Journal of Economic Entomology, 112(5), 2019, 2236–2252
doi: 10.1093/jee/toz141
Advance Access Publication Date: 28 May 2019
Research

OXFORD

Forest Entomology

The Role of Minor Pheromone Components in Segregating 14 Species of Longhorned Beetles (Coleoptera: Cerambycidae) of the Subfamily Cerambycinae

Lawrence M. Hanks,[1,6] Judith A. Mongold-Diers,[1] Robert F. Mitchell,[1,2] Yunfan Zou,[3] Joseph C. H. Wong,[1,4] Linnea R. Meier,[1,5] Todd D. Johnson,[1] and Jocelyn G. Millar[3]

[1]Department of Entomology, University of Illinois at Urbana-Champaign, 320 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, [2]Present address: Department of Biology, University of Wisconsin, Oshkosh, WI 54901, [3]Departments of Entomology and Chemistry, University of California, Riverside, CA 92521, [4]Present address: USDA-APHIS, El Segundo, CA 90245, [5]Present address: Otis Laboratory, USDA-APHIS-PPQ-CPHST, Buzzards Bay, MA 02542, and [6]Corresponding author, email: hanks@illinois.edu

Subject Editor: Brian Sullivan

Received 13 December 2018; Editorial decision 2 May 2019

Abstract

We present research on the chemical ecology of 14 species of longhorned beetles (Coleoptera: Cerambycidae), in four tribes of the subfamily Cerambycinae, conducted in east-central Illinois over 8 yr. Adult males produce aggregation-sex pheromones that attract both sexes. Twenty independent field bioassays explored the pheromone chemistry of the species and tested the possible attractive or antagonistic effects of compounds that are not produced by a given species, but are pheromone components of other species. Analyses of beetle-produced volatiles revealed compounds that had not been reported previously from several of the species. The most common pheromone component was (*R*)-3-hydroxyhexan-2-one, but pheromones of some species included isomers of the related 2,3-hexanediols. Males of the congeners *Phymatodes amoenus* (Say) and *Phymatodes testaceus* (L.) produced pure (*R*)-2-methylbutan-1-ol. Enantiomers of 2-methylbutan-1-ol also proved to be powerful synergists for *Megacyllene caryae* (Gahan), *Sarosesthes fulminans* (F.), and *Xylotrechus colonus* (F.). The major components of pheromone blends were consistently present in collections of headspace volatiles from male beetles, and only the major components were inherently attractive to a subset of species when tested as single components. Minor components of some species acted as powerful synergists, but in other cases appeared not to influence attraction. Among the minor components identified in headspace extracts from males, 2,3-hexanedione and 2-hydroxyhexan-3-one appeared to be analytical artifacts or biosynthetic by-products, and were neither attractants nor synergists. The antagonistic effects of minor compounds produced by heterospecific males suggest that these compounds serve to maintain prezygotic reproductive isolation among some species that share pheromone components.

Key words: Cerambycidae, semiochemical, aggregation, reproductive isolation

Sympatric species of longhorned beetles (Coleoptera: Cerambycidae) may have pheromones of similar or even identical composition, with the result that traps baited with a shared component will attract several species over the course of a season (e.g., Hanks et al. 2018). Some of these compounds are shared among species native to different continents, notably 2- or 3-hydroxyalkanones and the related 2,3-alkanediols produced by species in the large subfamily Cerambycinae (Millar and Hanks 2017). Pheromones of cerambycines are produced by males and attract beetles of both sexes, and thus are considered aggregation-sex pheromones (sensu Cardé 2014). Conservation of pheromone structures across continents has inspired research on developing generic trap lures consisting of blends of pheromones from multiple species, that could be used in monitoring for new incursions of a broad taxonomic range of exotic cerambycid species (Hanks et al. 2018, Fan et al. 2019).

Field bioassays of synthesized pheromones have revealed that many cerambycid species are attracted to traps baited with the major pheromone component produced by males, even when blended with pheromones of other species (Hanks et al. 2018). However, the response of a species to a blend, or lack thereof, may provide

© The Author(s) 2019. Published by Oxford University Press on behalf of Entomological Society of America. All rights reserved.
For permissions, please e-mail: journals.permissions@oup.com.

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

important insight into its pheromone chemistry. For example, the fortuitous baiting of a trap in east-central Illinois with separate lures that contained racemic 3-hydroxyhexan-2-one and 2-methylbutan-1-ol resulted in the sudden influx of a species which had rarely been trapped previously, the cerambycine *Phymatodes lengi* Joutel (L. M. Hanks, unpublished data). It subsequently was determined that males of this species produce a blend of (*R*)-3-hydroxyhexan-2-one and (*R*)-2-methylbutan-1-ol (Mitchell et al. 2015), and here we confirm that beetles of this species are not attracted to the individual components, only to the binary blend.

In this article, we report on the semiochemistry of 14 species of cerambycid beetles from a compilation of data from 20 independent field bioassays conducted in east-central Illinois during an 8-yr period. The bioassays were designed to address three basic objectives: 1) confirm the pheromone chemistry of each species; 2) explore the responses of species to analogs of their pheromones, such as stereoisomers of their pheromone components; 3) test for antagonistic effects of compounds which are not produced by a given species, but which are produced by heterospecific males.

Materials and Methods

Sources of Chemicals

Compounds that were used in field bioassays were purchased from commercial sources if available, or were synthesized (Table 1). For convenience, compound names are abbreviated so as to specify stereochemistry, assuming six-carbon chain lengths unless stated otherwise (e.g., [*R*]-3-hydroxyhexan-2-one is henceforth referred to as 3*R*-ketol, racemic 3-hydroxyoctan-2-one is referred to as 3-C8ketol, while the mixture of *syn*- and *anti*-2,3-hexanediol is referred to as 2,3-diol; Table 1). We also tested compounds that are common pheromone components of species in the subfamily Lamiinae (Hanks and Millar 2016), including fuscumol, fuscumol acetate, and geranylacetone (abbreviated fusc, fuscac, geran, respectively). Synthesized compounds included racemic 3-C8ketol, *syn*- and *anti*-2,3-alkanediols, and enantiomerically enriched 2,3-alkanediols and 3-hydroxyalkan-2-ones (94 to >98% stereoisomerically pure; see Lacey et al. 2004, 2007a; Millar et al. 2009). 1-(1H-Pyrrol-2-yl)-1,2-propanedione (henceforth semanopyrrole) was made as described in Zou et al. (2016).

(*R*)-2-Methylbutan-1-ol (henceforth 2*R*-MB) was prepared from (*R*)-2-methylbutanoic acid by slight modifications to previously reported methods (Mori 2009, Mori et al. 2010). Thus, a solution of (*R*)-2-methylbutanoic acid (8.58 g, 84 mmol; T. Hasegawa Co., Ltd., Tokyo, Japan; e.e. > 99.0% as analyzed by chiral stationary-phase gas chromatography) in dry Et_2O (40 ml) was added dropwise to a stirred, ice-cooled suspension of $LiAlH_4$ (4.78 g, 126 mmol) in dry Et_2O (120 ml). The mixture was stirred for 3 h while warming to room temperature, then cooled to 0°C and quenched by sequential addition of water (4.8 ml), 15% aqueous NaOH (4.8 ml), and water (14.4 ml). After stirring for 10 min, the mixture was filtered through celite to remove the granular precipitate, and the filtrate was dried

Table 1. Sources of synthesized pheromones and other chemicals tested in field bioassays, their abbreviations, and purities (e.e. = enantiomeric excess)

Chemical	Abbreviation	% Purity (e.e.)	Source
racemic 3-hydroxyhexan-2-one	3-ketol	91	Bedoukian Inc.
(*R*)-3-hydroxyhexan-2-one	3*R*-ketol	91 (94)	See Methods
(*S*)-3-hydroxyhexan-2-one	3*S*-ketol	95 (94)	See Methods
racemic 2-hydroxyhexan-3-one	2-ketol	99	See Methods
2,3-hexanedione	2,3-dione	90	Aldrich Chemical
racemic *syn*-2,3-hexanediol	*syn*-diol	>98	See Methods
racemic *anti*-2,3-hexanediol	*anti*-diol	>98	See Methods
(2*R*,3*R*)-2,3-hexanediol	*RR*-diol	99 (96)	See Methods
(2*S*,3*S*)-2,3-hexanediol	*SS*-diol	99 (96)	See Methods
(2*R*,3*S*)-2,3-hexanediol	*RS*-diol	99 (88)	See Methods
(2*S*,3*R*)-2,3-hexanediol	*SR*-diol	99 (82)	See Methods
racemic 3-hydroxyoctan-2-one	3-C8ketol	91	See Methods
racemic *syn*-2,3-octanediol	*syn*-C8diol	99	See Methods
racemic *anti*-2,3-octanediol	*anti*-C8diol	96	See Methods
racemic 2-methylbutan-1-ol	2-MB	98	Aldrich Chemical
(*R*)-2-methylbutan-1-ol	2*R*-MB	99 (98)	See Methods
(*S*)-2-methylbutan-1-ol	2*S*-MB	99 (99)	Aldrich Chemical
racemic fuscumol	fusc	99	Bedoukian Inc.
racemic fuscumol acetate	fuscac	99	Bedoukian Inc.
geranylacetone	geran	>97	Sigma-Aldrich
(*E*)-β-farnesene	β-farnesene	>95	Bedoukian Inc.
(*R*)-(+)-limonene	N/A	97 (97)	Sigma-Aldrich
(*S*)-(−)-limonene	N/A	95 (97)	Sigma-Aldrich
2-phenylethanol	N/A	99	Sigma-Aldrich
(−)-α-terpineol	N/A	96[a]	Sigma-Aldrich
nerol	N/A	>90	Sigma-Aldrich
citral (= neral + geranial)	N/A	95	Sigma-Aldrich
3-methylphenylacetate	3-methylphen.	>99	Aldrich Chemical
sulcatone	N/A	98	Alfa Aesar
1-(1*H*-pyrrol-2-yl)-1,2-propanedione	semanopyrrole	87	See Materials and Methods

Commercial sources: Aldrich Chemical (Milwaukee, WI), Alfa Aesar (Haverhill, MA), Bedoukian Inc. (Danbury, CT), Sigma-Aldrich (St. Louis, MO).
[a]Enantiomeric excess not provided by manufacturer.

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

over anhydrous Na_2SO_4 and carefully concentrated without heating under mild vacuum (~400 Torr). The crude product was purified by Kugelrohr distillation (25 Torr, 55–60°C) to yield 2*R*-MB as a colorless liquid (7.18 g, 97%).

High release rate ethanol and α-pinene lures were obtained from Synergy Semiochemicals Corp., Burnaby, BC, Canada (~80 g of 95% ethanol, releasing ~0.4 g/d, model IP036-100; ~170 g α-pinene, releasing ~2 g/d, model IP037-75). Alternatively, lures with similar release rates were prepared from polyethylene sachets (10 × 15 cm, 0.05 mm wall thickness, Cousin Corp., Largo, FL) loaded with 100 ml of ethanol (95%, Synergy Semiochemicals Corp.) or 50 ml of methanol (100%, Fisher Scientific, Waltham, MA), and with smaller sachets (5.1 × 7.6 cm, 0.05 mm wall thickness, Cousin Corp.) loaded with 10 ml of α-pinene (Synergy Semiochemicals Corp.).

Study Species

The community of cerambycine beetles in east-central Illinois where our studies were conducted comprises at least 48 species in 16 tribes (Hanks et al. 2014). Most of the study species for the present article are in the tribe Clytini (Table 2), including the congeners *Neoclytus acuminatus acuminatus* (F.), *Neoclytus caprea* (Say), *Neoclytus mucronatus mucronatus* (F.), and *Neoclytus scutellaris* (Olivier), as well as *Megacyllene caryae* (Gahan), *Sarosesthes fulminans* (F.), and *Xylotrechus colonus* (F.). The remaining study species were *Anelaphus pumilus* (Newman) (Elaphidiini), *Curius dentatus* Newman (Curiini), *Euderces pini* (Olivier) (Tillomorphini), and the congeners *Phymatodes aereus* (Newman), *Phymatodes amoenus* (Say), *P. lengi*, and *Phymatodes testaceus* (L.) (Callidiini). The last species was introduced from Eurasia and is broadly distributed in the eastern United States (Swift and Ray 2010). Species which are considered generally abundant in the eastern United States include *A. pumilus*, *N. a. acuminatus*, *N. m. mucronatus*, *N. scutellaris*, *P. amoenus*, and *X. colonus*, whereas those considered rare or uncommon include *C. dentatus*, *P. aereus*, *P. lengi*, and *S. fulminans* (Lingafelter 2007). Although *N. scutellaris* is generally considered common in the eastern United States, it appears to be rare in the area of our studies (Hanks et al. 2014).

For most of the study species, the larvae are woodborers and polyphagous on deciduous trees, with oaks (*Quercus* species) and hickory (*carya* species) being favored (for host plant relationships, see Linsley 1963, 1964; Lingafelter 2007). However, larvae of *N. m. mucronatus* specialize on species of hickories. Most of the species develop in weakened, dying, or freshly killed trees, although *N. caprea* prefers seasoned wood, and *N. scutellaris* may feed within decomposing hosts. Larvae of *P. amoenus* develop in stems of wild grape (*Vitis* species), but host plants of *P. lengi* remain unknown.

The flight period of each study species is limited to 3–8 wk, and they vary in seasonal activity period (Hanks and Millar 2013, Hanks et al. 2014, Mitchell et al. 2015). The earliest species to emerge in spring (typically late March) are *N. caprea* and *M. caryae*, followed later in spring by *A. pumilus*, *E. pini*, *P. aereus*, *P. amoenus*, and *P. testaceus*. The remaining species became active in summer. The study species also varied as to the daily flight period of adults (Lacey et al. 2009, Mitchell et al. 2015; L. M. Hanks, unpublished data), with none flying before 11:00 am, some flying in late morning to mid-afternoon (*M. caryae*, *N. caprea*, *N. scutellaris*), some in late afternoon to evening (*E. pini*, *N. a. acuminatus*, *N. m. mucronatus*, *P. amoenus*, *P. lengi*), and some into the night (between sundown and midnight; *A. pumilus*, *P. aereus*, *P. testaceus*, *S. fulminans*, *X. colonus*). Daily flight period of *C. dentatus* is not known, to our knowledge.

Table 2. Total number of collections of headspace volatile from males of 14 cerambycid species, number of aeration extracts that contained male-specific volatiles in detectable quantities (success), volatile compounds (in order of relative abundance; see Supp Table 3 [online only]), and reference for the original pheromone identification

Species	No. of aerations (success)	Pheromone components (trace amt)[a]	Reference
Anelaphus pumilus	15 (8)	3*R*-ketol[b]; 2,3-dione	Mitchell et al. 2015
Curius dentatus	4 (0)	*RS*- and/or *SR*-diol[c]	Lacey et al. 2004
Euderces pini	31 (19)	3*R*-ketol; 2,3-dione	Mitchell et al. 2015
Megacyllene caryae	149 (113)	neral; 2*S*-MB; (−)-α-terpineol; geranial; 2-phenylethanol; (*S*)-(−)-limonene; (nerol; *RS*-, *SR*-diol)	Lacey et al. 2008; Mitchell et al. 2012, 2017
Neoclytus a. acuminatus	17 (7)	*SS*-diol	Lacey et al. 2004, Ray et al. 2015
Neoclytus caprea	54 (22)	3*R*-ketol; 2,3-dione	Ray et al. 2015
Neoclytus m. mucronatus	26 (16)	3*R*-ketol; 2,3-dione (2-ketol; 2,3-diol)	Lacey et al. 2007a, Ray et al. 2015
Neoclytus scutellaris	4 (3)	3*R*-ketol; 2,3-dione	Ray et al. 2015
Phymatodes aereus	5 (4)	3*R*-, 3*S*-ketol	Mitchell et al. 2015
Phymatodes amoenus	50 (13)	2*R*-MB[d]	Mitchell et al. 2015
Phymatodes lengi	31 (10)	3*R*-ketol; 2*R*-MB; (2,3-dione)	Mitchell et al. 2015
Phymatodes testaceus	23 (11)	2*R*-MB	Present article
Sarosesthes fulminans	9 (6)	3*R*-ketol; *SR*-diol; 2*R*-MB; 2,3-dione	Lacey et al. 2009
Xylotrechus colonus	77 (62)	3*R*-, 3*S*-ketol; 2,3-dione; *SS*-, *RR*-diol; 2*R*-MB; (*RS*-, *SR*-diol)	Lacey et al. 2009

Underlined compounds have not been reported previously from a given species.
[a]Less than 1% of primary component.
[b]Chemical abbreviations as in Table 1.
[c]Based on attraction to racemic *anti*-diol during field bioassays.
[d]Pheromone originally misidentified as 3*R*-ketol + 2*R*-MB (Mitchell et al. 2015).

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Cerambycine species vary as to whether they feed or not as adults (Hanks and Wang 2017). Feeding can complicate pheromone identifications if beetles produce frass that emits odors during collection of headspace volatiles. Feeding can be determined simply by retaining field-caught beetles in glass vials and checking for frass after 24 h. Most of the study species do not feed as adults, the exceptions being *A. pumilus*, *E. pini*, and *P. caryae*, which feed on pollen (L. M. Hanks, unpublished data). Regardless of whether they feed or not, males and females of the study species probably aggregate and mate on host plants of the larvae.

General Methods of Trapping

Field bioassays were conducted at eight study sites in east-central Illinois (Table 3), most of which were mature second-growth or successional forests dominated by deciduous trees, including species of oak, hickory, maple (*Acer*), and ash (*Fraxinus*). The private residence was in a suburban area developed ~60 yr earlier with mature deciduous and coniferous trees of many native and exotic species.

The black cross-vane panel traps, lures, and trapping methods have been previously described (e.g., Millar et al. 2017, Hanks et al. 2018). Traps were modified to capture live beetles for identification of pheromones by replacing the supplied collecting basin with a ~1 liter plastic jar vented with holes covered with aluminum window screen. Traps used in field bioassays used the standard collecting basins. Lures consisted of the 5.1 × 7.6 cm transparent polyethylene sachets described above, loaded with 25 mg of each chiral compound or 50 mg of racemic compounds (i.e., 25 mg of each enantiomer), either as single components are as blends, diluted to 1 ml with isopropanol. Lures loaded with 1 ml of neat isopropanol served as controls. Lures were replaced as needed, usually every 2 wk. Lures for ethanol and α-pinene were as described above, and did not require replacement during bioassays.

Analysis of Insect-Produced Volatiles

Male-produced compounds of most of the study species had been reported previously (Table 2), but further detailed analyses revealed additional possible pheromone components in headspace extracts. Although all indications are that pheromones are produced sex-specifically by males of cerambycine species (Millar and Hanks 2017), odors from both sexes were analyzed. Most of the beetles used for collection of volatiles were caught with traps baited with generic blends of cerambycid pheromones that attract multiple species (Hanks et al. 2014), or with synthetic reconstructions of their known pheromones (see Table 2).

To collect insect-produced volatiles, beetles were held in glass aeration chambers (0.5 liter Mason-style canning jars) lined with aluminum window screen so that beetles could move freely. Lids of jars were fitted with two threaded brass pipes (9.5 mm diameter, 6.35 mm i.d.) seated with brass nuts and Teflon washers. One pipe extended ~2 cm into the jar and was connected to a charcoal scrubber through which purified air was drawn. The other pipe extended ~6 cm into the jar and connected to an adsorbent cartridge, a 10-cm glass tube containing ~150 mg HayeSepQ (Sigma-Aldrich, St. Louis, MO) held between plugs of silanized glass wool, through which air was drawn by vacuum at ~1 liter/min. The various components were connected with Teflon tubing. Chambers were positioned adjacent to closed exterior windows (natural photoperiod; ~14:10 (L:D) h, ~20°C) and headspace volatiles were collected for ~24 h. Insect-produced chemicals were recovered by extracting the cartridges with 1.5 ml of dichloromethane spiked with the internal standard eicosane (38 µg). After each collection session, beetles were returned to cages for at least 24 h before being reused.

Extracts of headspace samples were analyzed on an Agilent 7890B GC coupled to a 5977A mass selective detector (Agilent Technologies, Santa Clara, CA), in splitless mode with He carrier gas. The GC was fitted with an HP-5 column (30 m × 0.25 mm i.d., Agilent Technologies), which was temperature programed from 30°C for 1 min, 10°C/min to 250°C, hold for 5 min. The injector temperature was 110°C. Mass spectra were recorded with electron impact ionization (EI, 70 eV). Compounds were conclusively identified by matching their retention times and mass spectra with those of authentic standards, obtained from sources as described above and listed in Table 1.

Absolute configurations of chiral compounds were determined with an HP 5890 GC fitted with a Cyclodex-B column (30 m × 0.25 mm i.d., 0.25 µm film; Agilent Technologies). Enantiomers were identified by comparisons of their retention times with those of authentic standards of known absolute configuration. Injector temperature was 110°C, and oven temperature was held at 50°C for 1 min, increased at 5°C/min to 130°C, and held 10 min.

Compounds were identified by matching retention times, Kovat's indices, and mass spectra with those of authentic standards (Supp Table 1 [online only]). Headspace collections usually were made from individual males, but in some cases collections were made from groups of males, or mixed sex pairs, in case interactions among beetles enhanced pheromone production. The number of headspace samples per species (Table 2) depended on the number of males available, which was greatest for common species that were strongly attracted to lures. For *C. dentatus*, only a few males were available for headspace collection, and we have not yet found any insect-produced compounds in extracts from this species. The pheromone of this species has been predicted to be composed of *RS*-diol and/or *SR*-diol based on earlier field bioassays (Lacey et al. 2004, Millar et al. 2018).

Table 3. Study sites for field bioassays conducted in east-central Illinois during 2011–2018

County	Site	GPS coordinates (latitude, longitude)	Area (ha)
Champaign	Brownfield Woods[a]	40.1452, −88.1655	26
	Nettie Hart Memorial Woods[a]	40.2278, −88.3589	16
	Private residence (city of Urbana)	40.0971, −88.2032	N/A
	Trelease Woods[a]	40.1335, −88.1414	29
	Univ. Illinois Operations and Maintenance	40.0855, −88.2165	9
Piatt	Robert Allerton Park[a]	39.9853, −88.6501	600
Vermilion	Forest Glen Preserve[b]	40.0152, −87.5677	728
	Vermilion River Observatory[a]	40.0655, −87.5613	192

[a]University of Illinois natural area (http://research.illinois.edu/cna/).
[b]Vermilion County Conservation District (http://www.vccd.org/).

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Field Bioassays

Attraction of beetles to particular compounds, or blends of compounds, was tested with field bioassays at the eight study sites during 2011–2018 (for details about the timing of bioassays at each study site, see Supp Table 2 [online only]). For each bioassay, traps were deployed ~10 m apart in linear transects with one trap per treatment, usually at multiple field sites. Traps were serviced at intervals of 1–4 d, at which time treatments were shifted down transects to control for positional bias. Beetles were identified using keys in Lingafelter (2007) and taxonomy follows Monné and Hovore (2005). Specimens are available from the laboratory collection of L.M.H., and voucher specimens have been deposited at the Illinois Natural History Survey, Champaign, IL.

The 20 field bioassays were categorized based on the pheromone chemistry of particular species that were targeted, including three independent bioassays for *N. a. acuminatus* (bioassays Na1–3; Table 4), four for *M. caryae* (Mc1–4; Table 5), eight bioassays based on ketols for the various species that have 3*R*-ketol as their sole or major pheromone component (KT1–8; Table 6), and five bioassays based on 2-MB for species that use that compound as a major pheromone component (MB1–5; Table 7). The primary objectives were to confirm pheromone chemistry of *M. caryae* (Mc3), *N. caprea* (KT1), *P. aereus* (MB5), *P. lengi* (MB1–3), *S. fulminans* (KT6), and *X. colonus* (KT3, KT4, MB4).

Some of the test chemicals were identified as candidate pheromone components based on analyses of beetle-produced volatiles (see Table 2; Supp Table 3 [online only]). In particular, due to its volatility, 2-MB had been overlooked in the original GC-MS analyses of extracts from males of *M. caryae*, *S. fulminans*, and *X. colonus*, and its influence on attraction was tested in bioassays Mc1, Mc2, MB2, and MB4. 2,3-Dione had not been reported as a pheromone component of several species because it had been considered an artifact (Millar and Hanks 2017), but its potential role was evaluated here for *N. m. mucronatus* and *X. colonus* (bioassay KT8). Other new candidate components had been detected in only a few headspace extracts, but nevertheless were absent in controls, including 6-methyl-5-hepten-2-one (sulcatone) for *P. aereus* (MB5) and (*E*)-β-farnesene for *M. caryae* (Mc3; see Mitchell et al. 2017). Sulcatone had been detected previously in volatiles collected from males of *Phymatodes decussatus* (LeConte) (J. G. Millar, unpublished data), a species native to the Pacific coast of the United States (Linsley 1964). It should be noted that (*E*)-β-farnesene was only detected in headspace collections from males of *M. caryae* that had been deliberately agitated during aeration, by vigorously shaking aeration jars, to encourage production of defensive spiroacetals (see Mitchell et al. 2017). Thus, that compound was not considered a component of the aggregation-sex pheromone. (*E*)-β-Farnesene is a known pheromone component of many types of insects, including beetles of other families (El-Sayed 2018), and the isomer (3*E*,6*E*)-α-farnesene has been suggested as a pheromone component of the lamiine *Anoplophora glabripennis* (Motschulsky), the Asian longhorned beetle (Crook et al. 2014).

Most of the bioassays were designed to test multiple hypotheses, such as documenting attraction to male-produced compounds while also testing for antagonism by compounds that are not produced by the species in question, including stereoisomers of pheromone components and/or compounds that are pheromones of more distantly related cerambycids (i.e., in different tribes or subfamilies). The latter compounds are henceforth abbreviated as COHs (i.e., compounds of heterospecifics). Conversely, it also is possible that novel blends of compounds could by chance simulate the previously unknown pheromone blend of another species, or at least be similar enough to trap beetles for proper identification of their male-produced compounds. Some of these COHs were structurally related to pheromone components of a particular species, including diastereomers (bioassays Na1, Na2), positional isomers (KT4), and chain-length analogues (Na2, Na3, KT2, KT5), as well as ketols for species that produce only diols (Mc, Na1, Na3) and diols for those that produce only ketols (KT2). Other COHs tested against species which do not produce them included: 1) citral, an isomeric blend of neral and geranial, which are pheromone components of *M. caryae* (Table 2; KT2, KT5), 2) common pheromones of lamiine species (fusc, fuscac, and geran; Millar and Hanks 2017; KT2, KT4, KT5, Mc3, Mc4), and 3) semanopyrrole, a major pheromone component or synergist of some cerambycines native to North America, South

Table 4. Treatments for 'Na' field bioassays which tested the response of *Neoclytus a. acuminatus* to its pheromone, (2*S*,3*S*)-2,3-hexanediol ('*SS*-diol'), or the racemic version of its pheromone (= *syn*-diol), and the influence of potential antagonists

Chemicals[a]	Na1	Na2	Na3
solvent control	X	X	X
syn-diol		X	X
SS-diol	X		
syn-C8diol		X	X
anti-diol		X	
anti-C8diol		X	
anti-diol + *anti*-C8diol		X	
Combined with *SS*-diol:			
3-ketol	X		
3*R*-ketol	X		
3*S*-ketol	X		
RS-diol	X		
SR-diol	X		
anti-diol	X		
Combined with *syn*-diol:			
syn-C8diol		X	X
3-ketol			X
3*R*-ketol			X
3*S*-ketol			X

[a]Chemical abbreviations as in Table 1.

Table 5. Treatments for 'Mc' field bioassays which tested the response of *Megacyllene caryae* to candidate pheromone components and the influence of potential antagonists

Chemicals[a]	Mc1	Mc2	Mc3	Mc4
solvent control	X	X	X	X
citral	X	X	X	X
2-MB		X		
β-farnesene			X	
pheromone blend[b]	X			
pheromone blend[b] + 2-MB	X			
Combined with citral:				
β-farnesene			X	
3-ketol + 3-C8ketol				X
syn-diol				X
anti-diol				X
2-MB	X	X		
fuscac + geran				X

[a]Chemical abbreviations as in Table 1.
[b]Pheromone blend = (*S*)-(−)-limonene + 2-phenylethanol + (−)-α-terpineol + nerol + citral + *anti*-diol + 2*S*-MB.

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Table 6. Treatments for 'KT' field bioassays which tested the response of cerambycid beetles to racemic 3-hydroxyhexan-2-one ('3-ketol') and the influence of potential synergists and antagonists

Chemicals[a]	KT1	KT2	KT3	KT4	KT5	KT6	KT7	KT8
solvent control	X	X	X	X	X	X	X	X
3-ketol	X	X	X	X	X		X	X
3*R*-ketol	X							
3*S*-ketol	X							
3-C8ketol					X			
syn-diol			X				X	
anti-diol			X					
2,3-dione								X
semanopyrrole							X	
syn-diol + semanopyrrole							X	
ethanol								X
methanol								X
Combined with 3-ketol:								
2-ketol				X				
3-C8ketol		X			X			
syn-diol			X	X				
anti-diol			X	X		X		
2,3-dione								X
syn-diol + *anti*-diol		X	X					
anti-diol + 2-MB						X		
citral + 2-MB		X						
semanopyrrole							X	
fusc				X				
fuscac				X				
fusc + fuscac + geran		X						
3-methylphen.				X				
ethanol								X
methanol								X

[a]Chemical abbreviations as in Table 1.

Table 7. Treatments for 'MB' field bioassays which tested the response of cerambycid beetles to 2-methylbutan-1-ol ('2-MB') and the influence of potential synergists and antagonists

Chemicals[a]	MB1	MB2	MB3	MB4	MB5
solvent control	X	X	X	X	X
2-MB	X		X	X	X
2*R*-MB	X				
3-ketol	X	X	X	X	X
syn-diol				X	
anti-diol				X	
sulcatone					X
2*R*-MB + 3-ketol	X	X			
2*S*-MB + 3-ketol		X			
Combined with 2-MB:					
3-ketol	X	X	X	X	X
3-ketol + ethanol			X		
3-ketol + α-pinene			X		
3-ketol + ethanol + α-pinene			X		
3-ketol + sulcatone					X
syn-diol				X	
anti-diol				X	

[a]Chemical abbreviations as in Table 1.

America, and Asia (Zou et al. 2016, Diesel et al. 2017, Silva et al. 2017, Millar et al. 2019; KT7).

Bioassays MB3, KT4, and KT8 also tested for synergistic effects of plant volatiles that have been shown to influence attraction to cerambycid pheromones, including ethanol and α-pinene (e.g., Miller et al. 2015), and the floral volatile 3-methylphenylacetate (Nakamuta et al. 1997). Methanol also was tested because it has been reported to be produced by some fungi associated with wood decay (Lewis and Yamamoto 1990) and it attracts wood-boring beetles of other families (e.g., Da Silva et al. 2006).

Statistical Analyses

For each bioassay, treatment effects were tested separately for any species that was represented by at least 10 specimens. Emphasis was placed on testing for attraction to candidate pheromone components, and on how attraction was influenced by the presence of COHs and plant volatiles. We do not present analyses that contribute no new information about the pheromone chemistry of a species. For example, during bioassay K2 more than 400 adults of *M. caryae* were captured by traps baited with a blend that contained the powerful attractant citral (see Results), which was tested as a potential COH antagonist for other species (see Results). However, the design of that bioassay allowed no assessment of how attraction of *M. caryae* was influenced by other compounds, and thus the data for that species are not presented.

Trap catch data were analyzed using a statistical method which has proven reliable and consistent in many publications (e.g., Millar et al. 2018). That is, replicates were defined by the number of collection events (i.e., at intervals of 1–4 d) and the number of transects (i.e., replication over time, and usually space). Differences between treatment means, blocked by site and collection date, were tested with the nonparametric Friedman's test (PROC FREQ, option CMH; SAS Institute 2011) because data violated assumptions of ANOVA (Sokal and Rohlf 1995). Replicates with no beetles of

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

the focal species in any trap were dropped from analyses so that results were not skewed by periods when beetles were not active. Replicates with the greatest numbers of beetles (i.e., that represented the independent responses of multiple beetles to bioassay treatments) were lent greater weight by dropping from analyses those replicates having fewer than a threshold number of beetles. These threshold numbers were selected, separately for each analysis, so as to maximize the number of beetles captured while maintaining sufficient replication for a robust statistical test (at least 12 replicates; range of threshold numbers 2–5). The significance level of *P* was set at 0.0025 to control Type I errors without being excessively conservative, maintaining an α = 0.05 per experiment (Quinn and Keough 2002). For analyses meeting this overall level of significance, pairs of treatment means were compared using the Ryan-Einot-Gabriel-Welsch Q multiple comparison test (REGWQ; SAS Institute 2011).

The rarity of *P. aereus* and *P. testaceus* necessitated combining data from bioassays MB3 and MB5 for a robust statistical test for attraction, retaining only the four treatments in common: 3-ketol, 2-MB, 3-ketol + 2-MB, and the solvent control.

Results

Analysis of Insect-Produced Volatiles

For most of the study species, volatiles collected from male beetles included compounds that were absent in extracts from females and controls (Table 2; Supp Table 3 [online only]). However, males often did not emit volatiles under laboratory conditions. For example, male-produced volatiles were present in detectable levels in only 113 of 149 (76%), 10 of 31 (32%), and 62 of 77 (81%) collections from *M. caryae*, *P. lengi*, and *X. colonus*, respectively. Feeding by adults was only a problem with *M. caryae* in terms of frass from both sexes apparently releasing miscellaneous volatiles (e.g., see Lacey et al. 2008). Although *A. pumilus* and *E. pini* also feed on pollen (see *Study Species* section, above), their relatively small body size resulted in minimal frass production and thus negligible contamination of aeration extracts with frass volatiles. Extracts from males of the nonfeeding species either contained pheromone components or were completely blank except for a few peaks from system contaminants.

The only male-produced compound found in extracts of volatiles from males of *P. testaceus* was 2*R*-MB. Extracts from males of several other species were found to contain compounds which had not been reported in the original identifications of their pheromones (Table 2; Supp Table 3 [online only]). A notable example is 2,3-dione, which was detected in headspace extracts from nearly all the species that had 3*R*-ketol as the major component, as previously reported for the congeners *N. caprea*, *N. m. mucronatus*, and *N. scutellaris* (Lacey et al. 2007a, Ray et al. 2015). We did not detect 2,3-dione in collections of volatiles from *P. aereus*, but it may have been missed due to the small sample size for that species. Two of the species that produced 3*R*-ketol also had small quantities of 2-ketols or 2,3-diols in extracts of their headspace volatiles, including *N. m. mucronatus* and *X. colonus*.

Extracts of volatiles from males of *M. caryae*, *S. fulminans*, and *X. colonus* revealed small amounts of 2-MB which had not been reported previously (see Lacey et al. 2008, 2009). 2*R*-MB was the only compound detected from males of *P. amoenus* and *P. testaceus*, as mentioned above, but was paired as a minor component with 3*R*-ketol in the congener *P. lengi*.

For species that had multiple pheromone components, males varied considerably in the ratios of components they emitted, but usually produced the dominant component most consistently. For example, of 113 extracts from males of *M. caryae* that contained any candidate pheromone components, citral was present in 101 (~89%), but (−)-α-terpineol, 2-MB, 2-phenylethanol, (*S*)-(−)-limonene, and the *anti*-diols were detected in only 77, 61, 59, 36, and 5 extracts, respectively. The component nerol was detected only in trace quantities in five extracts, and only one extract contained all six of the minor compounds. Similarly, each of 62 successful extracts from males of *X. colonus* contained 3-ketol (primarily the *R*-enantiomer), but diols and 2*R*-MB were detected in only 11 and 17 extracts, respectively, and only two extracts contained all three components. Lastly, of 10 extracts from males of *P. lengi* that contained any possible pheromone components, eight contained 3*R*-ketol, six contained 2*R*-MB, and only three contained both in detectable quantities.

Field Bioassays

A total of 9,634 cerambycid beetles of 79 species were captured during the 20 bioassays, representing 22 tribes of six subfamilies (Supp Table 4 [online only]). Also captured were 39 specimens of a species in the closely related Disteniidae, *Elytrimitatrix undata* (F.). Because most of the tested compounds were known pheromone components of cerambycines, ~94% of the captured beetles were of that subfamily. However, as expected, some lamiines were captured during bioassays that tested for antagonism of cerambycine pheromones by common pheromones of that subfamily (fusc, fuscac, geran). Species in other subfamilies probably represent random encounters with traps irrespective of how they were baited.

The species caught in greatest numbers were among the more common cerambycids in east-central Illinois (Hanks et al. 2014), and their pheromones were often represented in bioassays. Among the three most numerous species were the two that specifically were targeted with bioassays, *N. a. acuminatus* (nearly 800 specimens) and *M. caryae* (nearly 3,000 specimens). Other species that were represented by more than 100 specimens were common species that are strongly attracted to 3-ketol (*A. pumilus*, *E. pini*, *N. caprea*, *N. m. mucronatus*, *N. scutellaris*, *X. colonus*), to 2-MB (*P. amoenus*), or to the blend of 3-ketol and 2-MB (*P. lengi*; see below).

The following summarizes statistically significant treatment effects for the 14 beetle species caught in significant numbers (Table 8; Supp Table 5 [online only] summarizes the findings for all the species):

1) *Anelaphus pumilus*: Bioassays Na1 and KT1 showed that attraction to *R*-ketol was not influenced by *S*-ketol, while bioassays MB3 and MB5 showed that attraction to racemic 3-ketol was not influenced by the COH 2-MB, nor by ethanol, but was antagonized by sulcatone. The lack of an effect by 2-MB is consistent with the findings of Mitchell et al. (2015).
2) *Curius dentatus*: Attraction to *anti*-diol in bioassay KT3 is consistent with earlier studies (Lacey et al. 2004, Millar et al. 2018). Addition of 3-ketol antagonized attraction to *anti*-diol (KT-3).
3) *Euderces pini*: Attraction to 3*R*- and racemic 3-ketol was confirmed by bioassays KT1, KT2, and MB2, which also showed that attraction was not affected by 3*S*-ketol and the COH 3-C8ketol. Attraction was antagonized by both enantiomers of 2-MB and the racemic compound (MB-2), as previously reported by Mitchell et al. (2015).
4) *Megacyllene caryae*: In bioassay Mc3, adults of this species were attracted to citral alone (the isomeric blend of neral and geranial), and attraction was strongly antagonized by (*E*)-β-farnesene. Bioassays Mc1 and Mc2 showed that racemic 2-MB was a strong synergist, and that attraction to citral + 2-MB was

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Table 8. Mean (± SE) number of beetles captured per replicate during field bioassays and results of Friedman's *Q* analyses for 14 species of cerambycid beetles

Species, bioassay, Friedman's Q^a	Treatments (contents of trap lures)[b] and mean (± SE) number of beetles caught[c]							
Anelaphus pumilus								
Na1	control	*SS*-diol	*SS*-diol 3*R*-ketol	*SS*-diol 3*S*-ketol	*SS*-diol 3-ketol	*SS*-diol *RS*-diol	*SS*-diol *SR*-diol	*SS*-diol *anti*-diol
$Q_{7,96}$ = 54.4***	0b	0b	**1.0 ± 0.4a**	0.1 ± 0.1b	**1.7 ± 0.5a**	0b	0b	0b
KT1	control	3-ketol	3*R*-ketol	3*S*-ketol				
$Q_{3,16}$ = 13.1*	0b	**5.0 ± 0.4a**	**6.0 ± 2.7a**	0b				
MB3	control	2-MB	3-ketol	2-MB 3-ketol	2-MB 3-ketol ethanol	2-MB 3-ketol α-pinene	2-MB 3-ketol ethanol α-pinene	
$Q_{6,91}$ = 39.2***	0c	0.1 ± 0.1c	**2.9 ± 0.7a**	**2.5 ± 0.7ab**	**2.2 ± 0.6ab**	1.1 ± 0.5bc	0.8 ± 0.2bc	
MB5	control	2-MB	3-ketol	sulcatone	2-MB 3-ketol	3-ketol sulcatone		
$Q_{5,83}$ = 49.0***	0b	0b	**1.9 ± 0.6a**	0b	**1.6 ± 0.4a**	0.5 ± 0.2b		
Curius dentatus								
KT3	control	3-ketol	*syn*-diol	*anti*-diol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol *syn*-diol *anti*-diol	
$Q_{6,42}$ = 30.4***	0c	0c	0c	**2.5 ± 0.6a**	0c	0.7 ± 0.5bc	**1.5 ± 0.5ab**	
Euderces pini								
KT1	control	3-ketol	3*R*-ketol	3*S*-ketol				
$Q_{3,52}$ = 17.0***	0b	**1.7 ± 0.9a**	**1.4 ± 0.4a**	0.2 ± 0.1b				
KT2	control	3-ketol	3-ketol 3-C8ketol	3-ketol *syn*-diol *anti*-diol	3-ketol 2-MB citral	3-ketol fusc fuscac geran		
$Q_{5,48}$ = 20.7***	0b	**1.5 ± 0.4a**	**1.8 ± 0.5a**	0.6 ± 0.3ab	0.1 ± 0.1b	0.5 ± 0.2ab		
MB2	control	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol	2*S*-MB 3-ketol			
$Q_{4,29}$ = 22.4***	0b	**3.3 ± 1.1a**	0b	0b	0.3 ± 0.2b			
Megacyllene caryae								
Mc1	control	citral	citral 2-MB	blend	blend minus 2-MB			
$Q_{4,50}$ = 20.2***	0.2 ± 0.2c	9.9 ± 4.3bc	**52.4 ± 17.8a**	**36.4 ± 12.7ab**	17.7 ± 8.2bc			
Mc2	control	citral	2-MB	citral 2-MB				
$Q_{3,24}$ = 16.6***	0b	0.3 ± 0.2b	0.8 ± 0.8b	**12.8 ± 5.7a**				
Mc3	control	citral	β-farnesene	citral β-farnesene				
$Q_{3,20}$ = 16.0*	0b	**3.8 ± 1.6a**	0b	0.2 ± 0.2b				
Mc4	control	citral	citral 3-ketol 3-C8ketol	citral *syn*-diol	citral *anti*-diol	citral fuscac geran		
$Q_{5,126}$ = 77.3***	0b	2.4 ± 1.4b	7.2 ± 2.0b	**23.7 ± 5.0a**	**17.7 ± 3.8a**	1.1 ± 0.4b		

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Table 8. Continued

Species, bioassay, Friedman's Q^a	Treatments (contents of trap lures)[b] and mean (± SE) number of beetles caught[c]						
Neoclytus a. acuminatus							
Na1	control	*SS*-diol	*SS*-diol 3*R*-ketol	*SS*-diol 3*S*-ketol	*SS*-diol 3-ketol	*SS*-diol *RS*-diol	*SS*-diol *SR*-diol	*SS*-diol *anti*-diol
$Q_{7,144}$ = 37.2***	0c	**2.6 ± 0.4a**	0.6 ± 0.2bc	**1.6 ± 0.4b**	0.7 ± 0.2bc	1.1 ± 0.4bc	0.7 ± 0.3bc	0.8 ± 0.3bc
Na2	control	*syn*-diol	*syn*-C8diol	*syn*-diol *syn*-C8diol	*anti*-diol	*anti*-C8diol	*anti*-diol *anti*-C8diol	
$Q_{6,84}$ = 52.0***	0b	**2.8 ± 1.1a**	0b	**3.3 ± 0.7a**	0.1 ± 0.1b	0.1 ± 0.1b	0.1 ± 0.1b	
Na3	control	*syn*-diol	*syn*-C8diol	*syn*-diol *syn*-C8diol	*syn*-diol 3-ketol	*syn*-diol 3*R*-ketol	*syn*-diol 3*S*-ketol	
$Q_{6,76}$ = 39.3***	0.1 ± 0.1c	**5.5 ± 1.2a**	0.1 ± 0.1c	**2.7 ± 0.8b**	0.7 ± 0.3bc	1.5 ± 0.6bc	1.6 ± 0.4bc	
KT3	control	3-ketol	*syn*-diol	*anti*-diol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol *syn*-diol *anti*-diol	
$Q_{6,105}$ = 58.0***	0.1 ± 0.1b	0.2 ± 0.1b	**6.1 ± 1.0a**	0.5 ± 0.2b	1.6 ± 0.5b	0.1 ± 0.1b	1.1 ± 0.3b	
KT7	control	3-ketol	*syn*-diol	semanopyrrole	*syn*-diol semanopyrrole	3-ketol semanopyrrole		
$Q_{5,90}$ = 63.3***	0.1 ± 0.1b	0.1 ± 0.1b	**2.9 ± 0.7a**	0b	**2.3 ± 0.4a**	0.1 ± 0.1b		
MB4	control	2-MB	3-ketol	*syn*-diol	*anti*-diol	2-MB 3-ketol	2-MB *syn*-diol	2-MB *anti*-diol
$Q_{7,56}$ = 37.9***	0b	0b	0b	**2.9 ± 0.9a**	0b	0b	1.1 ± 0.6b	0b
Neoclytus caprea								
KT1	control	3-ketol	3*R*-ketol	3*S*-ketol				
$Q_{3,40}$ = 22.5***	0.2 ± 0.1b	**4.8 ± 0.9a**	**5.2 ± 1.1a**	0.6 ± 0.3b				
KT2	control	3-ketol	3-ketol 3-C8ketol	3-ketol *syn*-diol *anti*-diol	3-ketol 2-MB citral	3-ketol fusc fuscac geran		
$Q_{5,114}$ = 25.6***	0b	**1.7 ± 0.5a**	**2.0 ± 0.6a**	**2.8 ± 1.1a**	**1.8 ± 0.3a**	1.5 ± 0.5ab		
Neoclytus m. mucronatus								
KT1	control	3-ketol	3*R*-ketol	3*S*-ketol				
$Q_{3,36}$ = 23.6***	0.1 ± 0.1b	**1.7 ± 0.3a**	**2.0 ± 0.5a**	0b				
KT3	control	3-ketol	*syn*-diol	*anti*-diol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol *syn*-diol *anti*-diol	
$Q_{6,84}$ = 29.7***	0.1 ± 0.1c	**2.3 ± 0.4a**	0c	0.2 ± 0.1c	0.7 ± 0.3bc	**1.3 ± 0.5b**	0.8 ± 0.3bc	
KT5	control	3-ketol	3-C8ketol	3-ketol 3-C8ketol				
$Q_{3,52}$ = 25.1***	0b	**1.5 ± 0.4a**	0b	**1.0 ± 0.3a**				
KT6	control	3-ketol *anti*-diol	3-ketol *anti*-diol 2-MB					
$Q_{2,48}$ = 16.9***	0b	**1.2 ± 0.3a**	0.4 ± 0.1b					
KT7	control	3-ketol	*syn*-diol	semanopyrrole	*syn*-diol semanopyrrole	3-ketol semanopyrrole		
$Q_{5,66}$ = 43.5***	0b	**2.2 ± 0.8a**	0.1 ± 0.1b	0b	0b	**3.4 ± 0.9a**		
KT8	control	3-ketol	ethanol	methanol	2,3-dione	3-ketol ethanol	3-ketol methanol	3-ketol 2,3-dione

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Table 8. Continued

Species, bioassay, Friedman's Q^a	Treatments (contents of trap lures)[b] and mean (± SE) number of beetles caught[c]							
$Q_{7,104}$ = 26.8***	0b	0.3 ± 0.1b	0.4 ± 0.2b	0.1 ± 0.1b	0b	**1.4 ± 0.3a**	0.3 ± 0.2b	0.3 ± 0.2b
MB1	control	2-MB	2*R*-MB	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol		
$Q_{5,24}$ = 17.4**	0b	0b	0b	**1.3 ± 0.3a**	0b	0.50 ± 0.5b		
MB2	control	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol	2*S*-MB 3-ketol			
$Q_{4,195}$ = 29.1***	0.1 ± 0.1b	**1.6 ± 0.4a**	0.5 ± 0.1b	0.4 ± 0.1b	0.6 ± 0.1b			
MB3	control	2-MB	3-ketol	2-MB 3-ketol	2-MB 3-ketol ethanol	2-MB 3-ketol α-pinene	2-MB 3-ketol ethanol α-pinene	
$Q_{6,85}$ = 46.2***	0b	0b	1.0 ± 0.6b	0.2 ± 0.1b	**3.4 ± 0.8a**	0.4 ± 0.2b	1.1 ± 0.5b	
Neoclytus scutellaris								
KT3	control	3-ketol	*syn*-diol	*anti*-diol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol *syn*-diol *anti*-diol	
$Q_{6,84}$ = 26.8***	0.2 ± 0.1b	**2.5 ± 0.7a**	0.1 ± 0.1b	0.2 ± 0.1b	0.7 ± 0.3b	1.2 ± 0.3b	0.8 ± 0.3b	
Phymatodes aereus								
MB3/5	control	2-MB	3-ketol	2-MB 3-ketol				
$Q_{3,32}$ = 20.0***	0b	0b	**1.1 ± 0.2a**	0.3 ± 0.2b				
Phymatodes amoenus								
MB2	control	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol	2*S*-MB 3-ketol			
$Q_{4,39}$ = 32.7***	0b	0b	0.3 ± 0.2b	**3.6 ± 1.1a**	0b			
MB3	control	2-MB	3-ketol	2-MB 3-ketol	2-MB 3-ketol ethanol	2-MB 3-ketol α-pinene	2-MB 3-ketol ethanol α-pinene	
$Q_{6,91}$ = 40.4***	0b	**2.6 ± 0.9a**	0.1 ± 0.1b	0.2 ± 0.1b	0.4 ± 0.2b	0b	0.5 ± 0.3b	
MB5	control	2-MB	3-ketol	sulcatone	2-MB 3-ketol	3-ketol sulcatone		
$Q_{5,53}$ = 35.2***	0b	**7.2 ± 5.0a**	0b	0b	1.4 ± 1.1b	0b		
Phymatodes lengi								
MB1	control	2-MB	2*R*-MB	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol		
$Q_{5,66}$ = 45.6***	0.1 ± 0.1b	0.1 ± 0.1b	0.3 ± 0.1b	0b	**9.2 ± 3.0a**	**6.6 ± 1.5a**		
MB2	control	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol	2*S*-MB 3-ketol			
$Q_{4,64}$ = 37.4***	0b	0b	**1.0 ± 0.3a**	**1.5 ± 0.4a**	0b			
MB3	control	2-MB	3-ketol	2-MB 3-ketol	2-MB 3-ketol ethanol	2-MB 3-ketol α-pinene	2-MB 3-ketol ethanol α-pinene	
$Q_{6,99}$ = 54.7***	0b	0.2 ± 0.1b	0.3 ± 0.2b	**4.7 ± 1.0a**	**5.9 ± 1.4a**	**4.0 ± 0.7a**	**4.1 ± 0.7a**	

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Table 8. Continued

Species, bioassay, Friedman's Q^a	Treatments (contents of trap lures)[b] and mean (± SE) number of beetles caught[c]							
MB5	control	2-MB	3-ketol	sulcatone	2-MB 3-ketol	3-ketol sulcatone		
$Q_{5,53}$ = 31.7***	0.1 ± 0.1b	0.4 ± 0.3b	0.4 ± 0.3b	0.1 ± 0.1b	**5.3 ± 1.0a**	0.1 ± 0.1b		
Phymatodes testaceus								
MB3/5	control	2-MB	3-ketol	2-MB 3-ketol				
$Q_{3,64}$ = 13.5**	0.1 ± 0.1b	**1.1 ± 0.3a**	0.3 ± 0.1b	0.6 ± 0.2ab				
Sarosesthes fulminans								
KT4	control	3-ketol	3-ketol 2-ketol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol fusc	3-ketol fuscac	3-ketol methylphen.
$Q_{7,47}$ = 37.5***	0b	0b	1.0 ± 0.3ab	0b	**1.7 ± 0.5a**	0b	0.2 ± 0.2b	0b
KT6	control	3-ketol *anti*-diol	3-ketol *anti*-diol 2-MB					
$Q_{2,30}$ = 12.1**	0b	0.3 ± 0.2b	**1.6 ± 0.4a**					
Xylotrechus colonus								
Na1	control	*SS*-diol	*SS*-diol 3*R*-ketol	*SS*-diol 3*S*-ketol	*SS*-diol 3-ketol	*SS*-diol *RS*-diol	*SS*-diol *SR*-diol	*SS*-diol *anti*-diol
$Q_{7,144}$ = 74.0***	0.1 ± 0.1b	0.4 ± 0.2b	**3.2 ± 0.6a**	1.7 ± 0.3b	**3.9 ± 1.0a**	0.1 ± 0.1b	0.4 ± 0.2b	0.7 ± 0.3b
Na3	control	*syn*-diol	*syn*-C8diol	*syn*-diol *syn*-C8diol	*syn*-diol 3-ketol	*syn*-diol 3*R*-ketol	*syn*-diol 3*S*-ketol	
$Q_{6,63}$ = 27.1***	0.2 ± 0.2c	0.9 ± 0.3bc	0.1 ± 0.1c	0.4 ± 0.2c	**2.7 ± 0.8a**	**2.3 ± 0.6ab**	**2.2 ± 0.5ab**	
KT1	control	3-ketol	3*R*-ketol	3*S*-ketol				
$Q_{3,48}$ = 13.0**	0.2 ± 0.1b	**1.1 ± 0.2a**	**1.3 ± 0.4a**	0.3 ± 0.1b				
KT3	control	3-ketol	*syn*-diol	*anti*-diol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol *syn*-diol *anti*-diol	
$Q_{6,70}$ = 21.6**	0.1 ± 0.1b	**2.6 ± 0.9a**	0.1 ± 0.1b	0.6 ± 0.2b	1.1 ± 0.5ab	0.7 ± 0.3b	1.9 ± 0.4ab	
KT4	control	3-ketol	3-ketol 2-ketol	3-ketol *syn*-diol	3-ketol *anti*-diol	3-ketol fusc	3-ketol fuscac	3-ketol methylphen.
$Q_{7,151}$ = 33.8***	0.1 ± 0.1b	**1.9 ± 0.4a**	1.2 ± 0.4ab	**2.3 ± 0.6a**	1.2 ± 0.4ab	**2.2 ± 0.4a**	**2.6 ± 0.6a**	1.6 ± 0.5ab
KT5	control	3-ketol	3-C8ketol	3-ketol 3-C8ketol				
$Q_{3,76}$ = 35.2***	0b	**1.0 ± 0.3a**	0b	**1.5 ± 0.4a**				
KT6	control	3-ketol *anti*-diol	3-ketol *anti*-diol 2-MB					
$Q_{2,30}$ = 14.2***	0b	**2.3 ± 0.7a**	**1.8 ± 0.6a**					
KT7	control	3-ketol	*syn*-diol	semanopyrrole	*syn*-diol semanopyrrole	3-ketol semanopyrrole		
$Q_{5,72}$ = 24.8**	0b	**1.3 ± 0.7a**	0.8 ± 0.3ab	0.3 ± 0.1b	0b	**1.8 ± 0.4a**		
KT8	control	3-ketol	ethanol	methanol	2,3-dione	3-ketol ethanol	3-ketol methanol	3-ketol 2,3-dione
$Q_{7,96}$ = 42.1***	0.1 ± 0.1b	0.4 ± 0.2b	0.7 ± 0.5b	0	0.1 ± 0.1b	**3.8 ± 0.4a**	0.8 ± 0.2b	0.8 ± 0.2b
MB1	control	2-MB	2*R*-MB	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol		
$Q_{5,84}$ = 30.8***	0.3 ± 0.2b	0.1 ± 0.1b	1.1 ± 0.7ab	3.4 ± 1.7ab	**4.7 ± 2.4a**	3.8 ± 0.9ab		

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Table 8. Continued

Species, bioassay, Friedman's Q^a	Treatments (contents of trap lures)[b] and mean (± SE) number of beetles caught[c]							
MB2	control	3-ketol	2-MB 3-ketol	2*R*-MB 3-ketol	2*S*-MB 3-ketol			
$Q_{4,100} = 32.7^{***}$	0.2 ± 0.1b	**3.2 ± 0.8a**	2.7 ± 0.6ab	2.3 ± 0.6ab	**4.5 ± 1.0a**			
MB3	control	2-MB	3-ketol	2-MB 3-ketol	2-MB 3-ketol ethanol	2-MB 3-ketol α-pinene	2-MB 3-ketol ethanol α-pinene	
$Q_{6,133} = 67.4^{***}$	0.2 ± 0.1c	0.3 ± 0.2c	1.2 ± 0.4c	1.2 ± 0.3c	**5.5 ± 1.0a**	0.7 ± 0.3c	**3.4 ± 0.7b**	
MB4	control	2-MB	3-ketol	*syn*-diol	*anti*-diol	2-MB 3-ketol	2-MB *syn*-diol	2-MB *anti*-diol
$Q_{7,96} = 35.5^{***}$	0b	0.1 ± 0.1b	**4.5 ± 2.2a**	0.5 ± 0.2b	0.4 ± 0.2b	**4.6 ± 2.0a**	0.1 ± 0.1b	0.3 ± 0.1b
MB5	control	2-MB	3-ketol	sulcatone	2-MB 3-ketol	3-ketol sulcatone		
$Q_{5,90} = 62.8^{***}$	0.1 ± 0.1b	0.2 ± 0.1b	**2.9 ± 0.4a**	0b	**4.3 ± 1.3a**	**3.5 ± 0.8a**		

Means in bold were significantly different from those of controls.

[a]Asterisks indicate significance level of Friedman's Q: $^*P < 0.005$, Q: $^{**}P < 0.001$, $^{***}P < 0.0001$.

[b]Chemical names abbreviated as in Table 1, and blends are as described in Tables 4–7.

[c]Means within species with different letters are significantly different (REGWQ test, $P < 0.05$).

not influenced by the addition of the remaining compounds present in headspace extracts from males, including (*S*)-(−)-limonene, 2-phenylethanol, (−)-α-terpineol, and nerol. Bioassay Mc4 showed that attraction to citral was strongly synergized by both *anti*-diol and *syn*-diol, even though only the former was present in headspace extracts from males.

5) *Neoclytus a. acuminatus*: Bioassays Na1, Na3, and KT3 confirmed attraction to *SS*-diol and racemic *syn*-diol, and showed that attraction was antagonized by both enantiomers of 3-ketol and both enantiomers of *anti*-diol. The influence of *syn*-C8diol on attraction was not consistent across bioassays, having no effect in Na2, but being antagonistic in Na3. Bioassays KT7 and MB4 showed that attraction to *syn*-diol was not influenced by semanopyrrole, but was antagonized by 2-MB.
6) *Neoclytus caprea*: Bioassay KT1 showed that adults of this species were attracted to 3*R*-ketol and not influenced by the *S*-enantiomer, consistent with the findings of Ray et al. (2015). In bioassay KT2, attraction to racemic 3-ketol was not influenced by 3-C8ketol, nor by the blends of *syn*- + *anti*-diols and 2-MB + citral.
7) *Neoclytus m. mucronatus*: The abundance of this species resulted in statistically significant treatment effects for many of the KT and MB bioassays. Bioassay KT1 showed that attraction to 3*R*-ketol was not influenced by 3*S*-ketol, and bioassays KT5 and KT7 showed that attraction to racemic 3-ketol was not influenced by 3-C8ketol nor by semanopyrrole. Attraction to racemic 3-ketol was antagonized by both *syn*- and *anti*-diols in bioassay KT3, and by 2*R*-MB, 2*S*-MB, and racemic 2-MB in bioassays KT6, MB1, and MB2. Bioassays KT8 and MB3 further showed that attraction to 3-ketol was synergized by ethanol, but apparently not influenced by α-pinene, methanol, and 2,3-dione.
8) *Neoclytus scutellaris*: Bioassay KT3 showed that attraction to 3-ketol was antagonized by both *syn*- and *anti*-diols.
9) *Phymatodes aereus*: The combined bioassays MB3 and MB5 showed that attraction to 3-ketol was antagonized by racemic 2-MB, as reported previously by Mitchell et al. (2015).
10) *Phymatodes amoenus*: Bioassay MB2 confirmed attraction of this species to 2*R*-MB (despite its being blended with 3-ketol), and that attraction was antagonized by 2*S*-MB. Bioassays MB3 and MB5 further showed that attraction to racemic 2-MB was antagonized by 3-ketol, and this antagonism was not altered by the addition of ethanol or α-pinene.
11) *Phymatodes lengi*: Bioassays MB1, MB2, MB5 confirmed that this species is attracted only by the blend of 2*R*-MB and 3-ketol, not to the individual components, and was not influenced by 2*S*-MB. In bioassay MB3, attraction to the blend of 2-MB and 3-ketol was not influenced by ethanol nor by α-pinene.
12) *Phymatodes testaceus*: The combined bioassays MB3 and MB5 confirmed attraction to racemic 2-MB.
13) *Sarosesthes fulminans*: Bioassay KT4 confirmed that this species is attracted by the blend of 3-ketol and *anti*-diols, and bioassay KT6 showed that attraction was synergized by 2-MB.
14) *Xylotrechus colonus*: As with *N. m. mucronatus*, the abundance of this species resulted in statistically significant treatment effects in nearly all of the Na, KT1, and MB bioassays, yielding a wealth of information about its semiochemistry. Beetles were attracted in significant numbers to 3-ketol in several bioassays, and bioassays Na1 and KT1 suggested that the (*R*)-enantiomer was inherently attractive while the (*S*)-enantiomer was neither attractive nor synergistic. However, bioassay Na3 suggested that beetles were attracted by a blend of 3*S*-ketol with *syn*-diol.

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Taken together, bioassays Na1, Na3, and KT3 showed that *SS*-, *syn*-diol, and *anti*-diol were not inherently attractive as individual compounds. Bioassays KT3 and KT4 suggested that attraction to racemic 3-ketol apparently was not influenced by *syn*-diol, but was antagonized by *anti*-diol. Bioassays KT6 and MB1–5 showed that attraction to 3-ketol was not influenced by the 2*R*-MB present in headspace extracts, nor by the COH 2*S*-MB, or racemic 2-MB. Similarly, bioassays KT4, KT5, KT7, KT8, and MB5 showed that attraction to 3-ketol was not influenced by 2,3-dione, nor by the COHs fusc, fuscac, 3-C8ketol, semanopyrrole, and sulcatone. Semanopyrrole also was shown to have no effect on attraction of *X. colonus* to 3-ketol in an earlier study (Diesel et al. 2017). Plant volatiles varied in how they influenced attraction of *X. colonus* to 3-ketol, with bioassay KT8 showing synergism by ethanol and no effect by methanol, and bioassay MB3 showing synergism by ethanol being reduced by α-pinene. The opposite effects of ethanol and α-pinene on attraction were consistent with earlier research (e.g., Hanks et al. 2012).

Discussion

Headspace extracts from males of *M. caryae*, *S. fulminans*, and *X. colonus* contained single enantiomers of 2-MB that had been overlooked in their original pheromone identifications (Lacey et al. 2008, 2009). This omission was due to the early elution of this volatile compound from the GC column and its peak being obscured by the solvent front, necessitating a cooler initial oven temperature. Such highly volatile pheromone components may be detected more reliably by solventless collection methods such as solid phase microextraction (Millar and Sims 1998), as has been demonstrated for *M. caryae* (e.g., Mitchell et al. 2017). Recognition of 2-MB as a potential cerambycid pheromone led to its subsequently being identified as a pheromone component of three *Phymatodes* species. That is, males of *P. lengi* were known to produce a blend of 3*R*-ketol and 2*R*-MB (Mitchell et al. 2015), but evidence of attraction had been limited to bioassays of a multispecies generic blend which fortuitously included both components (Hanks et al. 2012). Here, we document attraction to the specific blend of racemic 3-ketol and 2*R*-MB.

The pheromone of *P. amoenus* originally was misidentified by Mitchell et al. (2015) as a blend of 3*R*-ketol and 2*R*-MB due to taxonomic confusion with the morphologically similar *P. lengi* (see Yanega 1996). Here we demonstrate that the pheromone of *P. amoenus* consists solely of 2*R*-MB. Similarly, males of the *P. testaceus*, native to Europe but introduced into the United States, produced 2*R*-MB, and attraction to racemic 2-MB is documented here and in other publications (e.g., Hanks et al. 2018). Taken together, these findings suggest that only single enantiomers of 2-MB are used as pheromone components, consistent with earlier research on other cerambycine species (e.g., Hanks et al. 2007, Silva et al. 2018). The COH 2*S*-MB antagonized attraction of *P. amoenus*, but had no effect on *P. lengi*.

2,3-Dione was present in extracts of volatiles from most species that had 3*R*-ketol as the major pheromone component. 2,3-Alkanediones often are detected in headspace extracts from cerambycids that use either 2- or 3-ketols as pheromones (Millar and Hanks 2017). Chain lengths of the 2,3-diones invariably match those of the associated ketols (i.e., 6, 8, or 10 carbons; e.g., Schröder et al. 1994, Hall et al. 2006, Ray et al. 2009), suggesting that the 2,3-diones are either biosynthetic precursors or artifacts, or that they arise from degradation of ketols during collection, storage, or analysis (Millar and Hanks 2017). Isomerization of ketols also may have given rise to the trace quantities of 2-ketols seen in the extracts from some of the ketol-producing species; thermal isomerization of these compounds is well known (e.g., Sakai et al. 1984). The probability of detecting trace quantities of these artifactual compounds, and other minor components, increases with the number of extracts analyzed, which in turn depends on the natural abundance of a species and the ease with which beetles are trapped. Thus, males of *N. m. mucronatus* and *X. colonus* appear to produce a greater diversity of volatiles than do males of *P. aereus*, possibly in part due to the much larger number of extracts analyzed from the former two species.

The apparent artifactual nature of 2,3-dione and 2-ketols suggests they are not components of aggregation-sex pheromones. Indeed, no cerambycid species have been attracted to traps baited solely with racemic 2-ketol in repeated field bioassays deployed in the same area over a number of successive field seasons (L. M. Hanks, unpublished data). Furthermore, bioassay KT8 showed that the 2,3-dione was not inherently attractive and had no effect on attraction of *N. m. mucronatus* and *X. colonus* to 3-ketol. Bioassays in California similarly revealed no effect of 2,3-dione on attraction of *Rusticoclytus* (formerly *Xylotrechus*) *nauticus* (Mannerheim) to 3-ketol (Hanks et al. 2007). Assuming that 2,3-dione is artifactual, then pheromones of several of the study species are effectively comprised solely of 3*R*-ketol, including *A. pumilus*, *E. pini*, *N. caprea*, *N. m. mucronatus*, and *N. scutellaris*. Thus, of our 14 study species, the only ketol-producing species with truly multicomponent pheromones are likely to be *P. aereus*, *P. lengi*, *S. fulminans*, and *X. colonus*.

Variation among conspecific males in whether they produced any compounds at all during collection of headspace volatiles suggests that emission may vary with the physiological state of the field-collected insects, which in turn may be influenced by the laboratory environment, and/or that pheromone release is under active control by the insect. This finding is consistent with what is known of the calling behavior of cerambycine males, first described for *N. a. acuminatus* (Lacey et al. 2004). Males of this species aggregate on host plants of the larvae where they adopt a characteristic stationary pose, with the front legs extended, termed the 'pushup stance'. This behavior has never been observed in females. It coincides with pheromone release and apparently serves to elevate the thorax above the substrate, facilitating dispersion of pheromone from the prothoracic glands (Lacey et al. 2007b). The same calling behavior also has been reported for *M. caryae* (Lacey et al. 2008), *N. m. mucronatus* (Lacey et al. 2007a), *X. colonus* (Lacey et al. 2009), and other species of cerambycines (Ray et al. 2009, Zou et al. 2016).

Conspecific males also varied in the relative ratios of pheromone components they released, which could again be due to the artificial laboratory environment under which beetles were held during headspace collections, and/or to our using field-captured males that varied in body size, age, and mating status. Similar variation in ratios of components has been reported previously for other cerambycid species (e.g., Meier et al. 2016, Silva et al. 2018). For the ketol-producing study species that have multicomponent pheromones, the (*R*)-enantiomer was always dominant. Research on other species of cerambycines, native to many regions of the world, also suggests that 3*R*-ketol is always either the sole enantiomer or strongly dominant over the *S*-enantiomer, while the reverse is true of pheromone components of the 2-ketol configuration (summarized in Millar and Hanks 2017).

The minor components identified from extracts of males of the ketol-producing species varied in activity. For example, extracts

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

from males of *S. fulminans* contained 3*R*-ketol and *anti*-diol (Lacey et al. 2009), and subsequent bioassays showed that beetles were attracted to traps baited with either racemic 3-ketol or *anti*-diol if the blend of the two was not also present (Hanks and Millar 2013, Millar et al. 2018). Here, we found that only the blend attracted significant numbers of this species, and that males also produced 2*R*-MB, which synergized attraction to the blend. Similarly, *P. lengi* did not respond to 3-ketol alone, nor to 2*R*-MB alone, but only to the synergistic binary blend. In contrast, none of the minor components of *X. colonus*, including 2*R*-MB, appeared to be inherently attractive nor synergistic, consistent with the findings of other studies on this species (e.g., Lacey et al. 2009, Hanks et al. 2012, Hanks and Millar 2013, Wong et al. 2017, Millar et al. 2018). In fact, the *anti*-diols (present in trace amounts) appeared to be antagonistic during the present studies. Diol isomers may play a variable role for *Xylotrechus* species in general, as suggested by two species native to Asia. That is, males of *Xylotrechus quadripes* Chevrolat produce 2*S*-C10ketol and *SS*-C10diol, and attraction to the ketol was antagonized by the diol in laboratory and field bioassays (Hall et al. 2006). In contrast, males of *Xylotrechus pyrrhoderus* Bates produce 2*S*-C8ketol and *SS*-C8diol, and both components are necessary for attraction (Narai et al. 2015).

As with the ketol-producing species, males of *M. caryae* appeared to vary considerably in the suite of components produced by any given male. This species initially was thought to respond only to the complete eight-component blend (Lacey et al. 2008), although attraction to citral alone was suggested by later studies (Mitchell et al. 2012, Handley et al. 2015). Here, we confirm that *M. caryae* is attracted to citral alone, and attraction is strongly synergized by 2-MB. Both sexes of *M. caryae* also produce small amounts of various spiroacetals that may contribute an odor component to their mimicry of vespid wasps (Mitchell et al. 2017). During the present studies, a spiroacetal also was detected in headspace extracts from adults of *E. pini*, which could play a role in its mimicry of ants (see Francke and Kitching 2001).

Males of a congener of *M. caryae* native to the southwestern United States, *Megacyllene antennata* White, produce a similarly complex blend of compounds, sharing (−)-α-terpineol, 2-phenylethanol, and (*S*)-(−)-limonene with *M. caryae* (Mitchell et al. 2018). Males of *M. antennata* also produce quite variable ratios of components, but the major two compounds, (−)-α-terpineol and (*E*)-hexenal, were present in all headspace extracts from males, and beetles were attracted to traps baited with this binary blend. In contrast, males of the South American congener *Megacyllene acuta* (Germar) produce compounds more typical of cerambycines, including 3*R*-ketol along with small amounts of *SS*-diol and 2*S*-MB, both of which were synergists of 3*R*-ketol (Silva et al. 2018).

Attraction of *M. caryae* to citral also was strongly synergized by *anti*-diols, which were detected in some headspace extracts. Unexpectedly, attraction also was synergized by *syn*-diols, which have not been detected in extracts from males. This species previously had been attracted to *syn*-diols in some parts of its range (Georgia, Pennsylvania; Hanks and Millar 2013, Miller et al. 2015), but only to *anti*-diols in other areas (Texas; Millar et al. 2018), suggesting genetic variation in the responses of different populations to 2,3-hexanediol diastereomers, if not in which diastereomers the males produce. (*E*)-β-Farnesene appeared to antagonize attraction of *M. caryae*, despite its being detected in a few aeration extracts. This finding is consistent with this compound only being produced by agitated males of *M. caryae*, along with the spiroacetals that apparently serve a defensive function (Mitchell et al. 2017).

The lack of inherent attractiveness or synergism by minor components produced by males of some species raises the question as to what function these compounds might serve. It is possible that they elicit some response from conspecific males, or from females, such as by rendering females more receptive to mating. Minor components also may play a role in reproductive isolation by discouraging attraction of other species to shared components, and this effect may be reciprocal. For example, *X. colonus* and *N. a. acuminatus* overlap in both seasonal and daily flight periods, as well as in hosts for their larvae (Lingafelter 2007, Hanks et al. 2014). *SS*-Diol is a minor component of *X. colonus*, but the sole pheromone component of *N. a. acuminatus*. Because adults of *X. colonus* are not attracted to diols, they would not be drawn to calling males of *N. a. acuminatus*, whereas the ketols and *anti*-diols of *X. colonus* would inhibit attraction of *N. a. acuminatus*. Similarly, the diols and 2*R*-MB of *X. colonus* would discourage attraction of *N. m. mucronatus* to the 3*R*-ketol component they share, but it is not yet known what factors would inhibit attraction of *X. colonus* to the pure 3*R*-ketol produced by males of *N. m. mucronatus*.

Mitchell et al. (2015) demonstrated that both phenological and chemical mechanisms served to inhibit interspecific attraction among cerambycine species which are active in spring in the area where the present studies were conducted, including *A. pumilus*, *E. pini*, *N. caprea*, *P. aereus*, *P. amoenus*, and *P. lengi*. Here, we further show that attraction between the latter two congeners, during their brief period of seasonal overlap (Mitchell et al. 2015), would be discouraged because males of *P. amoenus* produce only 2*R*-MB, whereas *P. lengi* responds only to the synergistic blend of 2*R*-MB and 3*R*-ketol, with the latter compound antagonizing attraction of *P. amoenus*. In another example, males of both the exotic species *P. testaceus* and the native *P. amoenus* produce 2*R*-MB, and they overlap broadly in seasonal flight period, both flying during early May through June (Hanks and Millar 2013, Hanks et al. 2014, Handley et al. 2015). However, interspecific attraction is averted because adults of *P. amoenus* are active in mid- to late afternoon, whereas *P. testaceus* flies in late evening (L. M. Hanks, unpublished data). The latter species shows a similar phenology in its native Europe, flying at night during May through August (Bílý and Mehl 1989, Bense 1995), suggesting that its phenology in North America has not been shaped by selection against interspecific attraction with North American congeners. It is likely that the phenology of *P. testaceus* in Europe is the product of coevolution with sympatric species that have 2*R*-MB as a pheromone component, such as *Phymatodes pusillus pusillus* (F) (Molander and Larsson 2018). Thus, *P. testaceus* may have been preadapted to colonize North America because it differed in phenology from native congeners which have similar pheromones.

Responses of the study species to COHs, or other compounds which are not produced by their males, prove that they have olfactory mechanisms for detecting those compounds, or at least compounds of similar structure. Such olfactory mechanisms presumably are of some adaptive significance. In contrast, beetles should not respond to compounds that have no bearing on their fitness, and may lack the ability to detect them. For example, attraction of *N. caprea* to its 3-ketol pheromone was not influenced by COHs such as *syn*-diols, *anti*-diols, and 2-MB, which is consistent with its flying so early in the season that it is temporally isolated from all the other 3-ketol-producing species in the study area. Similarly, adults of *M. caryae* were unaffected by 3-ketol, as would be expected because none of the 3-ketol-producing species have citral as a pheromone component. Furthermore, no species in the area of our studies appear to produce C8ketols or semanopyrrole as pheromone

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

components, and those compounds did not influence attraction of any of the study species. It should be noted, however, that the pheromone of the native cerambycine *Dryobius sexnotatus* Linsley (tribe Dryobiini) is composed of semanopyrrole and 3*R*-ketol. This species is limited in its distribution within Illinois to the southern counties (Diesel et al. 2017), and so is geographically separated from the area of our studies.

All our study species also appeared to be 'odor blind' to the lamiine compounds fusc, fuscac, and geran, suggesting that these compounds have no biological relevance to cerambycines. A possible exception is the cerambycine *Obrium maculatum* (Olivier), which is at least attracted by fusc and/or fuscac (e.g., Millar et al. 2018), but its pheromone has not yet been identified. Sulcatone also is produced by males of some lamiine species native to the area (Meier et al. 2019), and its antagonistic effect on *A. pumilus* may be evidence that it serves as a pheromone component for one or more unknown cerambycine species that also use *R*-ketol as a dominant pheromone component. As already mentioned, sulcatone is known to be produced by males of the cerambycine *P. decussatus*, native to the western United States.

Finally, none of the plant volatiles attracted significant numbers of any species, and they varied in how they affected attraction to synthesized pheromones. For example, attraction of *N. m. mucronatus* and *X. colonus* was synergized by ethanol and antagonized by α-pinene, as reported earlier (Hanks et al. 2012, Miller et al. 2015), but in other species these plant volatiles appeared not to influence attraction. The response of beetles to plant volatiles probably is associated with the condition of host plants required by their larvae (i.e., whether alive, moribund, dead, or decaying; Hanks 1999), as well as their specific host requirements (e.g., gymnosperms vs angiosperm trees). However, not enough is known of the larval hosts of the study species to explain, for example, why ethanol is a synergist for *N. m. mucronatus*, but has no effect on *A. pumilus*, and why α-pinene is an antagonist for *A. pumilus*, but has no effect on *P. lengi*. It is possible that antagonism by α-pinene plays a role in preventing interspecific attraction between conifer-feeding species and those that develop in deciduous hosts (e.g., Collignon et al. 2016), particularly among species that use 3*R*-ketol as a pheromone.

Taken together, these results from 20 field bioassays, with 14 species of cerambycid beetles, suggest five general conclusions, as follows:

1) 2-MB is a pheromone component for numerous species.
2) Both 2,3-dione and 2-ketol (when present in trace quantities) are likely biosynthetic artifacts, or artifacts of collection and analysis procedures, because there was no indication that they have roles as components of aggregation-sex pheromones.
3) Some species use pheromones consisting of a single component, whereas for other species, blends are clearly necessary for attraction. Furthermore, it is clear that minor components can play roles as synergists, or as antagonists for heterospecifics, or both. One can speculate that these different scenarios are a result of natural selection, whereby the counterproductive effects of attraction of two or more species to a single shared component can be mitigated by the evolution of species-specific blends. This scenario would in turn suggest the hypothesis that blends should be most common among closely related species which are both sympatric and synchronic.

However, there are several points relating to the production of blends of compounds which remain obscure. For example, it is not clear why some species, such as *M. caryae* and *M. antennata*, produce complex blends containing multiple components, whereas only a subset of these compounds appears to be involved in attraction. Furthermore, it is unclear why the blend ratios produced by males were so variable, both qualitatively and quantitatively, in some cases appearing to lack important synergists. That is, one would predict that males should produce consistent ratios of compounds in the interest of producing a clear and reliable signal. It also is unclear why attraction of *M. caryae* to its pheromone was enhanced by *syn*-diols, which this species appears not to produce, unless the *syn*-diols were acting as mimics of the *anti*-diols produced by males.

It also seems counterintuitive that species with pheromones composed of multiple components were often attracted to traps baited with only the major component, or incomplete reconstructions of their pheromones, in the absence of the complete blend. Nevertheless, these findings have important implications for developing multispecies generic lures, because many species share the same major component, notably 3*R*-ketol, and each could be attracted to traps baited with either a single component, or a carefully selected blend of components that are unlikely to interfere with each other.

4) Although attraction of a particular species was often antagonized by compounds produced by closely related heterospecifics (e.g., in the same genus or tribe), compounds from more distantly related heterospecifics (e.g., in different subfamilies) appeared to have little effect. This is not unexpected given the major differences in the structures and chemistries of the pheromone components from the different subfamilies (Millar and Hanks 2017). However, it will be useful for practical applications because it suggests that multispecies blends that are composed of pheromone components of species in different subfamilies can be formulated, with minimal risk of antagonistic effects.
5) Species varied as to how attraction to pheromone lures was influenced by concomitant release of the host volatiles ethanol and α-pinene, and in a manner that was difficult to predict because of our poor understanding of host plant relationships of the study species. However, ethanol either synergized attraction or had no effect, suggesting that releasing ethanol with pheromone lures may improve trap catch of some species, while having minimal or no negative effects on other species. In contrast, α-pinene may antagonize attraction, particularly of species that develop in deciduous hosts, as has been shown previously (Collignon et al. 2016).

Overall, the work summarized here lends further support to the hypothesis that, analogous to other insect taxa, differences between sympatric cerambycids in their pheromone chemistry, as well as in seasonal and daily flight periods, are the product of selection against unproductive interspecific attraction. Thus, the cerambycine community in east-central Illinois, which is representative of the eastern United States (Hanks et al. 2014), is a closely coevolved system, with each species occupying a specific niche as defined by its pheromone chemistry and phenology. Many of the same compounds serve as pheromone components of cerambycine species in other parts of the world (Millar and Hanks 2017), where there is growing evidence of similar niche partitioning (e.g., Narai et al. 2015; Wickham et al. 2016; Silva et al. 2017, 2018). This conservation of pheromone compounds among species from different continents, coupled with coevolution in pheromone chemistry within native beetle communities, may pose a barrier to establishment of invasive species that have pheromones similar to those of native fauna (Hanks and Millar 2016, 2018). That is, such exotic species will encounter a community

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

in which their pheromone channels, vital to reproduction and establishment, already have been partitioned among the endemic fauna with which they have not coevolved, and the resulting interference in their mate location behavior will reduce the chances that the exotic species will establish.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Acknowledgments

We thank Steve Buck, the University of Illinois Committee on Natural Areas, and the Vermilion County Conservation District for access to field sites. For assistance with field and laboratory work we thank Jodie Ellis and former graduate students Natalie Diesel, Pete Reagel, Tom Schmeelk, and Christina Silliman, and undergraduate technicians Lorenzo D'Alessio, Kate Johnson, Heidi Pierce, Victoria Rodriguez, Suzanne Vachula, and Alexandra Wirtas. Thanks to Professor Kenji Mori for the gift of (*R*)-2-methylbutanoic acid used in the synthesis of 2*R*-MB. We appreciate financial support from the Alphawood Foundation of Chicago (to L.M.H.), and from the following USDA agencies (all to J.G.M. and L.M.H.): National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (grant 2009-35302-05047), National Institute of Food and Agriculture (award 2012-67013-19303, 2015-67013-23173), and APHIS (grant [10–17]-8130-1422-CA).

References Cited

Bense, U. 1995. Longhorn beetles. Illustrated key to the Cerambycidae and Vesperidae of Europe. Margaf Verlag, Weikersheim, Germany.

Bílý, S., and O. Mehl. 1989. Longhorned beetles (Coleoptera: Cerambycidae) of Fennoscandia and Denmark. E. J. Brill, Leiden, The Netherlands.

Cardé, R. T. 2014. Defining attraction and aggregation pheromones: teleological versus functional perspectives. J. Chem. Ecol. 40: 519–520.

Collignon, R. M., I. P. Swift, Y. Zou, J. S. McElfresh, L. M. Hanks, and J. G. Millar. 2016. The influence of host plant volatiles on the attraction of longhorn beetles to pheromones. J. Chem. Ecol. 42: 215–229.

Crook, D. J., D. R. Lance, and V. C. Mastro. 2014. Identification of a potential third component of the male-produced pheromone of *Anoplophora glabripennis* and its effect on behavior. J. Chem. Ecol. 40: 1241–1250.

Da Silva, F., M. U. Ventura, and L. Morales. 2006. Capture of *Hypothenemus hampei ferrari* (Coleoptera, Scolytidae) in response to trap characteristics. Sci. Agric. 63: 567–571.

Diesel, N. M., Y. Zou, T. D. Johnson, D. A. Diesel, J. G. Millar, J. A. Mongold-Diers, and L. M. Hanks. 2017. The rare north American cerambycid beetle *Dryobius sexnotatus* shares a novel pyrrole pheromone component with species in Asia and South America. J. Chem. Ecol. 43: 739–744.

El-Sayed, A. M. 2018. The pherobase: database of pheromones and semiochemicals. http://www.pherobase.com. Accessed 10 December 2018.

Fan, J., O. Denux, C. Courtin, A. Bernard, M. Javal, J. G. Millar, L. M. Hanks, and A. Roques. 2019. Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests. J. Pest Sci. 92: 281–297.

Francke, W., and W. Kitching. 2001. Spiroacetals in insects. Curr. Org. Chem. 5: 233–251.

Hall, D. R., A. Cork, S. J. Phythian, S. Chittamuru, B. K. Jayarama, M. G. Venkatesha, K. Sreedharan, P. K. Vinod Kumar, H. G. Seetharama, and R. Naidu. 2006. Identification of components of male-produced pheromone of coffee white stemborer, *Xylotrechus quadripes*. J. Chem. Ecol. 32: 195–219.

Handley, K., J. Hough-Goldstein, L. M. Hanks, J. G. Millar, and V. D'Amico. 2015. Species richness and phenology of cerambycid beetles in urban forest fragments of northern Delaware. Ann. Entomol. Soc. Am. 108: 251–262.

Hanks, L. M. 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. Annu. Rev. Entomol. 44: 483–505.

Hanks, L. M., and J. G. Millar. 2013. Field bioassays of cerambycid pheromones reveal widespread parsimony of pheromone structures, enhancement by host plant volatiles, and antagonism by components from heterospecifics. Chemoecology. 23: 21–44.

Hanks, L. M., and J. G. Millar. 2016. Sex and aggregation-sex pheromones of cerambycid beetles: basic science and practical applications. J. Chem. Ecol. 42: 631–654.

Hanks, L. M., and J. G. Millar. 2018. Conservation of pheromone chemistry within the Cerambycidae on a global scale, and implications for invasion biology. Proceedings of the 29th USDA Interagency Research Forum on Invasive Species, 9–12 January 2018, Annapolis, MD.

Hanks, L. M., and Q. Wang. 2017. Reproductive biology of cerambycids, pp. 133–159. *In* Q. Wang (ed.), Cerambycidae of the world: biology and pest management. CRC Press/Taylor & Francis, Boca Raton, FL.

Hanks, L. M., J. G. Millar, J. A. Moreira, J. D. Barbour, E. S. Lacey, J. S. McElfresh, F. R. Reuter, and A. M. Ray. 2007. Using generic pheromone lures to expedite identification of aggregation pheromones for the cerambycid beetles *Xylotrechus nauticus*, *Phymatodes lecontei*, and *Neoclytus modestus modestus*. J. Chem. Ecol. 33: 889–907.

Hanks, L. M., J. G. Millar, J. A. Mongold-Diers, J. C. H. Wong, L. R. Meier, P. F. Reagel, and R. F. Mitchell. 2012. Using blends of cerambycid beetle pheromones and host plant volatiles to simultaneously attract a diversity of cerambycid species. Can. J. For. Res. 42: 1050–1059.

Hanks, L. M., P. F. Reagel, R. F. Mitchell, J. C. H. Wong, L. R. Meier, C. A. Silliman, E. E. Graham, B. L. Striman, K. P. Robinson, J. A. Mongold-Diers, *et al.* 2014. Seasonal phenology of the cerambycid beetles of east-central Illinois. Ann. Entomol. Soc. Am. 107: 211–226.

Hanks, L. M., J. A. Mongold-Diers, T. H. Atkinson, M. K. Fierke, M. D. Ginzel, E. E. Graham, T. M. Poland, A. B. Richards, M. L. Richardson, and J. G. Millar. 2018. Blends of pheromones, with and without host plant volatiles, can attract multiple species of cerambycid beetles simultaneously. J. Econ. Entomol. 111: 716–724.

Lacey, E. S., M. D. Ginzel, J. G. Millar, and L. M. Hanks. 2004. Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus*. J. Chem. Ecol. 30: 1493–1507.

Lacey, E. S., J. A. Moreira, J. G. Millar, A. M. Ray, and L. M. Hanks. 2007a. Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus mucronatus mucronatus*. Entomol. Exp. Appl. 122: 171–179.

Lacey, E. S., A. M. Ray, and L. M. Hanks. 2007b. Calling behavior of the cerambycid beetle *Neoclytus acuminatus acuminatus* (F.). J. Insect Behav. 20: 117–128.

Lacey, E. S., J. A. Moreira, J. G. Millar, and L. M. Hanks. 2008. A male-produced aggregation pheromone blend consisting of alkanediols, terpenoids, and an aromatic alcohol from the cerambycid beetle *Megacyllene caryae*. J. Chem. Ecol. 34: 408–417.

Lacey, E. S., J. G. Millar, J. A. Moreira, and L. M. Hanks. 2009. Male-produced aggregation pheromones of the cerambycid beetles *Xylotrechus colonus* and *Sarosesthes fulminans*. J. Chem. Ecol. 35: 733–740.

Lewis, N. G., and E. Yamamoto. 1990. Lignin: occurrence, biogenesis and biodegradation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 455–496.

Lingafelter, S. W. 2007. Illustrated key to the longhorned woodboring beetles of the eastern United States. Special Publication No. 3. Coleopterists Society, North Potomac, MD.

Linsley, E. G. 1963. The Cerambycidae of North America, Part IV. Taxonomy and classification of the subfamily Cerambycinae, tribes Elaphidionini through Rhinotragini. Univ. Calif. Publ. Entomol. 21: 1–165.

Linsley, E. G. 1964. The Cerambycidae of North America. Part V. Taxonomy and classification of the subfamily Cerambycinae, tribes Callichromini through Ancylocerini. Univ. Calif. Publ. Entomol. 22: 1–197.

Meier, L. R., Y. Zou, J. G. Millar, J. A. Mongold-Diers, and L. M. Hanks. 2016. Synergism between enantiomers creates species-specific pheromone blends and minimizes cross-attraction for two species of cerambycid beetles. J. Chem. Ecol. 42: 1181–1192.

Meier, L. R., J. G. Millar, and L. M. Hanks. 2019. Sulcatone and sulcatol are pheromone component for two species of cerambycid beetles in the subfamily Lamiinae. J. Chem. Ecol. (in press).

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022

Millar, J. G., and L. M. Hanks. 2017. Chemical ecology of cerambycids, pp. 161–196. *In* Q. Wang (ed.), Cerambycidae of the world: biology and pest management. CRC Press/Taylor & Francis, Boca Raton, FL.

Millar, J. G., and J. J. Sims. 1998. Preparation, cleanup, and preliminary fractionation of extracts, pp. 1–37. *In* J. G. Millar and K. F. Haynes (eds.), Methods in chemical ecology: volume 1. Chemical methods. Chapman and Hall, New York, NY.

Millar, J. G., L. M. Hanks, J. A. Moreira, J. D. Barbour, and E. S. Lacey. 2009. Pheromone chemistry of cerambycid beetles, pp. 52–79. *In* K. Nakamuta and J. G. Millar (eds.), Chemical ecology of wood-boring insects. Forestry and Forest Products Research Institute, Ibaraki, Japan.

Millar, J. G., R. F. Mitchell, L. R. Meier, T. D. Johnson, J. A. Mongold-Diers, and L. M. Hanks. 2017. (2*E*,6*Z*,9*Z*)-2,6,9-Pentadecatrienal as a male-produced aggregation-sex pheromone of the cerambycid beetle *Elaphidion mucronatum*. J. Chem. Ecol. 43: 75–83.

Millar, J. G., R. F. Mitchell, J. A. Mongold-Diers, Y. Zou, C. E. Bográn, M. K. Fierke, M. D. Ginzel, C. W. Johnson, J. R. Meeker, T. M. Poland, *et al.* 2018. Identifying possible pheromones of cerambycid beetles by field testing known pheromone components in four widely separated regions of the United States. J. Econ. Entomol. 111: 252–259.

Millar, J. G., A. B. Richards, S. Halloran, Y. Zou, E. A. Boyd, K. N. Quigley, and L. M. Hanks. 2019. Pheromone identification by proxy: identification of aggregation-sex pheromones of North American cerambycid beetles as a strategy to identify pheromones of invasive Asian congeners. J. Pest Sci. 92: 213–220.

Miller, D. R., C. M. Crowe, P. D. Mayo, P. J. Silk, and J. D. Sweeney. 2015. Responses of Cerambycidae and other insects to traps baited with ethanol, 2,3-hexanediol, and 3,2-hydroxyketone lures in North-Central Georgia. J. Econ. Entomol. 108: 2354–2365.

Mitchell, R. F., D. T. Hughes, C. W. Luetje, J. G. Millar, F. Soriano-Agatón, L. M. Hanks, and H. M. Robertson. 2012. Sequencing and characterizing odorant receptors of the cerambycid beetle *Megacyllene caryae*. Insect Biochem. Mol. Biol. 42: 499–505.

Mitchell, R. F., P. F. Reagel, J. C. H. Wong, L. R. Meier, W. D. Silva, J. Mongold-Diers, J. G. Millar, and L. M. Hanks. 2015. Cerambycid beetle species with similar pheromones are segregated by phenology and minor pheromone components. J. Chem. Ecol. 41: 431–440.

Mitchell, R. F., T. Curkovic, J. A. Mongold-Diers, L. Neuteboom, H. M. Galbrecht, A. Tröger, J. Bergmann, W. Francke, and L. M. Hanks. 2017. Evidence that cerambycid beetles mimic vespid wasps in odor as well as appearance. J. Chem. Ecol. 43: 75–83.

Mitchell, R. F., A. M. Ray, L. M. Hanks, and J. G. Millar. 2018. The common natural products (*S*)-α-terpineol and (*E*)-2-hexenol are important pheromone components of *Megacyllene antennata* (Coleoptera: Cerambycidae). Environ. Entomol. 47: 1547–1552.

Molander, M. A., and M. C. Larsson. 2018. Identification of the aggregation-sex pheromone of the cerambycid beetle *Phymatodes pusillus* ssp. *pusillus* and evidence of a synergistic effect from a heterospecific pheromone component. J. Chem. Ecol. 44: 987–998.

Monné, M. A., and F. T. Hovore. 2005. Checklist of the Cerambycidae (Coleoptera) of the Western Hemisphere. BioQuip, Rancho Dominguez, CA.

Mori, K. 2009. Pheromone synthesis. Part 240: cross-metathesis with Grubbs I (but not Grubbs II) catalyst for the synthesis of (*R*)-trogodermal (14-methyl-8-hexadecenal) to study the optical rotatory powers of compounds with a terminal *sec*-butyl group. Tetrahedron. 65: 3900–3909.

Mori, K., T. Tashiro, B. Zhao, D. M. Suckling, and A. M. El-Sayed. 2010. Pheromone synthesis. Part 243: synthesis and biological evaluation of (3*R*,13*R*,1′*S*)-1′-ethyl-2′-methylpropyl 3,13-dimethylpentadecanoate, the major component of the sex pheromone of Paulownia bagworm, *Clania variegata*, and its stereoisomers. Tetrahedron. 66: 2642–2653.

Nakamuta, K., W. S. Leal, T. Nakashima, M. Tokoro, M. Ono, and M. Nakanishi. 1997. Increase of trap catches by a combination of male sex pheromones and floral attractant in the longhorn beetle, *Anaglyptus subfasciatus*. J. Chem. Ecol. 23: 1635–1640.

Narai, Y., Y. Zou, K. Nakamuta, J. A. Mongold-Diers, L. M. Hanks, and J. G. Millar. 2015. Candidate aggregation pheromones of two potentially invasive Asian cerambycid species in the genus *Xylotrechus*. J. Econ. Entomol. 108: 1444–1446.

Quinn, G. P., and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, United Kingdom.

Ray, A. M., I. P. Swift, J. A. Moreira, J. G. Millar, and L. M. Hanks. 2009. (*R*)-3-hydroxyhexan-2-one is a major pheromone component of *Anelaphus inflaticollis* (Coleoptera: Cerambycidae). Environ. Entomol. 38: 1462–1466.

Ray, A. M., J. G. Millar, J. A. Moreira, J. S. McElfresh, R. F. Mitchell, J. D. Barbour, and L. M. Hanks. 2015. North American species of cerambycid beetles in the genus *Neoclytus* share a common hydroxyhexanone-hexanediol pheromone structural motif. J. Econ. Entomol. 108: 1860–1868.

Sakai, T., Y. Nakagawa, J. Takahashi, K. Iwabuchi, and K. Ishii. 1984. Isolation and identification of the male sex pheromone of the grape borer, *Xylotrechus pyrrhoderus* Bates (Coleoptera: Cerambycidae). Chem. Lett. 1984: 263–264.

SAS Institute. 2011. SAS/STAT users guide release 9.3. SAS Institute, Cary, NC.

Schröder, F., R. Fettköther, U. Noldt, K. Dettner, W. A. König, and W. Francke. 1994. Synthesis of (3*R*)-3-hydroxy-2-hexanone, (2*R*,3*R*)-2,3-hexanediol and (2*S*,3*R*)-2,3-hexanediol, the male sex pheromone of *Hylotrupes bajulus* and *Pyrrhidium sanguineum* (Cerambycidae). Liebigs Ann. Chem. 1994: 1211–1218.

Silva, W. D., Y. Zou, J. M. S. Bento, L. M. Hanks, and J. G. Millar. 2017. Aggregation-sex pheromones and likely pheromones of 11 South American cerambycid beetles, and partitioning of pheromone channels. Frontiers Ecol. Evol. 5: article 101.

Silva, W. D., J. G. Millar, L. M. Hanks, C. M. Costa, M. O. G. Leite, M. Tonelli, and J. M. S. Bento. 2018. Interspecific cross-attraction between the South American cerambycid beetles *Cotyclytus curvatus* and *Megacyllene acuta* is averted by minor pheromone components. J. Chem. Ecol. 44: 268–275.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd ed. W. H. Freeman, New York, NY.

Swift, I. P., and A. M. Ray. 2010. Nomenclatural changes in North American *Phymatodes* Mulsant (Coleoptera: Cerambycidae). Zootaxa 2448: 35–52.

Wickham, J. D., W. Lu, L. W. Zhang, Y. Chen, Y. Zou, L. M. Hanks, and J. G. Millar. 2016. Likely aggregation-sex pheromones of the invasive beetle *Callidiellum villosulum*, and the related Asian species *Allotraeus asiaticus*, *Semanotus bifasciatus*, and *Xylotrechus buqueti* (Coleoptera: Cerambycidae). J. Econ. Entomol. 109: 2243–2246.

Wong, J. C. H., L. R. Meier, Y. Zou, J. A. Mongold-Diers, and L. M. Hanks. 2017. Evaluation of methods used in testing attraction of cerambycid beetles to pheromone-baited traps. J. Econ. Entomol. 110: 2269–2274.

Yanega, D. 1996. Field guide to northeastern longhorned beetles (Coleoptera: Cerambycidae). Manual 6, Illinois Natural History Survey, Champaign, IL.

Zou, Y., C. E. Rutledge, K. Nakamuta, C. T. Maier, L. M. Hanks, A. B. Richards, E. S. Lacey, and J. G. Millar. 2016. Identification of a pheromone component and a critical synergist for the invasive beetle *Callidiellum rufipenne* (Coleoptera: Cerambycidae). Environ. Entomol. 45: 216–222.

Downloaded from https://academic.oup.com/jee/article/112/5/2236/5499365 by guest on 20 August 2022