CHEMICAL ECOLOGY

(R)-3-Hydroxyhexan-2-one Is a Major Pheromone Component of Anelaphus inflaticollis (Coleoptera: Cerambycidae)

A. M. RAY,1,2 I. P. SWIFT,3 J. A. MOREIRA,4 J. G. MILLAR,4 AND L. M. HANKS1,5


ABSTRACT  We report the identification and field bioassays of a major component of the male-produced aggregation pheromone of Anelaphus inflaticollis Chemsak, an uncommon desert cerambycine beetle. Male A. inflaticollis produced a sex-specific blend of components that included (R)-3-hydroxyhexan-2-one, (S)-2-hydroxyhexan-3-one, 2,3-hexanedione, and (2R,3R) - and (2R,3S)-2,3-hexanediols. Field trials with baited bucket traps determined that the reconstructed synthetic pheromone blend and (R)-3-hydroxyhexan-2-one alone attracted adult A. inflaticollis of both sexes, with significantly more beetles being attracted to the blend. We conclude that (R)-3-hydroxyhexan-2-one is a major pheromone component of A. inflaticollis, and our results suggest that one or more of the minor components may further increase attraction of conspecifics. Scanning electron microscopy showed that male A. inflaticollis have pores on the prothorax that are consistent in structure with sex-specific pheromone gland pores in related species. Males also displayed stereotyped calling behavior similar to that observed in other cerambycine species. This study represents the first report of volatile pheromones for a cerambycine species in the tribe Elaphidiini.

KEY WORDS  Mojave desert, longhorned beetle, mating behavior, sex pheromone, (R)-3-hydroxyhexan-2-one

Sex or aggregation pheromones produced by males have been identified for 14 species in four tribes of the cerambycid beetle subfamily Cerambycinae to date (reviewed by Ray et al. 2006, 2009; Hanks et al. 2007). The pheromone components of most of these species share a similar motif, consisting of compounds that are 6, 8, or 10 carbons long with hydroxyl or carbonyl groups at C2 and C3 (Hanks et al. 2007). Furthermore, males of these species have sex-specific gland pores in the prothorax that release the pheromone (Ray et al. 2006; Hanks et al. 2007; Lacey et al. 2008, 2009; and references therein). When releasing pheromone, males adopt a characteristic calling posture, termed the “pushup stance,” in which they remain still with only their prothoracic legs extended, elevating the anterior portion of the body (Lacey et al. 2007a, b). This posture may enhance release of pheromone into the airstream (Lacey et al. 2007b).

We report on the chemical ecology of the cerambycine species Anelaphus inflaticollis Chemsak. A. inflaticollis apparently is limited in distribution to a few areas in the western Mojave Desert (Cope 1984, Solomon 1995). It is rarely collected, being represented by only two specimens in the insect collection of the Smithsonian National Museum of Natural History (S. W. Lingafelter, personal communication), one specimen in the California Academy of Sciences, and no specimens in either the Entomology Research Museum at the University of California, Riverside, or the Los Angeles County Museum of Natural History (A.M.R. and I.P.S., unpublished data). Larvae of A. inflaticollis bore within and eventually girdle branches of box thorn (Lycium cooperi Gray, Solanaceae) and black greasewood [Sarcobatus vermiculatus (Hook) Torrey, Chenopodiaceae; Hovore et al. 1978, Cope 1984]. In the process, they produce a linear series of small holes through which they expel frass (Hovore and Giesbert 1976, Solomon 1995). Larvae may require 1–2 yr to complete development (Solomon 1995). The crepuscular adults are cryptically colored and have been collected in May (Linsley 1963). There is no published information on the behaviors of adult A. inflaticollis (see Solomon 1995).

We provide evidence that (R)-3-hydroxyhexan-2-one, the most abundant sex-specific compound produced by male A. inflaticollis, attracted both sexes in field trials. The addition of a blend of the minor components [(S)-2-hydroxyhexan-3-one, 2,3-hexanedi-one, and (2R,3R)- and (2R,3S)-2,3-hexanediol] to (R)-3-hydroxyhexan-2-one further increased attraction of beetles.
Materials and Methods

We reared adult *A. inflaticollis* from branches of box thorn that were collected on 16 March 2007 from a site in the Mojave Desert (~56 km northeast of Mojave, Kern Co., CA, State Hwy. 14; 35°28′12″ N, 117°57′43″ W, 964-m elevation). Other dominant perennial plants at the site were creosote bush [*Larrea tridentata* (de Candolle) Coville, Zygophyllaceae], Joshua tree (*Yucca brevifolia* Engelman, Agavaceae), and cholla cactus (*Cylindropuntia* sp., Cactaceae). Branches of box thorn were placed in a 40-liter plastic storage container on a laboratory bench (12:12-h L:D, 20°C). Ten box thorn were placed in a 40-liter plastic storage container on a laboratory bench (12:12-h L:D, 20°C).

Adult beetles that emerged were caged individually in the laboratory in 0.1-m³ cylindrical cages of aluminum screen with glass petri dishes at top and bottom. Beetles were provided 10% sucrose solution in 8-ml vials plugged with cotton rolls. Males in cages were observed to adopt a body posture analogous to the pushup stance of calling males of other cerambycine species (see Lacey et al. 2007a, b).

Headspace volatiles were collected from three female and three male *A. inflaticollis* held individually in ~0.3-liter glass vacuum traps lined with aluminum screen to provide a perch. One nipple of each chamber was connected with Teflon tubing to a collector consisting of a glass tube (6 cm by 4 mm ID) that contained 100 mg of 80/100 mesh SuperQ (Alltech Associates, Deerfield, IL) held between plugs of silanized glass wool. Charcoal-purified air was pulled through the apparatus with a water aspirator (0.7 liter/min). One male and one female were aerated simultaneously on a laboratory windowsill for 24 h, with different beetles aerated on 3 consecutive d. Collectors were eluted with three 0.5-ml aliquots of methanol; or (3) 1 ml of ethanol (solvent control). Each trap was buried such that the lip of the bucket was ~8 cm above ground level to exclude vertebrates. Traps were placed ~10 m apart in three northeasterly transects that were ~0.4–0.8 km apart.

Lures consisted of clear self-seal polyethylene bags (Bagette model 14770, 5.1 by 7.6 cm, 0.05-mm wall thickness; Cousin, Largo, FL) that were sealed and suspended with wire from the handle of the bucket over the center of the funnel. Lures were loaded with (1) 1 ml of an ethanol solution of the reconstructed blend of pheromone components found in aeration extracts from male *A. inflaticollis* (see Results), including (per ml of solution) 2,3-hexanedione (19 µl), (R)-3-hydroxyhexan-2-one (100 µl), (S)-2-hydroxyhexan-3-one (6 µl), (2R,3R)-2,3-hexanediol (11 µl), and (2R,3S)-2,3-hexanediol (6 µl); or (3) 1 ml of ethanol (solvent control). Each transect included one trap for each treatment.

We checked traps for beetles every 3–4 d, at which time lures were replaced and treatments were rotated one position along the transect to control for positional effects. Differences between treatments in numbers of beetles captured were tested with the two-way nonparametric Friedman's test (blocked by day; PROC FREQ with CMH option; SAS Institute 2001) because assumptions of analysis of variance (ANOVA) were violated by heteroscedasticity (Sokal and Rohlf 1995). Differences between pairs of means were tested with the REGWQ means-separation test.
to control maximum experimentwise error rates (SAS Institute 2001). We include in the analysis data from only two trapping periods during which we captured adult *A. inflaticollis* (see Results).

Voucher specimens of *A. inflaticollis* have been deposited in the Entomology Research Museum at the University of California, Riverside, and the Smithsonian National Museum of Natural History, Washington DC.

**Results**

Comparisons of extracts of headspace volatiles collected from male and female *A. inflaticollis* showed that there were five male-specific compounds. These compounds were tentatively identified (in order of elution) as 2,3-hexanediol, 2-hydroxyhexan-3-one, (2R*,3R*)-2,3-hexanediol, and (2S,3S*)-2,3-hexanediol by matches of their mass spectra with spectra from our library of known cerambycid pheromone components. The identifications were confirmed by matching the mass spectra and retention times with those of authentic standards on DB-5 and Cyclodex B columns (see below). Having determined the gross structures of the two hydroxyhexanones and the two 2,3-hexanediols, the absolute configurations of the insect-produced compounds were determined by analysis of the extracts on a chiral stationary phase Cyclodex-B column (Table 1). Identifications were confirmed by coinjection of aliquots of extract with the racemic hydroxyhexanones or diols, to show conclusively that one of the two peaks in a racemate was enhanced by coinjection of the extract. In total, these analyses unambiguously identified the five male-specific compounds as (R)-3-hydroxyhexan-2-one (∼70%), (S)-2-hydroxyhexan-3-one (4%), 2,3-hexanediol (14%), (2R,3R)-2,3-hexanediol (8%), and (2R,3S)-2,3-hexanediol (4%). Extracts of headspace aerations of female *A. inflaticollis* did not contain any of these compounds in detectable quantities.

The prothoraces of male and female *A. inflaticollis* are strongly dimorphic in punctuation and shape of the pronotum (Hovore and Giesbert 1976). Pronota and pleura of males had groups of pores within indentations, whereas females had indentations but lacked the pores (Fig. 1).

We captured adult *A. inflaticollis* during only two 2-d trapping periods (17–19 and 19–21 May 2008) of the 34 d that bucket traps were in place in the Mojave Desert (with the exception of one female that was captured on 1 June). A total of 27 beetles were captured (13 females, 13 males; one specimen was damaged and could not be sexed), with a maximum of seven adults captured in one trap that was baited with the blend of pheromone components. Treatment means were significantly different from one another (Fig. 2; sexes combined, Friedman’s $Q_{2,17} = 11.7, P = 0.003$). Traps baited with the *A. inflaticollis*-specific blend and those baited with (R)-3-hydroxyhexan-2-one as a single component captured significantly more beetles than did controls, and the blend attracted more than twice as many beetles as (R)-3-hydroxyhexan-2-one alone (Fig. 2). Traps baited with synthetic pheromone did not capture any other insect species in consistent or significant numbers.

**Discussion**

(R)-3-hydroxyhexan-2-one is known to be an important component or the sole component of male-produced pheromones of 14 species of cerambycine beetles (Hanks et al. 2007). In fact, adults of many

<table>
<thead>
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<th>Compound</th>
<th>Retention time (min)</th>
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<tr>
<td>(R)-2-hydroxyhexan-3-one</td>
<td>10.49</td>
</tr>
<tr>
<td>(R)-3-hydroxyhexan-2-one</td>
<td>10.56</td>
</tr>
<tr>
<td>(S)-2-hydroxyhexan-3-one</td>
<td>10.67</td>
</tr>
<tr>
<td>(S)-3-hydroxyhexan-2-one</td>
<td>11.10</td>
</tr>
<tr>
<td>(2S,3S)-2,3-hexanediol</td>
<td>15.12</td>
</tr>
<tr>
<td>(2R,3R)-2,3-hexanediol</td>
<td>15.31</td>
</tr>
<tr>
<td>(2R,3S)-2,3-hexanediol</td>
<td>15.77</td>
</tr>
<tr>
<td>(2S,3R)-2,3-hexanediol</td>
<td>15.87</td>
</tr>
</tbody>
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![Fig. 1.](image-url) Scanning electron micrographs (dorsal view) of the prothorax of adult female (A) and male (B) *A. inflaticollis*. The prothorax of the male has pores lying within indentations (inset in B); females also have indentations, but lack pores (inset in A).
cerambicine species respond as strongly to (R)-3-hydroxyhexan-2-one alone as they do to more complete reconstructions of blends of headspace volatiles collected from live males (Hanks et al. 2007). In this context, the markedly stronger behavioral response of adult A. inflaticollis to the blend compared with their responses to (R)-3-hydroxyhexan-2-one alone is noteworthy and indicates that one or more of the minor components also forms part of the pheromone blend for A. inflaticollis. The 3-hydroxyhexan-2-one, 2-hydroxyhexan-3-one, and 2,3-hexanediol structures of the major and minor components produced by male A. inflaticollis (tribe Elaphidiini) match the structural motif shared by the pheromones identified for cerambicine species in the tribes Anaglyptini, Callidini, and Clytini (Lacey et al. 2004, 2007a, 2008, 2009; Ray et al. 2006; Hanks et al. 2007).

We have not attempted to further optimize the blend and narrow down the specific minor component(s) that contribute to the increased attraction because of the scarcity of the species and the very short time window that adult beetles apparently are active every year (e.g., we captured beetles during only two trapping periods). Thus, field trials to determine the role of each of the minor components and the exact nature of the full pheromone blend could require many field seasons. It also is important to note that the minor component (S)-2-hydroxyhexan-3-one was not simply an artifact of isomerization of the rather thermolabile (R)-3-hydroxyhexan-2-one (Leal et al. 1995), because isomerization would have resulted in approximately equivalent amounts of (R)- and (S)-2-hydroxyhexan-3-one, as well as lesser amounts of (S)-3-hydroxyhexan-2-one from isomerization in the reverse direction.

The pores on the prothorax of male A. inflaticollis are similar in structure to the pheromone gland pores in males of other cerambicine species that are also known to produce hydroxyketone and/or 2,3-alkanediol pheromones (Ray et al. 2006; Hanks et al. 2007, Lacey et al. 2009). It therefore seems likely that these pores have the same function in male A. inflaticollis. Male A. inflaticollis displayed the characteristic “pushup” stance that has been associated with calling behaviors of other cerambicines (Lacey et al. 2007a, b).

The small numbers of A. inflaticollis that were captured at our field site during only two trapping periods during the 48 d that intercept or bucket traps were deployed is consistent with literature reports that A. inflaticollis is uncommon and has a short adult activity period (Cope 1984, Solomon 1995). Attraction of both sexes of A. inflaticollis in field bioassays to lures baited with the species-specific blend, or (R)-3-hydroxyhexan-2-one alone, suggests that males produce an aggregation rather than a sex pheromone according to the accepted definitions of these terms (Wyatt 2003), as seems to be true for many other cerambicine species (Hanks et al. 2007). However, it should be pointed out that the male-produced pheromone may actually be a sex pheromone that attracts females, but which is exploited by other males as an indirect method of finding females. This “satellite male” strategy, whereby noncalling males intercept females responding to calling males, has been reported for other types of insects [e.g., the cockroach Nauphoeta cinerea (Olivier); Moore et al. 1995]. Alternatively, the pheromone signal may be increased when calling males aggregate, thereby improving the chances of attracting receptive females and allowing females to choose among a number of males. Analogous types of mating aggregations are known for insects as diverse as arctiid moths (Wunderer et al. 1986) and true fruit flies (Aluja and Norbom 2000).

In summary, we identified and conducted bioassays of a male-produced aggregation pheromone blend for a rare desert-dwelling cerambicid beetle. The most abundant component of the pheromone, (R)-3-hydroxyhexan-2-one, conforms to the structural motif characteristic of pheromones for related species in several tribes of the Cerambycinae, lending further support to our working hypothesis that male-produced pheromones are common and may be conserved across this large and diverse subfamily, which is represented by nearly 5,000 species and 65 tribes in the New World alone (Monné and Hovore 2006).

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