

## MALE-PRODUCED AGGREGATION PHEROMONE OF THE CERAMBYCID BEETLE *Neoclytus acuminatus acuminatus*

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**Abstract**—This is the first fully verified report of an aggregation pheromone produced by a cerambycid beetle species. Field bioassays with adult *Neoclytus acuminatus acuminatus* (F.) (Coleoptera: Cerambycidae) revealed that males produce a pheromone that attracts both sexes. Extracts of odors from males contained a single major male-specific compound, (2*S*,3*S*)-hexanediol. Field trials determined that both sexes were attracted by the racemic blend of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediols and that activity was similar to enantiomerically enriched (2*S*,3*S*)-hexanediol (e.e. 80.2%). However, the blend of all four 2,3-hexanediol stereoisomers attracted few beetles, indicating inhibition by one or both of the (2*R*<sup>\*</sup>,3*S*<sup>\*</sup>)-stereoisomers. Females of the cerambycid *Curius dentatus* Newman were attracted to traps baited with the four component blend, suggesting that a male-produced sex pheromone for this species may contain (2*R*,3*S*)-hexanediol and/or (2*S*,3*R*)-hexanediol. The pheromone of *N. a. acuminatus*, and presumed pheromone of *C. dentatus*, bear structural similarities to those produced by males of six other species in the Cerambycinae (straight chains of 6, 8, or 10 carbons with hydroxyl or carbonyl groups at C<sub>2</sub> and C<sub>3</sub>). It is likely that males of other species in this large subfamily produce pheromones that are variations on this structural motif.

**Key Words**—SPME, red-headed ash borer, *Neoclytus*, *Curius dentatus*, sex pheromone, (2*S*,3*S*)-hexanediol, wood-borer.

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## INTRODUCTION

The red-headed ash borer, *Neoclytus acuminatus acuminatus* (F.) (Coleoptera: Cerambycidae, Cerambycinae, tribe Clytini), is endemic to North America, where it infests a variety of hardwood tree species (Linsley, 1964). It also was accidentally introduced into the Adriatic region of southern Europe, where it continues to expand its range (Bense, 1995). The larvae develop in woody tissues of stressed, moribund, and dead trees and can degrade hardwood lumber (Linsley, 1964; Solomon, 1995). Adult *N. a. acuminatus* are present from spring through late summer and are most active between ~11:00 and 17:00 hr (authors' personal observation). They commonly aggregate on larval hosts in groups of more than 40 individuals, and females begin ovipositing immediately after mating (ESL, personal observation). Adults apparently do not feed, and usually live for fewer than 16 d (ESL, personal observation).

During observations of adult *N. a. acuminatus* on the larval hosts in the field and caged beetles in the laboratory, we observed a behavior performed only by males. They would periodically stop walking, fully extend their legs and elevate their body above the substrate, and remain motionless in this position for as long as 30 min. To our knowledge, this behavior has not been reported for any other cerambycid species, and it provided the impetus to investigate the pheromone chemistry of this species. We report here the identification of (2*S*,3*S*)-hexanediol as a male-produced aggregation pheromone for *N. a. acuminatus*.

## METHODS AND MATERIALS

*Testing for Attractant Pheromones.* To test for attractant pheromones in *N. a. acuminatus*, we conducted a field cage bioassay in a ~28 ha arboretum on the campus of the University of Illinois at Urbana—Champaign (UIUC; Champaign Co.). Bioassays were conducted from 13:00 to 16:30 hr on 25 and 26 June 2002; skies clear, air temperatures ~29°C, wind speeds ~8–16 kph. We set three aluminum screen cages (60 × 60 × 20 cm) on the ground, 2 m apart, and 20 m upwind from a pile of ~50 freshly cut logs of green ash (*Fraxinus pennsylvanica* Marshall) that initially harbored at least 40 adult beetles per day. Each cage contained four bolts (~40 × 12 cm) of freshly cut hackberry (*Celtis occidentalis* L.), a natural host of *N. a. acuminatus* (Linsley and Chemsak, 1997). One cage also contained six adult male *N. a. acuminatus*, a second cage contained six adult females, and the last contained only hackberry bolts. We switched positions of cages every hour so that each treatment occupied all positions at least once per day. Beetles used as baits had been captured on larval host material at a different site (Champaign Co., IL) and, prior to the experiment, sexes were housed separately in aluminum screen cages under laboratory conditions (~24°C and ~12L:12D) and provided water. Only beetles that were active and apparently healthy were used. For each

trial, we visually monitored field cages and recorded the number of adult *N. a. acuminatus* landing on cages. These beetles were retained but not sexed. Numbers of beetles responding to cage treatments were compared with a  $\chi^2$  goodness-of-fit test (Sokal and Rohlf, 1995).

In an independent bioassay, we used a horizontal glass Y-tube olfactometer (6 cm diam, main tube 26 cm long, arm length 22 cm, 70° angle between arms). We conducted bioassays outdoors because beetles were either sedentary or appeared agitated in the olfactometer under laboratory conditions. Bioassays were performed in partial shade between 13:00 and 17:00 hr on 14, 15, and 18 May 2003; skies clear, air temperatures ~30°C. We attached a 2 l plastic chamber containing a cylinder of aluminum screen for a perch to each arm of the Y-tube. One chamber held either six males or six females (field captured, as described above) whereas the other was empty. We pulled air through the olfactometer (~2.5 l/min) with a 1.0 hp vacuum cleaner connected to a variable power supply. A beetle was released at the base of the Y-tube and responded to an odor source by crossing a line 18 cm down an arm within 10 min. We bioassayed 20 individuals of each sex for a response to the opposite sex and alternated chambers between Y-tube arms every three trials to control for any positional bias. Numbers of beetles responding to treatments were compared with a  $\chi^2$  goodness-of-fit test.

*Identification of Pheromone.* To identify attractants, we sampled volatile compounds produced by adult *N. a. acuminatus* by both solid phase microextraction (SPME) and by collection of headspace volatiles on an adsorbent. Adults had been reared indoors from infested *F. pennsylvanica* logs, caged as described above for 4–8 d postemergence, and were active and apparently healthy. To sample volatiles by SPME, we placed 10 males and 10 females in separate clean Erlenmeyer flasks, sealed the flasks with corks, and left them on a north-facing laboratory windowsill from 13:00 to 18:00 hr to allow volatiles to accumulate in the flasks. Headspace volatiles were sampled by inserting the SPME fiber (100  $\mu$ m polydimethylsiloxane, Supelco®, Cat. No. 57300-U, Bellefonte, PA) through the cork and exposing it for 15 min. We desorbed the fiber in the injection port of a Hewlett-Packard® (HP) 5973 mass spectrometer interfaced to an HP 6890 gas chromatograph, using an HP-5MS column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film) in splitless mode with helium carrier gas. The injection port temperature was 250°C, and the oven temperature was programmed from 40°C for 1 min, 10°C/min to 300°C, and held for 5 min. We sampled volatiles three times from each sex on 17–19 May 2003.

To collect headspace volatiles, we placed 20 adult beetles of the same sex in a glass vacuum trap (~0.3 l) lined with aluminum screen as a perch. A disposable glass pipette containing 100 mg of 80/100 mesh SuperQ® (Alltech Associates, Deerfield, IL) was attached to one nipple of the chamber with a 2-cm long section of Tygon® tubing, and charcoal-purified air was pulled through the apparatus with a vacuum pump at a rate of ~1 l/min. Beetles were aerated on the windowsill

(see above) from 12:00 to 16:00 hr with males sampled on 24 June and females on 25 June 2003. The SuperQ<sup>®</sup> collectors were eluted with three 0.5-ml aliquots of methylene chloride, and the resulting extracts were analyzed by GC-MS as described above. An initial absence of chromatogram peaks was attributed to volatiles adhering to glass, and the problem was resolved by sonicating vials for 5 min and withdrawing a sample for injection during sonication. The extracts were reanalyzed at the University of California, Riverside, using an HP 6890 GC interfaced to an HP 5973 mass selective detector. The GC was fitted with a DB5-MS column (30 m × 0.25mm, programmed from 40°C/1 min, 10°C/min to 250°C), and injections were made in splitless mode.

The absolute configuration of the insect-produced 2,3-hexanediol was determined by analysis with an HP 5890 GC fitted with a chiral stationary phase Cyclodex B column (30 m × 0.25 mm ID, J&W Scientific), programmed from 50°C/0 min, 5°C/min to 150°C. Under these conditions, a racemic standard was resolved to baseline ( $\alpha = 1.015$ ) with the (2*S*,3*S*)-enantiomer eluting first. The absolute configuration of the insect-produced compound was confirmed by coinjection of an aliquot of the insect extract spiked with the racemic compound.

*Synthesis of Pheromone.* A solution of OsO<sub>4</sub> (2.5% in *t*-butanol, 5 ml, 0.5 mmol; Aldrich Chemical Co.) was added to an ice-bath cooled mixture of *N*-methylmorpholine oxide (18.9 g, 140 mmol) and (*E*)-2-hexene (12.6 g, 150 mmol; GFS Chemicals, Columbus, OH) in 200 ml of a 3:1 mixture of THF and water. The mixture was warmed to room temperature and stirred for 2 d. The mixture was quenched by addition of 160 ml of a 1 M solution of Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> in water and stirring for 3 hr. The resulting mixture was extracted ×3 with EtOAc, and the combined extracts were backwashed with 20 ml 3M H<sub>2</sub>SO<sub>4</sub> and brine. The solution was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated, and Kugelrohr distilled at 2 mm Hg (oven temp. ~60–70°C), yielding 5.84 g of racemic (2*R*\*, 3*R*\*)-hexanediol as a colorless viscous oil (33%; 96% pure by GC). (2*R*\*, 3*S*\*)-Hexanediol was prepared in analogous fashion by oxidation of (*Z*)-2-hexene (GFS Chemical Co.). Spectral data agreed with those previously reported (Schröder et al., 1994).

AD-Mix  $\alpha$  (1.4 g, Aldrich Chemical Co.) and methanesulphonamide (95 mg) were dissolved in a two-phase mixture of water (5 ml) and *t*-butanol (5 ml) (Kolb et al., 1994). The mixture was chilled in an ice-bath, and (*E*)-2-hexene (0.125 ml, 1 mmol) was added. The resulting mixture was transferred to a cold room (4°C) and stirred for 44 hr, then quenched by addition of sodium metabisulfite (1.5 g) to the cooled solution, stirred for 30 min, then extracted twice with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated, and purified by vacuum flash chromatography on silica gel, eluting with 50% EtOAc in hexanes, yielding enantiomerically enriched (2*S*,3*S*)-hexanediol (e.e. 80.2%, determined on Cyclodex-B column, see above) quantitatively. The (2*R*,3*R*)-enantiomer was made in analogous fashion by using AD-Mix  $\beta$  (Aldrich Chem. Co.) and otherwise identical reaction conditions (e.e. 90.0%).

*Field Bioassays of Synthetic Pheromone.* We conducted a preliminary field bioassay of racemic (2*R*\*, 3*R*\*)-hexanediol in an area adjacent to the UIUC arboretum and in proximity to a pile of freshly cut wood containing sectioned trunks and main branches of several tree species. Logs of white ash (*Fraxinus americana* L.) harbored a population of at least 30 adult *N. a. acuminatus* during the bioassay. We stapled sticky card traps (20.3 × 27.9 cm, Pherocon® AM, Trécé® Inc., Adair, OK) to 1.5 m tall wooden stakes, with the middle of the card ~1 m above the ground. A pair of cards was positioned 2 m apart at distances of 3 and 12 m upwind of the ash logs (*N* = 4 cards). One card of each pair was baited with a rubber septum (10 × 20 mm, Wheaton Science Products, Millville, NJ) loaded with 10 μl of a ~260 mg/ml solution of a racemic blend of (2*R*,3*R*)- and (2*S*,3*S*)-hexanediol in EtOAc clipped to the top of the card, and the other card was baited with a control septum loaded only with EtOAc. We monitored cards for captured beetles continuously because beetles could eventually free themselves. Captured beetles were removed, and the number and sex responding to treatments were recorded. We conducted the bioassay between 14:00 and 17:00 hr on 19 and 20 July 2003; skies clear, air temperature ~27–30°C, wind speed ~8–20 kph. Traps and lures were replaced on the second day. We compared total numbers of beetles responding to the treatment and control with the  $\chi^2$  goodness-of-fit test.

To determine the optimum dose of synthetic pheromone for capturing adult *N. a. acuminatus*, we conducted bioassays at four sites. Three sites were within the UIUC arboretum and were separated by at least 200 m, and the remaining site was in Trelease Woods, a ~25 ha mixed hardwood forest, ~11 km NE of the UIUC campus. We expected adult *N. a. acuminatus* would be present at these sites because there was a mature stand of green ash in the arboretum and a variety of host tree species in Trelease Woods. There were no large point sources of beetles, such as recently fallen trees, at any site that could have resulted in positional effects in trap catches. We used “mouse glue trap” cards (Model M180, 12.6 × 22 cm, Victor® Pest Control Products, Lititz, PA) from which the beetles could not escape. Cards were mounted on wooden stakes as described above. At each site we positioned eight stakes 10 m apart in a straight line and approximately perpendicular to the prevailing wind with the sticky surface of cards facing downwind. The pheromone release device was a cotton dental wick (Patterson Dental Supply, Inc., St. Paul, MN) stuck to the card center. The bioassay included eight dosage treatments randomly assigned to traps at each site: 0, 4, 16, 64, 250, 1000, 4000, and 16000 μg of a racemic mixture of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediol in 500 μl of EtOAc. The bioassay was replicated 13 times between 26 July and 18 August 2003; daily air temperatures ~18–35°C, variable cloud cover, negligible precipitation. For each replication, traps were set up at 14:00 hr, and captured beetles were counted, sexed, and removed from cards after 4, 22, 26, 42, and 46 hr. Differences between numbers of females and males captured were tested with the  $\chi^2$  goodness-of-fit test. Differences between treatments in

numbers of beetles captured were tested with the nonparametric Kruskal–Wallis test (PROC NPAR1WAY; SAS Institute, 2001). To test differences between pairs of treatment means, we compared 95% confidence intervals (absence of overlap of intervals is a conservative test of statistical significance; Payton et al., 2003). Replications that captured one or no beetles were excluded from the statistical analysis (four of 13 replications).

To determine whether stereoisomeric mixtures were as effective as enantiomerically enriched (2*S*,3*S*)-hexanediol in capturing *N. a. acuminatus*, we conducted field bioassays with an experimental design identical to the previous bioassay, and at the same field sites, but with four traps positioned 15 m apart in each replication. Treatments were loaded on cotton dental wicks and included a solvent control (500  $\mu$ l of EtOAc), (2*S*,3*S*)-hexanediol of 80.2% e.e., a racemic blend of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediol, and a blend of equal parts of all four 2,3-hexanediol stereoisomers. We based our dosages on the lowest effective dose in the previous study (2000  $\mu$ g of (2*S*,3*S*)-hexanediol per treatment dose, see Results). The bioassay was replicated nine times between 23 and 28 August 2003; daily temperatures 22–35°C with variable cloud cover, negligible precipitation. Data were analyzed as described above. One replication that captured no beetles was excluded from the analysis.

To determine the effective longevity of pheromone lures, we pooled the data from the two latter field bioassays and compared numbers of beetles captured on the first, second, and third days with the  $\chi^2$  goodness-of-fit-test.

Voucher specimens of cerambycid species have been submitted to the Insect Collection of the Illinois Natural History Survey (Champaign, IL).

## RESULTS

*Testing for an Attractant Pheromone.* In the initial bioassay, 32 adult *N. a. acuminatus* arrived on cages containing live males and hackberry logs compared to only five beetles arriving on cages with females and logs, and two beetles on cages with only logs (totals significantly different,  $\chi^2_{2,39} = 42.0$ ,  $P < 0.001$ ). These findings confirmed that males produced an attractant pheromone. During the bioassay, we also observed caged males displaying the characteristic “calling” behavior described in the Introduction.

In Y-olfactometer bioassays conducted outdoors, 16 females responded to odors emitted by males compared to only two responding to the control ( $\chi^2_{1,18} = 10.9$ ,  $P < 0.001$ ). Males showed no significant response to females (7 and 6 males responding to females vs. control, respectively,  $\chi^2_{1,13} = 0.08$ ,  $P > 0.05$ ). In addition, during bioassays using males as the odor source, we captured three wild male and four wild female *N. a. acuminatus* that were attracted to the exhaust vent of the olfactometer vacuum cleaner, indicating that males released a pheromone that was attractive to both sexes.

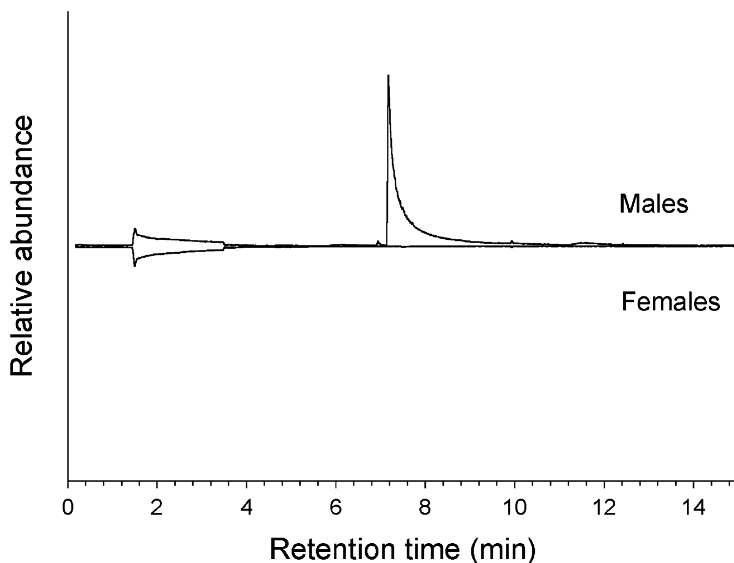


FIG. 1. Representative total ion chromatograms of SPME samples (HP-5MS GC column) of head-space volatiles produced by adult male (top trace) and female (bottom, inverted trace) *N. a. acuminatus*.

*Identification of Pheromone.* GC-MS analysis of SPME-trapped headspace volatiles from males revealed a single major peak in the total ion chromatogram that was absent in analogous headspace extracts from females (Figure 1). The rather simple mass spectrum had a base peak at  $m/z$  55, and significant fragments at  $m/z$  73 (61), 45 (36), 43 (35), 72 (34), 75 (16), and 57 (20). The highest mass fragment observed was at  $m/z$  103 (1). Although a search of the NIH-NIST computerized mass spectral database gave 4,5-octanediol as the best match, the retention time of an authentic standard was considerably longer than that of the insect-produced compound. The shorter retention time of the insect-produced compound and the similarities of its mass spectrum to that of 4,5-octanediol suggested that the natural compound might be a 2,3-hexanediol. This was confirmed by synthesis of authentic standards. Specifically, the  $(2R^*,3R^*)$ - and  $(2R^*,3S^*)$ -diastereomers were completely separated on a DB-5MS column, and the insect-produced compound coeluted with the earlier eluting  $(2R^*,3R^*)$  enantiomeric pair. The absolute configuration of the insect-produced compound was then determined by analysis of standards and the insect extract on a chiral phase Cyclodex-B column. The insect-produced compound coeluted with the earlier-eluting  $(2S,3S)$ -enantiomer on this column, with no trace of the opposite enantiomer. The fact that this compound

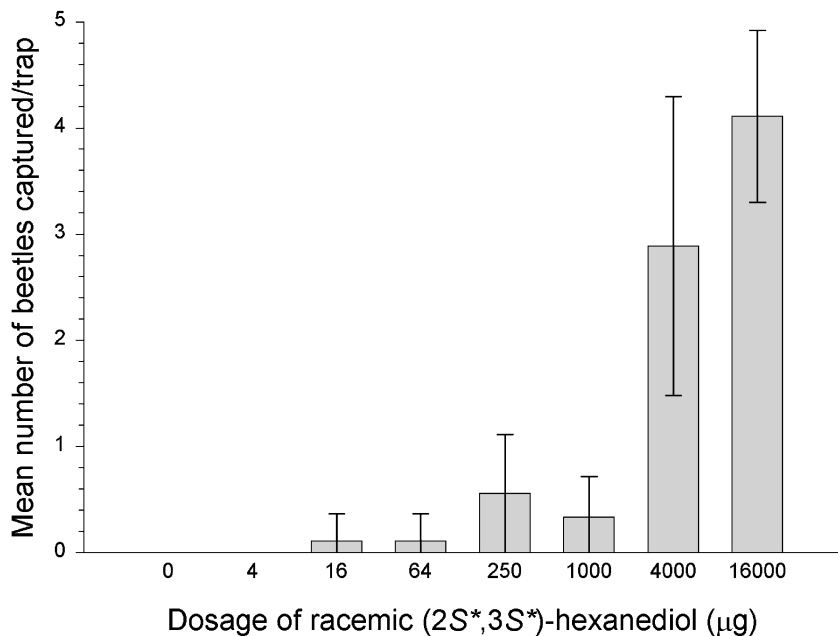


FIG. 2. Relationship between the mean number of adult *N. a. acuminatus* captured on sticky cards (sexes combined) over a 2-d period and dosage of a racemic blend of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediols. Error bars represent 95% confidence intervals ( $N = 73$ ).

was the only compound detected in both SPME and Super Q-collected volatiles from males suggested that the pheromone consisted of a single component.

*Field Bioassays of Synthetic Pheromone.* Traps baited with the racemic blend of (2*R*,3*R*)- and (2*S*,3*S*)-hexanediols captured six female and six male *N. a. acuminatus* whereas no beetles were captured on control traps (sexes combined,  $\chi^2_{1,12} = 12.0$ ,  $P = 0.001$ ). Beetles responded rapidly to baited traps, with the first beetle arriving within 5 min.

In a subsequent test for optimal dosage, traps captured 42 female and 31 male *N. a. acuminatus* (totals for sexes not different,  $\chi^2_{1,73} = 1.66$ ,  $P > 0.05$ ). There was a significant dose-response relationship (Figure 2; sexes combined, Kruskal-Wallis  $\chi^2_{7,73} = 50.2$ ,  $P < 0.001$ ), with the control and the lowest dosage treatments attracting no beetles, dosages between 16 and 1000  $\mu\text{g}$  attracting  $< 0.6$  beetles/trap (not different from control as indicated by overlap of 95% CI with 0), and greater numbers of beetles ( $> 2.8$  beetles/trap) captured with dosages of 4000 and 16000  $\mu\text{g}$ .

In bioassays to test the efficacy of the enantiomerically enriched (2*S*,3*S*)-enantiomer compared to other blends of the stereoisomers, greater numbers of



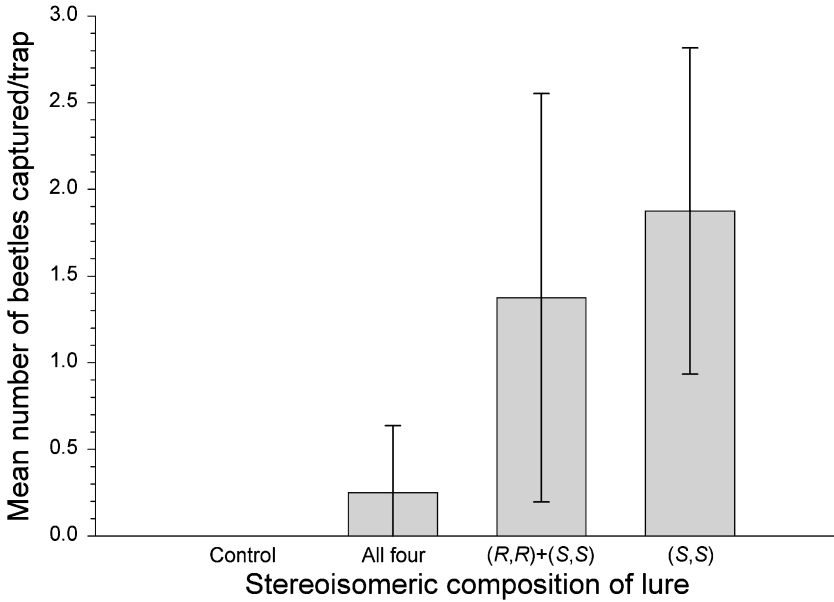


FIG. 3. Relationship between the mean number of adult *N. a. acuminatus* captured on sticky traps (sexes combined) and stereoisomeric composition of the lure: solvent control, blend of equal parts of all four 2,3-hexanediol stereoisomers, racemic blend of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediol, and 80.2% e.e. (2*S*,3*S*)-hexanediol. Error bars represent 95% confidence intervals ( $N = 28$ ).

females were captured on traps than males (20 vs. 8, respectively;  $\chi^2_{1,28} = 5.14$ ,  $P < 0.05$ ). Pheromone treatments differed in average numbers of beetles captured per trap (Figure 3; Kruskal–Wallis  $\chi^2_{3,28} = 17.4$ ,  $P < 0.001$ ), with control traps capturing no beetles, the blend of four stereoisomers attracting numbers of beetles that were no different from the controls (95% CI overlapping with 0), and greater numbers of beetles responding to the racemic blend of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediols and enantiomerically enriched (2*S*,3*S*)-hexanediol (means for the latter two treatments not different). Furthermore, 14 adult females of another cerambycid species, *Curius dentatus* Newman, were attracted to the blend of four stereoisomers, whereas only one was attracted to the enantiomerically enriched (2*S*,3*S*)-hexanediol, and none were attracted to the racemic (2*R*\*,3*S*\*) blend or the solvent control (totals different;  $\chi^2_{3,15} = 37.5$ ,  $P < 0.001$ ).

Pheromone traps in both of the latter field bioassays captured totals of 80, 21, and 0 beetles on the first, second, and third days, respectively (totals different,  $\chi^2_{2,101} = 102$ ,  $P < 0.001$ ). These data suggest that the cotton wick release devices were most effective on the first day, and became depleted by evaporation thereafter.

However, the possibility remains that reduced trap captures over time were due to local depletion of the beetle population due to trapping.

#### DISCUSSION

Both males and females of many cerambycid species are attracted to host plants by their odors, and once on the host, males locate and recognize females by contact chemoreception (Hanks, 1999; Ginzal and Hanks, 2003; Ginzal et al., 2003a,b). Nevertheless, behavioral observations and laboratory bioassays have suggested that volatile pheromones also play a role in mate location in cerambycid species of at least three subfamilies: Prioninae (Edwards, 1961; Benham and Farrar, 1976; Gwynne and Hostetler, 1978), Cerambycinae (Itami and Craig, 1989; Fauziah et al., 1992), and Lamiinae (Wang et al., 1991; Kim et al., 1992). The putative attractants have yet to be identified for any of these species. More detailed studies of pheromone chemistry using electroantennography, laboratory bioassays, and/or field bioassays have been carried out with another seven cerambycid species in two subfamilies. For these species, pheromone components have been identified, and their activity has been confirmed in some (Table 1). Pheromones were produced by males in all of these species.

Within the subfamily Lamiinae, male-specific volatile compounds have been identified only for *Anoplophora glabripennis* Motsch. (Table 1), and both sexes responded to the synthetic compounds in olfactometer bioassays (Zhang et al., 2002). Male-specific compounds identified from the six species of the Cerambycinae (tribes Anaglyptini, Callidiini, and Clytini) are structurally similar and composed of one to three compounds that have hydroxyl or carbonyl groups at C<sub>2</sub> and C<sub>3</sub> of 6, 8, or 10 carbon chains (Table 1). The biological activity of these compounds as attractant pheromones has been confirmed for four of these species, suggesting that similar compounds in the remaining two are also pheromones. The male-produced pheromone, (2*S*,3*S*)-hexanediol, that attracted both sexes of *N. a. acuminatus* in our study, fits this general pattern of pheromone production (Table 1). The structures of these compounds are entirely different from the female-produced pheromones of species in families closely related to the cerambycids. For example, N-(2'*S*)-methylbutanoyl-2-methylbutylamine is a pheromone component for *Migdolus fryanus* Westwood (Coleoptera: Anoplodermatidae, sensu Svacha and Danilevsky, 1987; Bento et al., 1992, 1993; Leal et al., 1994), and (*S*)-10-oxoisopiperitenone has been identified as a pheromone of *Vesperus xatarti* Dufour (Coleoptera: Vesperidae; Boyer et al., 1997).

Whereas the equivalent activity of enantiomerically enriched (2*S*,3*S*)-hexanediol and the (2*R*<sup>\*</sup>,3*R*<sup>\*</sup>) racemic blend suggests that (2*R*,3*R*)-hexanediol is not inhibitory to *N. a. acuminatus*, the negligible attractiveness of the blend of four stereoisomers indicates that one or both of the (2*R*<sup>\*</sup>,3*S*<sup>\*</sup>) stereoisomers

TABLE 1. SUMMARY OF PUBLISHED RESEARCH ON ATTRACTANT PHEROMONES OF MALE CERAMBYCIDAE AND BIOASSAYS USED TO CONFIRM ACTIVITY

Subfamily (Tribe)	Species	Pheromone components	Activity of candidate pheromone	References
Lamiinae (Monochamini)	<i>Anoplophora glabripennis</i> Motsch.	4-( <i>n</i> -heptyloxy)butanal, 4-( <i>n</i> -heptyloxy)butan-1-ol	Antennae of both sexes respond; both sexes attracted in lab bioassays	Zhang et al., 2002
Cerambycinae (Anaglyptini)	<i>Anaglyptus subfasciatus</i> Pic	(3 <i>R</i> )-hydroxy-2-hexanone; (3 <i>R</i> )-hydroxy-2-octanone	♀ weakly attracted in field bioassays; response of ♂ not significant	Nakamura et al., 1994, 1997; Leal et al., 1995
Cerambycinae (Callidiini)	<i>Hylotrupes bajulus</i> (L.)	(3 <i>R</i> )-hydroxy-2-hexanone; (2 <i>R</i> ,3 <i>R</i> )- and (2 <i>S</i> ,3 <i>R</i> )-hexanediol	♀ attracted in lab bioassays; response of ♂ not tested	Schröder et al., 1994; Fettköther et al., 1995
Cerambycinae (Callidiini)	<i>Pyrrhidium sanguineum</i> (L.)	(3 <i>R</i> )-hydroxy-2-hexanone; (2 <i>R</i> ,3 <i>R</i> )- and (2 <i>S</i> ,3 <i>R</i> )-hexanediol	Activity not tested	Schröder et al., 1994
Cerambycinae (Clytini)	<i>Xylotrechus pyrrhoderus</i> Bates	(2 <i>S</i> ,3 <i>S</i> )-octanediol; (2 <i>S</i> )-hydroxy-3-octanone	Antennae of both sexes respond; ♀ attracted in lab bioassays	Iwabuchi, 1982, 1988; Sakai et al., 1984; Iwabuchi et al., 1985, 1986
Cerambycinae (Clytini)	<i>Xylotrechus chinensis</i> Chevrolat	2,3-octanediol; 2-hydroxy-3-octanone, 3-hydroxy-2-octanone	Activity not tested	Iwabuchi et al., 1987; Kuwahara et al., 1987
Cerambycinae (Clytini)	<i>Xylotrechus quadripes</i> Chevrolat	(2 <i>S</i> )-hydroxy-3-decanone	Antennae of both sexes respond; ♀ weakly attracted in lab bioassays, ♂ not attracted; weak or no response by either sex in field bioassays	Hall et al., 1998; Rhainds et al., 2001

inhibits attraction. Our serendipitous capture of female but not male *Curius dentatus* (Cerambycinae, tribe Curiini), in traps baited with the mixture of the four 2,3-hexanediol stereoisomers but not with (2*S*,3*S*)-hexanediol or the (2*R*\*,3*R*\*) racemic blend, indicated that (2*R*,3*S*)- and/or (2*S*,3*R*)-hexanediol probably constitute a male-produced sex pheromone of this species. With this evidence that species representing four tribes of the Cerambycinae use C<sub>6</sub> to C<sub>10</sub> vicinal diol or hydroxyketone pheromones, it seems likely that males of other species in the large subfamily Cerambycinae also produce pheromones with this structural motif.

To our knowledge, this is the first fully verified report of an aggregation pheromone in the Cerambycidae. Male-produced aggregation pheromones have been identified in many insect orders (Schlyter and Birgersson, 1999), and in at least nine families of the Coleoptera, including several species in the Chrysomelidae (Rao et al., 2003), the group most closely related to the Cerambycidae (Farrell, 1998), and in other types of wood-borers, including the Bostrichidae, Platypodidae, and Scolytidae (Schlyter and Birgersson, 1999). Response of males to male-produced pheromones may be adaptive if multiple males are more effective in attracting females and acquiring mates than are individual males, although the mechanisms underlying evolution of group calling in insects remain controversial (see Shelly and Whittier, 1997).

There is some evidence of intrasexual activity of male-produced pheromones in other species of the Cerambycinae (see Table 1). For example, male-produced pheromones stimulated antennae of both sexes of *Xylotrechus pyrrhoderus* and *X. quadripes* (Iwabuchi et al., 1985; Rhains et al., 2001). Pheromones produced by male *Anaglyptus subfasciatus* attracted males as well as females in field bioassays (Nakamuta et al., 1997), whereas further research is needed to determine the full spectrum of activity of male-produced pheromones in *Hylotrupes bajulus*, *Pyrrhidium sanguineum*, *Xylotrechus pyrrhoderus*, and *X. chinensis* (see references in Table 1). Taken together, the fragmented body of literature summarized in Table 1 emphasizes the gaps in our knowledge of the role of pheromones in mate location in cerambycid beetles.

Although we captured fewer than five beetles/trap during 2-d bioassays, pheromone traps that have been tested for other cerambycid species have been even less effective, capturing only a few beetles over periods of several weeks to months (e.g., Nakamuta et al., 1997; Rhains et al., 2001). Moreover, our field bioassays revealed that adult *N. a. acuminatus* responded to pheromones over distances of at least 100 m, if not greater distances, because there was no source of adult beetles in proximity to traps in some of our field trials. In contrast, female *X. pyrrhoderus* were reported to respond to pheromones of males only over distances of less than 2 m (Iwabuchi, 1982). Furthermore, it is possible that attraction of *N. a. acuminatus* to traps could be enhanced by combining host plant volatiles with pheromones, as has been demonstrated with *A. subfasciatus* (Nakamuta et al., 1997).

*Curius dentatus* is a little-known species that previously had not been reported from east central Illinois, where we conducted our studies (see Linsley, 1963; Yanega, 1996), as evidenced by the absence of specimens from Illinois in extensive collections of the Field Museum of Natural History (Chicago, IL) and the Illinois Natural History Survey (Champaign, IL; personal observations). Our capture of this species demonstrates that pheromone trapping can be a sensitive method for detecting cerambycid species. Thus, cerambycid pheromones may prove to be valuable in developing detection and quarantine programs for invasive exotic cerambycid species. For example, the pheromone of *N. a. acuminatus* has already been used in a pheromone trap-based survey for this species in New Zealand, where it recently was introduced accidentally, and where it may well become a serious pest (D. M. Suckling, HortResearch, Lincoln, NZ, personal communication).

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