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Research Article

Blends of Pheromones, With and Without Host Plant Volatiles, Can Attract Multiple Species of Cerambycid Beetles Simultaneously

L. M. Hanks,[1,11] J. A. Mongold-Diers,[1] T. H. Atkinson,[2] M. K. Fierke,[3] M. D. Ginzel,[4] E. E. Graham,[5,6] T. M. Poland,[7] A. B. Richards,[8] M. L. Richardson,[9] and J. G. Millar[10]

[1]Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, [2]University of Texas Insect Collection, Lake Austin Center, Austin, TX 78703, [3]Department of Environmental and Forest Biology, State University of New York, Syracuse, NY 13210, [4]Department of Entomology, Purdue University, West Lafayette, IN 47907, [5]Department of Entomology, Michigan State University, East Lansing, MI 48824, [6]Present address: USDA Forest Service, Juneau, AK 99801, [7]USDA Forest Service, Lansing, MI 48910, [8]Aquatic Bioassessment Laboratory, California State University, Chico, CA 95929, [9]College of Agriculture, Urban Sustainability, and Environmental Sciences, University of the District of Columbia, Washington, DC 20008, [10]Department of Entomology, University of California, Riverside, CA 92521, and [11]Corresponding author, e-mail: hanks@life.illinois.edu

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Abstract

Pheromone components of cerambycid beetles are often conserved, with a given compound serving as a pheromone component for multiple related species, including species native to different continents. Consequently, a single synthesized compound may attract multiple species to a trap simultaneously. Furthermore, our previous research in east-central Illinois had demonstrated that pheromones of different species can be combined to attract an even greater diversity of species. Here, we describe the results of field bioassays in the northeastern, midwestern, southeastern, south-central, and southwestern United States that assessed attraction of cerambycids to a 'generic' pheromone blend containing six known cerambycid pheromone components, versus the individual components of the blend, and how attraction was influenced by plant volatiles. Nineteen species were attracted in significant numbers, with the pheromone blend attracting about twice as many species as any of the individual components. The blend attracted species of three subfamilies, whereas individual components attracted species within one subfamily. However, some antagonistic interactions between blend components were identified. The plant volatiles ethanol and α-pinene usually enhanced attraction to the blend. Taken together, these experiments suggest that blends of cerambycid pheromones, if selected carefully to minimize inhibitory effects, can be effective for sampling a diversity of species, and that plant volatiles generally enhance attraction. Such generic pheromone blends may serve as an effective and economical method of detecting incursions of exotic, potentially invasive species.

Key words: chemical ecology, pheromones, monitoring

The large beetle family Cerambycidae includes many species that are among the most important insect pests of woody plants in natural and managed ecosystems worldwide (Haack 2017, Wang 2017). Because the larvae of these species develop within wood and wooden products, they are readily transported around the world by international commerce and so are among the most common potentially invasive species intercepted in international quarantine (Eyre and Haack 2017). Traps baited with synthesized pheromones offer a means of monitoring for new incursions of exotic species of cerambycids, as well as for managing populations of native pest species, and monitoring threatened species (e.g., Rassati et al. 2012, Dutcher and Bactawar 2016, Larsson 2017, Sweeney et al. 2017).

Research to date has suggested that cerambycids use either male- or female-produced attractant pheromones whose function appears to break out along subfamily lines. Thus, aggregation-sex pheromones, produced by males and attracting both sexes, are used by species in the subfamilies Cerambycinae, Lamiinae, and Spondylidinae, whereas female-produced sex pheromones that attract only males are found in the Prioninae and Lepturinae (Hanks and Millar 2016). There also is growing evidence that several pheromone structures have been conserved within the family and are shared among many related species that may be sympatric, or native to different continents (Millar and Hanks 2017). For example, the aggregation-sex pheromones of many cerambycine species comprise individual or blends of stereoisomers of

3-hydroxyalkan-2-ones and/or the related 2,3-alkanediols, whereas those of lamiines and spondylidines are often composed of enantiomers of fuscumol ([*E*]-6,10-dimethylundeca-5,9-dien-2-ol) and/or fuscumol acetate ([*E*]-6,10-dimethylundeca-5,9-dien-2-yl acetate), or monochamol (2-[undecyloxy]ethanol; Hanks and Millar 2016). Similarly, the female-produced sex pheromone prionic acid ([3*R*,5*S*]-3,5-dimethyldodecanoic acid) appears to be shared by at least several genera of prionines in different parts of the world (Barbour et al. 2011, Wickham et al. 2016).

Because of the conservation of pheromone structures within the Cerambycidae, it often is possible to attract multiple species to single-component lures (Hanks et al. 2012, Sweeney et al. 2014, Wickham et al. 2014, Miller et al. 2017). The number and diversity of species that are attracted may be increased further by combining the pheromones of multiple species. Such blends could be advantageous for surveillance monitoring for exotic species, because they would reduce the number of traps necessary to attract species of multiple subfamilies and so reduce costs for materials and labor. In addition, blends may be advantageous because some cerambycid species respond most strongly to synergistic combinations of their pheromone components, and less so or not at all to the individual components (e.g., Lacey et al. 2009, Meier et al. 2016, Zou et al. 2016, Miller et al. 2017). Conversely, chemicals which are not components of the pheromone of a particular species may antagonize attraction of that species. Such antagonism may be an adaptation to avert unproductive attraction to pheromones of heterospecifics (Mitchell et al. 2015, Meier et al. 2016).

As a first step toward devising a multipurpose mixture of synthesized pheromones which would attract the maximum number of cerambycid species, we previously had conducted field tests in east-central Illinois with a blend of three chemicals that are typical pheromones of cerambycines (racemic 3-hydroxyhexan-2-one, *syn*-2,3-hexanediol, racemic 2-methylbutan-1-ol) with three common pheromone components of lamiines (fuscumol, fuscumol acetate, monochamol), versus the individual components (Hanks et al. 2012). Those experiments demonstrated that attraction of a given species to its dominant pheromone components in the blend may indeed be affected by the presence of other components, with attraction of several species being either synergized or antagonized by certain components. A second experiment revealed that attraction to the pheromone blend usually was enhanced by the host plant volatiles ethanol and α-pinene. In an independent study, the two experiments were replicated in counties across the state of Pennsylvania (Hanks and Millar 2013), with more than 30 cerambycid species responding to particular treatments.

Here, we describe results from conducting the same two experiments in a number of widely separated geographic regions of the United States, with the goal of more broadly assessing the efficacy of the same pheromone blend in attracting multiple cerambycid species, with and without host plant volatiles as potential synergists. The experiments were replicated independently at study sites in the northeastern (New York), midwestern (Michigan, Indiana), southeastern (Florida), south-central (Texas), and southwestern (California) United States. A companion article (Millar et al. 2017) summarizes complementary field bioassays in which some of the same cerambycid pheromones were field tested individually and in binary blends at some of the same study sites.

Materials and Methods

Sources of Chemicals

Compounds purchased from commercial sources included racemic 3-hydroxyhexan-2-one (henceforth '3-ketol'), racemic fuscumol, racemic fuscumol acetate, and monochamol (all from Bedoukian Research, Inc., Danbury, CT), and racemic 2-methylbutan-1-ol (Aldrich Chemical, Milwaukee, WI). Racemic *syn*-2,3-hexanediol ('*syn*-diol') was synthesized as described in Lacey et al. (2004), and prionic acid as described in Rodstein et al. (2009). See General Methods of Trapping section below for doses and blends of these compounds. Plant volatiles were emitted from high-release lures (95% ethanol, ~0.4 g/d, model IP036-100; α-pinene, ~2 g/d, model IP037-75; Synergy Semiochemical Corp., Burnaby, British Columbia, Canada).

Study Sites

Suitable sites for bioassays were located by collaborators in the six states, consisting of stands of mixed hardwood and/or coniferous trees in natural or managed environments (Table 1). The number of transects per experiment and duration of experiments were subject to the logistical and time constraints of the individual collaborators (Table 1).

General Methods of Trapping

Bioassays at all study sites used cross-vane panel traps (black corrugated plastic; AlphaScents, Portland, OR) that had been coated with a liquid fluoropolymer lubricant (Fluon, Northern Products, Inc., Woonsocket, RI) to improve trapping efficiency (Graham et al. 2010). In most cases, trap basins contained either diluted propylene glycol or saturated aqueous NaCl with a few drops of detergent to kill and preserve captured beetles. However, at the Florida site the basins contained only water with detergent, because traps usually were checked for beetles every few days.

Trap lures were polyethylene sachets (press-seal bags, Bagette model 14770, 5.1 × 7.6 cm, 0.05-mm thick, Cousin Corp., Largo, FL) that were loaded individually with the six test compounds, or the same six compounds combined in a blend, in both cases using 50 mg of the racemic compounds (i.e., 25 mg of each enantiomer of 3-ketol, *syn*-diol, 2-methylbutan-1-ol, fuscumol, and fuscumol acetate) or 25 mg of the achiral monochamol, in 1 ml of isopropanol (estimated release rates: ~0.77, 0.4, 0.22, 0.4, 0.48, and 0.07 mg/d, respectively). However, individual lures for the sex pheromone prionic acid were loaded with only 1 µl of the synthesized chemical in 1 ml of isopropanol (release rate ~1.8 µg/d), because even minute doses of that compound are highly attractive to *Prionus* species (Rodstein et al. 2011). Solvent control lures contained 1 ml of isopropanol. At most of the sites, traps were suspended <2 m above the ground from steel poles or frames of polyvinyl chloride irrigation pipe. The exceptions were two sites where traps were suspended from tree branches: Texas (traps <2 m above the ground) and California (traps at least 4 m above the ground to prevent damage by bears).

Traps were deployed 5–15 m apart in linear transects with treatments assigned randomly to traps on the day of setup, with one trap per treatment in each transect (see Table 1 for dates of experiments). Treatments for experiment 1 were as follows: 1) 3-ketol, 2) *syn*-diol, 3) 2-methylbutan-1-ol, 4) fuscumol, 5) fuscumol acetate, 6) monochamol, 7) the complete blend of these compounds, and 8) solvent control. At the study site in Florida, fuscumol and fuscumol acetate were blended together, and not tested separately, due to the limited space available for the transect.

Treatments for experiment 2 were as follows: 1) the same blend of six pheromone components, 2) the pheromone blend with the plant volatiles ethanol + α-pinene, 3) plant volatiles alone, and 4) solvent control, with one trap per treatment in each transect. Both experiments were conducted at study sites in New York, Michigan, and Florida, experiment 1 only in Indiana and Texas, and experiment 2 only in California (Table 1). The number of trap transects

Table 1. Study sites for two field experiments that tested attraction of cerambycid beetles to a blend of synthesized pheromones (experiment 1) and the influence of plant volatiles (experiment 2) in various areas of the United States during 2011, and the nature of the surrounding forests

State, experiment, study site	GPS (lat., long.)	Habitat type
New York		
Experiment 1 (25 May to 20 Aug.) and 2 (16 June to 20 Aug.)		
SUNY-ESF James F. Dubuar Memorial For., Adirondack Park, St. Lawrence Co.	44.163, –74.908	Mature hardwoods, managed conifers
Frank E. Jadwin State For., Lewis Co.	44.076, –75.381	Mature northern hardwoods
SUNY-ESF Lafayette Road Field Station, Onondaga Co.	42.991, –76.132	Arboretum: many hardwood and conifer species
SUNY-ESF Heiberg Memorial For., Cortland Co.	42.768, –76.072	Mixed hardwoods and managed conifers
Allegany State Park, Cattaraugus Co.	42.091, –78.851	Mixed hardwoods and conifers
Michigan		
Experiment 1 (23 Jun. to 9 Aug.)		
Michigan State Univ. Tree Res. Center, Lansing, Ingham Co.	42.672, –84.475	Mixed hardwoods
Experiment 2 (21 Jun. to 11 Aug.)		
MSU W. K. Kellogg Experimental Forest, Kalamazoo Co.	42.363, –85.358	Mixed hardwoods and conifers
Lakeshore Park, Novi, Oakland Co.	42.511, –83.485	Mixed hardwoods and conifers
Tollgate Educational Center, Novi, Oakland Co.	42.499, –83.459	Mixed hardwoods
Indiana		
Experiment 1 (10 May to 9 Aug.)		
Martell For., Tippecanoe Co. (2 sites)	40.435, –87.034; 40.442, –87.035	Mixed hardwoods
Florida		
Experiments 1 (24 Apr. to 31 Jul.) and 2 (7 Aug. to 16 Oct.)		
Private residence, Port St. Lucie, St. Lucie Co.	27.231, –80.383	Urban landscape with hardwoods and conifers of many species
Texas		
Experiment 1 (25 Apr. to 5 Jul.)		
Brackenridge Field Lab., Travis Co.	30.281, –97.777	Mixed hardwoods
Stengl Field Lab., Bastrop Co.	30.089, –97.166	Mixed hardwoods and conifers
California		
Experiment 2 (20 Aug. to 16 Oct.)		
Slaughterhouse Ravine, Butte Co.	39.839, –121.617	Mixed oak-conifer
Childs Meadows, Tehama Co.	40.345, –121.484	Conifers

Study sites are ordered so as to progress from states in the northeast to the midwest, south, and west.

per experiment varied from one to four, and transects within a site were widely separated (Table 1). Insects were collected from traps at intervals of 3–14 d, and on those days, treatments were rotated down transects to control for positional effects.

In addition to experiments 1 and 2, a single sentinel trap baited with prionic acid was deployed at study sites in New York, Michigan, Indiana, Florida, and Texas to determine whether there were *Prionus* species present (dates as in Table 1). This prionic acid treatment was not included in the statistical analysis of treatment effects.

Statistical Analysis

For individual species at each study site, overall treatment effects were tested with the nonparametric Friedman's test (PROC FREQ, option CMH; SAS Institute 2011), with the number of replicates based on the number of trap transects (if more than one) and the number of collection dates. Replicates that contained no specimens of the species in question in any of the treatments, which would contribute nothing to testing treatment effects, were dropped from analyses. Data were analyzed only for species that were represented by at least eight specimens at a study site, the minimum number that could result in statistically significant treatment effects. Although the collection date term was highly significant in each analysis, it is not reported here, because trap catches invariably changed with date due to the vagaries of weather and other abiotic and biotic factors. Pairwise comparisons of treatments were tested with the REGWQ test (controlling experiment-wise error rates; SAS Institute 2011) and were protected (i.e., assuming a significant overall Friedman's test).

Captured beetles were identified according to Monné and Hovore (2005) and Lingafelter (2007). Voucher specimens of the captured species were retained by the individual collaborators (those from New York and Florida are available from the laboratory collection of LMH).

Results and Discussion

Bioassays in the six different states resulted in capture of 6,539 cerambycid beetles and a few beetles from the related family Disteniidae (Supp. Table 1 [online only]). These comprised 142 species of cerambycids, including 48 species in 14 tribes of the subfamily Cerambycinae, 55 species in 10 tribes of the Lamiinae, 27 species in 2 tribes of the Lepturinae, 4 species in 2 tribes of the Prioninae, 7 species in 1 tribe of the Spondylidinae, and 1 species of the Parandrinae. Traps in Indiana and Texas also caught 11 adults of the disteniid *Elytrimitatrix undata* (F.). The greater species diversity and/or numbers per species of cerambycines, lamiines, and spondylidines is due to the fact that bioassay treatments included common pheromone components of species in those subfamilies but no known pheromones of lepturines, parandrines, or disteniids (see below in this section). Sentinel traps baited with prionic acid caught males of *Prionus imbricornis* (L.) in Texas and *Prionus pocularis* Dalman in Florida, but no prionines were caught in New York, Michigan, or

Indiana, possibly due to the timing of bioassays in relation to flight periods (Supp. Table 1 [online only]).

For Experiment 1, there were 32 cases of significant treatment effects, with some of the more common species being attracted to the same compounds in multiple states (Table 2). Pheromones, or likely pheromones (i.e., confirmed attractants), already had been identified for most of these species (summarized in Hanks and Millar 2016). For example, the (*R*)-3-hydroxyhexan-2-one enantiomer of 3-ketol is a dominant or sole pheromone component of the cerambycine species (ordered as in Table 2): *Phymatodes aereus* (Newman), *Neoclytus m. mucronatus* (F.), and *Xylotrechus colonus* (F.). Due to earlier taxonomic confusion between *Phymatodes amoenus* (Say) and *Phymatodes lengi* Joutel, the pheromone of the former species has been erroneously identified as being composed of (*R*)-3-hydroxyhexan-2-one and (*R*)-2-methylbutan-1-ol (Mitchell et al. 2015, Hanks and Millar 2016). The pheromone of *P. amoenus* now is known to comprise only (*R*)-2-methylbutan-1-ol, which accounts for attraction of that species to racemic 2-methylbutan-1-ol in the present study ('2-Me-ol' in Table 2). The pheromone of the exotic congener *Phymatodes testaceus* (L.) also is composed solely of (*R*)-2-methylbutan-1-ol (Hanks and Millar 2016), to which it was attracted in the present study. Attraction of *Phymatodes dimidiatus* (Kirby) by 2-methylbutan-1-ol represents new information about its likely pheromone. Last, the cerambycine *Neoclytus acuminatus acuminatus* (F.) was attracted to the *syn*-diol treatment as expected, because it contained the pheromone (2*S*,3*S*)-2,3-hexanediol.

Some species of lamiines were attracted to the known lamiine pheromone compounds, as would be expected from previous research (Hanks and Millar 2016), with fuscumol acetate ('Fusc. acet.' in Table 2) attracting *Graphisurus fasciatus* (Degeer), *Lepturges angulatus* (LeConte), and *Aegomorphus modestus* (Gyllenhal), fuscumol ('Fusc.') attracting *Sternidius alpha* (Say), and monochamol ('Monoch.') attracting *Monochamus carolinensis* (Olivier) and its congener *Monochamus scutellatus scutellatus* (Say). A third congener, *Monochamus notatus* (Drury) was most strongly attracted to the pheromone blend in New York, with intermediate attraction to monochamol. However, an earlier field study, conducted at the same study sites in New York, showed that adults of *M. notatus* were significantly attracted to monochamol alone (Fierke et al. 2012). The lamiine *Styloleptus b. biustus* (LeConte) was attracted by the blend of fuscumol and fuscumol acetate in Florida, and both of the *Tetropium* species (subfamily Spondylidinae) by fuscumol alone, consistent with results reported in a complementary study (Millar et al. 2017). Attraction of *Oplosia nubila* (LeConte) to fuscumol acetate represents new information about its possible pheromone component.

Comparing the responses of the various species to the blend of six pheromones versus the individual components illustrated both synergistic and antagonistic effects among the blend components. The blend was significantly less attractive, or not at all attractive to several species, compared to the individual components (Table 2), suggesting that one or more components of the blend had inhibited responses. Notable examples are the strong antagonistic effect of the 3-ketol component of the blend on attraction of *P. amoenus* to its pheromone (*R*)-2-methylbutan-1-ol, and *N. a. acuminatus* to its pheromone (2*S*,3*S*)-2,3-hexanediol (Hanks et al. 2012, Miller et al. 2017; L.M.H., unpub. data). Other species showed similar evidence of antagonism, including the cerambycines *P. aereus* and *N. m. mucronatus*, and the lamiines *S. alpha*, *S. b. biustus*, *A. modestus*, *O. nubila*, and *M. s. scutellatus*. For the spondylidine species in the genus *Tetropium*, the blend attracted significant numbers of beetles, although fewer beetles than were attracted by fuscumol alone, suggesting some degree of antagonism by other blend components.

In contrast, there was evidence of synergism between components in the blend for only two species. Thus, the greater attraction of *X. colonus* to the blend is no doubt due to the pairing of *syn*-diol, a minor component of its pheromone, with 3-ketol, the dominant component (Lacey et al. 2009). Similarly, adult males of *Anelaphus inermis* (Newman) produce the (2*S*,3*R*)-enantiomer of the *anti*-diol along with a small amount of *syn*-diol, which accounts for its stronger attraction to the complete blend of chemicals versus the individual compounds.

The remaining species were similarly attracted to their individual pheromone (or attractant) components and to the complete blend, suggesting that the components of the blend that were not part of their pheromones neither synergized nor antagonized attraction. These species included the cerambycines *P. dimidiatus* and *P. testaceus*, and the lamiines *G. fasciatus*, *L. angulatus*, and the three *Monochamus* species.

Taken together, traps baited with the blend of six pheromone components attracted significant numbers of 10 species or about half of the cerambycid species in Table 2, but antagonism significantly reduced or even eliminated attraction of the remaining 9 species. Nevertheless, the blend was a better general attractant than any of the individual components. For example, 3-ketol attracted only three species of cerambycines and no lamiines, and fuscumol acetate attracted only four species of lamiines and no cerambycines.

In experiment 2, there were no cases in which the plant volatiles ethanol and α-pinene antagonized attraction of a species to the pheromone blend. Several species were attracted to traps baited only with the plant volatiles (Table 3). Among the few species that were attracted by plant volatiles alone, the pheromone blend strongly antagonized attraction of the cerambycine *Anelaphus villosus* (F.). Although the two spondylidine *Tetropium* species also were attracted by plant volatiles alone, attraction possibly was enhanced by fuscumol in the pheromone blend (Table 3) based on previous reports of synergism between their pheromones and host plant volatiles (Silk et al. 2007; Sweeney et al. 2010, 2014). Similarly, the spondylidine *Asemum striatum* (L.) was significantly attracted by the combination of the pheromone blend with plant volatiles, but not to either alone, as previously reported (Collignon et al. 2016). This species also was attracted by the blend of fuscumol and fuscumol acetate in the companion article (Millar et al. 2017), but the present findings suggest that attraction to those chemicals in the blend was antagonized by other components. All six of the *Monochamus* species and subspecies were attracted only by the combination of pheromone blend and plant volatiles, the key blend component being monochamol (Millar and Hanks 2017). Plant volatiles are known to be important synergists of monochamol for at least some species of *Monochamus* (e.g., Teale et al. 2011, Allison et al. 2012, Hanks et al. 2012). Lastly, nothing is known of the pheromone chemistry of the cerambycine *Euderces picipes* (F.) but attraction only to the combination of the pheromone blend and plant volatiles suggests that the blend contains one or more components of its pheromone.

For the remaining species in experiment 2, plant volatiles apparently did not influence their responses to particular components of the pheromone blend (Table 3), including the cerambycines *X. colonus* (probably responding to 3-ketol and diols in the blend), the lamiines *G. fasciatus* (responding to fuscumol acetate), and the lamiine *S. b. biustus* (responding to fuscumol + fuscumol acetate; Table 3). These findings were consistent with an earlier study (Hanks and Millar 2013). Nevertheless, Miller et al. (2015a,b) found that ethanol alone enhanced attraction of *X. colonus* to 3-ketol.

In summary, the experiments described here illustrate two important points. First, it is clear that blends of cerambycid pheromones can be deployed to catch multiple species of cerambycids

Table 2. Mean (±1 SE) number of cerambycid beetles captured per treatment and replicate during Experiment 1, and results of Friedman's *Q* analysis, for the cases in which overall treatment effects were statistically significant

Taxonomy/ State/ Friedman's *Q* (df)[a]	Blend[b]	3-Ketol	*syn*-Diol	2-Me-ol	Fusc.[c]	Fusc. acet.	Monoch.	Control
Cerambycinae								
Callidiini								
Phymatodes aereus								
IN: 17.3 (7,32)*	0b	**4.0 ± 2.5a**	1.0 ± 1.0b	0b	0b	0b	0b	0b
NY: 26.8 (7,48)**	0.17 ± 0.17b	**3.0 ± 2.2a**	0b	0b	0b	0b	0b	0.33 ± 0.3b
Phymatodes amoenus								
IN: 24.8 (7,64)**	10.8 ± 8.4ab	0.25 ± 0.16b	0b	**31.5 ± 17a**	3.1 ± 2.9b	0.5 ± 0.5b	1.6 ± 1.5b	0b
Phymatodes dimidiatus								
NY: 21.2 (7,32)**	**2.3 ± 1.1a**	0b	0b	**1.5 ± 0.6a**	0b	0b	0b	0b
Phymatodes testaceus								
NY: 21.2 (7,40)**	1.2 ± 0.6ab	0.6 ± 0.4ab	0b	**2.0 ± 0.8a**	0b	0b	0b	0b
Clytini								
Neoclytus a. acuminatus								
IN: 102.0 (7,288)***	0.39 ± 0.1b	0.72 ± 0.3b	**2.7 ± 0.6a**	0.03 ± 0.03b	0.08 ± 0.05b	0.06 ± 0.05b	0.056 ± 0.04b	0.03 ± 0.03b
MI: 71.0 (7,112)***	0.36 ± 0.17b	0.64 ± 0.4b	**9.2 ± 1.4a**	0.07 ± 0.07b	0b	0.14 ± 0.1b	0.07 ± 0.07b	0b
NY: 78.6 (7,80)***	0b	0b	**3.4 ± 0.9a**	0b	0b	0b	0b	0b
TX: 47.2 (7,63)***	5.3 ± 1.5b	3.4 ± 3b	**29.5 ± 7.8a**	0b	0b	0b	0b	0.13 ± 0.1b
Neoclytus m. mucronatus								
MI: 22.1 (7,32) **	0.75 ± 0.5b	**7.0 ± 2.9a**	0b	0b	0b	0.5 ± 0.5b	0b	0b
TX: 43.3 (7,63)***	2.1 ± 0.4b	**12.5 ± 4.1a**	5.5 ± 1.8b	0.13 ± 0.1b	0.43 ± 0.3b	0.63 ± 0.5b	0.25 ± 0.2b	0b
Xylotrechus colonus								
IN: 92.4 (7,344)***	**5.0 ± 0.9a**	**3.0 ± 0.7b**	1.1 ± 0.38bc	0.19 ± 0.06c	0.44 ± 0.26c	0.37 ± 0.2c	1.2 ± 0.6bc	0.09 ± 0.09c
MI: 51.8 (7,120)***	**7.6 ± 3.1a**	**4.7 ± 2.3ab**	1.5 ± 1.5bc	0.33 ± 0.2c	0c	0.13 ± 0.1c	0c	0.33 ± 0.2c
NY: 100.7 (7,203)***	**3.8 ± 0.9a**	**2.2 ± 0.6b**	0.08 ± 0.06c	0.08 ± 0.06c	0.04 ± 0.04c	0.04 ± 0.04c	0.04 ± 0.04c	0.12 ± 0.09c
Elaphidiini								
Anelaphus inermis								
FL: 27.3 (6,70)***	**1.2 ± 0.3a**	0b	0.2 ± 0.1b	0.1 ± 0.1b	0.3 ± 0.15b		0b	0b
Lamiinae								
Acanthocinini								
Graphisurus fasciatus								
MI: 18.5 (7,72)**	**1.0 ± 0.3a**	0.22 ± 0.15b	0b	0.22 ± 0.15ab	0.11 ± 0.1b	**1.0 ± 0.4a**	0.44 ± 0.2b	0.11 ± 0.1b
NY: 35.8 (7,91)***	1.1 ± 0.3ab	0.33 ± 0.2b	0b	0.09 ± 0.09b	0b	**2.1 ± 0.85a**	0b	0.27 ± 0.14b
Lepturges angulatus								
NY: 18.5 (7,32)**	**1.3 ± 0.5a**	0b	0b	0b	0b	0.50 ± 0.3b	0b	0b
TX: 29.2 (7,63)***	**4.4 ± 2.6a**	0.38 ± 0.2b	0b	0b	0b	**6.4 ± 2.5a**	0b	0.13 ± 0.1b
Sternidius alpha								
MI: 33.5 (7,80)***	1.7 ± 0.9b	0.1 ± 0.1b	0.2 ± 0.1b	0b	**7.4 ± 2.9a**	0.3 ± 0.2b	0b	0.1 ± 0.1b
Styloleptus b. biustus								
FL: 24.2 (6,70)**	0.2 ± 0.1b	0.4 ± 0.2b	0b	0.1 ± 0.1b		**2.1 ± 0.6a**	**0b**	0.3 ± 0.2b

Table 2. *Continued*

Taxonomy/ State/ Friedman's Q (df)[a]	Blend[b]	3-Ketol	*syn*-Diol	2-Me-ol	Fusc.[c]	Fusc. acet.	Monoch.	Control
Acanthoderini								
Aegomorphus modestus								
IN: 30.3 (7,64)***	0.38 ± 0.38b	0.13 ± 0.1b	0b	0b	0b	**2.0 ± 0.53a**	0b	0.25 ± 0.2b
MI: 44.2 (7,64)***	0.9 ± 0.55b	0b	0b	0b	0.13 ± 0.13b	**1.8 ± 0.6a**	0b	0b
NY: 20.0 (7,59)**	0.50 ± 0.3b	0b	0b	0.14 ± 0.1b	0b	**2.5 ± 1.5a**	0.29 ± 0.2b	0b
Oplosia nubila								
IN: 32.2 (7,48)***	0b	0b	0b	0b	0.17 ± 0.17b	**1.2 ± 0.4a**	0b	0b
Monochamini								
Monochamus carolinensis								
TX: 22.5 (7,32)**	**4.0 ± 0.9a**	0b	0b	0.25 ± 0.2b	0.25 ± 0.2b	3.0 ± 2ab	**5.0 ± 1.7a**	0b
Monochamus notatus								
NY: 35.8 (7,80)***	**0.9 ± 0.3a**	0b	0b	0b	0b	0b	0.5 ± 0.2ab	0b
Monochamus s. scutellatus								
MI: 20.9 (7,32)**	1.0 ± 0.6b	0b	0b	0b	0b	0.5 ± 0.3b	**5.3 ± 3.6a**	0b
NY: 41.1 (7,176)***	**1.2 ± 0.3a**	0.09 ± 0.6b	0.045 ± 0.4b	0.046 ± 0.04b	0.045 ± 0.4b	0.045 ± 0.4b	**1.7 ± 0.3a**	0.09 ± 0.06b
Spondylidinae								
Asemini								
Tetropium cinnamopterum								
NY: 106.1 (7,264)***	**1.9 ± 0.46b**	0c	0.09 ± 0.09c	0.06 ± 0.04c	**3.4 ± 0.8a**	0.09 ± 0.09c	0.09 ± 0.09c	0.12 ± 0.1c
Tetropium schwarzianum								
NY: 70.6 (7,184)***	**0.70 ± 0.2b**	0c	0.04 ± 0.04c	0.04 ± 0.04c	**1.2 ± 0.3a**	0.09 ± 0.06c	0c	0.09 ± 0.06c

Means in bold were significantly different from controls. Abbreviations for chemicals: Blend = blend of all the following chemicals; 3-Ketol = racemic 3-hydroxyhexan-2-one; *syn*-Diol = *syn*-2,3-hexanediol; 2-Me-ol = racemic 2-methybutan-1-ol; Fusc. = racemic fuscumol; Fusc. Acet. = racemic fuscumol acetate; Monoch. = monochamol; Control = solvent control.

[a]Asterisks indicate significance level of Friedman's Q: *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.

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

[c]Merged cells for fuscumol and fuscumol acetate for the Florida site indicates that only the blend had been tested, not the individual compounds.

Table 3. Mean (±1 SE) number of cerambycid beetles captured per treatment and replicate during Experiment 2, and results of Friedman's *Q* analysis, for the cases in which overall treatment effects were statistically significant

Taxonomy/State/Friedman's *Q* (df)[1]	Pheromone blend[2]	Blend + Plant volatiles	Plant volatiles	Solvent control
Cerambycinae				
Clytini				
Xylotrechus colonus				
NY: 25.0 (3,88)***	**1.4 ± 0.3a**	**1.7 ± 0.4a**	0.32 ± 0.2b	0.36 ± 0.3b
Elaphidiini				
Anelaphus villosus				
MI: 15.4 (3,24)**	0b	0.5 ± 0.3b	**4.0 ± 1.1a**	0.33 ± 0.2b
Tillomorphini				
Euderces picipes				
MI: 16.3 (3,28)**	0.7 ± 0.5b	**4.0 ± 1.4a**	0.43 ± 0.4b	0b
Lamiinae				
Acanthocinini				
Graphisuris fasciatus				
NY: 11.9**	**2.4 ± 0.6a**	**1.8 ± 0.6a**	0b	0.4 ± 0.4b
Styloleptus b. biustus				
FL: 16.2 (3,40)**	**4.5 ± 1.0a**	**1.8 ± 1.1ab**	0.2 ± 0.1b	0.2 ± 0.2b
Monochamini				
Monochamus carolinensis				
MI: 11.6 (3,20)**	1.4 ± 0.9ab	**7.8 ± 3.9a**	4.0 ± 1.7ab	0.2 ± 0.2b
Monochamus clamator				
CA: 22.4 (3,24)***	0b	**2.5 ± 0.4a**	0b	0b
Monochamus notatus				
NY: 44.0 (3,85)***	0.62 ± 0.2b	**4.4 ± 0.8a**	0.67 ± 0.3b	0.5 ± 0.48b
Monochamus obtusus				
CA: 20.7 (3,44)***	3.8 ± 2.0b	**27.5 ± 9.2a**	1.3 ± 0.7b	0.1 ± 0.1b
Monochamus s. oregonensis				
CA - 15.7 (3,20)**	0.2 ± 0.2b	**2.0 ± 0.6a**	0b	0b
Monochamus s. scutellatus				
NY: 39.2 (3,92)***	1.1 ± 0.4b	**10.5 ± 2.4a**	1.4 ± 0.4b	0.43 ± 0.2b
Spondylidinae				
Asemini				
Asemum striatum				
NY: 29.0 (3,72)***	0.5 ± 0.1b	**2.5 ± 0.6a**	0.8 ± 0.4b	0b
Tetropium cinnamopterum				
NY: 29.2 (3,120)***	2.6 ± 0.5bc	**8.1 ± 2.1a**	**4.6 ± 1.2b**	1.2 ± 0.8c
Tetropium schwarzianum				
NY: 32.0 (3,112)***	1.3 ± 0.3bc	**3.6 ± 0.6a**	**2.1 ± 0.5b**	0.4 ± 0.3c

Treatments included the pheromone blend alone (components listed in footnote of Table 2), the pheromone blend + plant volatiles (ethanol and α-pinene), plant volatiles alone, and solvent control.

[1]Asterisks indicate significance level of Friedman's *Q*: ** $P < 0.001$; *** $P < 0.0001$.

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

simultaneously, but it is equally clear that the choice of which compounds to combine can be critically important. Our data suggest that attraction of a given species to a synthesized reconstruction of its pheromone is most likely to be antagonized by compounds produced by close relatives. Examples include cerambycine species which are attracted by ketol components of their pheromones but antagonized by diols produced by other species of cerambycines, and vice versa (e.g., Millar et al. 2017). In contrast, it is unlikely that attraction would be antagonized by pheromone components of more distantly related species. For example, we know of no cases in which the lamiine and spondylidine pheromones fuscumol or fuscumol acetate have been shown to inhibit attraction of cerambycines to ketols or diols, and vice versa. Choices of possible future blends for testing should be guided by these two principles. It is likely that the optimal strategy, in terms of attracting the greatest diversity of cerambycid species, would be to use multiple traps, each baited with different blends which have been crafted to minimize antagonism among components. In comparison to using traps baited with single compounds, or blends optimized for a single species, such composite blends would still reduce the number of traps necessary to cast a broad net and so minimize costs of materials and labor.

Second, there may be considerable advantages to deploying simple blends of host plant volatiles such as ethanol and α-pinene with pheromones, in terms of increasing both the number of species and the number of individuals attracted. In the studies reported here, there was no indication that ethanol and α-pinene antagonized attraction of any species to pheromone lures. In another large study, Miller et al. (2017) similarly found that ethanol often enhanced attraction of cerambycids to pheromone lures, with no evidence of inhibition. However, Collignon et al. (2016) reported that attraction of some cerambycid species that infest hardwoods to their pheromones was interrupted by high release rates of volatiles that are typical of conifers. Thus, even with host plant compounds, the anticipated target species and their typical host plants must be considered when choosing which pheromones and host plant volatiles to formulate into optimal attractants for multiple species simultaneously.

Supplementary Data

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

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