

Male-Produced Aggregation Pheromone of the Cerambycid Beetle *Rosalia funebris*

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Abstract We report the identification, synthesis, and field bioassays of a volatile, male-produced aggregation pheromone of a long-horned beetle, the banded alder borer, *Rosalia funebris* Mots. Headspace collections from males contained a major male-specific compound, (*Z*)-3-decenyl (*E*)-2-hexenoate, and several minor components, identified as (*Z*)-3-decenol, (*Z*)-3-nonenyl (*E*)-2-hexenoate, and (*Z*)-3-decenyl (*E*)-3-hexenoate. The antennae of both males and females responded strongly to (*Z*)-3-decenyl (*E*)-2-hexenoate. We collected significant numbers of adult *R. funebris* in field bioassays using traps baited with this compound. This pheromone structure is unprecedented in

the literature of cerambycid pheromones and distinct from the more common diol/hydroxyketone pheromone motif of many other species of the diverse subfamily Cerambycinae. This is the first pheromone identified for a species in the tribe Rosaliini.

Keywords Aggregation pheromone · Sex pheromone · (*Z*)-3-decenyl (*E*)-2-hexenoate · Cerambycidae · Banded alder borer · Long-horned beetle · Woodborer

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Introduction

To date, male-produced sex or aggregation pheromones have been reported for 11 species in three tribes of the cerambycid subfamily Cerambycinae (reviewed by Ray et al. 2006; Hanks et al. 2007; Lacey et al. 2008b). Pheromones of most of these species are comprised of one to four compounds that are 6, 8, or 10 carbons in length and that share a common structural motif of hydroxyl or carbonyl groups at C₂ and C₃ (Hanks et al. 2007; Lacey et al. 2008b). Exceptions include *Phymatodes lecontei* Linsley, males of which appear to produce only (*R*)-2-methylbutan-1-ol (Hanks et al. 2007), and *Megacyllene caryae* (Gahan), in which males produce a pheromone blend comprised of 2,3-hexanediols, terpenoids, and an aromatic alcohol (Lacey et al. 2008b). Pheromones of male cerambycines of many species are emitted through male-specific gland pores in the prothorax (Ray et al. 2006; Hanks et al. 2007). In some cerambycine species, however, prothoracic gland pores are absent in both sexes, suggesting that adults either do not produce volatile pheromones or produce them from other sources (Ray et al. 2006).

Here, we report the identification, synthesis, and field bioassays of the male-produced aggregation pheromone of a cerambycine species, the banded alder borer *Rosalia funebris* Mots., that lacks male-specific prothoracic pores (Ray et al. 2006). This attractive black-and-white patterned species is endemic to western North America (Linsley 1964) and, in fact, was one of the first insects described from this region (Linsley 1995). Larvae of *R. funebris* develop in woody tissues of trees in the genera *Alnus*, *Fraxinus*, *Quercus*, *Salix*, and *Umbellularia* (Linsley 1964). Adults emerge from April to August (depending on latitude, elevation, and ambient temperature) and mate. Females oviposit on trees that have recently died (Linsley 1964, 1995). The adults are diurnal but rarely encountered in their natural habitat. Consequently, little is known of their biology and behavior (Essig 1943; Linsley 1964, 1995). Both sexes occasionally aggregate on hot days on buildings that have been recently painted, and these aggregations have been the subject of many reports in the popular press (Chemsak and Linsley 1971; Linsley 1995). In the work reported here, we provide evidence that (*Z*)-3-decenyl (*E*)-2-hexenoate is the major component of the male-produced aggregation pheromone of *R. funebris*.

Materials and Methods

Identification of Pheromone Components Adult *R. funebris* of both sexes (mating status unknown) were collected on 24–27 June 2007 from an aggregation on a building that was painted shortly prior to this period (Chipotle Mexican Grill, 3409 E. Foothill Blvd., Pasadena, CA, USA; 34°9'1" N, 118°04'49" W, 218 m elevation). Headspace volatiles were collected from six females and five males, held individually in either 3 cm diameter×20 cm long, glass aeration chambers or 263-ml glass canning jars with the screw-cap metal lids modified to accommodate air inlet and outlet tubes. Beetles in chambers were provided with water in 2-ml glass vials plugged with cotton wicks. Air entering the chambers was purified by passage through activated charcoal with the volatile chemicals trapped on a 5-mm plug of thermally desorbed activated charcoal (50–200 mesh) held in place by glass wool plugs in a section of 7-mm OD glass tubing attached to the chamber outlet. Air was pulled through the chambers at ~300 ml min⁻¹ by vacuum. Aeration chambers were conducted in an environmentally controlled room (25°C, 50% room temperature, 16:8 h ratio of light/dark, fluorescent lighting). Aeration chambers were run for 2–7 days, before collectors were extracted with three 200- μ l rinses of methylene chloride. The extracts were combined and analyzed as described below. In total, 24 and 33 beetle days of volatiles were collected from males and females, respectively.

Aliquots of crude extracts of volatiles collected from both sexes were analyzed by gas chromatography–electroantennogram detection (GC–EAD) with custom-built equipment as described in McElfresh and Millar (1999), using antennal sections from three male and three female *R. funebris* captured in 2007 (see above). Antennal preparations consisted of the terminal 1–2 cm of a beetle antenna, with the tip cut off with a razor blade, suspended between two saline-filled glass electrodes. Extracts were analyzed with Hewlett-Packard 5890A or 5890 series II GCs (Hewlett-Packard, Palo Alto, CA, USA) with helium carrier gas, splitless injection at 250°C, and a DB-5 column (30 m×0.25 mm ID, 0.25 μ m film; J&W Scientific, Folsom, CA, USA) programmed from 40°C (held for 1 min) to 275°C at 10°C min⁻¹. We confirmed that synthetic (*Z*)-3-decenyl (*E*)-2-hexenoate elicited strong responses from antennae of beetles of both sexes by GC–EAD, using beetles collected during field bioassays in 2008 (see below).

Electron impact mass spectra (EIMS; 70 eV) were recorded with an HP 6890 gas chromatograph interfaced to an HP 5973 mass selective detector (GC–MS). An HP5-MS column (30 m×0.25 mm ID) was used in splitless mode with helium carrier gas, programmed from 40°C (held for 1 min) to 250°C at 10°C min⁻¹, with injector and transfer line temperatures at 275°C and 280°C, respectively. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards. To further verify identifications, extracts and synthetic standards were analyzed on a polar DB-Wax column (30 m×0.25 mm ID×0.25 μ m film, J&W Scientific) programmed from 50°C (held for 1 min) to 250°C at 10°C min⁻¹.

Microchemical Reactions on Aeration Extracts Reduction was carried out by adding approximately 1 mg of 5% Pd on carbon to a 1.5-ml vial containing 100 μ l of a crude aeration extract from a male beetle. The vial was flushed with H₂ and sealed and held under H₂ atmosphere while stirring for 1 h. The mixture was then filtered through a plug of Celite® to remove the catalyst and analyzed by GC–MS.

Positions of double bonds were determined by epoxidation of an aliquot of the extract (Hogge and Millar 1987). Thus, an aliquot was treated with two drops of a CH₂Cl₂ solution of *m*-chloroperbenzoic acid (5 mg/ml), and the mixture held at room temperature for 1 h, swirling periodically. The mixture was diluted with 1 ml pentane and extracted twice with 0.5 M aqueous NaOH. The pentane layer was dried over Na₂SO₄, concentrated under a stream of N₂, and analyzed by GC–MS as described above. Samples of synthetic (*Z*)-3-decenol and (*Z*)-3-decenyl (*E*)-2-hexenoate were submitted to the same procedure to provide standards for comparison with aeration extracts.

Chemicals Decyl hexanoate and (*Z*)-4-decenol were available from our in-house library of insect semiochemicals. (*Z*)-3-Decenol and (*Z*)-3-decenyl (*E*)-2-hexenoate were synthesized as follows:

(*Z*)-3-Decenol This compound was prepared by P-2 nickel reduction of 3-decyn-1-ol (Brown and Ahuja 1973). Ni(OAc)₂·4H₂O (1.5 g, 6 mmol) was dissolved in 120 ml EtOH, and the solution degassed by bubbling N₂ for ~15 min. Then, still under N₂ atmosphere, 1 M NaBH₄ solution (6 ml of a solution prepared from 0.4 g NaBH₄, 9.5 ml EtOH, and 0.5 ml 1 M NaOH, filtered to remove suspended material) was added by syringe, with vigorous stirring. Ethylene diamine (2.4 ml) was added to the resulting black solution, the flask was fitted with a balloon filled with H₂, the N₂ line was removed, and the solution was stirred 15 min before adding 3-decyn-1-ol (10 g, 64 mmol, Alfa-Aesar, Ward Hill, MA, USA) dropwise over 10 min. The mixture was stirred until the starting material had been consumed completely (determined by GC, ~1.5 h). The flask then was flushed with N₂, and the mixture was filtered through a plug of Celite® (~2 cm) and activated charcoal (~1 cm top layer). The resulting violet colored solution was concentrated, taken up in ether, and extracted with 1 M HCl. The ether layer was washed with saturated aqueous NaHCO₃ and brine, concentrated, and Kugelrohr distilled (bp ~70°C, 0.2 mm Hg), giving a quantitative yield of the alcohol as a colorless oil EIMS (70 eV); *m/z* (percent abundance): 156 (1), 138 (12), 110 (20), 95 (59), 81 (81), 67 (100), 55 (75), and 41 (51); ¹H nuclear magnetic resonance (NMR; 400 MHz, CDCl₃): δ 5.55 (m, 1H, H-3), 5.34 (m, 1H, H-4), 3.62 (t, 2H, *J*=7.4 Hz, H-1), 2.31 (br overlapped dt, 2H, H-2), 2.04 (br overlapped dt, 2H, H-5), 1.47 (br s, 1H, OH), 1.4–1.2 (m, 8H, H6–H9), and 0.86 (t, 3H, *J*=7.0 Hz, H-10); and ¹³C NMR: δ 133.8, 125.1, 62.5, 31.9, 31.0, 29.9, 29.2, 27.6, 22.9, 14.3].

(*Z*)-3-Decenyl (*E*)-2-hexenoate A mixture of (*E*)-2-hexenoic acid (6.84 g, 60 mmol; Acros Organics, Morris Plains, NJ, USA), (*Z*)-3-decenol (6.2 g, 40 mmol), and *p*-toluenesulfonic acid (0.5 g) was refluxed overnight in 50 ml benzene, with a Dean-Stark trap to remove water. After cooling to room temperature, 100 ml of saturated aqueous NaHCO₃ were added, and the mixture was stirred 10 min. The layers were separated, and the organic layer was washed with aqueous NaHCO₃ and brine, dried over anhydrous Na₂SO₄, and concentrated. The residue was purified by Kugelrohr distillation (oven temperature ~105–120°C, 0.05 mm Hg), yielding 9.5 g of the ester as a colorless oil (94%, purity >96% by GC) EIMS (70 eV); *m/z* (percent abundance) 252 (trace), 138 (70), 115 (12), 110 (42), 97 (95), 81 (55), 68 (81), 55 (100), and 41 (45); ¹H NMR (400 MHz, CDCl₃) δ

6.94 (dt, 1H, *J*=15.6, 7 Hz, H-3'), 5.79 (d, 1H, *J*=15.6 Hz, H-2'), 5.48 (m, 1H, H-3), 5.35 (m, 1H, H-4), 4.11 (t, 2H, *J*=6.6 Hz, H-1), 2.38 (br overlapped dt, 2H, *J*=~7, 7 Hz, H-2), 2.16 (overlapped dtd, 2H, *J*=~7, 7, 1.6 Hz, H-4'), 2.02 (br overlapped dt, 2H, *J*=~7 Hz, H-5), 1.47 (overlapped tq, 2H, H-5'), 1.4–1.2 (m, 8H, H6–H9), 0.92 (t, 3H, *J*=7.6 Hz, H6'), and 0.87 (t, 3H, *J*=6.8 Hz, H-10); and ¹³C NMR δ 167.0, 149.5, 133.2, 124.6, 121.5, 63.9, 34.4, 32.0, 29.8, 29.2, 27.5, 27.1, 22.8, 21.5, 14.3, and 13.9].

Authentic samples of (*Z*)-3-nonenyl (*E*)-2-hexenoate and (*Z*)-3-decenyl (*E*)-3-hexenoate, for confirming the identities of the minor components, were synthesized by analogous esterification reactions between (*Z*)-3-nonenol (TCI America, Portland, OR, USA) and (*E*)-2-hexenoic acid and (*Z*)-3-decenol and (*E*)-3-hexenoic acid (Aldrich Chem. Co., Milwaukee, WI, USA).

Field Bioassays of Synthetic Compounds The responses of adult *R. funebris* to the major component of the male aeration, (*Z*)-3-decenyl (*E*)-2-hexenoate, were tested in field bioassays at Placerita Canyon Natural Area (Los Angeles Co., CA, USA). Black flight-intercept panel traps (1.2 high × 0.3 m wide, model PT Intercept™, APTIV, Portland, OR) were used in the trials. Traps were modified by replacing the collection basin with a 1.9-l plastic funnel that was attached to a clear plastic jar (1.9 l, General Bottle Supply Company, Los Angeles, CA, USA), in order to capture beetles alive. The funnel spout was cut off to leave a 3.5-cm-diameter hole at the bottom, a ~10-cm hole was cut into the threaded lid of the jar, and the funnel spout was hot-melt glued to the lid so that the spout was inside the jar. Traps were hung from tree branches ~2 m above the ground. Lures consisted of clear low-density polyethylene press-seal bags (Bagette model 14770, 5.1 × 7.6 cm, 0.05 mm wall thickness, Cousin Corp., Largo, FL, USA). The plastic bags were sealed and suspended with wire in the central open area of traps.

A sentinel trap baited with 100 μl of neat (*Z*)-3-decenyl (*E*)-2-hexenoate was used to monitor adult *R. funebris* at the Placerita Canyon Natural Area. The trap was set up on 18 June 2008, based on a report of an adult *R. funebris* observed in the Natural Area on that date in 2007 (AMR, unpublished data). The field bioassay was started on the day that the first adult was captured, 22 June 2008, and continued daily through 3 July 2008 (clear skies, no precipitation, maximum air temperatures 28–42°C). Traps were hung from tree branches, with traps separated by ~10 m, in a northeasterly transect through a canyon containing mature California sycamore, *Platanus racemosa* Nutt., and coast live oak, *Quercus agrifolia* Neé (position of first trap: 34°22' 39" N, 118° 28' 1" W, 466 m elevation). Many trees in the area had been burned by recent wildfires. We tested the responses of beetles to two concentrations of

(*Z*)-3-decenyl (*E*)-2-hexenoate: neat material (100 μ l) and a lower dose (10 μ l of neat material in 90 μ l absolute ethanol). The trap transect included one trap baited with the lower concentration and two traps baited with the neat material, all positioned to alternate with three control traps having lures that contained 100 μ l of ethanol. The experiment was replicated over 12 days. Lures were replaced every 3 days, and treatments were rotated one position along the transect every 4 days to control for positional effects. Traps were checked for beetles daily at ~1100 and 1800 hours.

The sex of adult *R. funebris* captured was determined by using the sexually dimorphic characters of antennal length and form of the fifth abdominal sternite (Linsley 1964). Differences between numbers of females and males captured were tested with the χ^2 goodness-of-fit test (Sokal and Rohlf 1995). Differences between treatments in numbers of beetles captured (sexes combined) were tested with the nonparametric Friedman's test (blocking by replicate; PROC FREQ with CMH option; SAS Institute 2001) because assumptions of analysis of variance were violated by heteroscedasticity (Sokal and Rohlf 1995). Differences between pairs of means were tested with the Ryan–Einot–Gabriel–Welsch (REGWQ) means-separation test to control maximum experiment-wise error rates (SAS Institute 2001). Six dates on which fewer than three beetles in total were captured were excluded from the analysis.

We also tested for the presence of adult *R. funebris* in a variety of different habitats in southern California (Table 1) by setting up pairs of traps: one baited with 100 μ l of the 10% solution of synthetic (*Z*)-3-decenyl (*E*)-2-hexenoate in a plastic bag lure and the other a control trap with a solvent lure. These bioassays were conducted for varying periods between 28 June and 18 August 2008. Traps were checked for beetles every 1–5 days, and lures were replaced before the liquid had completely evaporated. Voucher specimens

of *R. funebris* were deposited at the Entomology Research Museum at the University of California, Riverside.

Results

Identification of Pheromone Components Coupled GC–EAD analyses of headspace volatiles collected from live adult males of *R. funebris* showed that antennae of both sexes responded strongly to the major component in extracts from males (peak 4, Fig. 1). Several minor components in extracts elicited weaker responses from antennae of males (peaks 1–3, Fig. 1). The compounds (peaks 1–4) were produced in a ratio of $10.2 \pm 9.1:0.3 \pm 0.3:15.3 \pm 9.9:100$, respectively (mean \pm standard deviation of four extracts), and all of these compounds were present only in extracts from males. Three aeration extracts from female beetles also were analyzed by GC–EAD, using antennae from three different males. The antennae did not respond to any components in the extracts, indicating that females of this species do not produce volatile sex pheromones, at least under the aeration conditions used in these studies.

GC–MS analyses of extracts showed that peak 1 (Fig. 1; Kovats index [KI] 1256 on DB-5 column) gave a weak molecular ion at m/z 156 (trace), with the first significant ion occurring at m/z 138 (12%) from loss of water, and a base peak at m/z 68 (for full mass spectrum, see Supplementary data). From comparison with database spectra and the retention times and mass spectrum of a (*Z*)-4-decenol standard (KI 1259), it appeared likely that this compound was a straight-chain decenol, with (*Z*)-3-decenol being the best match to database spectra. The spectrum of one of the two other minor components (peak 3, Fig. 1; KI 1770) and the major component (peak 4, Fig. 1; KI 1823) were very similar to each other, suggesting that

Table 1 Additional study sites in southern California where field bioassays were conducted (one trap baited with dilute [*Z*]-3-decenyl [*E*]-2-hexenoate and one control trap) in 2008 and the number and sex of adult *R. funebris* captured in baited traps

Location	Habitat	GPS coordinates (elevation)	Time period (# days)	♂/♀ captured
Kern Co., Mt. Pinos Rd.	Oak-conifer woodland	34°49'27" N, 119°05'00" W (1,969 m)	Late June–mid August (40)	2/1
Kern Co., Mt. Pinos Rd.	Oak-conifer woodland	34°49'18" N, 119°05'27" W (2,127 m)	Mid July–mid August (34)	0/1
Los Angeles Co., Big Pines Hwy.	Riparian area, oak-conifer woodland	34°22'57" N, 117°42'00" W (1,998 m)	Early July–mid August (27)	0/0
Los Angeles Co., State Hwy. 2	Oak woodland	34°22'46" N, 117°43'44" W (2,147 m)	Mid July–mid August (23)	0/0
Riverside Co., Residence	Urban forest	33°57'33" N, 117°21'24" W (290 m)	Early July (2)	1/1
Riverside Co., University of California Box Springs Reserve	Riparian area, coastal sage scrub, chamise chaparral	33°59'7" N, 117°18'12" W (416 m)	Early July (6)	1/1
Ventura Co., Mutau Flat Rd.	Oak-piñon pine woodland	34°42'37" N, 119°07'15" W (1,663 m)	Late July (5)	0/1

No *R. funebris* were captured in control traps at any site

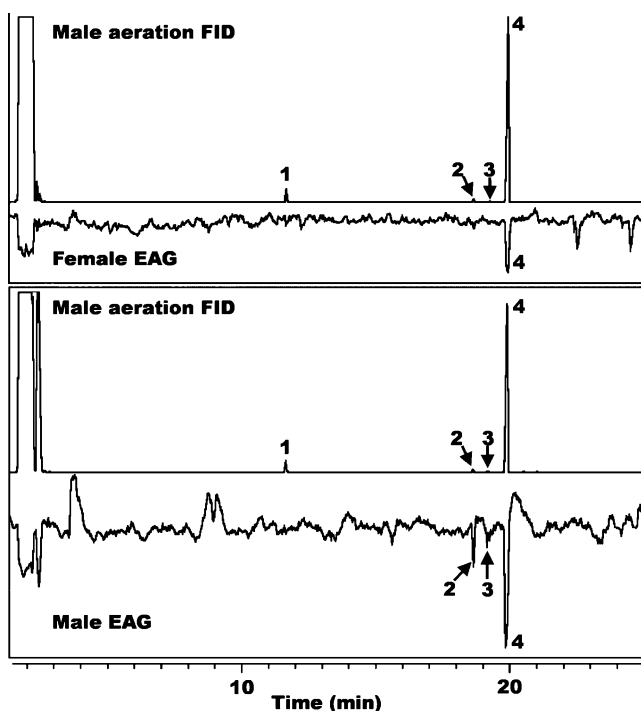


Fig. 1 Gas chromatogram (*top traces*) and corresponding electroantennogram (*EAG*) traces from antennae of female (*top*) and male (*bottom*) *Rosalia funebris*, in response to extract from a headspace collection of a male. A DB-5 column was used for coupled gas-chromatogram-EAD. A DB-5 column for the coupled gas chromatogram-EAG. *1* (*Z*)-3-decenol, *2* (*Z*)-3-nonenyl (*E*)-2-hexenoate, *3* (*Z*)-3-decenyl (*E*)-3-hexenoate, and *4* (*Z*)-3-decenyl (*E*)-2-hexenoate

they were isomers. The major, later-eluting isomer showed a weak molecular ion at m/z 252, with the first significant fragment at m/z 138 (74%), a strong peak at m/z 97 (95%), and a weaker fragment at m/z 115 (12%; for full mass spectrum, see [Supplementary data](#)). The latter two fragments would be expected from hexenoate esters so that, in total, these data suggested that the compound might be a decenyl hexenoate. Reduction of the crude extract with H_2 and a palladium-on-carbon catalyst gave a small, early-eluting peak (KI 1273) of which the mass spectrum and retention time matched those of decanol. The reduction collapsed peaks 3 and 4 into one peak (KI 1785) with the mass spectrum and retention time matching those of decyl hexanoate. These data provided support for the tentative identifications of peak 1 as a decenol and peaks 3 and 4 as decenyl hexenoate isomers. The fact that the retention time of the major component (peak 4) was significantly longer than that of decyl hexanoate on the nonpolar HP5-MS GC column suggested that the double bond in the hexenyl portion of the ester was conjugated with the carbonyl group.

The decenol component of the crude extract was confirmed as (*Z*)-3-decenol by epoxidation of crude

extract. The retention times and mass spectra of both the underivatized (*Z*)-3-decenol and the epoxidized (*Z*)-3-decenol matched those of synthetic standards. Although we did not know which stereoisomer was correct a priori, these matches confirmed both the position and the geometry of the double bond (Hogge and Millar 1987). These data also suggested that the major component in the extract was a (*Z*)-3-decenyl 2-hexenoate, and the retention time and mass spectrum of a synthesized standard of (*Z*)-3-decenyl (*E*)-2-hexenoate matched those of the major component on both nonpolar DB-5 and polar DB-Wax columns. As with the determination of the double bond stereochemistry of (*Z*)-3-decenol described above, we did not know the stereochemistry of the conjugated acid portion of the ester a priori, but the retention time and mass spectral matches with the synthetic standard provided good evidence for (*E*)-2 stereochemistry. This stereochemistry was verified by epoxidation of the ester components in the insect extract. Although the epoxidation of the extract did not proceed cleanly, possibly due to acid-catalyzed rearrangements of the initial diepoxide products, epoxidation of pure synthetic (*Z*)-3-decenyl (*E*)-2-hexenoate resulted in a pattern of peaks that was qualitatively similar to those in the epoxidized insect extract, with matching retention times and mass spectra. Furthermore, antennae from beetles of both sexes responded strongly to synthetic (*Z*)-3-decenyl (*E*)-2-hexenoate in GC-EAD bioassays. Finally, the retention indices of (*Z*)-3-decenol and (*Z*)-3-decenyl (*E*)-2-hexenoate on a polar DB-Wax column (KI 1767 and 2168, respectively) exactly matched those of standards. In sum, these data confirmed that the major male-specific compound in the aeration extracts from male *R. funebris* was (*Z*)-3-decenyl (*E*)-2-hexenoate and that the early-eluting trace component (peak 1) was (*Z*)-3-decenol.

The ester isomer with the slightly lower KI value than the major component (KI value 1770; peak 3, Fig. 1) was tentatively identified as a deconjugated analog of the main compound (for full mass spectrum, see [Supplementary data](#)) because it was generated as an impurity during attempted esterification of (*E*)-2-hexenoic acid with (*Z*)-3-decenol using 4-(*N,N*)-dimethylaminopyridine and dicyclohexylcarbodiimide (Hasner and Alexanian 1978). This tentative identification was confirmed by synthesis of an authentic standard of (*Z*)-3-decenyl (*E*)-3-hexenoate, the retention time and mass spectrum of which matched those of the insect-produced compound. Fortuitously, the (*E*)-3-hexenoic acid starting material was contaminated with a small amount of the (*Z*)-isomer, the ester of which had a different retention time, so that the identification of the stereochemistry in the natural compound was unequivocal.

A partial mass spectrum was obtained for peak 2, and the fragments suggested that it was a homolog of the main component, with a nine-carbon alcohol component to the

ester. A molecular ion was not seen, but significant ions included m/z 124 (54), 115 (8), 97 (76), 82 (66), 68 (74), 55 (100), and 41 (53). The ions at m/z 97 and 115, corresponding to fragments from a hexenoate ester, indicated that it was the alcohol rather than the acid portion of the ester that was one carbon shorter than the equivalent portion in the major component, and the KI value of 1725, almost exactly 100 KI units less than that of the main component, provided further evidence that it was probably a homolog that was shorter by one carbon. The identification was confirmed by synthesis of an authentic standard (for full mass spectrum, see [Supplementary data](#)), with spectral and retention time data matching those of the insect-produced compound.

Field Bioassays of Synthetic Compounds The sentinel trap at Placerita Canyon Natural Area captured the first adult *R. funebris* on 22 June 2008. We subsequently captured 48 adults in the field bioassay (four males and 44 females; sex ratio significantly different from 1:1, $\chi^2=33.3$, $P<0.001$), with a maximum of 12 adults (including three of the four males) over a 24-h period in one trap baited with neat (*Z*)-3-decenyl (*E*)-2-hexenoate. The number of beetles trapped per day reached a peak of 18 after 1 week but fell to one and zero over the last 4 days. Treatment means were significantly different (Fig. 2; sexes combined, Friedman's $Q_{2,35}=18.2$, $P<0.001$). Traps baited with diluted and neat (*Z*)-3-decenyl (*E*)-2-hexenoate captured significantly more beetles than did controls (Fig. 2), but the pheromone treatment means were not significantly different from one another. All but two of the beetles were captured between

1100 and 1800 hour. Traps baited with synthetic pheromone did not capture any other insect species in consistent or significant numbers.

Traps baited with dilute (*Z*)-3-decenyl (*E*)-2-hexenoate captured a total of nine adult *R. funebris* (four males, five females) at additional study sites (Table 1), with no beetles being caught in control traps. Some of these sites yielded no beetles, even though baited traps were in place for several weeks (Table 1).

Discussion

Attraction of both sexes of *R. funebris* in field bioassays to lures baited with synthetic (*Z*)-3-decenyl (*E*)-2-hexenoate suggested that this compound is an aggregation rather than a sex pheromone, as appears to be true for pheromones produced by many other cerambycine species (Hanks et al. 2007; Lacey et al. 2008b). The strong female bias of adult *R. funebris* captured in the replicated field trial suggests that the sexes differ either in their sensitivity or their responses to the compound. However, it is impossible to draw conclusions about differential responses of the sexes to the pheromone based on these data without information about the sex ratio of the local population. Also, males that were captured alive in the traps may have emitted pheromone and so could have influenced subsequent trap catches of conspecifics. Nevertheless, most of the females were captured in traps that did not contain males, so their response to the pheromone treatment was unequivocal.

The small numbers of beetles captured at the additional sites suggest that our choice of study site for the field bioassays, Placerita Canyon Natural Area, was fortuitous and attest to the general rarity of *R. funebris*, as has been previously noted (Essig, 1943; Linsley 1964, 1995). It is possible that *R. funebris* was more abundant in that area because of recent wildfires. Studies on this species suggest that females oviposit in burned trees (IPS, personal observation). The brief period of time that adult *R. funebris* were captured in traps at Placerita Canyon Natural Area (~9 days) is consistent with other reports and observations that adults of this species are only active for a short period (Chemsak and Linsley 1971; IPS, personal observation).

That we captured 12 beetles in one trap in a single day indicates that (*Z*)-3-decenyl (*E*)-2-hexenoate is sufficient to attract *R. funebris* in significant numbers when adults are active. Nevertheless, it is possible that the minor components identified may form part of the natural pheromone and that traps baited with a more complete reconstruction of the blend could attract greater numbers of beetles. Unfortunately, the full identification and syntheses of the minor components was not completed in time for these chemicals to be included in the field trials.

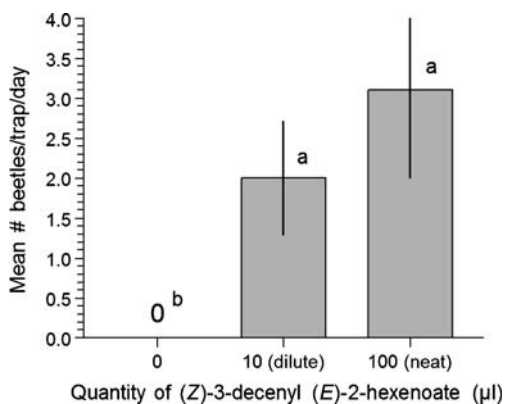


Fig. 2 Mean (\pm standard error) number of adult (sexes combined) *Rosalia funebris* captured from 22 June to 3 July 2008 at Placerita Canyon Natural Area in panel traps baited with 100 μ l neat (*Z*)-3-decenyl (*E*)-2-hexenoate, 10 μ l of (*Z*)-3-decenyl (*E*)-2-hexenoate in 90 μ l of ethanol, or 100 μ l of ethanol (solvent control). Bars with different letters are significantly different (REGWQ test $P<0.05$)

The function of the aggregation pheromone of *R. funebris* is probably similar to that of other cerambycid species. Both sexes of many cerambycid species are attracted to volatile compounds emanating from patches of larval host plants (Hanks 1999; Ginzl and Hanks 2005). Once in a patch of host plants, volatile pheromones may further facilitate mate location by bringing males and females together on hosts (Ginzl and Hanks 2005). Nonvolatile contact chemical signals also play a role, once the sexes are in the same vicinity, in males identifying conspecific females (Ginzl and Hanks 2003, Ginzl et al. 2003a, b; Lacey et al. 2008a). Consistent with these behaviors for other cerambycid species, adult *R. funebris* of both sexes aggregate on larval hosts (Linsley 1995). Aggregation also may expedite host colonization, a distinct adaptive advantage, because the quality of larval hosts (dying trees) declines rapidly as hosts are colonized by other types of wood-boring insects and saprophytes (Hanks 1999).

The structure of the male-produced pheromone of *R. funebris* is unprecedented among the cerambycid pheromones that have been reported to date and unlike the diol/hydroxyketone motif of the volatile pheromones of most other cerambycine species (see “Introduction”). Although the diol/hydroxyketone pheromone motif is common, it is by no means universal among members of this large and diverse subfamily, as further indicated by the pheromones of *P. lecontei* and *M. caryae* (Hanks et al. 2007; Lacey et al. 2008b). The unique structure of the pheromone of *R. funebris* is consistent with this species being relatively distantly related to other cerambycines for which pheromones have been identified. *Rosalia funebris* is the sole representative of the tribe Rosaliini in the Western Hemisphere, whereas the other cerambycine species for which pheromones have been identified are in the tribes Anaglyptini, Callidiini, and Clytini (Ray et al. 2006; Hanks et al. 2007; taxonomy according to Monné and Hovore 2005). Furthermore, *R. funebris* lacks the distinctive prothoracic glands and gland pores that are the source of pheromones of other cerambycine species (Ray et al. 2006), and we do not yet know either the source or the mechanism of release of the *R. funebris* pheromone. The only other known pheromone glands of cerambycids that are not in the prothorax are those on the ovipositor of females of the more distantly related species *Prionus californicus* Mots. (Barbour et al. 2006; Cervantes et al. 2006).

To our knowledge, (Z)-3-decenyl (E)-2-hexenoate or any of the minor components of the volatiles produced by male *R. funebris* are NOT similar in structure to volatile compounds released by house paint. Therefore, it seems likely that the attraction of both sexes to newly painted structures, which has been observed and documented a number of times, may be due to some structural similarity among volatile compounds emanating from paint and

compounds released by larval host plants, rather than similarity to the pheromone. It also seems unlikely that the observed attraction is due to color or other optical properties of the paint, which presumably persist over time, unlike the release of volatile chemicals from drying paint.

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