SPECIES-SPECIFIC BLENDS OF SHARED PHEROMONE COMPONENTS PREVENT CROSS ATTRACTION AMONG SYMPATRIC SPECIES OF CERAMBYCID BEETLES (SUBFAMILY LAMIINAE)

BY

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THESIS

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ABSTRACT

Research over the last decade has revealed extensive pheromonal parsimony within the large beetle family Cerambycidae, with closely related species producing the same or very similar pheromone components. Sympatric species in the subfamily Cerambycinae, that share pheromone components, are known to avoid cross attraction by differing in seasonal and/or circadian flight period, or due to minor pheromone components that serve as synergists for conspecifics or antagonists for heterospecifics. However, much less is known about the pheromones of species in the subfamily Lamiinae. In this article, I summarize research to identify pheromones of three species of lamiines that are native to eastern North America, and to test the hypothesis that cross attraction is averted by differences between species in pheromone chemistry. Males of the study species produced 6,10-dimethyl-5,9-undecadien-2-one ("geranylacetone"), as well as (*E*)-6,10-dimethyl-5,9-undecadien-2-ol ("fuscumol") and/or (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate ("fuscumol acetate"), but varied in the stereochemistry of the latter two compounds. That is, male *Astyleiopus variegatus* (Haldeman) produced *S*- fuscumol and *S*- fuscumol acetate, *Astylidius parvus* (LeConte) produced *R,S*-fuscumol and *R*-fuscumol acetate, while *Lepturges angulatus* (LeConte) produced *R,S* fuscumol acetate. Field bioassays showed that geranylacetone was a powerful synergist for *A. variegatus* and *L. angulatus*, and that attraction of beetles to traps was generally consistent with the chemical composition of volatiles released by males. For each of the species, cross attraction would be blocked by the absence of critical components, or presence of antagonistic compounds in pheromones of the other species, supporting my hypothesis.

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CHAPTER 1

INTRODUCTION

Research over the last 20 years has shown that semiochemicals play a powerful role in structuring insect communities by mediating species interactions (Vet 1999; Takken and Dicke 2006). For example, reproductive isolation among sympatric species that share pheromone components is facilitated by differing ratios of shared components that comprise species-specific blends, as is the case in many species of moths (e.g., Wakamura and Arakaki 2004, Groot et al. 2010). Aggregation pheromones of bark beetles also function as synomones, inhibiting cross attraction between sympatric species, and driving resource partitioning (Paine and Hanlon 1991, Savoie et al. 1998). Similarly, pheromones and other attractants play a key role in the ecology of longhorned beetles (Coleoptera: Cerambycidae) (Linsley 1961), which includes many important pest species that cause significant economic damage (e.g., MacLeod et al. 2002, Rhainds et al. 2002). Trap baited with pheromones have proven to be essential to quarantine monitoring for invasive cerambycid species, and eradication efforts of established species (Brockerhoff et al. 2006), providing impetus for thorough characterization of the chemical communication in this family.

Volatile aggregation pheromones produced by males, and sex pheromones produced by females, have been described for cerambycid species in the subfamilies Cerambycinae, Lamiinae, Lepturinae, Spondylidinae, and Prioninae (e.g., Ray et al. 2009, 2011; Hanks et al. 2013). This expanding body of knowledge has revealed widespread parsimony of pheromone structure and use within the family, with sympatric species sharing and producing very similar, and sometimes identical pheromones. For example, 3-hydroxyhexan-2-one and the analogous 2,3-hexanediols are common components of male-produced aggregation pheromones of species

in the subfamily Cerambycinae (Millar et al. 2009). Recent research has demonstrated that sympatric species in that subfamily, that share pheromone components, may avoid cross attraction by differing in seasonal and/or circadian flight period, or due to minor pheromone components that serve as synergists for conspecifics or antagonists for heterospecifics (Mitchell et al. 2015).

Much less is known about the pheromone chemistry of species in the subfamily Lamiinae, and mechanisms that would serve to prevent cross attraction. Many lamiine species that are native to the eastern United States overlap broadly in both seasonal and circadian flight periods, with most adults being active from June – August (Hanks et al. 2013) and between 20:00 and 22:00 (unpub. data). Moreover, it appears that many sympatric species in the tribes Acanthocinini and Acanthoderini have pheromones composed of (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (fuscumol) and/or its acetate (based on attraction of adult males and females in field trials; Mitchell et al. 2011, Hanks and Millar 2013). A third compound, 6,10-dimethyl-5,9-undecadien-2-one (geranylacetone), has also been detected in the headspace volatiles of males in two different subfamilies which are known to produce fuscumol and/or its acetate. Adults of the Palearctic congeners *Tetropium fuscum* (F.) and *T. cinnamopterum* Kirby (Spondylidinae) produce geranylacetone along with their major pheromone component, fuscumol (Mayo et al. 2013). In the subfamily Lamiinae, adults of the South American *Hedypathes betulinus* (Klug) produce fuscumol, its acetate, and geranylacetone (Fonseca et al. 2010).

I conducted research on the lamiines *Astyleiopus variegatus* (Haldeman), *Astylidius parvus* (LeConte), and *Lepturges angulatus* (LeConte) (Tribe Acanthocinini) with the objectives of 1) identifying or confirming pheromones, and characterizing stereochemistry, and 2) testing the hypothesis that sympatric species of lamiines use species-specific pheromone blends to

partition their pheromone channel. These three species are abundant in the areas of my studies (east-central Illinois), adults overlap in season and circadian flight period synchronous, and are attracted (*E*/*Z*)-fuscumol and/or its acetate, as described above. They also share tree species as larval hosts, including species of *Aesculus* (Hippocastanaceae), *Gleditsia* (Fabaceae), *Celtis* and *Ulmus* (Ulmaceae), and *Juglans* (Juglandaceae; Linsley 1997).

CHAPTER 2

MATERIALS AND METHODS

Sources of synthetic pheromones

(*E*)-Fuscumol and (*E*)-fuscumol acetate were purchased from Bedoukian Research (Danbury, CN, USA), and geranylacetone from Sigma-Aldrich (St. Louis, MO, USA). Chiral fuscumol and fuscumol acetate were synthesized as in Hughes et al. (2013).

Identification of pheromones

Earlier research had already shown that adult male *A. variegatus* produce the *S* enantiomer of both fuscumol and fuscumol acetate (Hughes et al. 2013). Live beetles of the remaining species were trapped for collection and analysis of insect-produced volatiles using pheromone-baited black cross-vane panel traps (AlphaScents, Portland, OR, USA) coated with Fluon® to improve capture efficiency (for details, see Graham et al. 2010). The supplied trap basins were replaced with 2-l plastic jars (General Bottle Supply Company, Los Angeles, CA) with bottoms replaced with aluminum screen to allow rain water to drain. Pheromone lures consisted of polyethylene sachets (Bagettes™ model 14770, 5.1 × 7.6 cm; Cousin Corp., Largo, FL, USA) that were loaded with 50 mg each of fuscumol and fuscumol acetate in 1 ml isopropanol. Beetles were collected during May – August of 2013 and 2014 with individual traps (at least 25 m apart) set up in seven wooded areas of Champaign, Piatt, and Vermillion counties, Illinois (Table 1). Traps were serviced every 1-2 d, and lures were replaced as needed, usually every 10-14 d.

aptured beetles were sexed by the relative lengths of the fourth and fifth abdominal sternites (Linsley 1995), but if that character was inconclusive, they were sexed by observing mating

behavior (i.e., males copulating with females). Beetles were housed separately by species in plastic containers with aluminum screen lids, under ambient laboratory conditions (~12:12 h L:D, ~20°C). Because adults of all three species feed on tender bark of oaks (pers. obs), caged beetles were provided sections of branches (2 – 5 cm in diameter and ~8 cm long) freshly cut from *Quercus alba* L. and *Q. rubra* L. at field sites (Table 1), as well as 10% aqueous sucrose solution as nourishment. Beetles were allowed at least 24 h to acclimate prior to being used for headspace collections.

Volatiles produced by beetles were collected by placing them in glass Mason-style jar chambers (for details, see Ray et al. 2011). Aeration collectors contained 150 mg HayeSepQ® (Sigma-Aldrich) and were connected to the aeration chamber and the vacuum source with Teflon® tubing. Air entering the chambers was purified with granulated charcoal. Headspace volatiles were collected for 24 h under natural photoperiod (aeration chambers positioned near closed windows; ~14:10 h L:D, ~20°C). Insects usually were aerated individually, but in some cases two or three beetles of the same sex were aerated together to improve the chances of collecting pheromones, as aggression between conspecific males has not been observed in any of these species. Beetles usually were aerated along with fresh twigs of oak (provided as food). Control aerations without insects, and with host materials when appropriate, were run simultaneously to check for system contaminants. Additional controls were chambers containing host materials that had been damaged mechanically, by scraping or gouging the bark, to simulate release of volatiles due to feeding by beetles. Collectors were extracted with 1.5 ml of dichloromethane into silanized glass vials that were stored at -20°C. Beetles were returned to cages after aerations for at least 24 h to before being reused.

Extracts were analyzed by coupled gas chromatography–mass spectrometry with an HP 6890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) fitted with an AT-5 ms column (30 m × 0.25 mm i.d., 0.25 μm film; Alltech Associates, Inc., Deerfield, IL, USA) coupled with an HP 5973 mass selective detector. Injector temperature was 250°C, and oven temperature was programmed from 35°C for 1 min, 10°C/min to 210°C, and held 3 min. Peaks were identified by comparing spectra and retention times with those of authentic standards.

The absolute configuration of fuscumol acetate was 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, Inc., Santa Clara, CA, USA). Injector temperature was 210°C, and oven temperature was held at 50°C for 1 min, increased at 2.5°C/min to 200°C, and held 5 min. *R*- and *S*-fuscumol acetate were resolved to baseline (retention times 51.33 and 51.72 min, respectively) and the identities of the enantiomers were confirmed by co-injection of *S*- fuscumol acetate with insect-produced samples. Fuscumol was derivatized prior to analysis to improve resolution (for details, see Hughes et al. 2013), by esterifying samples with O-acetyl lactic acid chloride, using pyridine and dimethylaminopyridine (DMAP) as a base. Samples of racemic and pure *R*-fuscumol were esterified under the same conditions to verify that the derivatizing reagent was enantiomerically pure, and that no racemization occurred under the reaction conditions. Following derivatization, samples were re-run on the Cyclodex B column and co-injected with a racemic fuscumol standard to identify enantiomers by retention time.

For all of the study species, identification of pheromones was challenging due to the fact that males would infrequently emit pheromone in aeration chambers. Thus, the number of aeration samples that contained sufficient quantities of chemicals for identification of molecular structure (i.e., without specifying stereochemistry) was limited to 7 of 9 aerations of male *A.*

variegatus, 5 of 15 aerations of *A. parvus*, and 8 of 14 aerations of *L. angulatus*. Sample sizes were still smaller for numbers of extracts that were suitable for characterizing stereochemistry, due to the greater amount of sample material required for successful determination of chirality.

Field Bioassays

All field experiments were designed based upon the findings of the pheromone identification phase of the present study. Attraction of beetles to test compounds in the field was assessed with the same type of panel traps, but with the supplied trap basins filled with ~300 ml of saturated sodium chloride solution as a killing agent and preservative. Traps were positioned 10 m apart in linear transects and checked for beetles every 1-3 d. Treatments were assigned randomly to positions within transects at the beginning of each bioassay, and rotated within transects after servicing to control for location effects. Lures were replaced as needed, usually every 10-14 days.

Field experiment 1 tested for attraction of beetles to (*E*/*Z*)-geranylacetone which had been detected in head-space aerations of males during the present study, and for interactions between the presence of geranylacetone and attraction to fuscumol and fuscumol acetate. Trap lures consisted of heat-sealed polyethylene sachets of varying thicknesses (Associated Bag, Milwaukee, WI), with or without cotton wicks (depending on the chemical) to yield consistent release rates. That is, a release rate of ~20 mg/day was achieved by loading 1 ml of neat fuscumol, fuscumol acetate, and geranylacetone into sachets with wall thicknesses of 1.5 mil (with wick), and 3 mil (with wick), and 3 mil (without a wick), respectively (unpub. data). Empty sachets served as blank controls. The experiment was conducted at Allerton Park and Brownfield Woods (Table 1) during 22 June – 6 August 2014. In this and subsequent

experiments, sex ratios of captured beetles were estimated for the study species by sampling individuals only from treatments that captured significantly more beetles than did the controls.

Experiment 2 evaluated attraction of *A. variegatus* to the individual enantiomers of fuscumol and fuscumol acetate, and blends of *R*-fuscumol with *R*-fuscumol acetate, and *S*-fuscumol with *S*-fuscumol acetate. In this and the following experiment, lures were loaded with diluted chemicals due to the great cost involved in synthesizing the chiral compounds. Thus, lures were the same Bagette™ model polyethylene sachets, loaded with 0.25 mg per enantiomer in 1 ml of isopropanol, with pure isopropanol serving as the control. The experiment was conducted at the Allerton Park and Hart Woods (Table 1) during 20 June – 25 July 2014.

Experiment 3 was designed to simultaneously evaluate attraction of *A. parvus* and *L. angulatus* to individual enantiomers of fuscumol and fuscumol acetate and blends thereof. Thus, treatments were: 1) fuscumol alone (i.e., racemic), 2) fuscumol acetate alone, 3) the blend of the two racemic chemicals, 4) fuscumol and *S*-fuscumol acetate, 5), fuscumol with *R*-fuscumol acetate, 6) fuscumol acetate with *R*-fuscumol, 7) fuscumol acetate with *S*-fuscumol, and 8) solvent control. The experiment was conducted at the Allerton Park, Brownfield Woods, and Trelease Woods field sites (Table 1) during 25 July – 3 September 2014.

Statistics

Differences between treatment means, blocked by site and date, were tested separately for each experiment and species using the nonparametric Friedman's Test (PROC FREQ, option CMH; SAS Institute 2001) because data violated assumptions of ANOVA (Sokal and Rohlf 1995). Differences between pairs of means were tested with Tukey's HSD means separation test to control maximum experiment-wise error rates (PROC GLM; SAS Institute, 2001). Date-site

combinations were included in the analysis based on a threshold number of specimens (3-6 depending on abundance of the species) so as to optimize sample size per replicate while maintaining sufficient replication for a robust analysis. Departure from the expected 1:1 sex ratio of captured beetles was tested with χ^2 tests.

Nomenclature follows Bezark (2014). Representative specimens of all species have been retained in the laboratory of LMH, and voucher specimens have been deposited with the collection of the Illinois Natural History Survey, Champaign, IL.

CHAPTER 3

RESULTS

Identification of pheromones

Aeration extracts from males of each species contained chemicals that were absent in extracts from females or controls, and included fuscumol, fuscumol acetate, and geranylacetone. The three species differed in the stereochemistry of fuscumol and fuscumol acetate, but all produced geranylacetone (Table 3). Male *A. variegatus* produced only the *S* enantiomers of fuscumol and fuscumol acetate, consistent with an earlier study (Hughes et al. 2013), with relative compound abundance ratios of fuscumol: fuscumol acetate: geranylacetone averaging ~3:3:1 (n = 3; Fig. 1). Male *A. parvus* produced both enantiomers of fuscumol and only one enantiomer of fuscumol acetate, and the ratio of the three components was ~3:1:1 (n = 4; Fig. 2), with the enantiomers of fuscumol in nearly equal proportions. Finally, male *L. angulatus* produced both enantiomers of fuscumol acetate (Fig. 3), with a fuscumol acetate: geranylacetone ratio of ~3:1 (n = 5), and the ratio of *R*:*S* fuscumol acetate being ~8:3.

I also identified chemicals emitted by male *Lepturges regularis* (LeConte), trapped during the present study. Headspace aerations contained a fuscumol:fuscumol acetate:geranylacetone ratio of ~1:4:0 (n = 2; Fig. 4), with *R*:*S* fuscumol approximating 1:1 and *R*:*S* fuscumol acetate averaging ~3:1. Beetles of this species were not caught in high enough numbers to gather meaningful data from field bioassays.

Field Bioassays

During field bioassays, panel traps captured 1,145 cerambycid beetles of 48 species, with the dominant species being the three focal species. Taxonomy and the total number of beetles

caught for the subfamily Lamiinae are detailed in Table 2. Beetles in the subfamilies Cerambycinae, Disteniinae, Lepturinae, Prioninae, and Spondylidinae were also caught, but in small numbers. For all of the study species, both sexes were attracted to traps baited with synthetic pheromones, although sex ratios were in some cases skewed (relative to an expected 1:1 ratio; see below).

In experiment 1, adult *A. variegatus* were significantly attracted only to the blend of all three components (Fig. 5), consistent with headspace samples of males (Table 3). The second highest mean was apparently for the blend of fuscumol and fuscumol acetate, consistent with findings of an earlier field study (Mitchell et al. 2011). The lack of statistical significance for that treatment, however, was due to dominance by the three-component blend (see experiment 2), suggesting that geranylacetone strongly enhances attraction to fuscumol and fuscumol acetate for this species. Adult *A. parvus* and *L. angulatus* also were trapped during this study. Adult *A. parvus* were equally attracted to the four treatments that contained racemic fuscumol, the dominant component in headspace aerations, and apparently not significantly influenced by either fuscumol acetate or geranylacetone (Fig. 6). Adult *L. angulatus* were most strongly attracted to traps baited with the blend of fuscumol acetate and geranylacetone (Fig. 7), consistent with males producing only those two chemicals (Table 3). The dominance by that blend is responsible for the insignificant mean for the fuscumol acetate treatment (see experiment 3), which was shown to be an attractant in the earlier study (Mitchell et al. 2011), again attesting to strong enhancement by geranylacetone. Sex ratios of beetles captured during experiment 1 were female biased for *A. parvus*, but not significantly different from 1:1 for *A. variegatus* or *L. angulatus* (see caption for Fig. 5,6, and 7).

During experiment 2, all of the 52 adult *A. variegatus* that were captured were in traps baited with *S*-fuscumol and *S*-fuscumol acetate (Friedman's $Q_{(6,84)} = 82.4$, $P < 0.001$), with no beetles in traps baited with *R*- or *S*-fuscumol alone, *R*- or *S*-fuscumol acetate alone, the blend of *R*-fuscumol and *R*-fuscumol acetate, and controls. The sex ratio of captured adult *A. variegatus* was significantly female biased (3:1, female:male; $\chi^2 = 5$, $P = 0.02$).

In experiment 3, *A. parvus* was most strongly attracted to traps baited with racemic fuscumol and either of the two enantiomers of fuscumol acetate, but less so to traps baited with fuscumol alone or combined with racemic fuscumol acetate (Fig. 8). Moreover, the individual enantiomers of fuscumol were not attractive when blended with racemic fuscumol acetate. These finding are difficult to explain, given that males produce only the *R* enantiomer of fuscumol acetate (Table 3). Male and female *A. parvus* were captured in approximately equal numbers (χ^2 test, $P > 0.05$).

In experiment 3, adult *L. angulatus* were significantly attracted only by treatments that contained racemic fuscumol acetate in the absence of racemic fuscumol (Fig. 9), suggesting that: 1) both enantiomers of fuscumol acetate were necessary, and 2) the individual enantiomers of fuscumol had no effect, but together antagonized attraction. Male and female *L. angulatus* were captured in approximately equal numbers (χ^2 test $P > 0.05$).

CHAPTER 4

DISCUSSION

The great number of beetles, representing many species in the Lamiinae, that were trapped during the field experiments is consistent with broad attraction of species in an earlier study (Mitchell et al. 2011), and further supports the notion that fuscumol and its acetate represent another common pheromone motif within the Cerambycidae. It therefore seems likely that these other species of lamiines caught have pheromones composed of fuscumol, fuscumol acetate, and/or geranylacetone. Low capture rates of these species may be due to the influence of minor components that have yet to be identified.

This study provides the first evidence of the important role of geranylacetone for certain lamiine species. Geranylacetone is a common plant volatile associated with insects of several orders (e.g., Hammack 1997, Hughes et al. 2010), including the South American lamiine *Hedypathes betulinus* (Klug), the male-produced pheromone of which is composed of fuscumol, fuscumol acetate, and geranylacetone (Fonseca et al. 2010). The pheromones of *H. betulinus* apparently attract only females (Fonseca and Zarbin 2009), while fuscumol and its acetate attract both sexes of many North American lamiine species (Mitchell et al. 2011, Hanks et al. 2013).

Although geranylacetone was present in headspace volatiles of male *A. variegatus* during the present study, it was not detected during initial research on its pheromone (Hughes et al. 2013). This discrepancy could be due to the fact the beetles were provided food (oak bark) during the present study, but not in the earlier study. Consistent with that explanation, male *H. betulinus* that were provisioned with plant host material during aeration produced geranylacetone (Fonseca et al. 2010). In fact, geranylacetone serves as a precursor in biosynthesis of fuscumol and its acetate in *H. betulinus* (Zarbin et al. 2013), and as an intermediate derived from farnesol

in biosynthesis of fuscumol for *Tetropium* species (Mayo et al. 2013). Further investigation is required to determine the link between adult diet and the production of geranylacetone in cerambycids.

Attraction of beetles to traps during our field bioassays was usually consistent with the pheromone composition of the study species as assessed by headspace aerations of males. The conspicuous exception was *A. parvus*. Although the pheromone of that species contained enantiomers of fuscumol and fuscumol acetate, along with geranylacetone, the latter two chemicals seemed to have no influence on attraction in experiment 1, while enantiomers of fuscumol and fuscumol acetate appeared to have non-additive effects on attraction in experiment 3. These findings emphasize the importance of carefully evaluating the individual effects of enantiomers, and provides further evidence that chirality plays an important role in species specificity of pheromones.

Results from this study support our hypothesis that sympatric and synchronous species of lamiines are using species-specific blends of shared components to partition the pheromone channel. That is, cross attraction of one species to another may be prevented if the first species is lacking critical components, or has components that antagonize attraction of the second species. In fact, the mechanisms appear to be complementary for our three study species, as summarized in Table 4. For each interaction between two species, our experiments have revealed mechanisms that would prevent cross attraction in both directions. For example, if male *A. variegatus* and *L. angulatus* were to be calling simultaneously, adult *L. angulatus* should not respond to the pheromone of the other species because they require both enantiomers of fuscumol acetate, while *A. variegatus* produces only the *S*-enantiomer. On the other hand, adult *A. variegatus* should not respond to the *R,S*-fuscumol acetate emitted by male *L. angulatus*

because the *R-enantiomer* inhibits attraction and because it lacks the essential *S*-fuscumol component. The complementary nature of these mechanisms are similar to those that recently have reported that prevent cross attraction among species of the subfamily Cerambycinae (Mitchell et al. 2015), and suggests that the pheromone systems of communities for lamiine species are the tightly integrated product of niche partitioning. Each species therefore occupies a unique communication "niche", which assures species specificity of pheromone signals that are used for locating mates.

CHAPTER 5

FIGURES AND TABLES

Table 1. Study sites for field bioassays conducted in east-central Illinois during 2013-2014.

County	Name	GPS coordinates	Area
Champaign	Brownfield Woods[1]	N40°8’42.8028” W88°9’55.659”	26 ha
Champaign	Nettie Hart Memorial Woods[1]	N40°13'46.833" W88°21'28.2594"	16 ha
Champaign	Private residence in Urbana, IL	N40°5'49.4412" W88°12'11.3832"	0.1 ha
Champaign	Trelease Woods[1]	N40°8'5.5422" W88°8'34.0656"	29 ha
Piatt	Allerton Park[1]	N39°59'7.2312" W88°39'0.5292"	600 ha
Vermilion	Forest Glen Preserve[3]	N40°0'54.576" W87°34'3.756"	3 ha
Vermilion	Vermilion River Observatory[1]	N40°3'55.9908" W87°33'40.7772"	192 ha

[1]University of Illinois (http://research.illinois.edu/cna/)
[2]Champaign County Forest Preserves District, Illinois (http://www.ccfpd.org/)
[3]Vermilion County Conservation District (http://www.vccd.org/)

Table 2. Taxonomy and numbers of beetles of the subfamily Lamiinae that were captured by panel traps in east-central Illinois during 2013. Focal species for field bioassays are in bold.

Tribe/species	No. specimens
Acanthocini	
Astyleiopus variegatus (Haldeman)	142
Astylidius parvus (LeConte)	252
Astylopsis macula (Say)	43
Graphisurus despectus (LeConte)	47
Graphisurus fasciatus (Degeer)	105
Leptostylus transversus (Gyllenhal in Schoenherr)	3
Lepturges angulatus (LeConte)	219
Lepturges confluens (Haldeman)	21
Lepturges pictus (LeConte)	9
Lepturges regularis (LeConte)	8
Lepturges symmetricus (Haldeman)	4
Sternidius alpha (Say)	106
Urgleptes querci (Fitch)	8
Urgleptes signatus (LeConte).	4
Acanthoderini	
Aegomorphus modestus (Gyllenhal in Schoenherr)	18
Dorcaschematini	
	6
	1
Lamiini	
	4
	1
Pogonocherini	
	1
	2
Saperdini	
Saperda tridentata Olivier	5
	5

Table 3. Chemical composition of extracts from headspace aerations of adult males of the three cerambycid study species, and the additional species *Lepturges regularis*.

	Fuscumol		Fuscumol acetate		Geranylacetone
	R	***S***	***R***	***S***	
Astyleiopus variegatus		+		+	+
Astylidius parvus	+	+	+		+
Lepturges angulatus			+	+	+
Lepturges regularis	+	+	+	+	

Table 4. Mechanisms that prevent cross attraction between the three study species based on pheromone composition and required pheromone components, or antagonistic components, as revealed by field experiments. Chemical abbreviations for fuscumol and fuscumol acetate are fusc., and fuscacet., respectively.

		Calling species (pheromone composition)		
		Astyleiopus variegatus (*S*-fusc., *S*-fuscacet.)	*Astylidius parvus* (*R*,*S*-fusc, *R*-fuscacet.)	*Lepturges angulatus* (*R*,*S*-fuscacet.)
Potential responding species	*Astyleiopus variegatus* (*S*-fusc., *S*-fuscacet.)		Inhibited by *R*-fusc, and *R*-fuscacet.	Requires *S*-fusc; inhibited by *R*-fuscacet.
	Astylidius parvus (*R*,*S*-fusc, *R*-fuscacet.)	Requires *R*- and *S*-fusc.		Requires fusc.
	Lepturges angulatus (*R*,*S*-fuscacet.)	Requires *R*- and *S*-fuscacet.	Inhibited by *R*,*S*-fusc.	

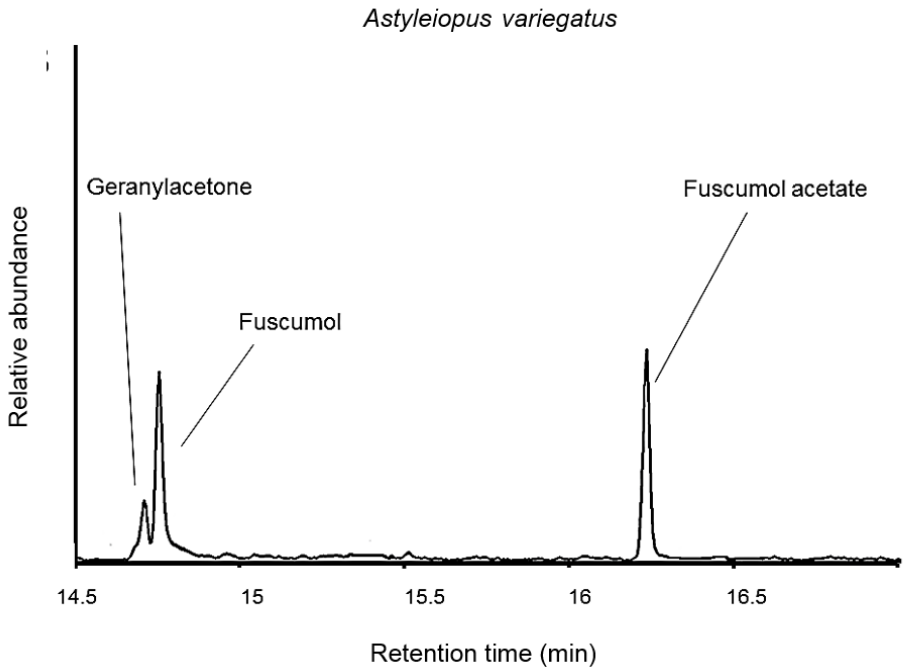


Figure 1. Representative total ion chromatogram of headspace extracts collected from male *A*. *variegatus.*

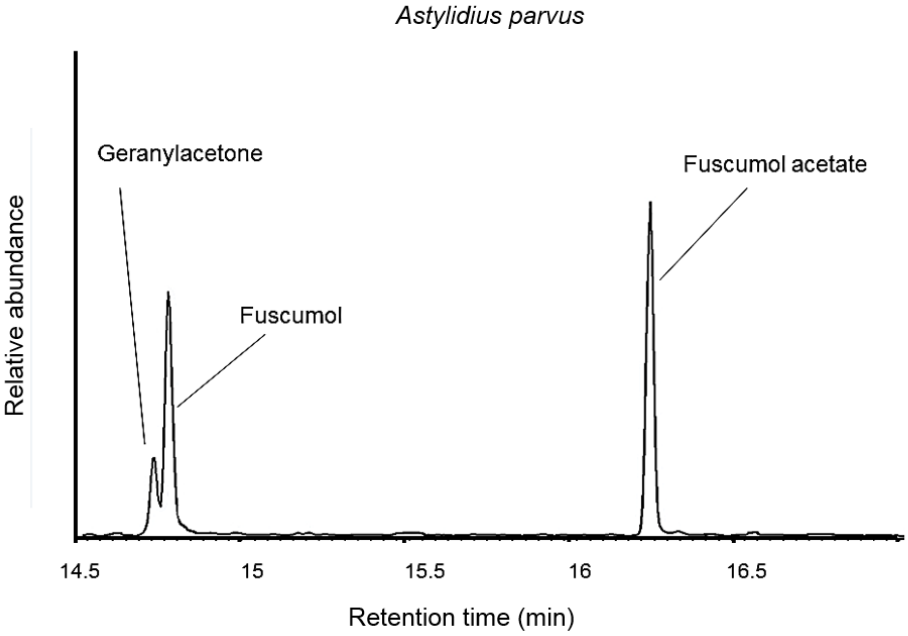


Figure 2. Representative total ion chromatogram of headspace extracts collected from male *A*. *parvus.*

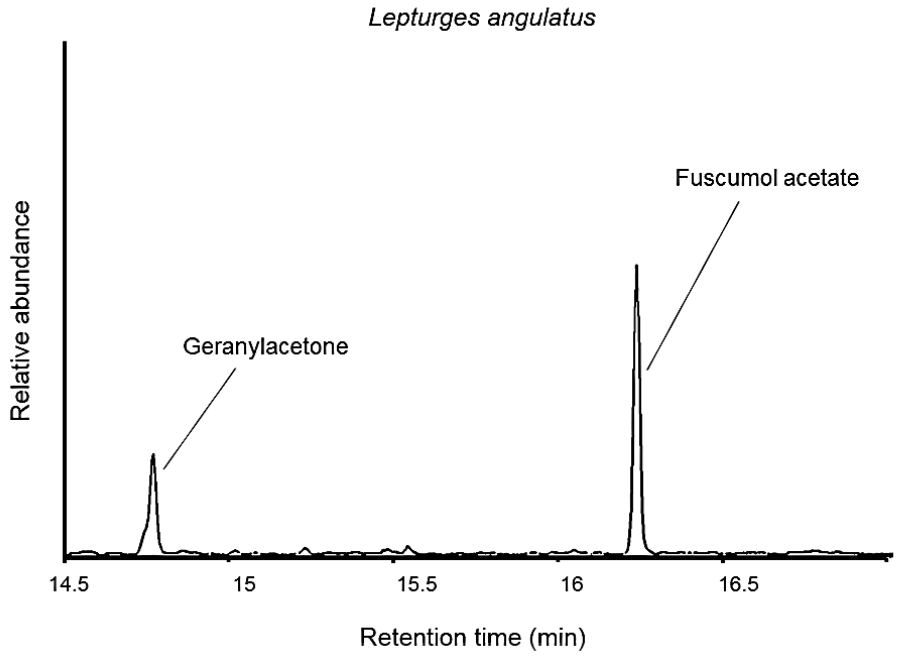


Figure 3. Representative total ion chromatogram of headspace extracts collected from male *L*. *angulatus*.

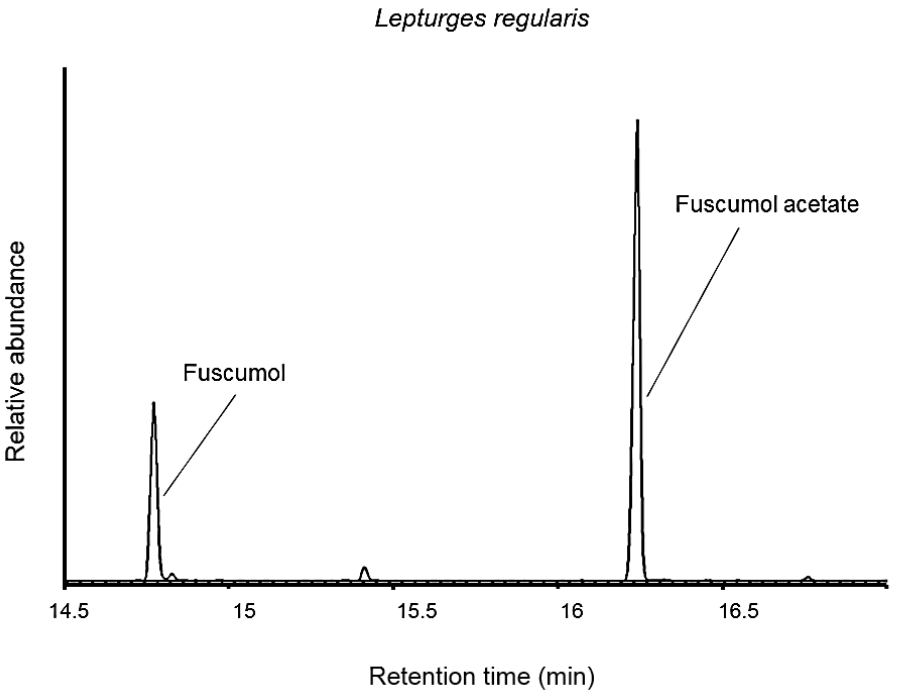


Figure 4. Representative total ion chromatogram of headspace extracts collected from male *L*. *regularis*.

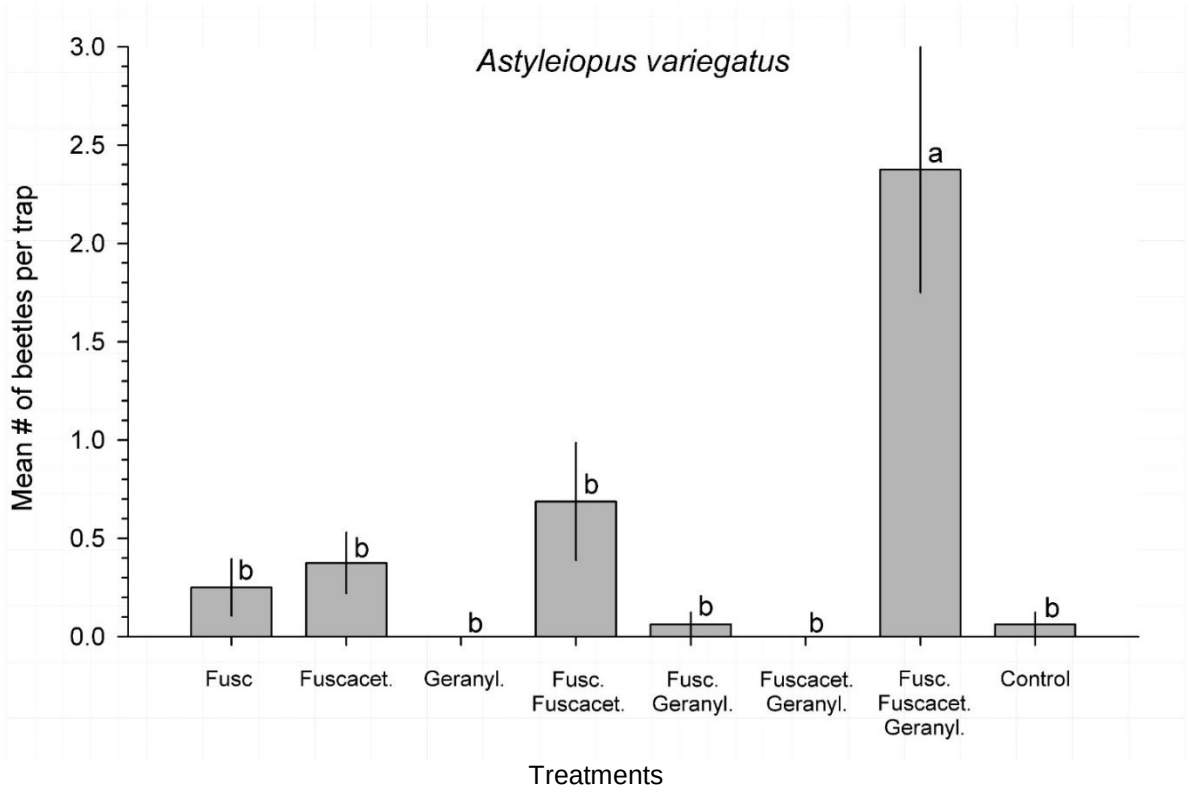


Figure 5. Mean (± SE) number of adult *A*. *variegatus* (N = 61) that were captured per trap during experiment 1 by traps baited with synthetic pheromone components. Means significantly different, Friedman's $Q_{(7,128)} = 42.2$, $P < 0.001$. Means with same letters are not significantly different (Tukey's HSD test; PROC GLM; SAS Institute 2001) at $P < 0.05$. Compound abbreviations for fuscumol, and fuscumol acetate, and geranylacetone, are fusc., and fuscacet., and geranyl., respectively. Sex ratio (female:male) of adult *A. variegatus* sampled from statistically significant treatments (N = 20) was 1.2:1 ($\chi^2 = 8.1$, $P = 0.66$).

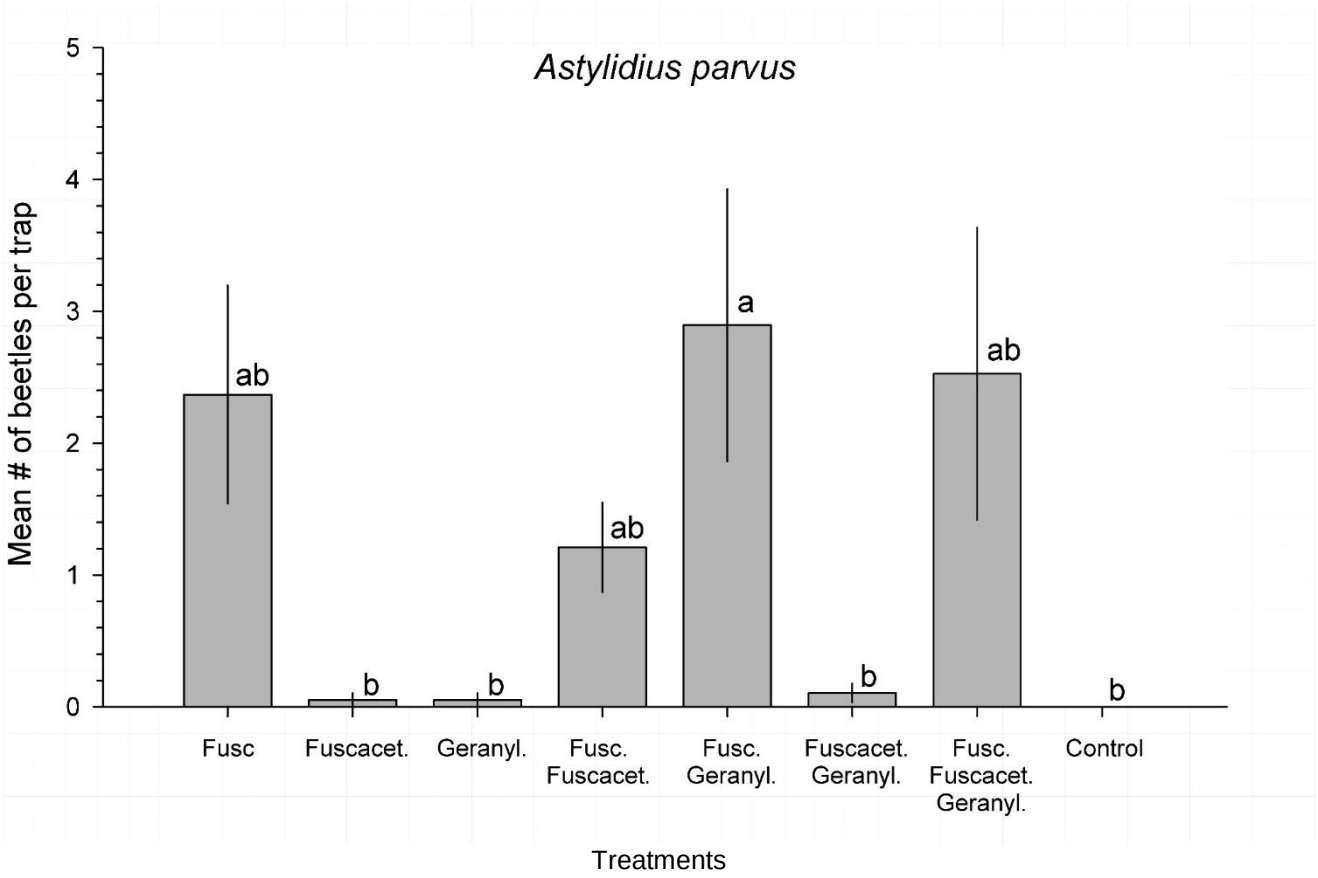


Figure 6. Mean (± SE) number of adult *A*. *parvus* (N = 175) that were captured per trap during experiment 1 by traps baited with synthetic pheromone components. Means significantly different, Friedman's $Q_{(7,152)} = 52.8$, $P < 0.001$. Means with same letters are not significantly different (Tukey's HSD test; PROC GLM; SAS Institute 2001) at $P < 0.05$. Compound abbreviations for fuscumol, and fuscumol acetate, and geranylacetone, are fusc., and fuscacet., and geranyl., respectively. Sex ratios (female:male) of adult *A. variegatus*, *A. parvus*, and *L. angulatus* sampled from statistically significant treatments (N = 20 per species) were 1.2:1 ($\chi^2 = 8.1$, $P = 0.66$), 4.25:1 ($\chi^2 = 15.4$, $P = 0.0005$), and 1.75:1 ($\chi^2 = 1.64$, $P = 0.2$).

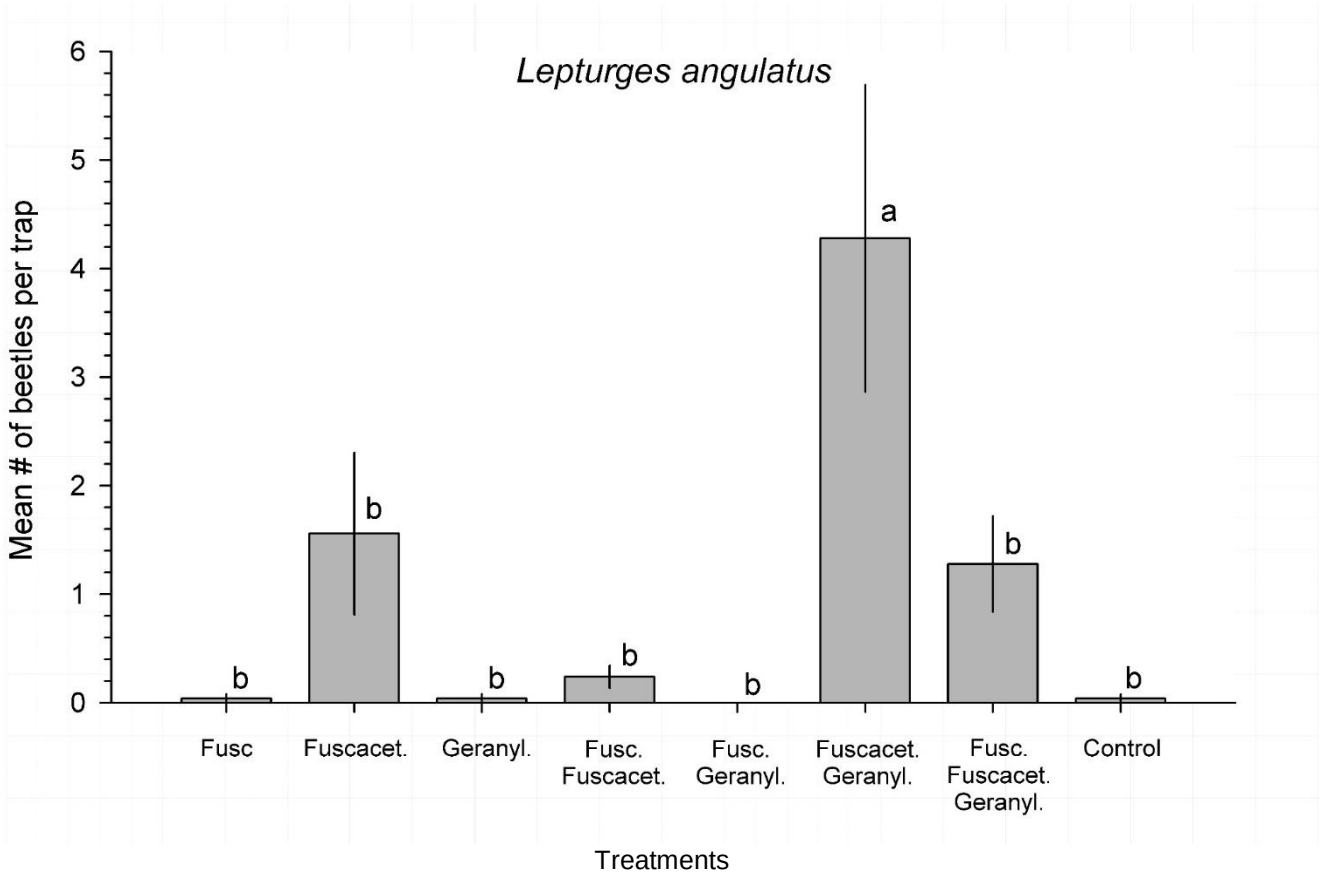


Figure 7. Mean (± SE) number of adult *L*. *angulatus* (N = 87) that were captured per trap during experiment 1 by traps baited with synthetic pheromone components. Means significantly different, Friedman's $Q_{(7,200)} = 99.3$, $P < 0.001$, respectively. Means with same letters are not significantly different (Tukey's HSD test; PROC GLM; SAS Institute 2001) at $P < 0.05$. Sex ratio (female:male) of adult *L. angulatus* sampled from statistically significant treatments (N = 20) was 1.75:1 ($\chi^2 = 1.64$, $P = 0.2$).

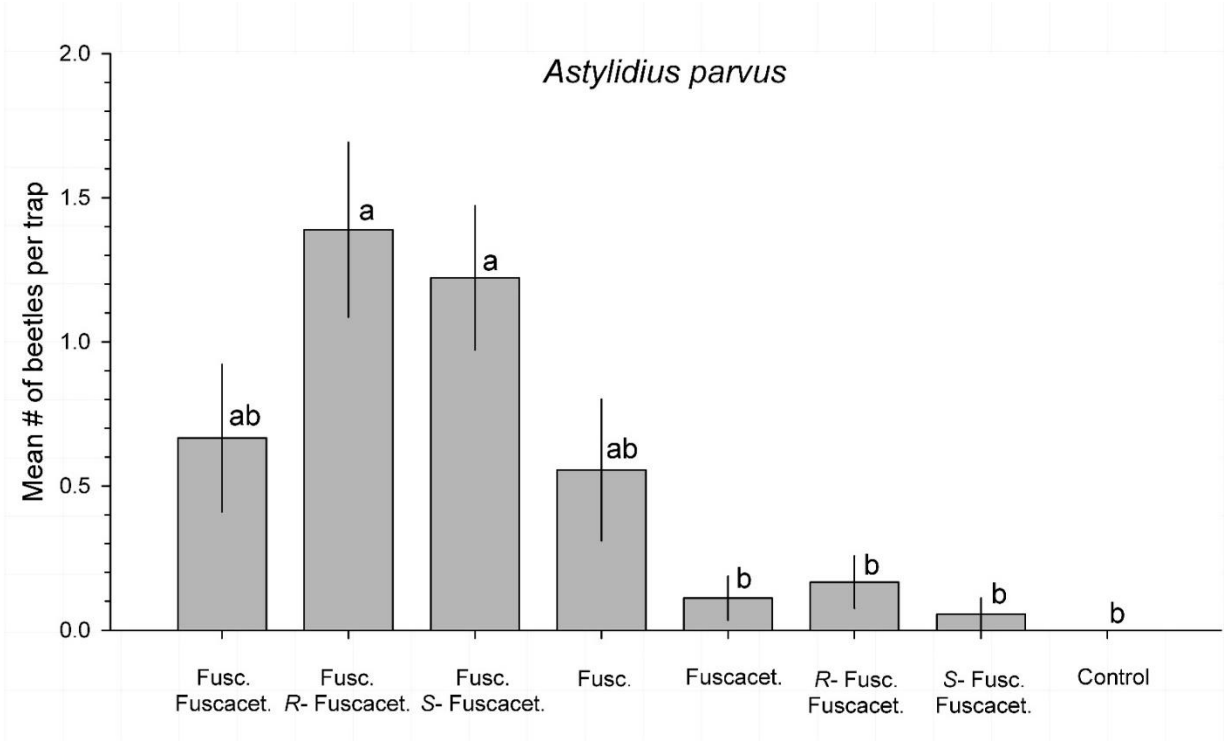


Figure 8. Mean (± SE) number of adult A) *A*. *parvus* (N = 75) captured per trap during experiment 3 by traps baited with pheromone components. Means significantly different, Friedman's $Q_{(7,144)} = 48.9$, $P < 0.001$. Means with same letters are not significantly different (Tukey's HSD test; PROC GLM; SAS Institute 2001) at $P < 0.05$.

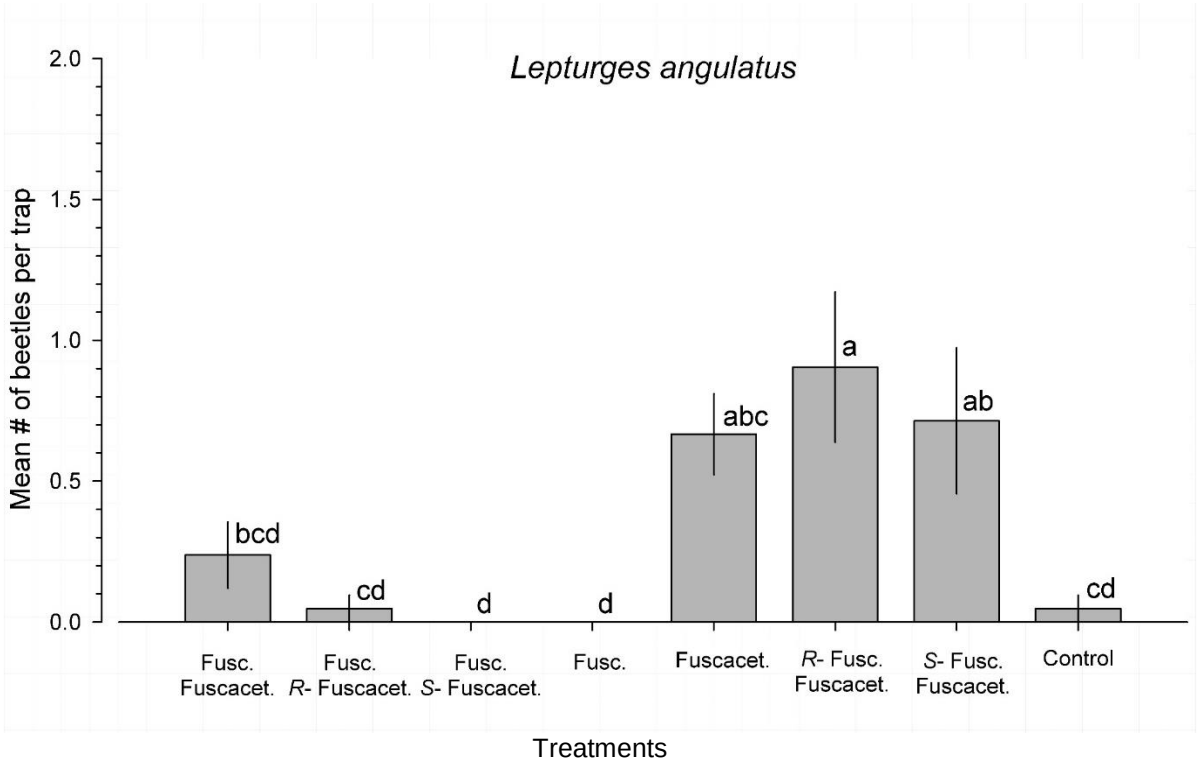


Figure 9. Mean (± SE) number of adult *L*. *angulatus* (N = 55) captured per trap during experiment 3 by traps baited with pheromone components. Means significantly different, Friedman's $Q_{(7,168)} = 47.2$, $P < 0.001$. Means with same letters are not significantly different (Tukey's HSD test; PROC GLM; SAS Institute 2001) at $P < 0.05$.

REFERENCES

Bezark, L. G. 2014. Checklist of the Oxypeltidae, Vesperidae, Disteniidae and Cerambycidae (Coleoptera) of the Western Hemisphere. (https://apps2.cdfa.ca.gov/publicApps/plant/bycidDB/checklists/WestHemiCerambycida 2014.pdf).

Brockerhoff, E. G., A.M. Liebhold, and H. Jactel. 2006. The ecology of forest insect invasions and advances in their management. Can. J. For. Res. 36: 263-268.

Fonseca M. G., D. M. Vidal, and P.H.G Zarbin. 2010. Male-produced sex pheromone of the cerambycid beetle *Hedypathes betulinus*: Chemical identification and biological activity. J. Chem. Ecol. 36: 1132-1139.

Graham E. E., R. F. Mitchell, P. F. Reagel, J. D. Barbour, J. G Millar and L. M. Hanks. 2010. Treating panel traps with a fluoropolymer enhances their efficiency in capturing cerambycid beetles. J. Econ. Entomol. 103: 641-647.

Groot A.T., M. Marr, D.G. Heckel, and G. Schoefl. 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecol. Entomol. 35: 105-118.

Hammack, L. 1997. Attractiveness of synthetic corn volatiles to feral northern and western corn rootworm beetles (Coloeoptera: Chrysomelidae). Environ. Entomol. 26: 311-317.

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., 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. Chemoecol. 23: 21-44.

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, and J. G. Millar. 2013. Seasonal phenology of the cerambycid beetles of east-central Illinois. Ann. Entomol. Soc. Am. 107: 211-226

Hughes, D. T., J. Pelletier, C. W. Luetje, and W. S. Leal. 2010. Odorant receptor from the southern house mosquito narrowly tuned to the oviposition attractant skatole. J. Chem. Ecol. 36: 797-800.

Hughes, G. P., Y. Zou, J. G. Millar, and M. D. Ginzel. 2013. (*S*)-fuscumol and (*S*)-fuscumol acetate produced by a male *Astyleiopus variegatus* (Coleoptera: Cerambycidae). Can. Entomol. 145: 1-6.

Linsley E. G. 1961. The Cerambycidae of North America, Part I. Introduction. Univ. Calif. Publ. Entomol. 20: 1-188.

Linsley, E. G. and J. A. Chemsak. 1995. The Cerambycidae of North America, Part VII, No. 2: Taxonomy and classification of the subfamily Lamiinae, tribes Acanthocini through Hemilophini. Univ. Calif. Publ. Entomol. 114: 1-292.

Linsley, E. G., and J. A. Chemsak. 1997. The Cerambycidae of North America, Part VIII. Bibliography, index, and host plant index. Univ. Calif. Publ. Entomol. 117. University of California Press, Berkeley, CA, USA.

MacLeod, A., H. F. Evans, and R.H.A. Baker. 2002. An analysis of pest risk from an Asian longhorn beetle (*Anoplophora glabripennis*) to hardwood trees in the European community. Crop. Prot. 21: 635-645.

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.

Mitchell, R. F., E. E. Graham, J.C.H. Wong, P. F. Reagel, B. L. Striman, G. P. Hughes, M. A. Paschen, M. D. Ginzel, J. G. Millar, and L. M. Hanks. 2011. Fuscumol and fuscumol acetate are general attractants for many species of cerambycid beetles in the subfamily Lamiinae. Entomol. Exp. Appl. 141: 71-77.

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. Cerambycid beetle species with similar pheromones are segregated by phenology and minor pheromone components. J. Chem. Ecol. (submitted Nov. 2014)

Mayo, P.D., P.J. Silk, M. Cusson, and C. Béliveau. 2013. Steps in the biosynthesis of fuscumol in the longhorn beetles *Tetropium fuscum* (F.) and *Tetropium cinnamopterum* Kirby. J. Chem. Ecol. 39: 377-389.

Paine, T. D. and C. C. Hanlon. 1991. Response of *Dendroctonus brevicomis* and *Ips paraconfusus* (Coleoptera: Scolytidae) to combinations of synthetic pheromone attractants and inhibitors verbenone and ipsdienol. J. Chem. Ecol. 17: 2163-2176.

Ray, A. M., J. G. Millar, J. S. McElfresh, I. P. Swift, J. D. Barbour, and L. M. Hanks. 2009. Male-produced aggregation pheromone of the cerambycid beetle *Rosalia funebris*. J. Chem. Ecol. 35: 96-103.

Ray, A. M., A. Žunič, R. L. Alten, J. S. McElfresh, L. M. Hanks, and J. G. Millar. 2011. Cis Vaccenyl acetate, a female-produced sex pheromone component of *Ortholeptura valida*, a longhorned beetle in the subfamily Lepturinae. J. Chem. Ecol. 37: 173-178.

Rhainds, M., L. ChinChiew, Z. MoLi, and G. Gries. 2002. Incidence, symptoms, and intensity of damage by three coffee stemborers (Coleoptera: Cerambycidae) in South Yunan, China. J. Econ. Entomol. 95: 106-112.

Rodstein, J., J. G. Millar, J. D. Barbour, J. S. McElfresh, I. M. Wright, K. S. Barbour, A. M. Ray, and L. M. Hanks. 2011. Determination of the relative and absolute configurations of the female-produced sex pheromone of the cerambycid beetle *Prionus californicus*. J. Chem. Ecol. 37: 114-124.

SAS Institute (2001). SAS/STATS user's guide, release 9.2. SAS Institute Inc., Cary

Savoie, A., J. H. Borden, H. D. Pierce, R. Gries, and G. Gries. 1998. Aggregation pheromone of *Pityogenes knechteli* and semiochemical-based interactions with three other bark beetles. J. Chem. Ecol. 24: 321-337.

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

Takken, W. and M. Dicke. 2006. Chemical ecology: from gene to ecosystem, pp. 1-8 in Takken, W. and M. Dicke (eds.) Chemical ecology: a multidisciplinary approach. Wageningen, Netherlands.

Vet, L.E.M. 1999. From chemical to population ecology: infochemical use in an evolutionary context. J. Chem. Ecol. 25: 31-49.

Wakamura, S., and N. Arakaki. 2004. Sex pheromone components of pyralid moths *Terastia subjectalis* and *Agathodes ostentalis* feeding on the coral tree, *Erithrina variegata*: two sympatric species share common components in different ratios. Chemoecology 14: 181 185.

Zarbin, P.H.G., M. G. Fonseca, D. Szczerbowski, and A.R.M Oliveira. 2013. Biosynthesis and site of production of sex pheromone components of the cerambycid beetle, *Hedypathes betulinus*. J. Chem. Ecol. 39: 358-363.