

Complex Blends of Synthetic Pheromones are Effective Multi-Species Attractants for Longhorned Beetles (Coleoptera: Cerambycidae)

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Abstract

The wood-boring larvae of longhorned beetles (Coleoptera: Cerambycidae) can be important pests of woody plants, particularly as invasive species introduced by international commerce. Previous research has revealed that cerambycid species native to different parts of the world often share the same aggregation-sex pheromones and that pheromones of different species can be combined to create multi-species attractants that would be advantageous for surveillance monitoring for a number of species simultaneously. To explore the extent to which these chemicals can be combined into single lures, we developed four different blends of six to eight compounds and tested their effects as attractants for a community of longhorned beetle species in Iowa. The blends included known pheromones of species native to the study site, as well as pheromones identified from cerambycid species native to other parts of the world. The experiment confirmed that several cerambycid species were attracted by specific blends, in accordance with their known pheromone chemistry, and despite the presence of pheromone components of heterospecifics. This finding lends further support to developing multi-component blends that can effectively monitor for new incursions of multiple exotic species concurrently.

Key words: semiochemical, surveillance, monitoring, insect trap

The wood-boring larvae of longhorned beetles (Coleoptera: Cerambycidae), when sequestered within wooden packing materials and products, are readily transported by international commerce, and the emerging adults can become economically important pests when introduced into new regions of the world (Eyre and Haack 2017). It is essential that strategies are developed to monitor for incursions of these exotic species at transportation hubs and other potential points-of-entry while their populations are still at low density and eradication is possible (Rassati et al. 2014).

Over the last decade, aggregation-sex pheromones, produced by males and attracting both sexes, have been identified for more than 100 species of cerambycid beetles in the two largest subfamilies, the Cerambycinae and Lamiinae (Hanks and Millar 2016). There is increasing evidence that pheromone structures of cerambycids are often conserved among congeners in different parts of the world, and even among species in different tribes or subfamilies (Millar and Hanks 2017). For example, 3-hydroxyhexan-2-one and the related 2,3-hexanediols comprise pheromones of cerambycine species native to all six inhabited continents (Hanks and Millar 2016). Similarly,

pheromone components of lamiines which are widely shared among species include the terpenoid fuscumol ([E]-6,10-dimethylundeca-5,9-dien-2-ol), also a pheromone of cerambycids in the subfamily Spondylidinae, and its corresponding acetate, as well as monochamol (2-[undecyloxy]ethanol; Hanks and Millar 2016).

Earlier studies have demonstrated that pheromones of different cerambycid species can be combined to create blends which attract multiple species, and that attraction of species to their pheromones within the blends is usually not antagonized by the presence of the other pheromone components from heterospecifics (e.g., Hanks et al. 2018, Millar et al. 2018, Fan et al. 2019). Such pheromone blends would be advantageous in surveillance monitoring for exotic species because they would dramatically reduce the number of traps required, while still providing effective monitoring for a broad range of cerambycid taxa.

Our objective was to test attraction of a community of cerambycid species native to the midwestern United States to their pheromones, when presented as complex blends of six to eight chemicals that included known or suspected pheromones of many native

species, as well as pheromones of cerambycid species native to other parts of the world.

Materials and Methods

The field experiment was conducted at Nine Eagles State Park in Decatur County, Iowa (lat./long. of trap transects: 40.59502, -93.77385; 40.58990, -93.76195; and 40.59730, -93.75936) that consists primarily of second-growth and old-growth hardwood forest dominated by oak (*Quercus* species) and hickory (*Carya* species), but the western-most location was bottomland with an additional mix of black walnut (*Juglans nigra* L.), honeylocust (*Gleditsia triacanthos* L.), common hackberry (*Celtis occidentalis* L.), American elm (*Ulmus americana* L.), and buckeye (*Aesculus* species). Beetles were caught with cross-vane panel traps (black corrugated plastic; AlphaScents, Portland, OR) coated with a lubricant (Fluon PTFE fluoropolymer; AGC Chemicals Americas, Inc., Exton, PA) to improve trapping efficiency (Graham et al. 2010). Traps were suspended from inverted L-shaped frames of polyvinylchloride irrigation pipe, with the base of the trap ~1 m above the ground. Trap basins were partially filled with commercial automotive coolant (50:50 ethylene glycol:water) to kill and preserve captured beetles.

Traps were baited with lures consisting of low-density polyethylene sachets (press-seal bags, Bagette model 14770, 5.1 × 7.6 cm, 0.05 mm thick, Cousin Corp., Largo, FL) containing a dental cotton roll and loaded with blends composed of 25 mg of nonracemic compounds, or 50 mg of racemic compounds (i.e., 25 mg of each enantiomer) in 1 ml of isopropanol. Previous studies have confirmed that the tested chemicals readily diffuse through the walls of these

sachets (Hanks et al. 2018, Millar et al. 2018). Solvent control lures contained 1 ml of isopropanol. Chemicals were purchased from commercial sources or were synthesized as described in earlier publications (Table 1).

The test blends represented combinations of compounds that were known to be pheromones or at least attractants of cerambycid species native to the Midwest, plus a variety of other pheromone components of cerambycid species native to other parts of the world (for references, see Hanks and Millar 2016, Millar and Hanks 2017, unless stated otherwise). These chemicals were as follows (Table 1):

- 1) Pheromones of individual cerambycid species, including the native species *Cyrtophorus verrucosus* (Olivier) ([R]-3-hydroxyhexan-2-one + nonan-2-one), *Dryobius sexnotatus* Linsley ([R]-3-hydroxyhexan-2-one + semanopyrrole), *Elaphidion mucronatum* (Say) ([2E,6Z,9Z]-2,6,9-pentadecatrienal; Millar et al. 2017), *Heterachthes quadrimaculatus* Haldeman (10-methyldodecanol + nonan-2-one), *Tylonotus bimaculatus* Haldeman (tylonotone; Zou et al. 2015), and the exotic species *Trichoferus campestris* (Faldermann) (trichoferone; Ray et al. 2019)
- 2) Chemicals targeting multiple species of cerambycines included racemic 3-hydroxyhexan-2-one (henceforth 3-ketol), racemic *anti*-(2,3)-hexanediol, and racemic 2-methylbutan-1-ol;
- 3) Compounds that are pheromone components of lamiine species in the area of our study, including species of *Monochamus* (monochemol) and species in the tribes Acanthocinini and Acanthoderini (fusicumol, fusicumol acetate, geranylacetone, sulcatol);

Table 1. Composition of four pheromone blends

Blend	Chemical name	Common name	Source/reference for synthesis
1	6-methyl-5-hepten-2-ol	Sulcatol	Alfa Aesar
1	2-methylbutan-1-ol		Aldrich Chemical
1	(E)-2-hydroxyoct-4-en-3-one	Tylonotone	Zou et al. (2015)
1	2-(undecyloxy)ethanol	Monochemol	Bedoukian
1	3-hydroxyoctan-2-one		Lacey et al. (2007)
1	(4R,9Z)-hexadec-9-en-4-olide	(R)-desmolactone	Ray et al. (2014)
2	(E)-6,10-dimethylundeca-5,9-dien-2-ol	Fusicumol	Bedoukian
2	(E)-geranylacetone	Geranylacetone	Bedoukian
2	2-methylbutan-1-ol		Aldrich Chemical
2	3-hydroxyhexan-2-one	3-Ketol	Bedoukian
2	3-methylthioprop-1-ol	Methionol	Oakwood Products Inc.
2	6-methyl-3,5-heptadien-2-one		Alfa Aesar
2	<i>anti</i> -(2,3)-hexanediol		Lacey et al. (2004)
3	(E)-6,10-dimethylundeca-5,9-dien-2-yl acetate	Fusicumol acetate	Bedoukian
3	(E)-geranylacetone	Geranylacetone	Aldrich Chemical
3	3-hydroxyhexan-2-one	3-Ketol	Bedoukian
3	(2R,4R)-2-hydroxy-4-methyl-1-phenylhexan-3-one	Trichoferone	Ray et al. (2019)
3	(Z)-8-pentadecen-1-ol		unpub. data ^a
3	(2E,6Z,9Z)-2,6,9-pentadecatrienal		Millar et al. (2017)
3	decan-2-one		TCI America
3	1-(1H-pyrrol-2-yl)-1,2-propanedione	Semanopyrrole	Chemtika
4	(E)-6,10-dimethylundeca-5,9-dien-2-ol	Fusicumol	Bedoukian
4	(E)-6,10-dimethylundeca-5,9-dien-2-yl acetate	Fusicumol acetate	Bedoukian
4	(E)-geranylacetone	Geranylacetone	Bedoukian
4	(R)-(+)-1-phenylethanol		Alfa Aesar
4	10-methyldodecanol		Silva et al. (2018)
4	nonan-2-one		Aldrich Chemical

Commercial sources: Aldrich Chemical (Milwaukee, WI), Alfa Aesar (Haverhill, MA), Bedoukian Research Inc. (Danbury, CT), Chemtika (Heredia, Costa Rica), Oakwood Products Inc. (Estill, SC), and TCI America (Portland, OR).

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4) Pheromone components of cerambycid species native to other parts of the United States or other parts of the world, including 3-hydroxyoctan-2-one, 6-methyl-3,5-heptadien-2-one, methionol, (R)-(+)-phenylethanol, (Z)-8-pentadecen-1-ol, and decan-2-one (Table 1).

In devising blends, consideration was given to combining potentially synergistic compounds and avoiding antagonistic effects. For example, 2-methylbutan-1-ol was paired with 3-ketol in Blend 2 because of synergism for some cerambycine species of the genus *Phymatodes*, but the two compounds were separated in Blends 1 and 3 because each antagonizes attraction of some species to the other (Hanks et al. 2019). Similarly, Blends 2, 3, and 4 contained various combinations of fuscumol, fuscumol acetate, and geranylacetone because of potential antagonistic effects for lamiine species that do not produce all three compounds (Meier et al. 2016, 2020). In addition, Blend 1 contained (R)-desmolactone ((4R,9Z)-hexadec-9-en-4-olide), the pheromone of lepturine species in the genus *Desmocerus* (Ray et al. 2014).

The experiment to assess the attraction of cerambycid species to the blends was run from 22 May to 3 August 2019. Five traps were set up ~5 m apart in each of three transects (separated by 50 to 400 m). Previous studies have demonstrated that this is an ample distance between traps to prevent interference between treatments within a transect (Wong et al. 2017). Blends were randomly assigned to traps on the day of setup, including one control trap baited with isopropanol. Insects were collected from traps every 2 wk, at which time new lures were added to traps.

Data from the field experiment were analyzed separately for all species represented by at least 15 specimens using a generalized linear mixed model (Proc GLIMMIX, SAS Inst. 2011) with the negative binomial distribution (log link; Bolker et al. 2009). Thus, the response variable was the number of beetles per replicate, trap transect and collection date were random effects, and the experiment was replicated over both space and time. Dates with no beetles of a particular species in any trap within a replicate, i.e., all treatments within a single replicate, were dropped from analyses to reduce unnecessary variance that would complicate the analyses. The cause of absence of a particular species from a replicate is unknown but may be due to one or several interacting factors, such as curtailed flight activity during inclement weather, or sampling outside the annual activity period of a particular species. The significance level of *P* was adjusted according to the sequential Bonferroni procedure in recognition of the 18 independent analyses (Quinn and Keough 2002). For analyses meeting this overall level of significance, pairs of treatment means were compared using the Tukey–Kramer method (Proc GLIMMIX; SAS Institute 2011).

Taxonomy of trapped beetles follows Monné and Hovore (2005). Representative specimens of each species have been retained in the personal collection of the first author.

Results and Discussion

During the field experiment, traps captured a total of 1,235 longhorned beetles of 48 species that represented five subfamilies of the Cerambycidae (Table 2; Fig. 1). Three specimens of a species in the closely related Disteniidae also were trapped. Most of the beetles were cerambycines and lamiines, as expected, given that most of the chemicals in the blends were known pheromones of species in those subfamilies. Species showing significant treatment effects were as follows.

Table 2. Taxonomy and numbers of cerambycid beetles captured during the field bioassay, Nine Eagles State Park, Decatur Co., Iowa

Taxonomy	Count
Cerambycinae	
<i>Anaglyptini</i>	
<i>Cyrtophorus verrucosus</i> (Olivier)	26
<i>Bothriospilini</i>	
<i>Knulliana c. cincta</i> (Drury)	25
<i>Callidiini</i>	
<i>Phymatodes lengi</i> Joutel	3
<i>Clytini</i>	
<i>Neoclytus a. acuminatus</i> (F.)	2
<i>Neoclytus m. mucronatus</i> (E.)	78
<i>Neoclytus scutellaris</i> (Olivier)	9
<i>Sarosesthes fulminans</i> (F.)	58
<i>Xylotrechus colonus</i> (F.)	149
<i>Eburiini</i>	
<i>Eburia quadrigeminata</i> (L.)	5
<i>Elaphidiini</i>	
<i>Anelaphus pumilus</i> (Newman)	250
<i>Anelaphus parallelus</i> (Newman)	2
<i>Anelaphus villosus</i> (F.)	19
<i>Elaphidion mucronatum</i> (Say)	18
<i>Parelaphidion aspersum</i> (Haldeman)	9
<i>Parelaphidion incertum</i> (Newman)	9
<i>Hesperophanini</i>	
<i>Tylonotus bimaculatus</i> Haldeman	20
<i>Neoibidionini</i>	
<i>Heterachthes quadrimaculatus</i> Haldeman	26
<i>Trachyderini</i>	
<i>Purpuricenax axillaris</i> Haldeman	1
Lamiinae	
<i>Acanthocinini</i>	
<i>Astyleiopus variegatus</i> (Haldeman)	16
<i>Astyliidius parvus</i> (LeConte)	6
<i>Astylopsis macula</i> (Say)	61
<i>Graphisurus despectus</i> (LeConte)	61
<i>Graphisurus fasciatus</i> (Degeer)	62
<i>Graphisurus triangulifer</i> (Haldeman)	5
<i>Leptostylus transversus</i> (Gyllenhal)	25
<i>Lepturges angulatus</i> (LeConte)	114
<i>Lepturges confluens</i> (Haldeman)	53
<i>Lepturges pictus</i> (LeConte)	1
<i>Lepturges regularis</i> (LeConte)	1
<i>Lepturges symmetricus</i> (Haldeman)	2
<i>Sternidius alpha</i> (Say)	11
<i>Urgleptes querci</i> (Fitch)	2
<i>Acanthoderini</i>	
<i>Acanthoderes quadrigibba</i> (Say)	1
<i>Aegomorphus modestus</i> (Gyllenhal)	62
<i>Desmiphorini</i>	
<i>Eupogonius pauper</i> LeConte	2
<i>Psenocerus supernotatus</i> (Say)	7
<i>Dorcaschematini</i>	
<i>Dorcaschema cinereum</i> (Olivier)	10
<i>Dorcaschema nigrum</i> (Say)	2
<i>Saperdini</i>	
<i>Saperda discoidea</i> F.	1
<i>Saperda imitans</i> Felt and Joutel	4
<i>Saperda l. lateralis</i> F.	3
Lepturinae	
<i>Lepturini</i>	
<i>Bellamira scalaris</i> (Say)	2
<i>Brachyleptura rubrica</i> (Say)	1
<i>Stenocorus cinnamopterus</i> (Randall)	2
<i>Typocerus v. velutinus</i> (Olivier)	2

Taxonomy	Count
Necydalinae	
<i>Necydalis mellita</i> (Say)	2
Parandrinae	
<i>Parandrini</i>	
<i>Neandra brunnea</i> (F.)	2
Disteniidae	
<i>Disteniini</i>	
<i>Elytrimitatrix undata</i> (F.)	3
Total	1,235

Cerambycinae

- 1) The pheromone of *Anelaphus pumilus* (Newman) is composed primarily of (*R*)-3-ketol (Mitchell et al. 2015), and adults of that species were strongly attracted to Blend 3, which contained racemic 3-ketol. However, the lack of significant attraction to Blend 2, which also contained 3-ketol, suggested antagonism by one or more of the other components of that blend.
- 2) The pheromone of *C. verrucosus* is composed of (*R*)-3-ketol + nonan-2-one (Mitchell et al. 2013), and consistent with earlier findings, adults were attracted by the 3-ketol present in Blend 3, but not to nonan-2-one in the absence of 3-ketol in Blend 4 (e.g., Mitchell et al. 2013). The lack of attraction to 3-ketol in Blend 2 suggests antagonism by one or more of the other components.
- 3) Adults of *E. mucronatum* were significantly attracted only by Blend 3, the only blend which contained its pheromone, (2*E*,6*Z*,9*Z*)-2,6,9-pentadecatrienal (Millar et al. 2017).
- 4) Adults of *H. quadrimaculatus* were attracted only by Blend 4, the only blend which contained the synergistic combination of its two pheromone components 10-methyl-dodecanol and nonan-2-one (unpublished data).
- 5) The pheromone of *Neoclytus m. mucronatus* (F.) is composed primarily of (*R*)-3-ketol, and racemic 3-ketol is a sufficient attractant (Millar et al. 2018, Hanks et al. 2019), accounting for attraction of this species to both blends 2 and 3.
- 6) Adults of *Sarosesthes fulminans* (F.) (Fig. 1A) were attracted by Blend 2, the only blend which contained the synergistic blend of its pheromone components, i.e., 3-ketol + *anti*-(2,3)-hexanediol + 2-methylbutan-1-ol (Lacey et al. 2009, Hanks et al. 2019).
- 7) Adults of *T. bimaculatus* were attracted only by Blend 1, containing its pheromone tylenotone (Zou et al. 2015).
- 8) Adults of *Xylotrechus colonus* (F.) were attracted by both Blends 2 and 3, consistent with attraction to the 3-ketol present in both blends (Lacey et al. 2009). Earlier research had shown that attraction of this species to the ketol was not influenced by at least some of the other components of these blends, including 2-methylbutan-1-ol, *anti*-(2,3)-hexanediol, and semanopyrrole (Diesel et al. 2017, Millar et al. 2018, Hanks et al. 2019).

Lamiinae

- 9) Males of both *Astyloopsis macula* (Say) and *Leptostylus transversus* (Gyllenhal) produce a blend of (*S*)-sulcatol and sulcatone, but racemic sulcatol is a sufficient attractant (Meier et al. 2019), accounting for attraction of both species to Blend 1, the only blend containing this compound.
- 10) The pheromone of *Astyleiopus variegatus* (Haldeman) is composed of (*S*)-fusicumol + (*S*)-fusicumol acetate + a trace of geranylacetone (Meier et al. 2020) and fusicumol acetate is a

sufficient attractant (Mitchell et al. 2011), accounting for attraction of this species to both Blends 3 and 4.

- 11) The pheromone of *Graphisurus deceptus* (LeConte) is not known; therefore, attraction to Blends 3 and 4 may be the first insight into its pheromone chemistry.

Six of the species represented by at least 15 specimens did not show significant treatment effects (Table 3), but in some cases, trends in the data are consistent with what is known about their pheromone chemistry. For example, methionol is among the compounds produced by males of *Knulliana c. cincta* (Drury) (unpublished data), and most of the beetles of that species were caught by traps baited with the blend that contained that compound, Blend 2. The pheromone of *Aegomorphus modestus* (Gyllenhal) is composed of both enantiomers of fusicumol acetate plus geranylacetone as a minor component, and aggregation to these compounds is antagonized by fusicumol (Meier et al. 2020), which explains the greater numbers coming to traps baited with Blend 3. The pheromone of *Graphisurus fasciatus* (Degeer) is not known, but earlier studies have shown that adults of this species can be attracted by fusicumol and related compounds such as those present in Blends 2, 3, and 4 (e.g., Schmeelk et al. 2016, Hanks and Millar 2018, Millar and Hanks 2018). Males of *Lepturges angulatus* (LeConte) and its congener *L. confluens* (Haldeman) produce a blend of both enantiomers of fusicumol acetate plus geranylacetone (Meier et al. 2016). Racemic fusicumol acetate alone is a sufficient attractant for both *Lepturges* species (Meier et al. 2016, 2020), which accounts for the greater numbers in traps baited with Blends 3 and 4. The pheromone of the cerambycine *Anelaphus villosus* (F.) also is not known.

It also should be noted that some of the chemicals in the blends probably failed to attract species for which they are known pheromones simply due to the absence of those species in the study area. For example, the exotic species *T. campestris* is not yet known to be present in Iowa, providing a likely explanation for why no beetles of this species were attracted by its pheromone trichiferone, present in Blend 3. Similarly, the pheromone of the native species *D. sexnotatus* ([*R*]-3-hydroxyhexan-2-one + semanopyrrole) in Blend 3 attracted no beetles, likely because the study site is outside the geographic range of this rare species (Perry et al. 1974). Some of the species that were significantly attracted by experimental treatments are among the most 'common' and 'abundant' cerambycids of the eastern United States (for information about the general abundance of species, see Yanega 1996, Lingafelter 2007, Hanks et al. 2014), including *C. verrucosus*, *E. mucronatum*, *X. colonus*, *A. modestus*, *A. variegatus*, *L. transversus*, *L. angulatus*, and *L. confluens*. Others are considered rare, including *S. fulminans*, *T. bimaculatus*, *H. quadrimaculatus*, and *G. despectus*. Furthermore, some of the species have only recently been reported from Iowa, including *A. pumilus*, *H. quadrimaculatus*, and *N. m. mucronatus* (Rice and Veal 2006). Attraction of significant numbers of such rare species testifies to the effectiveness of pheromone lures as tools for detection of species that probably are at low population densities, such as endangered species, or conversely, invasive species during the early stages of an invasion. Some of the species that did not show significant treatment effects also have been only recently reported from Iowa, including *Lepturges pictus* (LeConte) and *Lepturges regularis* (LeConte) (Fig. 1B; Rice and Veal 2006). *Graphisurus triangulifer* (Haldeman) was previously unreported from Iowa.

The results from this study and a number of other recent studies suggest that the deployment of generic blends of pheromones of a number of cerambycid species can be an effective strategy for attracting multiple target species simultaneously (e.g., Hanks et al.

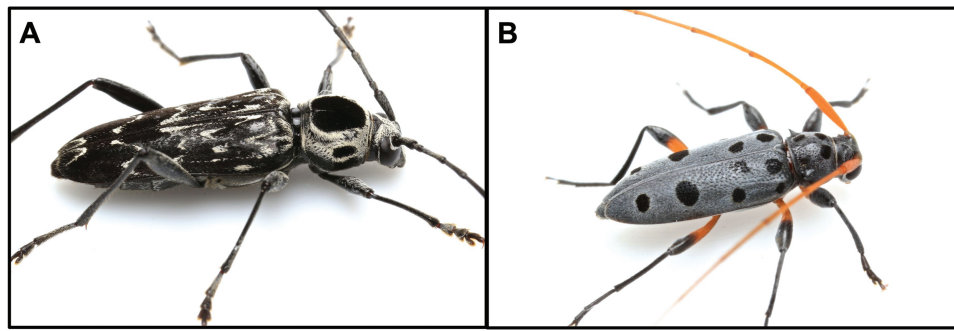


Fig. 1. Two of the cerambycid beetle species that were represented among specimens caught during the field experiment in Iowa: (A) the cerambycine *Sarosesthes fulminans* (F.), (B) the lamiine *Lepturges regularis* (LeConte).

Table 3. Mean (\pm SE) number of cerambycid beetles captured per treatment and replicate during the field bioassay in Decatur Co., Iowa in 2019, and results of generalized linear models test (Proc GLIMMIX), for the species represented by at least 15 specimens

Species	Blend 1	Blend 2	Blend 3	Blend 4	Control	Proc GLIMMIX (d.f.; P)	Bonferroni α
Cerambycinae							
<i>A. pumilus</i>	0b	4.4 \pm 1.2b	23.2 \pm 9.6a	0.1 \pm 0.1b	0b	7.85 (4,36; 0.0001)	0.003
<i>A. villosus</i>	0	0.14 \pm 0.1	0.43 \pm 0.2	2.1 \pm 1.1	0	4.0 (4,25; 0.012)	0.01
<i>C. verrucosus</i>	0.38 \pm 0.26b	0b	2.6 \pm 0.56a	0.25 \pm 0.16b	0b	23.0 (4,31; <0.0001)	0.004
<i>E. mucronatum</i>	0.17 \pm 0.17b	0.83 \pm 0.4ab	1.7 \pm 0.3a	0.3 \pm 0.3b	0b	6.8 (4,30; 0.0044)	0.007
<i>H. quadrimaculatus</i>	0b	0b	0b	2.6 \pm 0.65a	0.3 \pm 0.3b	13.5 (4,36; <0.0001)	0.003
<i>K. c. cincta</i>	0.1 \pm 0.1	2.3 \pm 1.2	0.86 \pm 0.3	0.1 \pm 0.1	0.1 \pm 0.1	4.2 (4,25; 0.0094)	0.008
<i>N. m. mucronatus</i>	0.7 \pm 0.7bc	3.2 \pm 1.3ab	3.8 \pm 0.7a	0.9 \pm 0.8abc	0.1 \pm 0.1c	5.5 (4,36; 0.0014)	0.006
<i>S. fulminans</i>	0.2 \pm 0.16b	3.9 \pm 1.0a	0.07 \pm 0.07b	0b	0b	19.3 (4,59; <0.0001)	0.003
<i>T. bimaculatus</i>	2.5 \pm 0.8a	0.83 \pm 0.5ab	0b	0b	0b	10.8 (4,21; <0.0001)	0.004
<i>X. colonus</i>	0.4 \pm 0.17b	4.3 \pm 0.9a	4.9 \pm 1.3a	0.86 \pm 0.3b	0.1 \pm 0.1b	12.7 (4,59; <0.0001)	0.003
Lamiinae							
<i>A. macula</i>	4.3 \pm 1.8a	1.8 \pm 1.0b	0.22 \pm 0.2b	0.11 \pm 0.1b	0.33 \pm 0.17b	4.7 (4,35; 0.0037)	0.006
<i>A. modestus</i>	0.89 \pm 0.68	2.1 \pm 1.6	3.0 \pm 0.8	0.89 \pm 0.65	0	2.5 (4,45; 0.06)	0.025
<i>A. variegatus</i>	0.14 \pm 0.14b	0b	1.0 \pm 0.38a	1.1 \pm 0.3a	0b	7.7 (4,26; 0.0003)	0.004
<i>G. despectus</i>	0.4 \pm 0.2b	0.8 \pm 0.5ab	2.4 \pm 0.8a	2.3 \pm 0.6a	0.2 \pm 0.2b	5.6 (4,40; 0.0011)	0.005
<i>G. fasciatus</i>	1.0 \pm 0.4	1.4 \pm 0.5	1.7 \pm 0.7	1.9 \pm 0.9	0.2 \pm 0.1	2.1 (4,40; 0.11)	0.05
<i>L. angulatus</i>	0	2.8 \pm 2.6	4.3 \pm 1.2	4.3 \pm 2.3	0	2.5 (4,40; 0.057)	0.02
<i>L. confluens</i>	0.4 \pm 0.4	0.8 \pm 0.25	1.8 \pm 0.6	2.3 \pm 1.1	0	3.5 (4,40; 0.015)	0.01
<i>L. transversus</i>	1.5 \pm 0.48a	0.5 \pm 0.2b	0.1 \pm 0.1b	0.2 \pm 0.1b	0.2 \pm 0.2b	6.3 (4,39; 0.0006)	0.005

Significance levels are adjusted according to the sequential Bonferroni procedure (Quinn and Keough 2002). Generalized linear model values shown in bold font are significant relative to the Bonferroni α , means in bold font are significantly different from controls, and means within species with the same letters are not significantly different (Tukey–Kramer test at $P < 0.05$).

2012, 2018; Millar et al. 2018; Fan et al. 2019; Rassati et al. 2019). This finding stands in marked contrast to other insect taxa such as Lepidoptera, in which even traces of contaminants, isomers of pheromone components, or pheromone components of related species can completely inhibit attraction to lures. The literature on lepidopteran pheromones is replete with examples of such inhibitory interactions, due in large part to the necessity for having a unique pheromone channel to avoid unproductive cross-attraction to sympatric heterospecifics which may have similar pheromone blends. For example, in bioassays of 75 binary combinations of lepidopteran pheromones, Brockerhoff et al. (2013) found evidence for some degree of inhibition of attraction in the majority of cases. Several factors may contribute to this marked difference between cerambycids and lepidopterans. First, with lepidopterans, pheromone lures are reconstructions of female-produced sex pheromones, which serve the clearly defined function of attracting mates by scramble competition, with a winner-take-all outcome. Hence, there is strong selection pressure to avoid mistaken cross-attraction to heterospecifics, or cross-attraction of heterospecifics. In contrast, with the exception

of prionic acid, a female-produced sex pheromone of species in the subfamily Prioninae (Millar and Hanks 2017), all cerambycid pheromones which are in common use as lures are male-produced aggregation-sex pheromones. Although it is known that these pheromones attract both sexes, their functional roles are not well understood. Clearly, they assist in bringing the sexes together, but the purpose of male-male attraction is less clear. Hence, the overall costs and benefits of using this type of pheromone remain obscure.

Second, there appears to be substantially more structural variation in the pheromone chemistry of cerambycids than Lepidoptera. For example, the majority of known lepidopteran pheromones are straight-chain compounds with chain lengths of 10–23 carbons, one or more double bonds, and terminal alcohol, acetate, or aldehyde functionality, or no functionality at all (Ando and Yamamoto 2019). All of these compounds are derived from modifications of fatty acid biosynthetic pathways. In contrast, the known cerambycid pheromones encompass a much broader array of structures, including straight- and branched-chain compounds, cyclic compounds, compounds with multiple functional groups, and compounds with

heteroatoms such as nitrogen or sulfur (reviewed in Hanks and Millar 2016). From their structures, it is also apparent that cerambycid pheromones are the products of a number of different biosynthetic routes, including fatty acid, terpenoid, and acetogenin pathways. Thus, this broad structural diversity provides a much larger chemical palette with which to work, and so may decrease the need for antagonists to minimize heterospecific attraction. Antagonistic interactions have been documented with some blends of cerambycid pheromones (e.g., Silva et al. 2018, Hanks et al. 2019, Meier et al. 2020) but they appear to be much less common than with the more highly conserved lepidopteran pheromones. Furthermore, within the Cerambycidae, different chemical classes of compounds generally appear to be restricted to specific subfamilies; for example, hydroxyketones have only been found from species in the subfamily Cerambycinae, whereas hydroxyethers have only been found from species in the subfamily Lamiinae (Hanks and Millar 2016). Consequently, the evidence suggests that it is generally possible to blend pheromone components from species in different cerambycid subfamilies, with minimal chance of antagonistic interactions.

Third, cerambycid species may make greater use of temporal segregation of daily activity cycles than moth species. That is, adults of most moth species are nocturnal, whereas adults of various cerambycid species may be diurnal, crepuscular, or nocturnal (e.g., Mitchell et al. 2015, Hanks et al. 2019). Several studies have shown that cerambycids are active during specific time windows within the daily cycle (reviewed in Hanks and Wang 2017). This temporal separation decreases the need for inhibitory compounds to prevent interspecific attraction, and even allows sympatric and seasonally synchronic species to utilize the same pheromones. When using pheromone-baited traps to sample cerambycid communities, these temporal segregation mechanisms are obscured because the lures release pheromone continuously.

In summary, a number of the study species were attracted by particular blends as would be predicted by what is known of their pheromone chemistry, and despite the presence of a number of other components in the test blends. These findings provide further evidence that judiciously chosen combinations of pheromones of cerambycid beetles can be 'stacked' to create complex blends that are efficient multi-species attractants. Although there were a few examples of specific combinations resulting in decreased attraction, in the majority of cases, significant attraction to blends containing as many as seven additional pheromone components of heterospecifics was still achieved. These results should help to guide the formulation of lure blends for effective and efficient monitoring of both native and exotic cerambycid species in the United States, and worldwide.

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