Western black oak (N = 2)		All oak samples (N = 7)	Synthetic oak blend
	Mean ± SE	Chirality	Mean ± SE	Chirality	Mean ± SE	Chirality	Mean ± SE	Rel. abundance
hexanal	0.24 ± 0.07	–	0.29 ± 0.11	–	0.08 ± 0.08	–	0.22 ± 0.06	0.87
1-hexanol	3.1 ± 0.8	–	2.3 ± 1.0	–	1.9 ± 1.2	–	2.4 ± 0.5	2.2
(E)-3-hexen-1-ol	18.6 ± 3.7	–	4.5 ± 1.5	–	7.3 ± 2.8	–	9.3 ± 2.7	5.9
(Z)-2-hexen-1-ol	0	–	1.2 ± 0.9	–	1.8 ± 1.0	–	1.4 ± 0.5	–
α-pinene	29.3 ± 3.3	racemic	9.9 ± 1.4	2:1 (−):(+)	9.4 ± 2.7	racemic	15.3 ± 3.8	16.6
(Z)-2-hexenal	2.2 ± 1.4	–	3.4 ± 2.6	–	4.1 ± 2.0	–	3.3 ± 1.2	11.4
camphene	1.1 ± 0.2	(−)[1]	0.30 ± 0.13	(−)	0.30 ± 0.14	(−)	0.53 ± 0.17	2.6
β-pinene	16.4 ± 2.9	2:1 (−):(+)	7.6 ± 5.5	2:1 (−):(+)	1.7 ± 1.0	(+)	8.4 ± 3.2	13.7
myrcene	4.0 ± 0.5	–	3.5 ± 2.9	–	1.1 ± 0.6	–	3.0 ± 1.2	8.4
3-carene	2.2 ± 0.3	–	1.3 ± 0.9	–	0.55 ± 0.38	–	1.3 ± 0.4	12.5
limonene	11.5 ± 2.3	(+)	9.4 ± 6.9	(+)	2.6 ± 1.0	2:1 (+):(−)	8.0 ± 3.0	9.1
β-phellandrene	2.7 ± 0.5	–	3.7 ± 2.7	–	2.9 ± 1.5	–	3.2 ± 1.1	–
1,8-cineole	5.2 ± 3.1	–	69.1 ± 9.1	–	56.0 ± 1.7	–	43.5 ± 11.8	14.4
benzaldehyde	0.13 ± 0.02	–	0.15 ± 0.09	–	0.09 ± 0.01	–	0.12 ± 0.03	0.77
4-allylanisole	0.55 ± 0.02	–	0.54 ± 0.29	–	0.34 ± 0.13	–	0.49 ± 0.12	1.1
methyl salicylate	0.19 ± 0.02	–	0.38 ± 0.21	–	3.3 ± 0.5	–	1.2 ± 0.6	0.55
(−)-trans-β-caryophyllene	0.10 ± 0.01	–	0.07 ± 0.07	–	0.08 ± 0.01	–	0.08 ± 0.03	–
unidentified	2.5 ± 1.2	–	5.5 ± 5.5	–	6.6 ± 2.1	–	4.9 ± 2.2	–

[1] Unless otherwise specified, indicates dominant isomer

monoterpene hydrocarbons was similar between conifers and oaks, the same ratio of these terpenes was used in the two blends to standardize this variable between the two blend compositions. The reconstructed blends are listed in Table 3.

Table 3 Synthetic blend compositions for the conifer and oak blends, with each compound's contribution expressed as milliliters per 100 ml. Ratio of enantiomers listed

Compound	Conifer blend		Oak blend	
	Enantiomeric ratio	ml / 100 ml	Enantiomeric ratio	ml / 100 ml
hexanal	–	–	–	0.4
hexanol	–	–	–	7.3
(Z)-3-hexen-1-ol	–	–	–	14.7
(E)-2-hexen-1-ol	–	–	–	1.7
α-pinene	2:1 (−):(+)	17.2	2:1 (−):(+)	7.3
(E)-2-hexenal	–	–	–	14.7
camphene	racemic	1	racemic	0.4
β-pinene	(−)	17.2	(−)	7.3
myrcene	–	17.2	–	7.3
3-carene	racemic	17.2	racemic	7.3
limonene	2:1 (+):(−)	17.2	2:1 (+):(−)	7.3
1,8-cineole	–	4	–	14.7
benzaldehyde	–	–	–	0.4
borneol	(−)	2	–	–
camphor	2:1 (−):(+)	2	–	–
4-allylanisole	–	1	–	3.7
methyl salicylate	–	–	–	3.7
(−)-trans-β-caryophyllene	–	4	–	1.8

To determine which compounds beetles can perceive, GC-EAD analyses were conducted with the HPV samples and with synthetic compounds. GC-EAD analyses were carried out with antennae of the oak-infesting species *Phymatodes obliquus* Casey, *Phymatodes grandis* Casey, *Brothylus conspersus* Leconte, *Brothylus gemmulatus* Leconte, and *Neoclytus modestus modestus* Fall, and the conifer-infesting species *Neospondylis upiformis* (Mannerheim), *Asemum striatum* (L.), *Asemum caseyi* (L.), *Asemum nitidum* (L.), and *Monochamus clamator* (Leconte). The objective was not to determine every compound that may or may not be perceived by the beetles, but to screen for low abundance compounds that elicited strong antennal responses that might otherwise have been overlooked. GC-EAD analyses were conducted on an HP 5890 Series II GC fitted with a DB-17 column (30 m × 0.25 mm ID × 0.25 μm film) programmed from 50 °C/1 min, then increased 5 °C/min to 250 °C, hold 10 min. The injector temperature was 250 °C, and injections were made in splitless mode. A glass X-cross split the effluent between the flame-ionization detector (FID) and EAD, with helium being added through the fourth arm of the cross as makeup gas. The column effluent was directed into a humidified air stream that then was directed over the antennal preparation. Antennae were prepared by excising the distal three to five segments of a live beetle's antenna and then removing the tip of the antenna with a razor blade to provide electrical contact. The antennal section was suspended between two saline-filled (7.5 g NaCl, 0.21 g CaCl$_2$, 0.35 g KCl, and 0.20 g NaHCO$_3$ in 1 L Milli-Q purified water) glass capillary electrodes with 0.2 mm diam gold wire connections between the electrodes and a custom-built electroantennogram amplifier. The signals from the GC and the EAD were recorded simultaneously using Peak-Simple software (SRI International, Menlo Park, CA, USA).

Bioassay of Pheromones with Reconstructed Blends of Host Plant Volatiles The goal of this experiment was to test synthetic blends of volatiles mimicking the odors of oaks and conifers, respectively (see above), with and without pheromones. Three release rates were tested: ~0.02, ~0.4, and ~3 g per day of the total blend for the low, medium, and high release rates, respectively. The release devices consisted of a screw-top 2 ml glass vial (low rate; 3 cm tall × 11 mm outer diam × 5 mm opening), a 7.5 ml glass vial (medium rate; 3.7 cm tall × 22 mm outer diam × 13 mm opening), and a 25 ml glass jar (high rate; 4.3 cm tall × 4.3 cm outer diam × 3.1 cm opening). All three release devices were left uncapped for deployment, and vials were never more than half-filled to control for higher release rates of compounds closer to the rim of the container (Weatherston et al. 1985). One and 5 ml, respectively, of the synthetic blends were loaded into the low and medium release devices weekly, and 10 ml were

loaded into the high release rate dispensers twice weekly. Before replenishing the tree volatile blends, the remaining blend in the vials was poured into a waste container for disposal.

Release rates from the devices were estimated in the laboratory by measuring weight loss from devices held in a fume hood. These approximations were confirmed by observing the amount of each blend that evaporated from the release devices in the field. The ratio of the compounds in the blend remained consistent until the meniscus of the blend reached the base of the vial, at which point lower volatility compounds were in higher abundance (determined by GC-MS analysis of blends sampled in the laboratory). During the experiment, the high-release rate device was occasionally reduced to the meniscus when site temperatures were high.

The field experiment was run from 15 April to 3 September 2013 at both field sites, with four spatial replicates. The treatments were: 1) pheromone blend + ethanol, 2) pheromone blend + ethanol + low release rate synthetic conifer blend, 3) pheromone blend + ethanol + medium release rate conifer blend, 4) pheromone blend + ethanol + high release rate conifer blend, 5) pheromone blend + ethanol + low release rate synthetic oak blend, 6) pheromone blend + ethanol + medium release rate oak blend, 7) pheromone blend + ethanol + high release rate oak blend, 8) ethanol + medium release rate conifer blend, 9) ethanol + medium release rate oak blend, 10) ethanol control. Ethanol was included in this experiment because it is known to occur in stressed trees and because of its known efficacy in attracting cerambycids (see Introduction). The ethanol was released from 10 × 15 cm low density polyethylene resealable bags (2 mil wall thickness; Fisher Scientific) loaded with 100 ml ethanol. These devices had a consistent release rate of ~0.2 g per day as long as the bags contained enough ethanol to coat the interior surface area of the bag. The release rate was determined in the laboratory gravimetrically.

Bioassay of Pheromones with Subsets of Host Plant Volatiles To minimize the overall number of traps that had to be deployed and to use the available field sites most efficiently, this experiment combined two parts. The objective of the first part was to test whether a relatively complex blend of HPVs could be sufficiently mimicked by a single major component, α-pinene, for the species infesting conifers. Thus, we tested the full synthetic conifer blend used in the second experiment vs. α-pinene, with and without ethanol. The second objective was to test whether ethanol increased attraction of oak-infesting species to their pheromones. The experiment was run from 29 April to 11 August 2014 at the Jenks Lake site, using three spatial replicates. The high release rate of the conifer blend was used because this had proven to be most attractive to conifer-infesting species in the second experiment. α-Pinene as a single component was released from the

high-release device, and gravimetric measurements in the laboratory confirmed that the release rate was comparable to that of the full synthetic conifer blend (i.e., ~3 g/day). α-Pinene was deployed in a 2:1 ratio of the (-)- and (+)-enantiomers, as was used in the full conifer blend. Ethanol was released from 100 ml plastic bags as described above. Thus, the treatments were: 1) pheromone blend + α-pinene, 2) pheromone blend + ethanol + α-pinene, 3) pheromone blend + full conifer blend, 4) pheromone blend + ethanol + full conifer blend, 5) pheromone blend, 6) pheromone blend + ethanol. Cerambycid species were classified as either conifer- or oak-infesting (Linsley and Chemsak 1997) and then analyzed for differences between treatments 1–4 or 5–6, respectively. The oak-infesting species do not necessarily specialize solely on oaks, but all typically utilize oaks as primary hosts in the SBNF.

Statistical Analyses Species with less than five individuals trapped were excluded from analysis. In the first experiment using chipped host plant material, our initial attempts to analyze the trap catch data by tree species were not successful because of the high variability among trap catches and relatively low numbers of beetle captured for some species. Thus, the data from the four conifer and three oak species, respectively, were pooled for analysis. Replicates for each experiment were based on both spatial and temporal replicates, with temporal replicates equalling the number of times the traps were checked. Temporal replicates where no beetles of a given species were trapped – usually due to inclement weather or being outside the species' flight period – were not included in analyses for that species. Data were not normally distributed as determined by the Shapiro-Wilks Test, and so were analyzed with the non-parametric Kruskal-Wallis Test followed by Dunn's Test due to rank-ties (Elliott and Hynan 2011; SAS 9.3 and 9.4, SAS Institute 2012), using $\alpha = 0.05$. For Dunn's Test, the q-value must be greater than the q (0.05)-value to be statistically significantly different. The q (0.05)-value is 2.94 for six treatments (first and third experiments), 3.26 for 10 treatments (second experiment), and 1.96 for two treatments (third experiment).

Results

Bioassays of Pheromone Blends with Crude Host Plant Volatiles A total of 1,046 cerambycid beetles of species that typically infest oak were caught, with four cerambycine species being caught in sufficient numbers to warrant statistical analysis, including *Brothylus conspersus* (23 beetles), *Brothylus gemmulatus* (20 beetles), *Phymatodes grandis* (98 beetles), and *Phymatodes obliquus* (905 beetles). The entire list of species caught is provided in the supplementary information (Table S1). There were no significant differences in the responses of any of the four species to pheromones alone vs. the pheromones plus chipped oak (*P. obliquus*, $q = 1.05$; *P. grandis*, $q = 0.07$;

B. conspersus, $q \leq 2.19$; *B. gemmulatus*, $q \leq 2.35$; Fig. 1a and b). Attraction of both *Phymatodes* species to the pheromone blend was inhibited by volatiles from the chipped conifer branches (*P. obliquus*, $q = 3.55$; *P. grandis*, $q = 4.11$), whereas there were no significant differences in the responses of either *Brothylus* species to the pheromone vs. pheromone + conifer material treatments. None of the four species were significantly attracted to the chipped oak or conifer materials alone.

Three conifer-infesting species were caught in significant numbers during this experiment: *Tetropium abietis* Fall (Spondylidinae) (20 beetles), *Monochamus clamator* (Lamiinae) (27 beetles), and *Tragosoma depsarium* sp. nov. Laplante (Prioninae) (38 beetles) (Table S1). *Tetropium abietis* were significantly more attracted to pheromone blend plus conifer chips than to any non-pheromone blend treatments ($q = 4.17$), but not to the pheromone alone or to the pheromone + chipped oak material ($q \leq 2.83$; Fig. 1c). *Monochamus clamator* were equally attracted to the pheromone + conifer and pheromone + oak treatments ($q = 0.83$), significantly more so than to all other treatments ($q \geq 3.01$; Fig. 1c). Male *T. depsarium* sp. nov. Laplante were equally attracted to pheromones alone or pheromones with either conifer or oak chips, but not to the remaining treatments ($q \leq 1.73$; Fig. 1c).

The spondylidine species *Neospondylis upiformis* appeared to be attracted to the pheromone plus conifer chips treatment. However, due to the unusual appearance of this species (Linsley 1962), it was not initially recognized as a longhorn beetle. By the time this error was realized, its flight period had nearly ended, and so this species was not included in the analysis.

Analysis of Host Plant Volatiles, and Reconstruction of Synthetic Blends Mimicking Host Plant Volatiles The extracts from both conifers and oaks shared a number of monoterpenes, including α-pinene, β-pinene, myrcene, 3-carene, limonene, and β-phellandrene (Fig. 2; Tables 1 and 2). However, only the oak samples contained green-leaf volatiles such as hexanal, hexanol, (Z)-3-hexenol, (E)-2-hexenol, and (E)-2-hexenal. The conifer and oak samples also showed qualitative differences among low-abundance sesquiterpenes and oxygenated monoterpenoids. Many of the chiral compounds were present as non-racemic and variable ratios of enantiomers, in which case we set the ratios in the synthetic blends to 2:1 to standardize the differences between samples. 3-Carene was not resolved on either the Cyclodex-B or the Beta-Dex column, and so racemic 3-carene was used in the synthetic blends. The final ratios of compounds used in the synthetic conifer and oak blends are shown in Table 3.

Among species trapped in the field bioassays, antennae of five oak-infesting species (*P. obliquus*, *P. grandis*, *B. conspersus*, *B. gemmulatus*, and *N. m. modestus*) and five conifer-infesting species (*N. upiformis*, *Asemum striatum*, *Asemum caseyi*, *Asemum nitidum*, and *M. clamator*) were tested for their responses to HPVs in GC-EAD analyses of

Fig. 1 Mean (±1 SE) numbers of beetles caught in traps baited with the pheromone blend and chipped host plant materials. **a)** Oak-infesting species, *Phymatodes obliquus*, **b)** oak-infesting species *Phymatodes grandis*, *Brothylus conspersus*, and *Brothylus gemmulatus* (subfamily Cerambycinae), **c)** conifer-infesting species *Tetropium abietis* (subfamily Spondylidinae), *Monochamus clamator* (subfamily Lamiinae), and *Tragosoma depsarium* sp. nov. Laplante (subfamily Prioninae). Within each species, means with the same letter are not significantly different (Dunn's test *P* > 0.05)
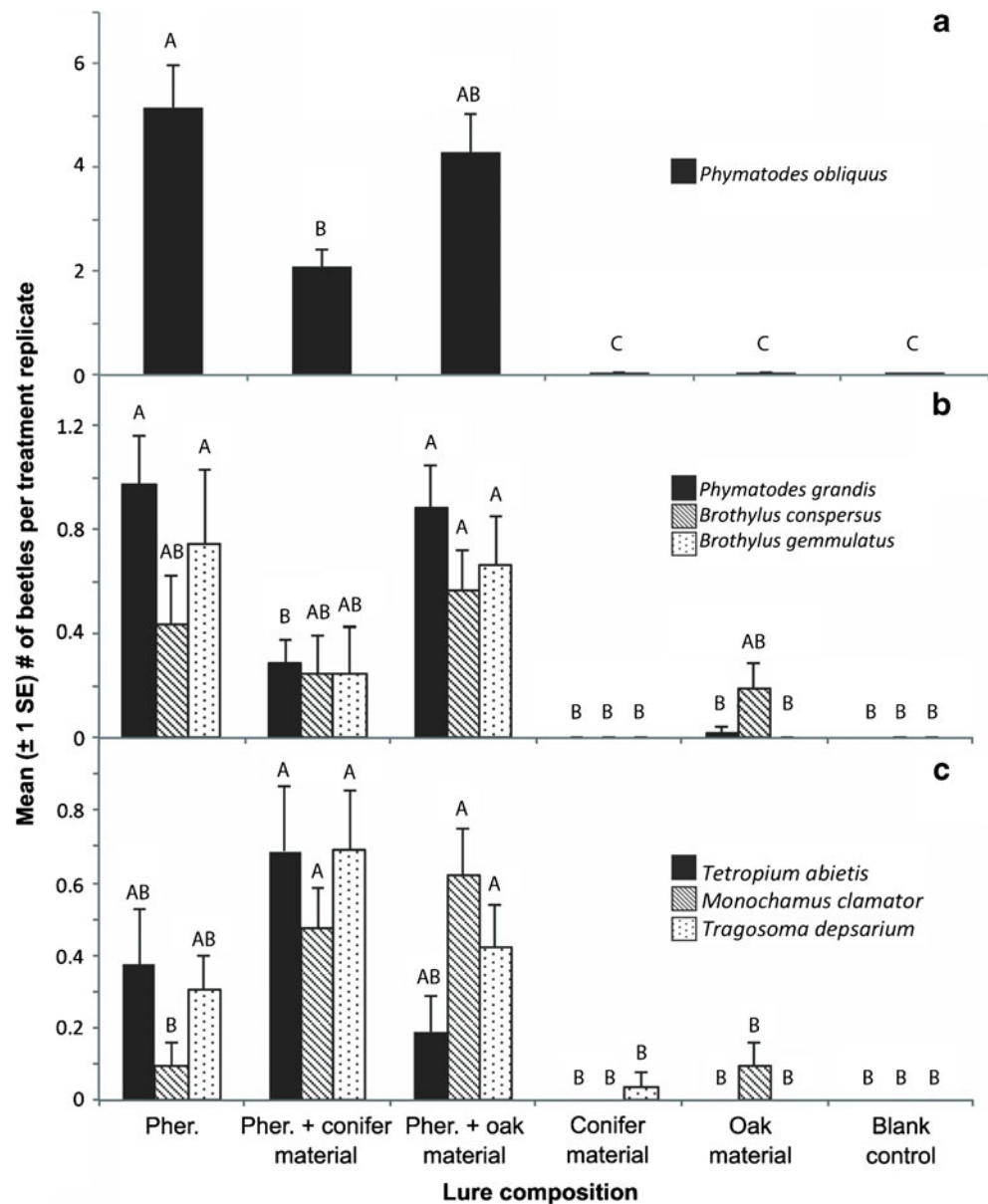
samples of the host plant volatiles collected by aeration of chipped host plant material. The results from these analyses clearly indicated that cerambycid species can detect non-host compounds (Tables S2 and S3). For example, borneol and camphor, both conifer-specific compounds, elicited responses from antennae of female *P. obliquus*, an oak-infesting species, whereas green leaf volatiles specific to oaks elicited responses from antennae of conifer-infesting *A. striatum* and *A. caseyi*.

Bioassays of Pheromones with Reconstructed Blends of Host Plant Volatiles Four oak-infesting species, *P. obliquus* (896 beetles), *P. grandis* (243 beetles), *B. gemmulatus* (322 beetles), and *N. m. modestus* (20 beetles), all in the subfamily Cerambycinae, were caught in significant numbers (Table S4) during bioassays of the pheromone blend with

the reconstructed blends of host plant volatiles. Trap catches of *P. obliquus* and *P. grandis* decreased with increasing release rates of both synthetic conifer and oak volatiles, with trap catches at the highest release rates of either being no different from catches in traps baited with the various control treatments without pheromones (Fig. 3a and b). *Brothylus gemmulatus* trap catches appeared to show similar trends, but high variability within the various treatments prevented any meaningful interpretation of the results (Fig. 3a). *Neoclytus m. modestus* were equally attracted to ethanol alone and the pheromone + ethanol treatment (*q* = 1.68), indicating that attraction was due primarily to ethanol. Addition of any of the host volatile blends at any rate to the pheromone + ethanol blend decreased captures (*q* ≥ 3.83; Fig. 3b).

Fig. 2 Representative gas chromatograms for conifers and oaks. *Top* chromatogram: sample of volatiles from Jeffrey pine; *bottom*: sample of volatiles from canyon live oak. Labelled peaks are as follows: *A*) hexanal, *B*) 1-hexanol, *C*) (*Z*)-3-hexen-1-ol, *D*) (*E*)-2-hexen-1-ol (co-eluted shoulder of (*Z*)-3-hexen-1-ol), *E*) α-pinene, *F*) (*E*)-2-hexenal, *G*) camphene, *H*) β-pinene, *I*) myrcene, *J*) 3-carene, *K*) limonene, *L*) β-phellandrene, *M*) 1,8-cineole, *N*) benzaldehyde, *O*) borneol, *P*) camphor, *Q*) 4-allylanisole, *R*) methyl salicylate, *S*) (−)-*trans*-β-caryophyllene
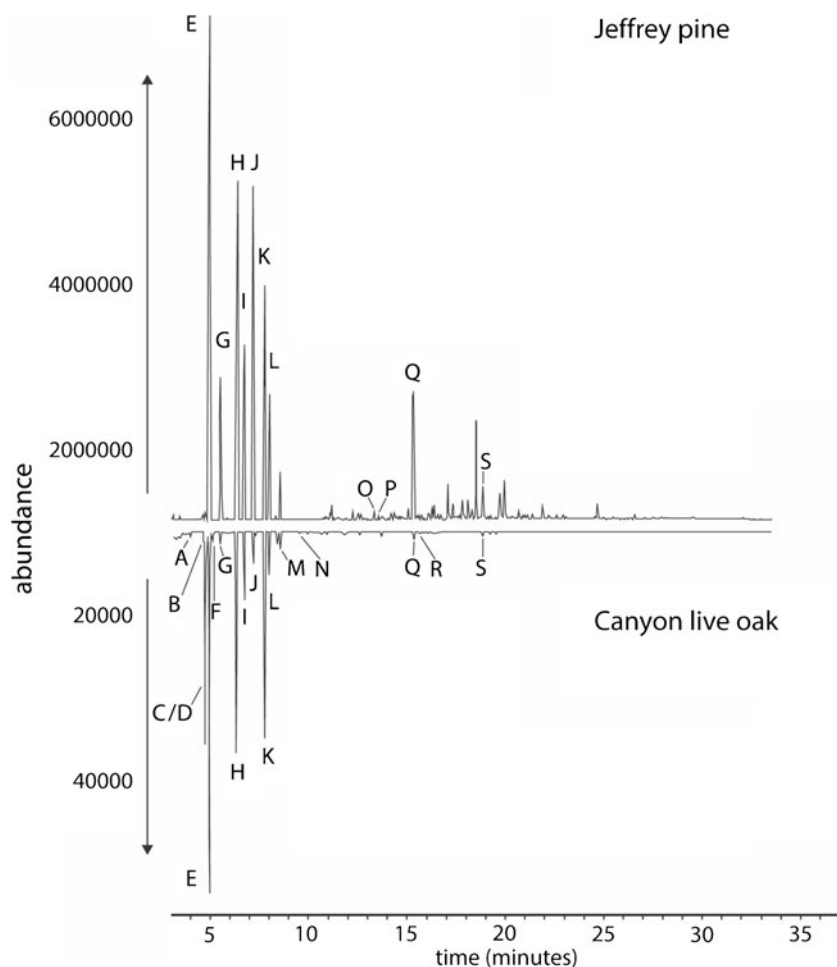
Conifer-infesting species were represented in this experiment in significant numbers by the spondylidines *N. upiformis* (51 beetles), *A. striatum* (20 beetles), *A. caseyi* (11 beetles), and *A. nitidum* (18 beetles), the lamiine *M. clamator* (18 beetles), and the cerambycine *Xylotrechus albonotatus* Casey (10 beetles) (Table S4). *Neospondylis upiformis* were most attracted by the pheromone + high release rate conifer volatiles treatment, with significant separation from all other treatments except pheromone plus medium release rate conifer ($q \geq 3.39$, $q = 2.38$; Fig. 3c). Responses of *M. clamator* followed a similar pattern, with significant separation of pheromone blend + high release rate conifer blend from all other treatments ($q \geq 3.36$; Fig. 3d); no other treatments were significantly different from the controls. For *X. albonotatus*, treatment means did not separate statistically, although a Kruskal-Wallis test indicated differences among all the treatments ($P = 0.024$; Fig. 3d). In this experiment, the spondylidines *A. striatum*, *A. caseyi*, and *A. nitidum* were caught in low numbers overall (see above), and their responses to the various treatments also did not separate statistically. However, all but *A. striatum* exhibited differential responses to the treatments when analyzed by Kruskal-Wallis tests (*A. nitidum*, $P = 0.015$; *A. striatum*, $P = 0.059$; *A. caseyi*, $P = 0.043$; Fig. S1).

Bioassay of Pheromones with or without Ethanol, for Oak-Infesting Species The oak-infesting cerambycines *P. grandis* (208 beetles), *P. obliquus* (458 beetles), *B. conspersus* (8 beetles), and *B. gemmulatus* (17 beetles) were trapped during this experiment (Table S5). The treatment effect was significant only for *P. grandis*, with attraction to the pheromone blend increased by ethanol ($q = 2.91$; Fig. 4). None of the remaining species discriminated between pheromone with or without ethanol (Fig. 4).

Bioassay of Pheromones with Conifer Volatiles or α-Pinene The spondylidines *A. nitidum* (142 beetles), *A. striatum* (25 beetles), *N. upiformis* (96 beetles), and *T. abietis* (13 beetles), the lamiine *M. clamator* (34 beetles), and the cerambycine *X. albonotatus* (8 beetles) were trapped during this experiment (Table S5). For *N. upiformis*, the pheromone + conifer blend + ethanol treatment was most attractive and statistically separated from all treatments except the pheromone plus conifer blend without ethanol ($q \geq 3.36$, $q = 1.68$; Fig. 5a); no other treatments separated from each other. *Asemum nitidum* and *A. striatum* were most strongly attracted to the pheromone + α-pinene treatments, particularly the

Fig. 3 Mean (±1 SE) numbers of beetles caught in traps baited with the pheromone blend, low (LR), medium (MR), and high (HR) release rates of reconstructed blends of host plant compounds, and ethanol. **a**) Oak-infesting species *Phymatodes obliquus* and *Brothylus gemmulatus*, **b**) oak-infesting species *Phymatodes grandis* and *Neoclytus m. modestus* (subfamily Cerambycinae), **c**) conifer-infesting *Neospondylis upiformis* (subfamily Spondylidinae), **d**) conifer-infesting *Xylotrechus albonotatus* (subfamily Cerambycinae) and *Monochamus clamator* (subfamily Lamiinae). Within each species, means with the same or no letter are not significantly different (Dunn's test *P* > 0.05)
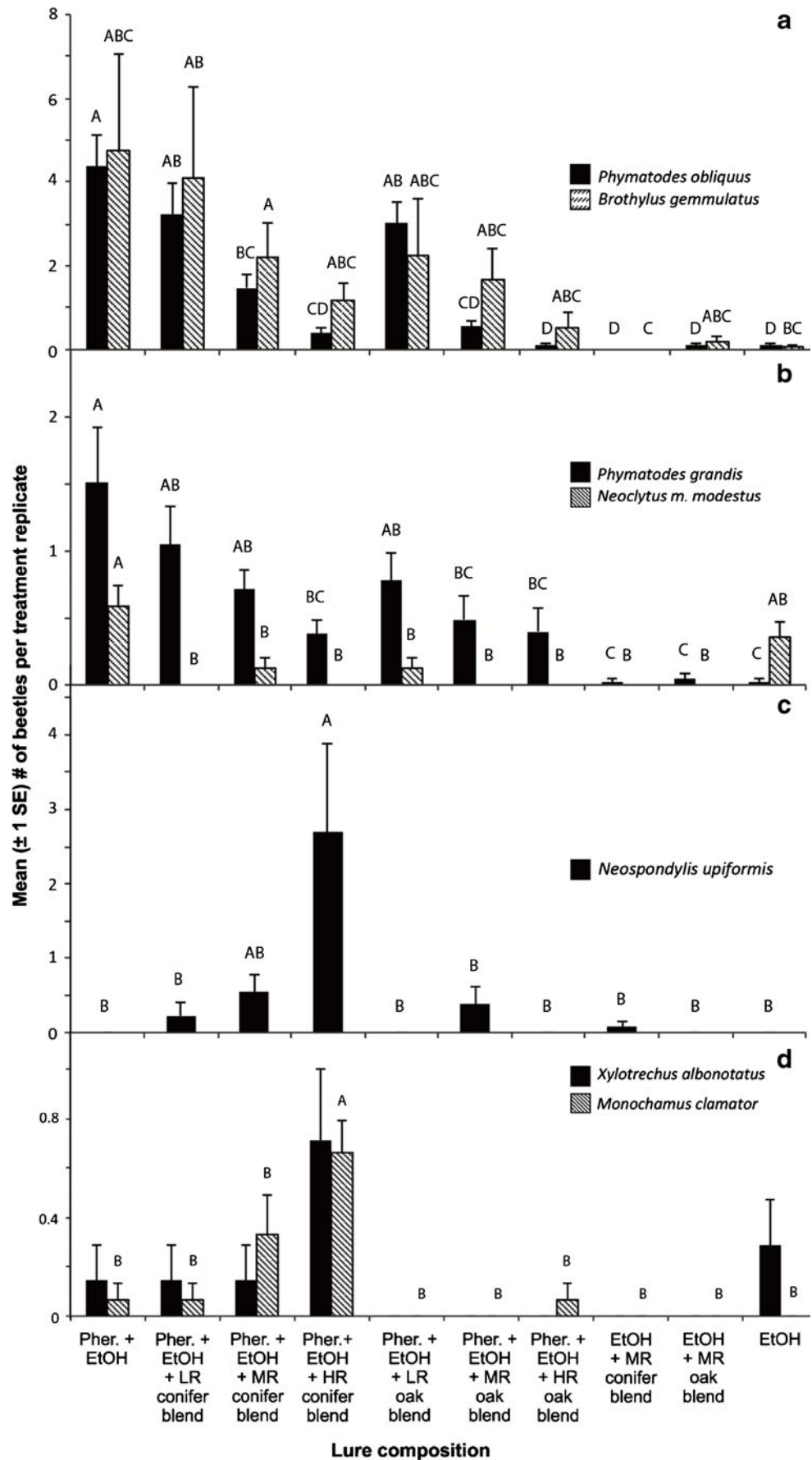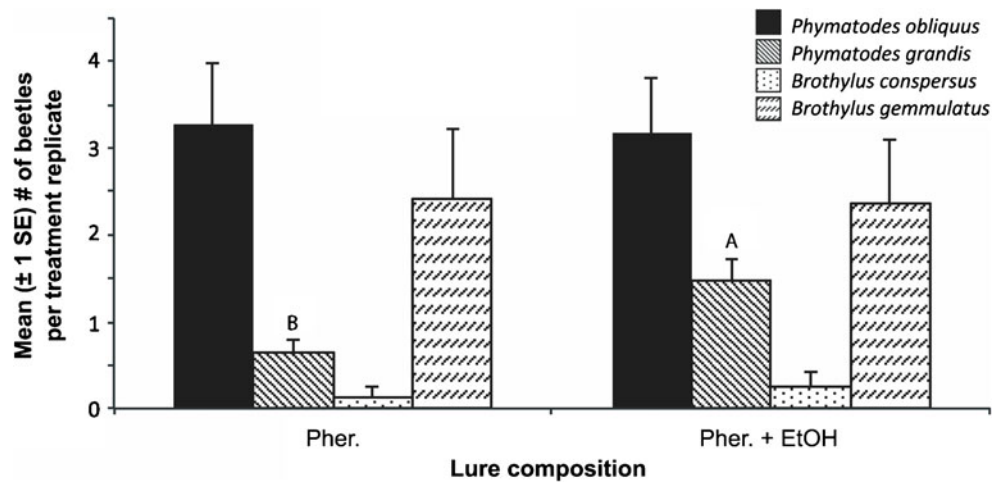
Fig. 4 Mean (±1 SE) numbers of oak-infesting *Phymatodes obliquus*, *Phymatodes grandis*, *Brothylus conspersus*, and *Brothylus gemmulatus* (subfamily Cerambycinae) beetles caught in traps baited with the pheromone blend with and without ethanol. Within each species, means with the same or no letter are not significantly different (Dunn's test $P > 0.05$)


pheromone + α-pinene without ethanol treatment, which was significantly more attractive than the two conifer blend treatments (*A. nitidum*, $q \geq 3.91$; *A. striatum*, $q = 2.87$; Fig. 5a and b). *Monochamus clamator* was most strongly attracted to pheromone blend + conifer blend, and addition of ethanol to this mixture had no significant effect; only pheromones + α-pinene + ethanol was significantly less attractive than pheromone + conifer blend ($q = 2.83$; Fig. 5b). There were no significant differences among treatments for *T. abietis* and *X. albonotatus*.

Discussion

In the first experiment, testing the influence of crude chipped host tree material on the attraction of cerambycids to their pheromones, the data suggested that HPVs enhanced attraction of conifer-infesting cerambycids to pheromone lures. In contrast, for oak-infesting species, attraction to pheromone lures was not influenced by oak volatiles. However, attraction of some of the oak-infesting species to the pheromone lures was inhibited by odors from non-host conifer material.

Fig. 5 Mean (±1 SE) numbers of conifer-infesting species in traps baited with the pheromone blend, high release rate (HR) conifer volatiles or α-pinene, and ethanol. **a**) Conifer-infesting *Neospondylis upiformis* and *Asemum nitidum* (subfamily Spondylidinae), **b**) conifer-infesting *Xylotrechus albonotatus* (subfamily Cerambycinae), *Asemum striatum*, and *Tetropium abietis* (subfamily Spondylidinae), and *Monochamus clamator* (subfamily Lamiinae). Within each species, means with the same or no letter are not significantly different (Dunn's test $P > 0.05$)
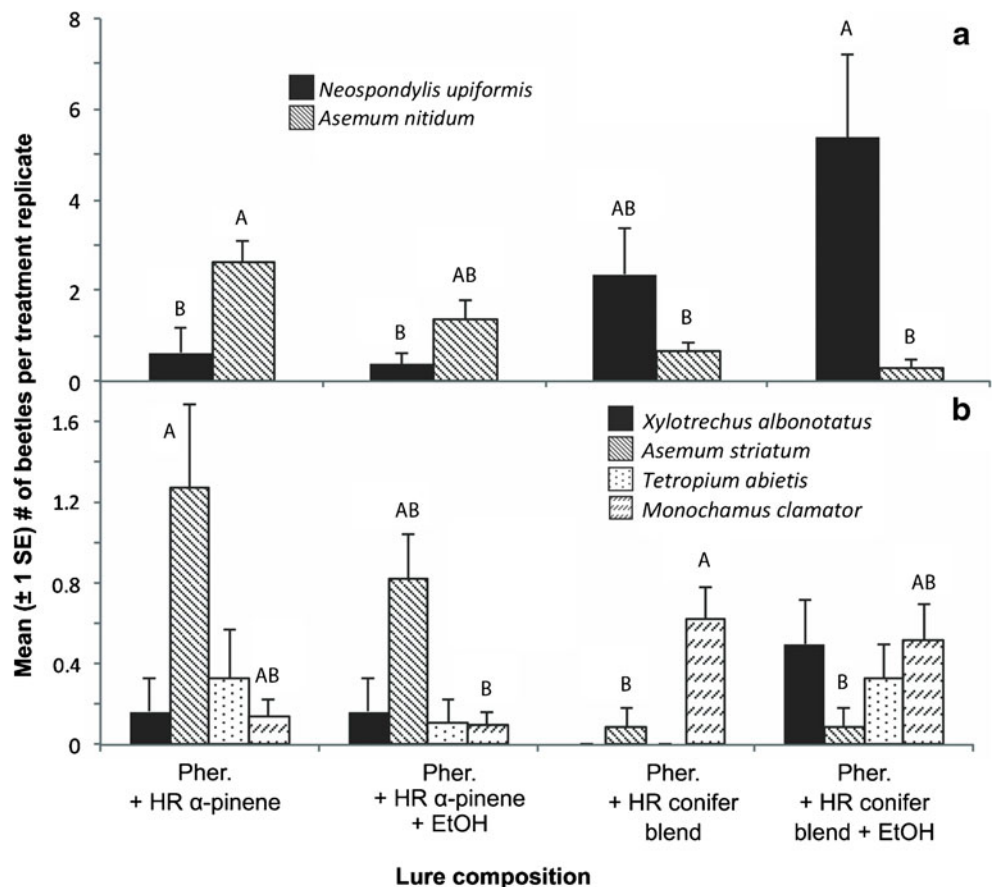
The reconstructed HPV blends developed for the second and third experiments were based on analyses of the volatiles collected from the same stock of chipped tree materials used in the first round of bioassays. Whereas we anticipated that the profiles of the various conifer and oak species, respectively, would be similar within these two groups, we found that qualitatively, the profiles of monoterpenes between these two groups were similar as well. However, in addition to monoterpenes, the chipped oak material produced substantial amounts of green leaf volatiles that were not present in the conifer volatiles, and the sesquiterpene profiles of the conifers and oaks also differed (Fig. 2). Furthermore, the overall abundance of volatile compounds differed between the two groups, as demonstrated by the two orders of magnitude difference in compound abundance seen in Fig. 2, and typical of the other conifer and oak volatile samples.

In the second round of bioassays testing reconstructed blends of HPVs with pheromones, the three HPV release rates were not intended to replicate exactly the emission rate from conifers or oaks, but to test a range (approximately three orders of magnitude) of release rates for practical deployment in traps. In these experiments, the reconstructed blend of conifer volatiles appeared to be a satisfactory mimic of the odors released by the crude plant material, with the conifer-infesting species generally exhibiting increased responses with increasing release rates of the conifer volatiles blend. In addition, two of the species, *N. upiformis* and *M. clamator*, showed minimal responses to treatments containing pheromone alone, clearly indicating that the HPVs form an important part of the overall attractant for these species.

In contrast, the responses of the oak-infesting species generally decreased with increasing release rates of the reconstructed blends of host volatiles, for either the conifer or oak volatile blends. Given that the oak-infesting species had shown no response to volatiles from chipped oak branches, and were in some cases repelled by the chipped conifer material, these results were not unexpected. However, the lack of response to oak volatiles in the first experiment and the repellence to high release rates of oak volatiles in the second experiment may be due in part to the emitted oak volatiles not being representative of appropriate hosts for these species. That is, the oak branches used as sources of volatiles were fresh, and were chipped shortly after collection. Consequently, they may not have been representative of the host condition typically utilized by our study species, which infest dying and dead oaks (Linsley and Chemsak 1997). For example, Dunn and Potter (1991) found that *Elaphidion mucronatum* (Say) (Cerambycinae), which attacks dying and dead oaks, were not attracted to freshly cut oak logs plus ethanol, but only to a cardboard log mimic from which ethanol was released.

In the first part of the third field experiment, we tested the hypothesis that the more complete reconstruction of the conifer HPV blend would be a better pheromone synergist than the single host component α-pinene, which has been used extensively as a generic lure for cerambycids (see Introduction). However, we found that different species responded differently to α-pinene vs. the more complex blend. Thus, adult *N. upiformis* were significantly more attracted to the blend of pheromone + conifer volatiles + ethanol than to the corresponding blend with α-pinene as a single host component. Similarly, *M. clamator* was significantly more attracted to the blend of pheromone + conifer volatiles than to pheromone + α-pinene. Thus, for these two species, minor components of the conifer volatiles result in increased attraction. In contrast, the two *Asemum* species were more attracted to lures with α-pinene alone than to those with the full conifer blend, indicating that one or more of the minor components may be antagonistic. This also could explain why these species did not show a preference for the conifer blend treatments in the second experiment. These differences among ecologically similar conifer-infesting species may be related to their specific host preferences. For example, *M. clamator* and the SBNF population of *N. upiformis* prefer pines, whereas the *Asemum* species all prefer fir (Linsley and Chemsak 1997). In prior work, Chénier and Philogène (1989) and Sweeney et al. (2004) tested reconstructed conifer blends in the absence of pheromones, and similarly found that *A. striatum* was more attracted to traps baited with only α-pinene than to a blend of conifer terpenes. Conversely, *Spondylis buprestoides* L. was more attracted to the conifer blend than to α-pinene (Sweeney et al. 2004). Further bioassays of subsets of conifer blends will be required to determine the specific components which are responsible for the added or decreased attraction, respectively, for the different cerambycid species.

The second part of the third experiment tested the hypothesis that oak-infesting cerambycid species utilized ethanol as a host cue (see Dunn and Potter 1991), and would be more attracted to pheromones when they were co-released with ethanol. We again found that the responses were nuanced, because ethanol significantly increased attraction to pheromones for *P. grandis*, but not for any of the other oak-infesting species. There were also indications that ethanol might mitigate the antagonistic effects of conifer volatiles or α-pinene on attraction of both *P. grandis* and *B. gemmulatus* to pheromones, with all pheromone treatments containing both ethanol + conifer volatiles or α-pinene attracting more than twice as many beetles as the corresponding treatments without ethanol (Table S5, Fig. S2).

To our knowledge, this is the first experiment that has tested the combined effects of reconstructed host plant volatiles with a generic blend of pheromones designed to attract a number of species within a community of cerambycids. Silk et al. (2007) had previously conducted a more focused study by

testing reconstructed HPV blends with the pheromones of two conifer-infesting *Tetropium* species. Similar to our results with conifer-infesting species, both *Tetropium* species were more strongly attracted to pheromone + conifer volatiles than to either lure component alone. Based on this and other studies in which HPVs have been shown to enhance attraction of conifer feeders to pheromone lures (see Introduction), increased attraction of conifer-infesting cerambycids to pheromones when they are released in combination with HPVs is likely to be a general phenomenon.

In contrast, we found no evidence that host plant volatiles, other than ethanol for *P. grandis*, increased the attraction to pheromones for the oak-infesting cerambycids described here. It also was interesting to note that the oak-infesting species in our study were generally repelled by conifer volatile blends, suggesting that these species are using olfactory cues to avoid non-hosts. GC-EAD analyses with several of the oak-infesting species confirmed that their antennae do indeed perceive non-host volatiles. Non-host volatiles have been reported to repel or inhibit orientation to otherwise attractive compounds for other wood-boring beetles, such as green-leaf volatiles inhibiting attraction of the conifer-infesting cerambycid *Arhopalus tristis* (F.) (Suckling et al. 2001) or conifer-infesting bark beetles (Zhang and Schlyter 2004). Thus, it is reasonable to assume that the reciprocal case is occurring with the oak-infesting species in our study.

For all of our test species, there was no indication that the HPVs were more attractive than controls in the absence of pheromones, despite the fact that HPVs like α-pinene and ethanol are commonly used as generic attractants for cerambycids (reviewed in Allison et al. 2004). Clearly, such blends are by no means attractive to all cerambycid species. However, in the first field experiment in which the chipped material by itself was not attractive, the lack of attraction may have been due in part to the relatively small amounts of chipped material deployed (~1 L of chips) and to the fact that the traps were deployed in field experiments where there would be competition from natural sources of volatiles. In addition, GC-MS analyses of aged chips showed that the release rate of volatiles declined rapidly within a few days (data not shown).

In summary, our results have shown that host plant volatiles are an important component of attractants for many cerambycid species that infest conifers, whereas they appear to be much less important for species infesting dying and dead oak trees. Release rates of HPVs will need to be considered when developing generic lures for cerambycids because conifer-infesting species are likely to be most strongly attracted to high release rates of HPVs, whereas species infesting angiosperms are likely to be increasingly repelled with increasing HPV release rates. Comparison of a reconstructed blend of conifer volatiles vs. α-pinene produced mixed results, with some species appearing to prefer the full blend, whereas others preferred α-pinene alone. The effect of ethanol on species infesting oak also was mixed, with attraction to the pheromone of one species clearly being synergized by ethanol, whereas other species were unaffected. Thus, the choice of which HPVs to use for trapping a given species or subset of species will require careful consideration. Nevertheless, for many species, due to the overlap in both pheromones and host plant volatiles, it should still be possible to formulate relatively generic lures that will attract a number of species simultaneously, as suggested by several previous studies of blends of cerambycid pheromones with α-pinene and ethanol (e.g., Hanks and Millar 2013).

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