

Blending Synthetic Pheromones of Cerambycid Beetles to Develop Trap Lures That Simultaneously Attract Multiple Species

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ABSTRACT We evaluated attraction of cerambycid beetle species to blends of known cerambycid pheromones to determine whether such blends could be used as effective trap lures for detecting and monitoring multiple species simultaneously. Pheromone-baited traps captured 1,358 cerambycid beetles of which 1,101 (81.1%) belonged to three species in the subfamily Cerambycinae: *Neoclytus acuminatus* (F.), *Neoclytus mucronatus* (F.), and *Xylotrechus colonus* (F.). Beetles of these species were significantly attracted to synthetic blends that contained their pheromone components (isomers of 3-hydroxy-2-hexanone, 2,3-hexanediol, or both), despite the presence of pheromone components of different species, including other isomers of 2,3-hexanediol, (*E/Z*)-6,10-dimethyl-5,9-undecadien-2-yl acetate, and citral. In some cases, attraction was partially inhibited by the pheromone components of heterospecific species, whereas for *N. acuminatus*, attraction was completely inhibited when blends contained (2*R**,3*S**)-hexanediol, the racemic mixture of diastereomers of its pheromone, (2*S*,3*S*)-hexanediol. Among the remaining beetles captured were three species in the subfamily Lamiinae: *Astyleiopus variegatus* (Haldeman), *Graphisurus fasciatus* (Degeer), and *Lepturges angulatus* (LeConte). All three lamiine species were previously known to be attracted to (*E/Z*)-6,10-dimethyl-5,9-undecadien-2-yl acetate and were captured in significant numbers by blends containing that compound. Our results suggest that different types of cerambycid pheromones can be combined to create effective multispecies lures for use in surveillance programs that target exotic cerambycid species.

KEY WORDS Cerambycidae, inhibition, 3-hydroxy-2-hexanone, 2,3-hexanediol, fuscumol acetate

Several exotic cerambycid beetle species have invaded North America in recent years and have become economically important pests of woody plants (Smith and Hurley 2000, Nowak et al. 2001, Paine and Millar 2002, Reddy et al. 2005, Maier 2007, Haack et al. 2010). It is essential that effective international quarantine procedures be developed to detect new incursions of exotic cerambycid pests before they become established, or as soon as possible after they have become established, to maximize the chances of eradication during the early stages of colonization. Current surveillance programs for cerambycids use traps baited with host plant volatiles (often ethanol and α -pinene) that attract conifer feeders but are less effective to completely ineffective for detecting species that attack deciduous trees (Brockerhoff et al. 2006, Witzgall et al. 2010, Miller et al. 2011).

There has been much recent progress in identifying volatile sex or aggregation pheromones produced by cerambycids, and these compounds show great promise for developing general attractants for quarantine

applications. For example, males of many species in the subfamily Cerambycinae produce pheromones composed of isomers of 3-hydroxy-2-hexanone, 2,3-hexanediol, or both to which both sexes are attracted (Lacey et al. 2004, 2007, 2008, 2009; Hanks et al. 2007). Similarly, the terpenoid alcohol (*E*)-6,10-dimethyl-5,9-undecadien-2-ol, termed “fuscumol,” is a male-produced pheromone of some species in the subfamily Spondylidinae (Silk et al. 2007), but the same compound and its acetate have recently been shown to attract many species in the subfamily Lamiinae as well (Mitchell et al. 2011).

To use expensive labor and resources most efficiently, state and federal agencies charged with operating surveillance programs for native and exotic species of cerambycids seek lures that attract many target species simultaneously. Our project goal was to test the feasibility of developing such multispecies lures by creating blends of pheromone components from a diversity of cerambycid species. This approach requires careful validation because there is at least one report that attraction of a cerambycid species to its pheromone is inhibited by one or more stereoisomers of the pheromone (Lacey et al. 2004). This phenomenon is common among other insect taxa, particularly the Lepidoptera, where small amounts of behavioral

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Table 1. Numbers of cerambycid beetles of two subfamilies that were captured by panel traps during experiments I, II, and III, according to lure treatment

Expt.	Treatment	Cerambycinae			Lamiinae		
		<i>N. acuminatus</i>	<i>N. mucronatus</i>	<i>X. colonus</i>	<i>A. variegatus</i>	<i>G. fasciatus</i>	<i>L. angulatus</i>
I	3R*-ketone	2	20	46	0	1	0
	R*R*-diol	30	2	18	0	4	0
	R*S*-diol	4	0	13	0	6	0
	Blend I: 3R*-ketone, R*R*- and R*S*-diol, fuscumol acetate, citral	1	9	72	0	13	0
	Control	0	0	7	0	4	0
II	3R*-ketone	3	77	41	0	10	1
	R*R*-diol	97	5	8	0	10	0
	R*S*-diol	4	10	21	0	7	0
	Blend II A: 3R*-ketone, R*R*-diol, fuscumol acetate, citral	34	63	51	1	32	4
	Blend II B: 3R*-ketone, R*S*-diol, fuscumol acetate, citral	9	54	58	2	24	14
III	Control	2	3	2	0	2	0
	3R*-ketone	5	57	20	0	0	3
	Blend III A: 3R*-ketone + fuscumol acetate	2	42	30	11	0	20
	Blend III B: 3R*-ketone + fuscumol acetate + citral	1	37	17	6	2	11
	RR*-diol	34	3	1	1	0	1
	Blend III C: R*R*-diol + fuscumol acetate	36	3	0	8	1	26
	Blend III D: R*R*-diol + fuscumol acetate + citral	36	3	4	12	0	18
	Control	2	1	1	2	0	0
Totals	302	389	410	43	116	98	

Treatment means (number of beetles per trap) are presented in Figs. 1–3 (cerambycines) and Figs. 4 and 5 (lamiines).

inhibitors in pheromone blends serve to prevent cross-attraction of congeners that share one or more pheromone components (e.g., Ando et al. 2004). Thus, the research described here evaluated the attraction of native cerambycid species to blends of some of the more common and well-known cerambycid pheromones, as proof of concept of using blends of pheromones to detect or monitor many cerambycid species simultaneously.

Materials and Methods

Field bioassays were conducted during 2 June–16 September 2010 at four locations in east central Illinois: 1) Allerton Park (Piatt Co.; 39.985342°N, –88.650147°W), a 600-ha forest composed primarily of oak (*Quercus* spp.) and hickory (*Carya* spp.) owned by the University of Illinois; 2) Trelease Woods (Champaign Co.; 40.134873°N, –88.142796°W), a 28.8-ha deciduous upland forest with a mix of primarily oak, ash (*Fraxinus* spp.), and maple (*Acer* spp.), also owned by the University of Illinois; 3) Forest Glen Seep (Vermilion Co.; 40.01516°N, –87.56771°W), 4.5 ha in area and forested primarily with beech (*Fagus* spp.), maple, oak, and hickory (within Forest Glen Preserve, a Vermilion Co. nature preserve); and 4) a residence in the city of Urbana (Champaign Co.; 40.097067°N, –88.203162°W) in a neighborhood with mature trees of many species, primarily deciduous (Dirr 1998). During the study, minimum and maximum temperatures averaged 17.7 and 30°C, respectively, and rainfall was unusually heavy (41 cm total,

9 cm greater than the 10-yr average; Weather Underground, Inc., Ann Arbor, MI).

We trapped beetles with black flight intercept panel traps (corrugated plastic, 1.2 m in height by 0.3 m in width; Alpha Scents Inc., West Linn, OR) that were suspended from frames of polyvinyl chloride irrigation pipe (for details, see Graham et al. 2010). The supplied trap basins were replaced with 2-liter plastic jars (P.E.T. model 55-650C, General Bottle Supply Company, Los Angeles, CA) that contained a killing solution (≈ 300 ml of propylene glycol). We cut a 7.5-cm hole in the threaded lid of each jar and hot-melt glued the lid to a plastic funnel (2 liter; spout cut to yield a 35-mm-diameter opening) such that the spout would be inside the jar when the lid was attached. The funnel-jar assembly was wired to each trap bottom. We treated the inner surfaces of the trap panels, bottoms, and funnels with Fluon (Thermo Fisher Scientific, Waltham, MA) to improve trapping efficiency (see Graham et al. 2010).

Our experiments targeted three native cerambycid species that were common in the study area, and for which the male-produced pheromones were known (Lacey et al. 2004, 2007, 2009): 1) *Neoclytus acuminatus* (F.), pheromone composed of (2S,3S)-hexanediol; 2) *Neoclytus mucronatus* (F.), (R)-3-hydroxyhexan-2-one; and 3) *Xylotrechus colonus* (F.), (R)- and (S)-3-hydroxyhexan-2-one and (2S,3S)- and (2R,3R)-hexanediol. Pheromones of all three species have been shown to attract both sexes (Lacey et al. 2004, 2007, 2009). We used racemic blends of these compounds because the enantiomers are expensive to synthesize

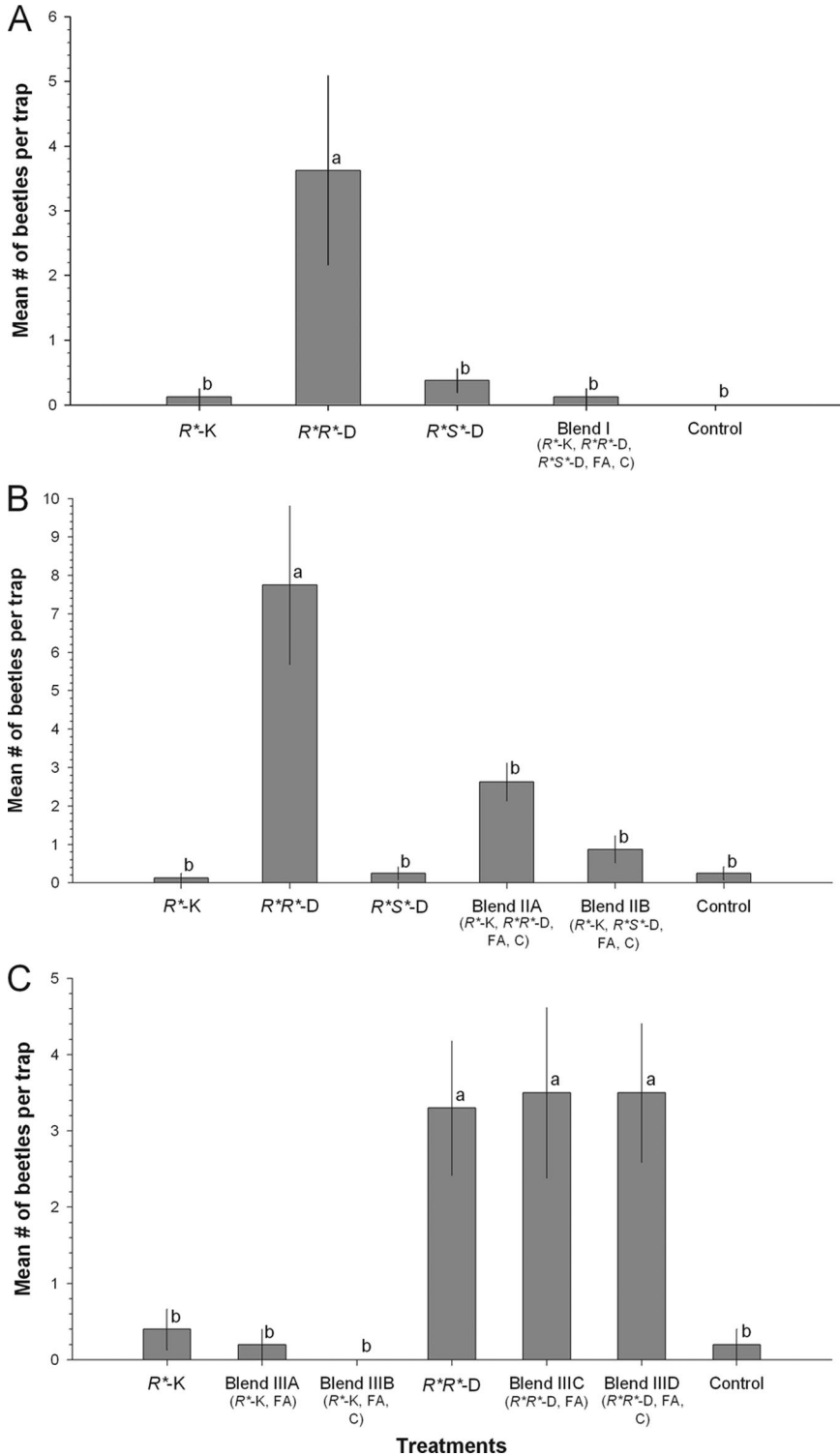


Fig. 1. Mean (± 1 SE) number of adult *N. acuminatus* captured per trap (sexes combined) with respect to composition of the lure during experiments I (A), II (B), and III (C). Means significantly different, Friedman's $Q_{4,39} = 18.8, P = 0.009$; $Q_{5,48} = 31.3, P < 0.0001$; and $Q_{6,70} = 37.2, P < 0.0001$, respectively. Means with the same letters are not significantly different (REGWQ means-separation test) at $P < 0.05$. Compound abbreviations: 3R*-ketone, R*-K; R*R*-diol, R*R*-D; R*S*-diol, R*S*-D; FA, fuscumol acetate; and C, citral.

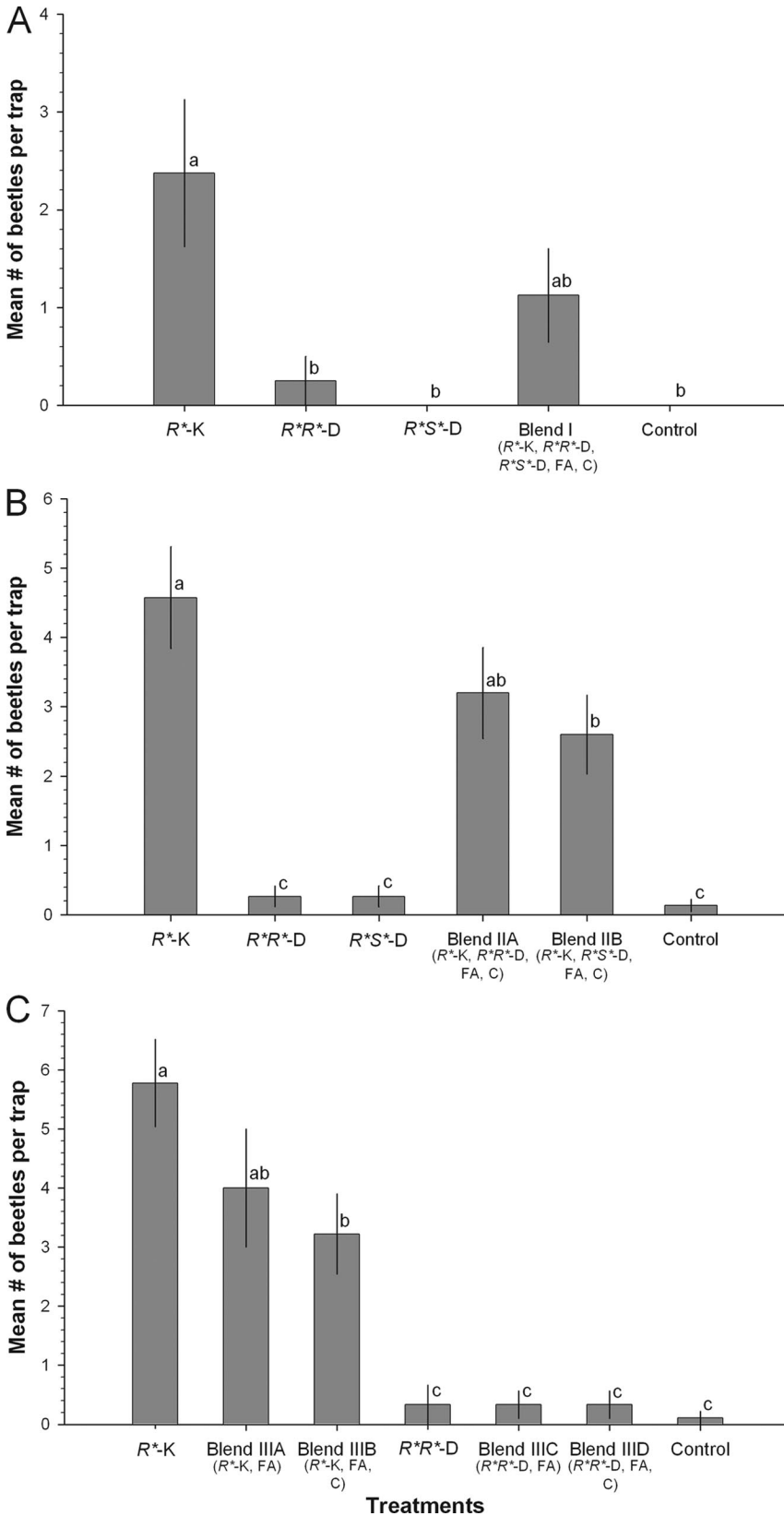


Fig. 2. Mean (± 1 SE) number of adult *N. mucronatus* captured per trap (sexes combined) with respect to composition of the lure during experiments I (A), II (B), and III (C). Means significantly different, Friedman's $Q_{4,39} = 18.5$, $P = 0.001$; $Q_{5,89} = 36.0$, $P < 0.0001$; and $Q_{6,63} = 42.6$, $P < 0.0001$, respectively. Means with the same letters are not significantly different (REGWQ means-separation test) at $P < 0.05$. Compound abbreviations as described in Fig. 1.

in quantities sufficient for general purpose trapping (for chemical syntheses, see Millar et al. 2009, Mitchell et al. 2011). Previous research had shown that (2*R*,3*R*)-hexanediol, the enantiomer of the *N. acuminatus* pheromone, did not inhibit attraction of this species, whereas it was inhibited by one or both of the diastereomeric (2*R*,3*S*)- and (2*S*,3*R*)-hexanediols (Lacey et al. 2004). Thus, our experiments were based on the test compounds (3*R**)-hydroxyhexan-2-one (henceforth 3*R**-ketone) and (2*R**,3*R**)-hexanediol (*R***R**-diol), but we also included (2*R**,3*S**)-hexanediol ("R*S*-diol") to evaluate potential inhibition by these diastereomers. These compounds were tested as single components, and in a five-component blend along with two other cerambycid pheromone chemicals that would be logical candidates for inclusion in multispecies lures (see Table 1 for experimental designs): 1) (*E*/*Z*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (henceforth fuscumol acetate), a known attractant for many lamiine species in our study area (Mitchell et al. 2011); and 2) citral (Sigma-Aldrich, St. Louis, MO), an isomeric blend of neral and geranial (≈3:5) that is a pheromonal attractant for another cerambycid species that is active in very early spring, *Megacyllene caryae* (Gahan) (Lacey et al. 2008).

Lure blends were formulated to contain 25 mg of each component per milliliter of solution in 95% ethanol. Ethanol is an efficient carrier for the pheromone components and does not itself attract cerambycid beetles at these volumes (Hanks et al. 2007), although it is possible that the solvent may have a slight synergistic influence. Emitters were clear polyethylene sachets (5.1 by 7.6 cm, 0.05-mm wall thickness, press-seal bags; Bagette model 14770, Cousin Corp., Largo, FL) that were hung in the center of traps. A single trap line was set up at each study site, including a control trap baited with a lure containing only ethanol. Traps were positioned 10 m apart in linear transects and checked for beetles every 1–3 d. Treatments were assigned randomly to positions within a block at the beginning of each bioassay, and they were rotated within transects weekly to control for location effects. Lures were replaced as necessary.

Experiment I assessed attraction to the three test compounds separately and to the five-component blend of all three of these compounds plus fuscumol acetate and citral (blend I; Table 1). The experiment was conducted during 2 June–6 July 2010 at Allerton Park.

Experiment II was similar in design to experiment I, but the *R***R**- and *R***S**-diols were not blended so as to avoid diastereomeric inhibition (see results for experiment I). Thus, the treatments for this experiment consisted of the three test compounds separately, blend II A (*R***R**-diol, 3*R**-ketone, fuscumol acetate, and citral), and blend II B (*R***S**-diol, 3*R**-ketone, fuscumol acetate, and citral; Table 1). Experiment II was conducted during 7 July–16 September 2010 at all four study sites.

Experiment III was designed to evaluate the cumulative influence of blending other components with the two test compounds that attracted the greatest

number of beetles, 3*R**-ketone and *R***R**-diol (see Results). The treatments were 3*R**-ketone alone, blend III A (3*R**-ketone and fuscumol acetate), blend III B (3*R**-ketone, fuscumol acetate, and citral), *R***R**-diol alone, blend III C (*R***R**-diol and fuscumol acetate), and blend III D (*R***R**-diol, fuscumol acetate, and citral; Table 1). Experiment III was conducted during 16 June–9 August 2010 at Trelease Woods.

During experiments II and III, we also captured three lamiine species in large enough numbers to allow us to determine whether their attraction to fuscumol acetate was inhibited by pheromone components of other species (in this case, 3-hydroxy-2-hexanone, 2,3-hexanediols, and citral). The species (all tribe Acanthocini) included *Astyleiopus variegatus* (Haldeman), *Graphisurus fasciatus* (Degeer), and *Lepturges angulatus* (LeConte) (see Results). For all three species, both sexes are attracted by fuscumol acetate (Mitchell et al. 2011), but their pheromones have yet to be formally identified.

Differences between treatment means, blocked by site and date, were tested separately for each experiment and species by using the nonparametric Friedman's test (PROC FREQ, option CMH, SAS Institute 2010). Differences between pairs of means were tested with the Ryan-Einot-Gabriel-Welsch Q (REGWQ) means-separation test that controls for maximum experiment-wise error rates (PROC GLM, SAS Institute 2008). Data for site and date replicates were included in the analysis based on a threshold number of specimens (two to eight specimens, depending on the total number captured) so as to optimize sample size per replicate while maintaining sufficient replication for a robust analysis ($N > 7$).

Results

Pheromone traps captured 1,358 of the targeted cerambycid beetles during the bioassays of which 1,101 (81.1%) were the three cerambycid species and 257 were the three lamiine species (Table 1; other species of cerambycids were captured in numbers too low for statistical analysis). In experiment I, the only traps that captured adult *N. acuminatus* in numbers significantly greater than controls were those baited with the racemic *R***R**-diol (Fig. 1A). The reduced captures of that species by traps baited with blend I, which contained *R***R**-diol plus the *R***S**-diol, confirmed the previous report that one or both of the *R***S**-diol diastereomers inhibit attraction of *N. acuminatus* to its pheromone (Lacey et al. 2004). When the diol diastereomers were tested in separate blends in experiment II (Fig. 1B), *N. acuminatus* again was strongly attracted to *R***R**-diol but showed reduced attraction to blend II A that contained a blend of *R***R**-diol, 3*R**-ketone, fuscumol acetate, and citral, demonstrating that one of the latter compounds also was inhibitory. That attraction to *R***R**-diol was not inhibited in the presence of fuscumol acetate and citral in experiment III (Fig. 1C) indicated that 3*R**-ketone probably was responsible for the decreased attraction to blend II A (Fig. 1B). We subsequently have con-

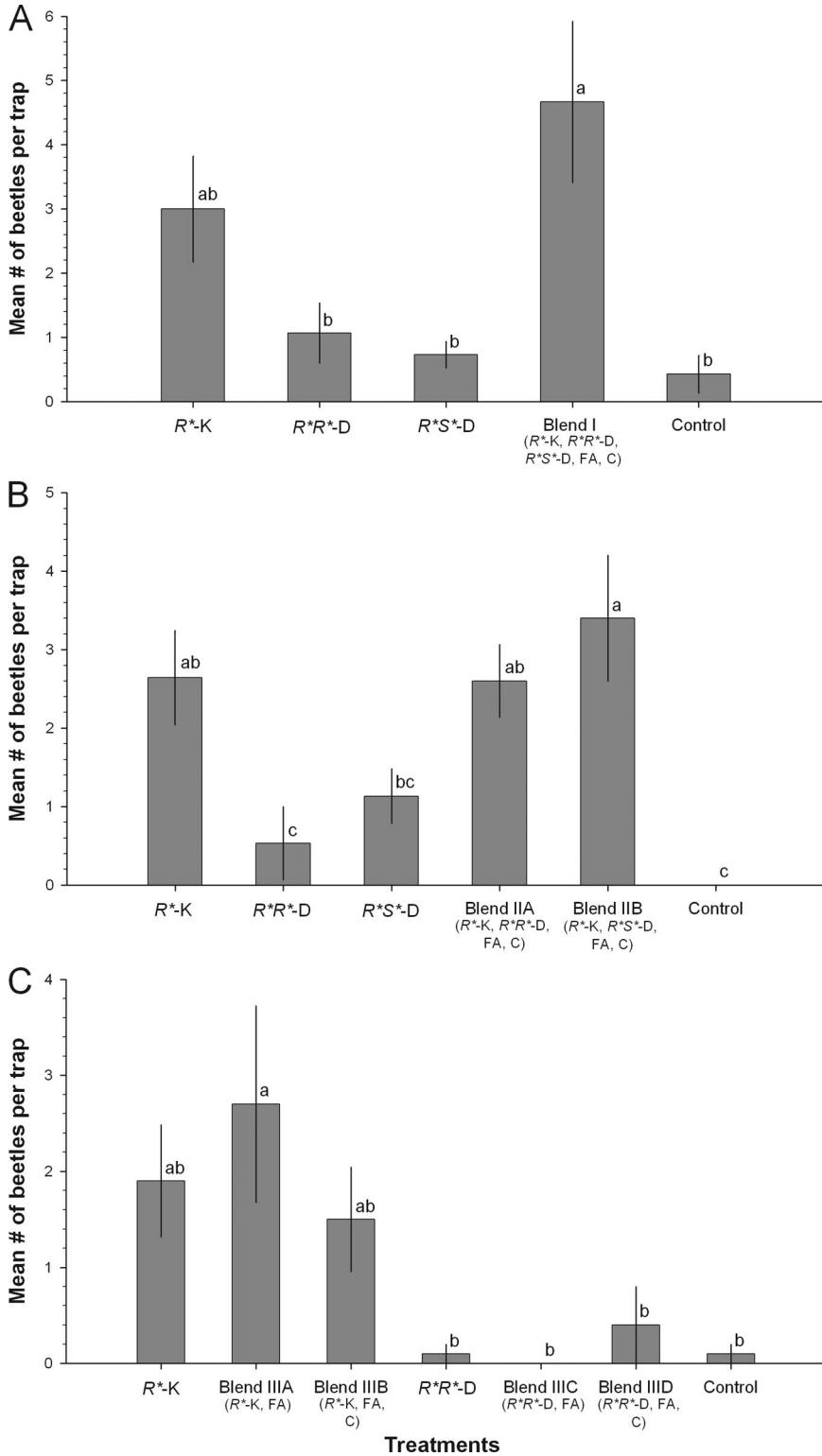


Fig. 3. Mean (± 1 SE) number of adult *X. colonus* captured per trap (sexes combined) with respect to composition of the lure during experiments I (A), II (B), and III (C). Means significantly different, Friedman's $Q_{4,74} = 18.4, P = 0.001$; $Q_{5,89} = 36.7, P < 0.0001$; and $Q_{6,70} = 29.5, P < 0.0001$, respectively. Means with the same letters are not significantly different (REGWQ means-separation test) at $P < 0.05$. Compound abbreviations as described in Fig. 1.

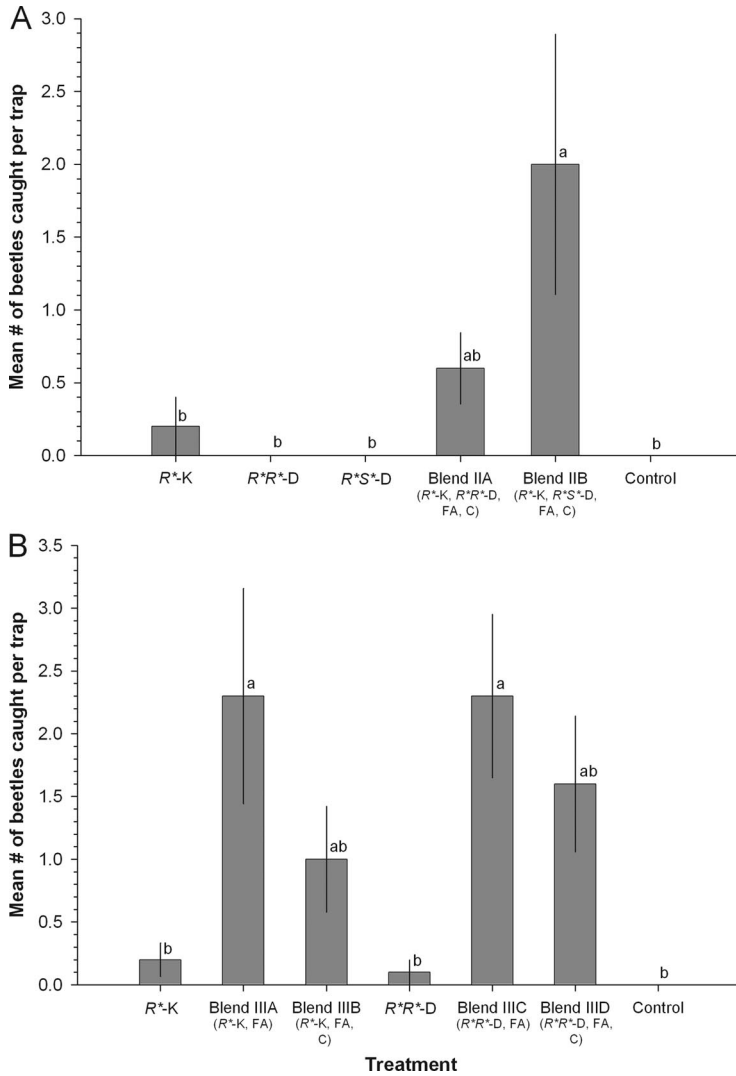


Fig. 4. Mean (± 1 SE) number of *L. angulatus* captured per trap (sexes combined) with respect to composition of the lure in experiment II (A) and III (B). Means significantly different, Friedman's $Q_{5,30} = 15.8$, $P = 0.0075$ and $Q_{6,70} = 27.3$, $P = 0.0001$, respectively. Means with the same letters are not significantly different (REGWQ means-separation test) at $P < 0.05$. Compound abbreviations as described in Fig. 1.

firmed with follow-up experiments that 3R*-ketone partially inhibits attraction of *N. acuminatus* to R*R*-diol (L.M.H., unpublished data).

In all three experiments, adult *N. mucronatus* were only significantly attracted by lures containing 3R*-ketone. Treatment means for blends I, IIA, and IIIA were not significantly different from those for 3R*-ketone as a single component (Fig. 2B and C) but still substantially more attractive than the control.

Adult *X. colonus* were captured in greatest numbers by traps baited with blend I in experiment I (Fig. 3A), the blend that contained four components identified from headspace odors of three species (i.e., [R]-3- and [S]-3-hydroxyhexan-2-ones, and [2S,3S]- and [2R,3R]-hexanediols; Lacey et al. 2009) plus R*,S*-

diol, fuscumol acetate, and citral. Attraction to 3R*-ketone as a single component was intermediate, and neither of the 2,3-hexanediols were attractive as single-component lures. In experiment II, combining 3R*-ketone with R*R*-diol (blend IIA; Fig. 3B) did not alter attraction compared with 3R*-ketone alone, and as in experiment I, the additional components in both blends IIA and IIB did not affect attraction of this species (Fig. 3B). Analogous results were obtained in experiment III, with the most important component of lures being 3R*-ketone and the absence of that compound resulting in insignificant trap catches (Fig. 3C).

The three lamiine species were attracted to blends containing fuscumol acetate, although small sample sizes resulted in weak statistical power (Table 1). *L.*

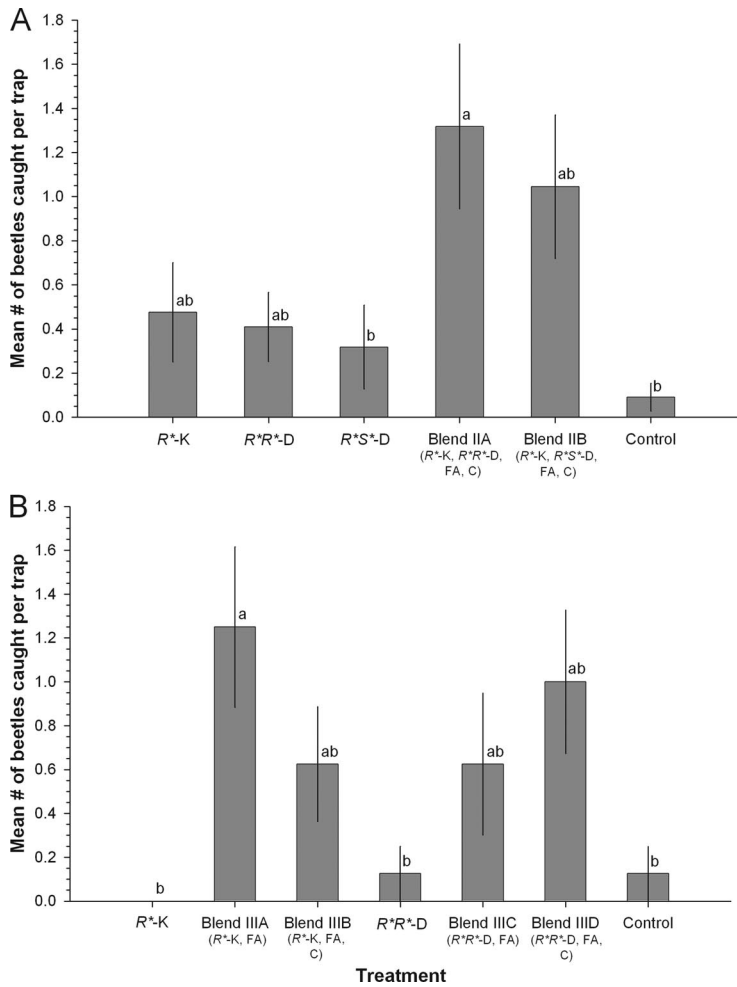


Fig. 5. Mean (± 1 SE) number of beetles of the subfamily Lamiinae captured per trap (sexes combined) with respect to composition of the lure in experiment II for *G. fasciatus* (A) and experiment III for *A. variegatus* (B). Means significantly different, Friedman's $Q_{5,131} = 19.5, P = 0.0015$ and $Q_{6,56} = 17.1, P = 0.009$, respectively. Means with the same letters are not significantly different (REGWQ means-separation test) at $P < 0.05$. Compound abbreviations as described in Fig. 1.

angulatus was captured in significant numbers in traps baited with blend IIB (Fig. 4A) and blends IIIA and IIIC (Fig. 4B). For *G. fasciatus*, the only treatment that was significantly different from the control was blend IIA (Fig. 5A), whereas for *A. variegatus* only blend IIIA differed from the control (Fig. 5B).

Discussion

The results described here provide further evidence that the attraction of some cerambycid beetles to their pheromones can be inhibited by pheromone components of sympatric species. For example, attraction of *N. acuminatus* to R*R*-diol was inhibited by both the diastereomeric R*S*-diol and by 3R*-ketone. Inhibition by isomers or other structural analogs of pheromone components may serve to maintain the species specificity of a semiochemical signal, as has been shown in other insect taxa (Tamaki 1985, Smadja and

Butlin 2009). Because *N. acuminatus* overlaps with *X. colonus* in seasonal and daily activity periods (Lacey et al. 2009), the 3R*-ketone in the pheromone blend of the latter species may inhibit attraction of *N. acuminatus* to the R*R*-diol component, preventing mistakes in mate location.

However, it should be noted that in the context of developing lure blends for monitoring multiple species, inhibition by blend components may be critically important only if it completely prevents attraction. That is, even if attraction to multicomponent blends were somewhat reduced by one or more components of the blend, the lures still could be effective for detection of a target species. For example, one or more of the blends tested in this study could be used for monitoring the three cerambycine species used as model species in this study, with the exception of blend I for *N. acuminatus*. Moreover, the significant attraction of the three lamiine species to one or more

of the blends further attests to the potential utility of blends as multispecies lures for cerambycids. Nevertheless, partial inhibition by certain components in blends could render blends less sensitive for detecting species that are at low densities, such as might occur during the initial stages of invasion by an exotic species. Thus, surveillance efforts will need to balance the need for sensitive detection methods with the substantially increased cost of deploying multiple traps baited with single pheromone components versus a single trap baited with a blend of pheromones of multiple species.

Extrapolating from our results, it seems likely that blends of pheromones could be effective for detecting exotic species. In particular, numerous cerambycid species in other parts of the world have pheromones composed of the same components that were tested in the trials described here, including the 3-hydroxyhexan-2-one and 2,3-hexanediols for cerambycine species in the tribes Anaglyptini and Callidiini (Fettköther et al. 1995, Leal et al. 1995, Schröder et al. 1994) and (*E*)-fusicumol acetate for lamiine species (Fonseca et al. 2010). Furthermore, it should be possible to extend this concept by incorporating additional, different classes of pheromone components into blends used for surveillance, particularly those with quite different chemistry and/or those which attract cerambycids of other taxonomic groups, where the chances of inhibition would be minimal.

Acknowledgments

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