BLENDING SYNTHETIC PHEROMONES OF CERAMBYCID BEETLES TO DEVELOP TRAP LURES THAT SIMULTANEOUSLY ATTRACT MULTIPLE SPECIES

BY

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THESIS

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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 and/or 2,3-hexanediol), 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 multi-species lures for use in surveillance programs that target exotic cerambycid species.

Acknowledgments

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TABLE OF CONTENTS

INTRODUCTION

Several exotic and invasive species of cerambycid beetles have established in North America in recent years and have become economically important pests of woody plants (e.g., 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 (e.g., see 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 and/or 2,3-hexanediol, 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 Aseminae (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).

In order 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 a large number of target species simultaneously. Our project goal was to test the

feasibility of developing such multi-species lures by creating blends of pheromone components from a diversity of cerambycid species. This approach requires careful validation as 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 also common among other insect taxa, particularly the Lepidoptera, where small amounts of behavioral 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.

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, -88.650147), a 600 ha forest composed primarily of oaks and hickories owned by the University of Illinois; 2) Trelease Woods (Champaign Co.; 40.134873, -88.142796), a 28.8 ha deciduous upland forest with a mix of primarily oak, ash, and maple, also owned by the University of Illinois; 3) Forest Glen Seep (Vermilion Co.; 40.01516, -87.56771), 4.5 ha in area and forested primarily with beech, maple, oak, and hickory (within Forest Glen Preserve, a Vermilion County nature preserve); and 4) a residence in the city of Urbana (Champaign Co.; 40.097067°, -88.203162°) in a neighborhood with mature trees of many species, primarily deciduous (see Dirr 1998). During the study period, minimum and maximum temperatures averaged 17.7 and 30 ^{o}C and rainfall was unusually heavy (41cm total, 9 cm greater than the ten-year average; Weather Underground, Inc., Ann Arbor, MI).

We trapped beetles with black flight intercept panel traps (corrugated plastic, 1.2 m high× 0.3 m wide, Alpha Scents, Inc., West Linn, OR) that were suspended from frames of PVC irrigation pipe (for details, see Graham et al. 2010). The supplied trap basins were replaced with 2-l plastic jars (P.E.T.; model 55-650C, General Bottle Supply Company, Los Angeles, CA). 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 l; 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® (Fisher Scientific, Pittsburg, PA) to improve trapping efficiency (see Graham et al. 2010).

Our experiments targeted three native cerambycine species that were common in the study area, and for which the male-produced pheromones were known (see Lacey et al. 2004,

2007, 2009): 1) *Neoclytus acuminatus* (F.); pheromone composed of (2*S*,3*S*)-hexanediol, 2) *Neoclytus mucronatus* (F.); (*R*)-3-hydroxyhexan-2-one, and 3) *Xylotrechus colonus* (F.) (*R*)- and (*S*)-3-hydroxyhexan-2-one, and (2*S*,3*S*)- and (2*R*,3*R*)-hexanediol). We used racemic blends of these compounds because the enantiomers are expensive to synthesize in quantities sufficient for general purpose trapping (for chemical syntheses, see Millar et al. 2009, Mitchell et al. 2011). Earlier 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 5-component blend along with two other cerambycid pheromone chemicals that would be logical candidates for inclusion in multi-species 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 cerambycine 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 ml 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). Emitters were clear polyethylene sachets (press-seal bags, Bagette model 14770, 5.1cm × 7.6cm, 0.05mm wall thickness, Cousin Corp., Largo, FL) that were hung in the center of traps. A control trap baited

with a lure containing only ethanol was included in each trap line. Traps were positioned 10 m apart in linear transects and checked for beetles every 1-3 d. Treatments were rotated within transects weekly to control for location effects, and lures were replaced as necessary.

Experiment I assessed attraction to the three test compounds separately and to the 5-component blend of all three of these compounds plus fuscumol acetate and citral (Blend I; see Table 1). The experiment was conducted during 2 June-6 July 2010.

Experiment II was similar in design to experiment I, but the *R*R**- and *R*S**-diols were not blended together 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 IIA (*R*R**-diol, 3*R**-ketone, fuscumol acetate, and citral), and blend IIB (*R*S**-diol, 3*R**-ketone, fuscumol acetate, and citral; see Table 1). Experiment II was conducted during 7 July –16 September 2010.

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 IIIA (3*R**-ketone and fuscumol acetate), blend IIIB (3*R**-ketone, fuscumol acetate, and citral), *R*R**-diol alone, blend IIIC (*R*R**-diol and fuscumol acetate), and blend IIID (*R*R**-diol, fuscumol acetate, and citral; see Table 1). Experiment III was conducted during 16 June - 9August 2010.

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, the 3-hydroxy-2-hexanones, 2,3-hexanediols, and citral). The species (all tribe Acanthocinini) included *Astyleiopus variegatus* (Haldeman), *Graphisurus fasciatus* (Degeer), and *Lepturges angulatus* (LeConte) (see Results).

All three species 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 using the nonparametric Friedman's Test (PROC FREQ, option CMH; SAS Institute 2001). Differences between pairs of means were tested with the REGWQ means-separation test which controls for maximum experiment-wise error rates (PROC GLM; SAS Institute, 2001). Data for site and date replicates were included in the analysis based on a threshold number of specimens (2-8 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 cerambycid beetles during the study, of which 1,101 (81.1%) were the three targeted cerambycine species and 257 were the three lamiine species (Table 1; the remaining cerambycid beetles represented 18 species, 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 earlier report that one or both of the *R*S**-diol diastereomers inhibit attraction of *N. acuminatus* to the 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 attraction to blend IIA, which contained a blend of *R*R**-diol, 3*R**-ketone, fuscumol acetate, and citral, demonstrated that one of the latter compounds also was inhibitory. The fact 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 was responsible for the decreased attraction to blend IIA (Fig. 1B). We subsequently have confirmed with follow-up experiments that 3*R**-ketone partially inhibits attraction of *N. acuminatus* to *R*R**-diol (unpub. data).

In all three experiments, adult *N. mucronatus* were only significantly attracted by lures containing 3*R**-ketone. Treatment means for blends I, IIA, and IIIA were not significantly different from those for 3*R**-ketone as a single component (Figs. 2A, 2B, 2C), whereas blends IIB and IIIB were less attractive than 3*R**-ketone (Fig. 2B, 2C), 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), which contained four components identified from headspace odors of

this species (i.e., [*R*]-3- and [*S*]-3-hydroxyhexan-2-ones,and [2*S*,3*S*]- and [2*R*,3*R*]-hexanediols; Lacey et al. 2009) plus *R*,S**-diol, fuscumol acetate, and citral. Attraction to 3*R**-ketone as a single component was intermediate, and neither of the 2,3-hexanediols were attractive as single-component lures. In Experiment II, combining 3*R**-ketone with *R*R**-diol (blend IIA in Fig. 3B) did not alter attraction compared to 3*R**-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 3*R**-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 (see Table 1). *L. 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 3*R**-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 (see Tamaki 1985, Smadja and Butlin 2009). Because *N. acuminatus* overlaps with *X. colonus* in seasonal and daily activity periods (see Lacey et al. 2009), the 3*R**-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 multi-component 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 multi-species lures for cerambycids.

Extrapolating from our results, it appears likely that blends of pheromones could be effective for detecting exotic species whose pheromones were included in a blend. It already has been shown that 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-ones and 2,3-hexanediols for cerambycine species in the tribes Anaglyptini and Callidiini (e.g., Schröder et al. 2004, Fettköther et al. 1995, Leal et al. 1995) and (*E*)-fuscumol 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.

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Tables and Figures

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

		Cerambycinae			Lamiinae		
	Treatment	***N. acuminatus***	***N. mucronatus***	***X. colonus***	***A. variegatus***	***G. fasciatus***	***L. angulatus***
Expt. I	**3*R**-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: 3*R**-ketone, *R*R**- and *R*S**-diol, fuscumol acetate, citral	**1**	**9**	**72**	**0**	**13**	**0**
	Control	**0**	**0**	**7**	**0**	**4**	**0**
Expt. II	**3*R**-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 IIA: 3*R**-ketone, *R*R**-diol, fuscumol acetate, citral	**34**	**63**	**51**	**1**	**32**	**4**
	Blend IIB: 3*R**-ketone, *R*S**-diol, fuscumol acetate, citral	**9**	**54**	**58**	**2**	**24**	**14**
	Control	**2**	**3**	**2**	**0**	**2**	**0**
Expt. III	**3*R**-ketone**	**5**	**57**	**20**	**0**	**0**	**3**
	Blend IIIA: 3*R**-ketone + fuscumol acetate	**2**	**42**	**30**	**11**	**0**	**20**
	Blend IIIB: 3*R**-ketone + fuscumol acetate + citral	**1**	**37**	**17**	**6**	**2**	**11**
	RR*-diol	**34**	**3**	**1**	**1**	**0**	**1**
	Blend IIIC: *R*R**-diol + fuscumol acetate	**36**	**3**	**0**	**8**	**1**	**26**
	Blend IIID: *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**

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 $P<0.05$.

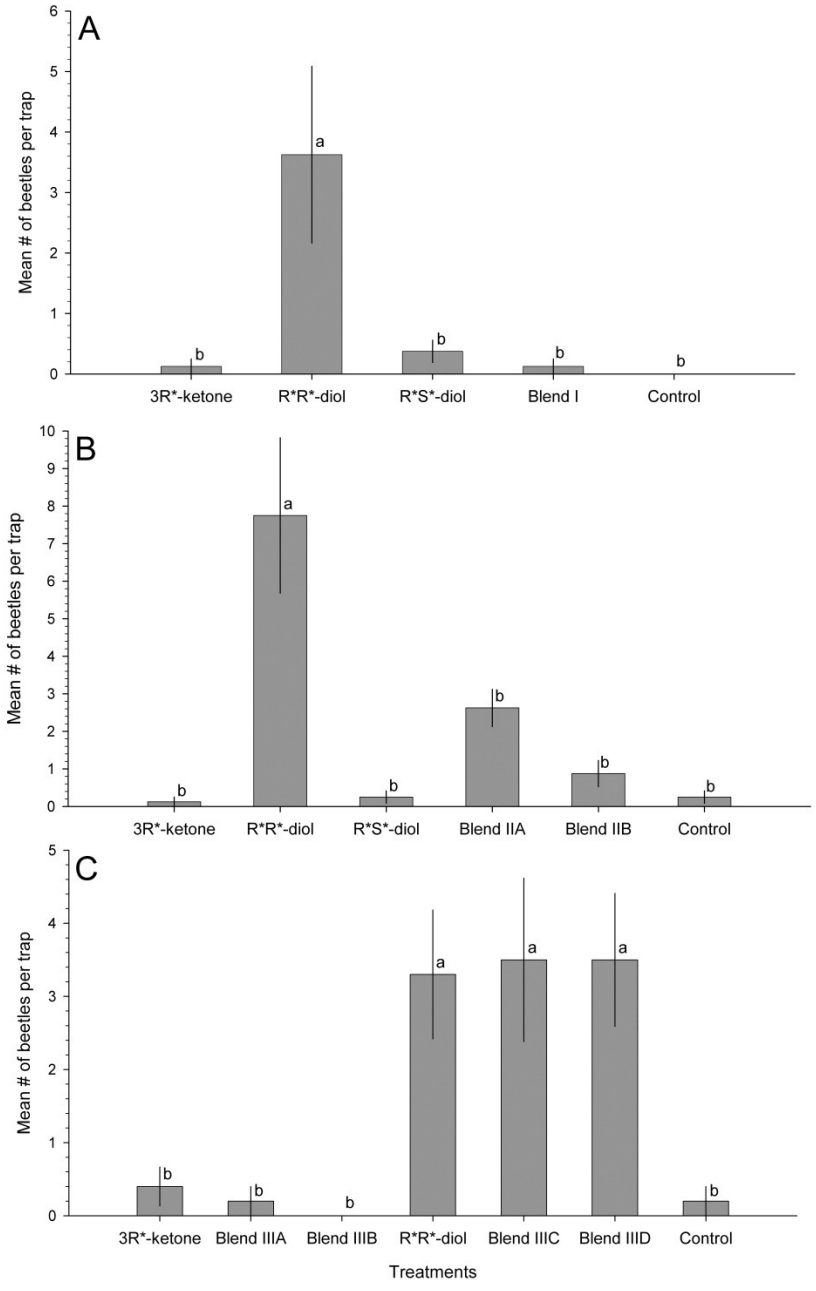

Fig. 2. Mean (± 1 SE) number of adult *N. mucronatus* captured per trap (sexes combined) with respect to composition of the lure during Experiments I (A), II (B), and III (C). Means significantly different, Friedman's $Q_{4,39} = 18.5, P = 0.001$, $Q_{5,89} = 36.0$, $P < 0.0001$, and $Q_{6,63} = 42.6$,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

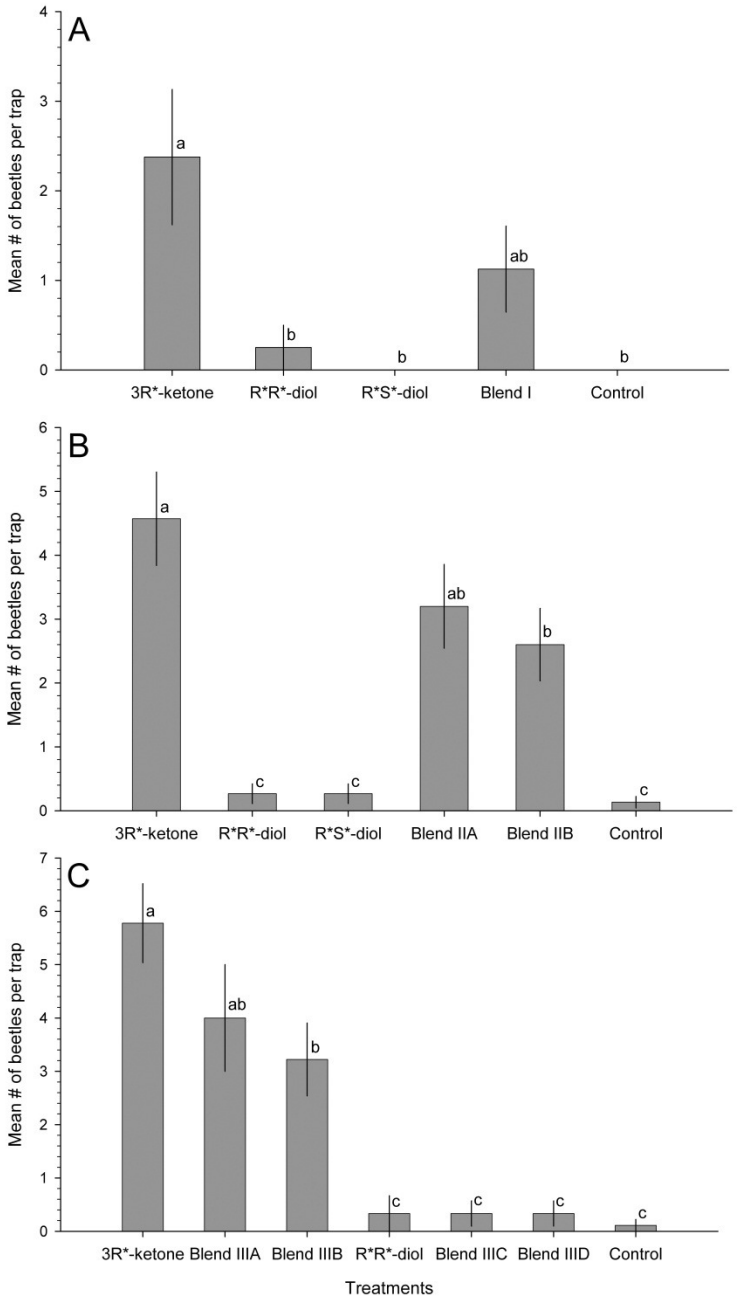

Fig. 3. Mean (± 1 SE) number of adult *X. colonus* captured per trap (sexes combined) with respect to composition of the lure during Experiments I (A), II (B), and III (C). Means significantly different, Friedman's $Q_{4,74} = 18.4$, $P = 0.001$, $Q_{5,89} = 36.7$, $P < 0.0001$, and $Q_{6,70} = 29.5$,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

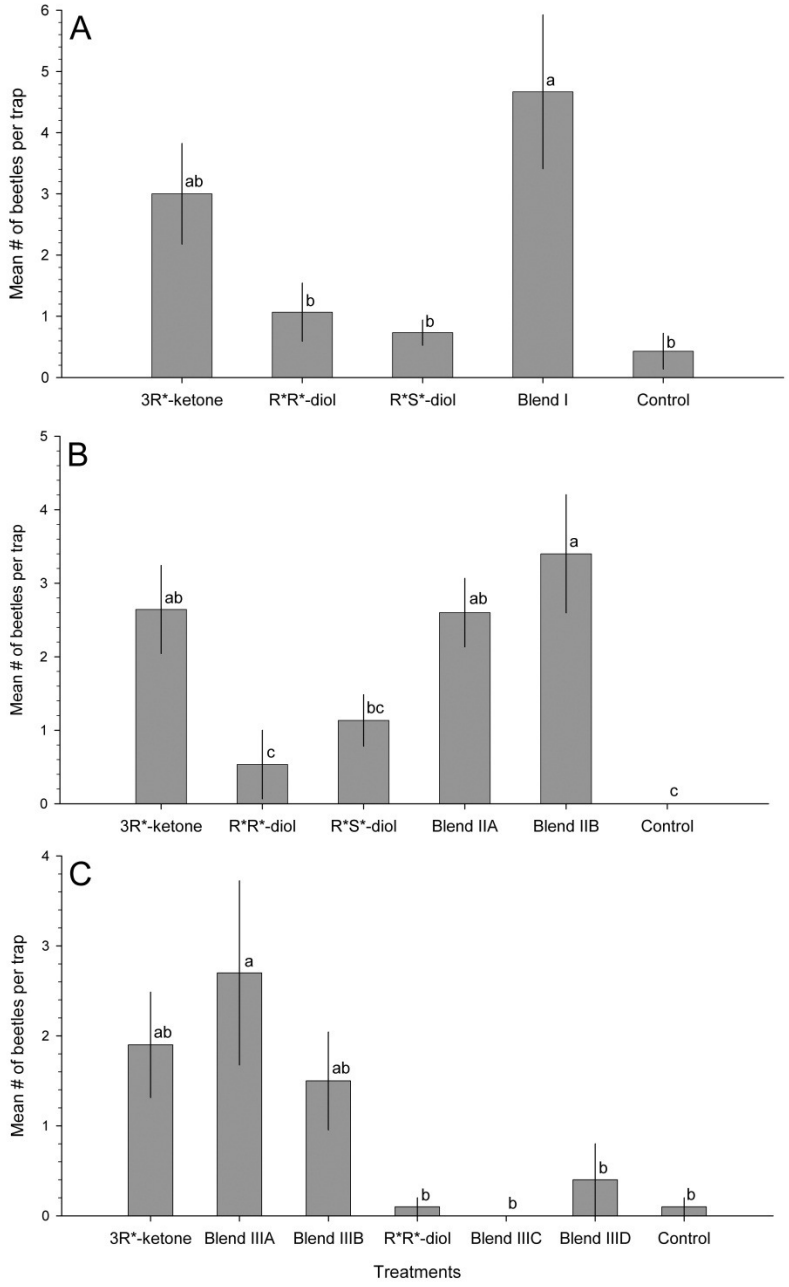

Fig. 4. Mean (± 1 SE) number of *Lepturges angulatus* captured per trap (sexes combined) with respect to composition of the lure in Experiment II (A), and III (B). Means significantly different, Friedman's $Q_{5,30} = 15.8$, $P = 0.0075$ and $Q_{6,70} = 27.3$, $P = 0.0001$, respectively. Means with the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

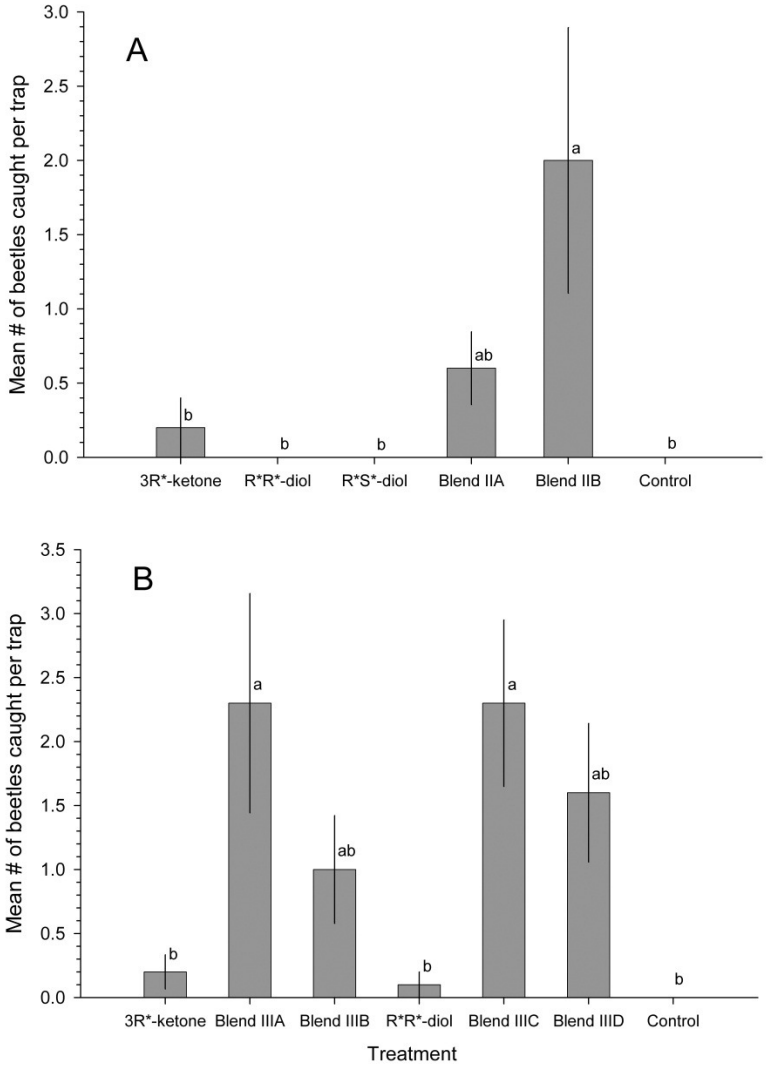

Fig. 5. Mean (± 1 SE) number of beetles of the subfamily Lamiinae captured per trap (sexes combined) with respect to composition of the lure in: A) Experiment II for *Graphisurus fasciatus* and B) Experiment III for *Astyleiopus variegatus*. Means significantly different, Friedman's $Q_{5,131}$= 19.5, 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 $P < 0.05$.