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Pheromone chemistry of the Neotropical cerambycid beetles *Achryson surinamum* and *Sphaerion inerme*

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Abstract

During field screening trials in Brazil, adults of both sexes of the cerambycid beetle *Achryson surinamum* (L.) (Cerambycinae: Achrysonini) were significantly attracted to racemic *anti*-2,3-octanediol, previously identified as a sex and aggregation-sex pheromone of various cerambycid species across different continents. Analyses of beetle-produced volatiles revealed that males of *A. surinamum* sex-specifically produce (2*S*,3*R*)-2,3-octanediol, as well as lesser amounts of (*S*)-2-methylbutan-1-ol. In field trials, both sexes of beetles were attracted by reconstructions of the species' pheromone blend with synthesized components, confirming males produce an aggregation-sex pheromone. During the trials, the cerambycine *Sphaerion inerme* White (Elaphidiini) was attracted to some of the test lures, providing leads to its attractant pheromone. Subsequent analysis of extracts of headspace volatiles from live adults of *S. inerme* revealed that males produce a blend of (*R*)-2-methylbutan-1-ol and (*R*)-2-methylpentan-1-ol. In field tests, blends of racemic 2-methylbutan-1-ol+2-methylpentan-1-ol attracted significant numbers of beetles of both sexes. This study provides further examples of how identification of attractant pheromones of cerambycid species can be expedited by leveraging prior knowledge of the pheromone chemistry of related species.

Keywords Coleoptera · Longhorn beetles · Pheromone chemistry · Aggregation-sex pheromone · Monitoring · Invasive species

Introduction

Over the past two decades, research has consistently revealed a noteworthy level of conservation of pheromone structures within the large beetle family Cerambycidae (Millar and Hanks 2017). This conservation phenomenon crosses taxonomic levels, ranging from genera to tribes and occasionally even subfamilies, with numerous demonstrated instances of species sharing the same or closely related pheromone components (reviewed in Millar and Hanks 2017). This parsimony in biosynthesis and utilization of shared structural motifs also extends across multiple continents, even though species have been geographically isolated for millions of years. An example is the compound 3-hydroxyhexan-2-one, which is a pheromone component for numerous cerambycid species from all habitable continents (Millar and Hanks 2017). A number of other compounds also are widely shared among cerambycid species (Hanks and Millar 2016).

From a practical perspective, this conservation of pheromone structures suggests traps baited with single components or blends of related pheromone compounds might attract multiple cerambycid species, and this hypothesis has been borne out in several studies (Bobadoye et al. 2019; Flaherty et al. 2019; Hayes et al. 2016; Millar et al. 2018; Rassati et al. 2019; Sweeney et al. 2014). As a result, traps baited with pheromones or pheromone blends are becoming pivotal tools in mapping geographic ranges of native cerambycids (Santos-Silva et al. 2020) and strengthening surveillance programs aimed at detecting incursions of invasive species (Fan et al. 2019; Roques et al. 2023). This is particularly critical due to the significant threat posed by invasive wood-boring cerambycids to orchards and natural and managed forests, because the long-lived larvae are easily transported between continents in

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wooden products and packing materials via global commerce (Meurisse et al. 2019). The widespread sharing of pheromone structures also holds promise for expediting identification of pheromones or their analogs for related target species, leveraging the identification and testing of pheromone components for one species into identification of pheromones of related species (e.g., Millar et al. 2018, 2019; Silva et al. 2017, 2018, 2020, 2021, 2024).

During field screening trials of known cerambycid pheromones in Brazil, adult males and females of *Achryson surinamum* (L.) (Cerambycinae: Achrysonini) were significantly attracted to traps baited with racemic *anti*-2,3-octanediol (Silva et al. 2024). Whereas previous studies have established 2,3-octanediols as sex and aggregation-sex pheromones, or candidates for pheromones of at least 10 cerambycid species endemic to Asia, Europe, and North America (Millar and Hanks 2017), to our knowledge this was the first time a South American species had been attracted to an isomer of 2,3-octanediol. The significant and specific attraction led us to predict that one of the enantiomers of *anti*-2,3-octanediol is a pheromone component for *A. surinamum*. It had been previously noted that males of this species have sex-specific pores on the tergum of the prothorax which in other cerambycine species are associated with the emission of volatile pheromones (Ray et al. 2006).

The range of *A. surinamum* encompasses southern South America, primarily Brazil, and extends northward to the southern United States (Monné 2024). The beetle has a wide host range, the larvae being associated with > 80 species of native and introduced woody plants in 21 families, especially Fabaceae (Monné 2024). This polyphagy underscores the potential invasiveness of *A. surinamum*, as evidenced by prior studies (Duffy 1953, 1960; Girard 1968; Maier 2017). Given the potential risk posed by this species, proactively developing pheromone-based attractants for incorporation into quarantine surveillance and monitoring programs could prove useful should it invade new regions.

Here, we present our findings on the identification and field testing of the attractant pheromone of *A. surinamum*. Serendipitously, our experiments also found significant attraction of the cerambycine *Sphaerion inerme* White (tribe Elaphidiini) to certain test compounds, providing an opportunity to identify pheromone components for this species as well. This species is restricted to South America, primarily Brazil, and little is known of its host preferences (Monné 2024).

Materials and methods

Source of chemicals

Racemic 2-methylbutan-1-ol (MeBuOH), (*S*)-2-methylbutan-1-ol (*S*-MeBuOH), and racemic 2-methylpentan-1-ol (MePeOH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). (*R*)-2-Methylbutan-1-ol (*R*-MeBuOH) was prepared by lipase-based kinetic resolution of racemic MeBuOH (Bello et al. 2015), and (*S*)-2-methylpentan-1-ol (*S*-MePeOH) was synthesized as described in Cossé et al. (2020). Racemic *anti*-2,3-octanediol (*anti*-C8-diol) was synthesized as described in Lacey et al. (2004), and (2*S*,3*R*)-2,3-octanediol as in Wickham et al. (2016).

Study sites

Field experiments to test synthetic attractant pheromones, and to collect live adults of target cerambycid species for collection of headspace volatiles, were conducted at two widely separated sites in the Brazilian state of Sao Paulo: Piracicaba (University of Sao Paulo campus, – 22.711 lat., – 47.625 long.) and Valentim Gentil (private agricultural property, – 20.372 lat., – 50.080 long.). The Piracicaba site spans a 28-ha regenerated fragment of Atlantic Forest, comprising native and exotic species of mature hard- and softwood trees. It primarily features tree species in genera such as *Inga*, *Leucaena*, *Libidibia*, *Piptadenia*, *Schizolobium*, *Tipuana* (all Fabaceae), *Cariniana*, *Lecythis* (Lecythidaceae), *Mangifera* (Anacardiaceae), and *Eucalyptus* (Myrtaceae). The understory is a mix of young trees, lianas, shrubs, and herbaceous vegetation. The Valentim Gentil site encompasses a 4.8-ha remnant of Cerrado (Brazilian savanna), bordered by pasture lands and rubber tree plantations. It is predominantly composed of mature mixed hardwood trees, featuring native species such as *Albizia*, *Anadenanthera*, *Hymenaea*, *Senegalia* (Fabaceae), *Handroanthus* (Bignoniaceae), and *Sterculia* (Malvaceae). The understory consists of young trees, lianas, shrubs, herbaceous vegetation, and scattered fallen decaying trees.

General field trapping methods

Synthetic attractant pheromones were tested using cross-vane panel traps custom-built from black corrugated plastic (1.2 m tall × 0.3 m wide). Internal surfaces of traps were coated with a 50% aqueous suspension of the lubricant Fluon® (Insect-a-Slip, BioQuip Products Inc., Rancho Dominguez CA, USA). Traps were suspended from PVC pipe frames (2.1 × 0.3 m long; 2 cm i.d.) mounted on 1-m steel reinforcing bar posts driven into the ground. Collection jars, suspended below the traps, were filled with 300 ml of saturated aqueous NaCl solution and a few drops of dish detergent to preserve captured beetles. Pheromone lures were prepared from clear polyethylene press-seal bags (5 × 7 cm, 50 µm wall thickness, BCIEEL S.A., Brazil), containing a dental cotton roll loaded with test compound solutions (50 mg for racemic compounds and 25 mg for pure enantiomers) in 1 ml of isopropanol. Release rates of compounds

from these lures generally range from 0.02 to 1.5 mg/d (Millar et al. 2018). Control lures comprised similar bags with cotton wicks loaded with 1 ml of isopropanol. Lures were hung in the central open slot of traps and replaced every 15 d. Lures were randomly assigned to traps within a block. Each block included one trap for each treatment and a control trap, spaced ~ 15 m apart, and blocks were ~ 30 m apart. The number of blocks varied with experiment (see below). To control for positional bias, treatments were rotated within blocks each time traps were serviced.

Sources of cerambycid species for collection of insect-produced volatiles

Live adults of *A. surinamum* and *S. inerme* were captured for collection of headspace volatiles using the aforementioned traps, but with minor modifications. Holes approximately 2 mm in diameter were drilled in the bottom of each trap's collection jar to facilitate rainwater drainage. One trap baited with *anti*-C8-diol was deployed in Piracicaba (1 October–6 November 2015), targeting *A. surinamum*. Adults of *S. inerme* were caught in Valentim Gentil (20–30 October 2016) with a trap baited with a six-component blend of cerambycid pheromones that has been shown to attract multiple cerambycid species across Brazil (for details, see Silva et al. 2024). Traps were checked daily for captured beetles. Adults of *A. surinamum* and *S. inerme* were sexed based on antennal length, the antennae being almost twice the body length in males and ~ equal to body length in females. Subsequently, beetles were held individually in plastic containers, each containing a glass vial filled with 10% sucrose solution to provide moisture and nourishment. Beetles captured in Valentim Gentil were transported to Piracicaba for aeration within the same collection week. All beetles underwent a 24 h acclimation period under laboratory conditions (25 ± 2 ℃, 60 ± 10% RH, 12:12 h L:D, and 5000 lx LED lights) before initiating volatile collections.

Collection of headspace volatiles from beetles

Beetles, either individually or in pairs of the same species and sex, were placed in 500 ml cylindrical glass chambers positioned horizontally on a laboratory bench. To offer perching places, half of the chamber's internal surface was lined with paper towels. Each chamber contained two glass vials filled with 10% sucrose solution for nourishment. Volatiles were trapped with collectors fabricated from glass pipettes (8.5 cm long × 0.5 cm i.d.) packed with 150 mg of 80/100 mesh HayeSep® Q adsorbent (Supelco, Bellefonte, PA, USA) held in place with glass wool plugs. Collectors were connected to chamber outlets using screw caps fitted with PTFE ferrules. A constant airflow of ~ 150 ml/min of activated charcoal-filtered air was passed through the chambers and pipettes, regulated by flowmeters. Beetles underwent continuous aeration for 1–4 periods, each lasting 24–72 h. Simultaneously, control chambers containing only paper towels and feeder vials were aerated to monitor for potential system contaminants. Three successive aliquots of 500 µl of dichloromethane were used to extract volatiles from collectors into 2 ml silanized amber glass vials. Vials were stored at − 30 ℃ until analysis. In total, we aerated eight males and three females of *A. surinamum* from 10 October to 6 November 2015 and eight males and four females of *S. inerme* from 24 to 31 October 2016.

Identification of candidate pheromone components of *A. surinamum* and *S. inerme*

Volatiles collected from *A. surinamum* (12 extracts from males and three from females) and *S. inerme* (six from males and three from females) were initially analyzed in Brazil by gas chromatography with flame ionization detection (GC–FID) or gas chromatography–mass spectrometry (GC–MS) to confirm they contained potential compounds of interest, i.e., sex-specific compounds. Two-microliter aliquots were injected into a GC–2010 gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with an Rtx–1 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film; Restek, Bellefonte, PA, USA). Injections were made splitless (purge valve off for 1 min) with an injector temperature of 250 ℃ and helium carrier gas at a linear velocity of 30 cm/s. The GC oven was programmed at 35 ℃ (hold 1 min), increased to 40 ℃ at 2 ℃/min (hold 1 min), and then increased to 250 ℃ at 10 ℃/min (hold 15 min). Similarly, 1 µl of the sample was injected splitless into a Shimadzu QP2010 Ultra GCMS fitted with an Rtx–1MS nonpolar column (30 m × 0.25 mm × 25 µm film; Restek). Injector and GC oven temperatures were set as described above, with helium carrier gas at 44 cm/s and 80.8 kPa inlet pressure. Ion source and quadrupole temperatures were 250 ℃. Mass spectra were recorded in electron impact mode (70 eV) from *m/z* 35–260 amu, with a 4-min solvent delay.

Samples that contained detectable quantities of sex-specific compounds were shipped to the University of California, Riverside. These samples were reanalyzed using an Agilent 7820A GC interfaced to a 5977E mass selective detector (Agilent Technologies, Santa Clara CA, USA) with helium as the carrier gas. The GC was equipped with an HP–5 column (30 m × 0.25 mm i.d. × 0.25 µm film; Agilent Technologies), and 1 µl aliquots of samples were injected in splitless mode. The GC oven was programmed from 40 ℃/5 min, with a ramp of 10 ℃/min to 280 ℃ (hold for 10 min). Quadrupole, ion source, and injector temperatures were set at 150, 230, and 250 ℃, respectively. Mass spectra were obtained using electron impact ionization (70 eV) with a mass

range from 40–400 amu. Absolute configurations of chiral compounds were determined by analysis of extracts on a Cyclodex-B chiral stationary phase GC column (30 m × 0.25 mm, 0.25 µm film, J&W Scientific, Folsom CA, USA), with a flame ionization detector, comparing retention times of insect-produced compounds with those of authentic standards. Identities of compounds were confirmed by coinjection of aliquots of insect extract, spiked with the appropriate standard. The oven was programmed from 50 ℃/1 min, 5 ℃/min to 200 ℃.

Field bioassays of identified candidate attractant pheromones of *A. surinamum* and *S. inerme*

Candidate synthetic pheromones of *A. surinamum* were bioassayed at the Piracicaba site. Three sequential bioassays were performed with treatments randomly assigned to traps across five blocks (Bioassay 1), six blocks (Bioassay 2), and seven blocks (Bioassay 3), with traps checked every 2–4 d for captured beetles.

Bioassay 1 was conducted from 23 October to 8 November 2021 (total of seven collection dates) and assessed attraction to individual compounds or 1:1 blends, including: 1) *SR*-C8-diol; 2) *anti*-C8-diol; 3) MeBuOH; 4) *SR*-C8-diol + MeBuOH; 5) *anti*-C8-diol + MeBuOH; and 6) Control.

Because bioassay 1 showed *A. surinamum* was most strongly attracted by *anti*-C8-diol + MeBuOH (see Results), bioassay 2 tested this blend, the analogous blend of *anti*-C8-diol with the insect-produced (*S*)-MeBuOH. as well as *anti*-C8-diol alone, (*S*)-MeBuOH alone, and the control. This bioassay ran from 9 to 24 November 2021 (seven collection dates).

Bioassay 3 tested ratios of *anti*-C8-diol:*S*-MeBuOH in a semilogarithmic dosage scale, as follows: (1) 100:0; (2) 100:3.3; (3) 100:10; (4) 100:33; (5) 100:100; and (6) control. This bioassay ran from 18 October to 1 November 2022 (four collection dates).

Candidate pheromone compounds for *S. inerme* were tested at the Valentim Gentil site, testing MeBuOH and MePeOH as individual compounds, the 1:1 blend, and the control. These treatments were allocated to traps across four blocks, with traps checked every 2–4 d from 8 October to 24 December 2017 (26 collection dates).

Cerambycid beetles were identified to species by Antonio Santos-Silva of the Museum of Zoology of the University of São Paulo (MZUSP). Voucher specimens of the study species have been deposited in the museum collection in the Department of Entomology and Acarology (USP/ESALQ), Piracicaba, SP, Brazil.

Statistical analyses

We evaluated treatment differences for species represented by at least eight specimens per bioassay using the nonparametric Friedman's test (PROC FREQ, option CMH; SAS Institute Inc. 2011) due to field data not meeting ANOVA assumptions due to heteroscedasticity (Sokal and Rohlf 1995). Replicates were defined by both spatial (block) and temporal (collection date) data. Spatial blocks with the greatest numbers of beetles (i.e., that represented the independent responses of multiple beetles to bioassay treatments) were lent greater weight by dropping from analyses those spatial blocks having fewer than a threshold number of the target species. These threshold numbers were selected, separately for *A. surinamum* (threshold 4, 2, and 31 for bioassays 1, 2 and 3, respectively), *S. inerme* (1), and non-target cerambycid species (1), so as to maximize the number of beetles captured per replicate while maintaining sufficient replication for a robust statistical test (at least eight replicates).

To address the multiple statistical tests of treatment effects, we adjusted significance levels in bioassays 1 ($\alpha = 0.017$; N = 3 analyses), 2 ($\alpha = 0.025$; N = 2), and 3 ($\alpha = 0.017$; N = 3) for *A. surinamum* using the Bonferroni procedure (Quinn and Keough 2002). When the Friedman's test showed overall significance, we conducted pairwise comparisons of treatment means using the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) which controls the Type I experiment-wise error rate (SAS Institute Inc. 2011).

Results

Identification of candidate pheromone components of *A. surinamum* and *S. inerme*

GC-FID analysis of aeration extracts from *A. surinamum* detected two volatile compounds emitted by males which were absent in extracts from conspecific females or control aerations (Fig. 1a). Among the 12 aeration samples from males, five contained both major and minor sex-specific compounds, five contained only the major compound, and two samples lacked these compounds altogether. The major compound was tentatively identified as a 2,3-octanediol from its mass spectrum, and was confirmed to be *anti*-C8-diol by comparing its retention time and mass spectrum with those of authentic standards of *syn*- and *anti*-diastereomers, which are readily separable and distinguishable on the nonpolar DB-5 GC column used for analyses. Further analyses on the chiral stationary phase Cyclodex B GC column determined the beetles produced exclusively the (2*S*,3*R*)-enantiomer.

The minor component, which eluted considerably earlier than *anti*-C8-diol, was tentatively identified as the known

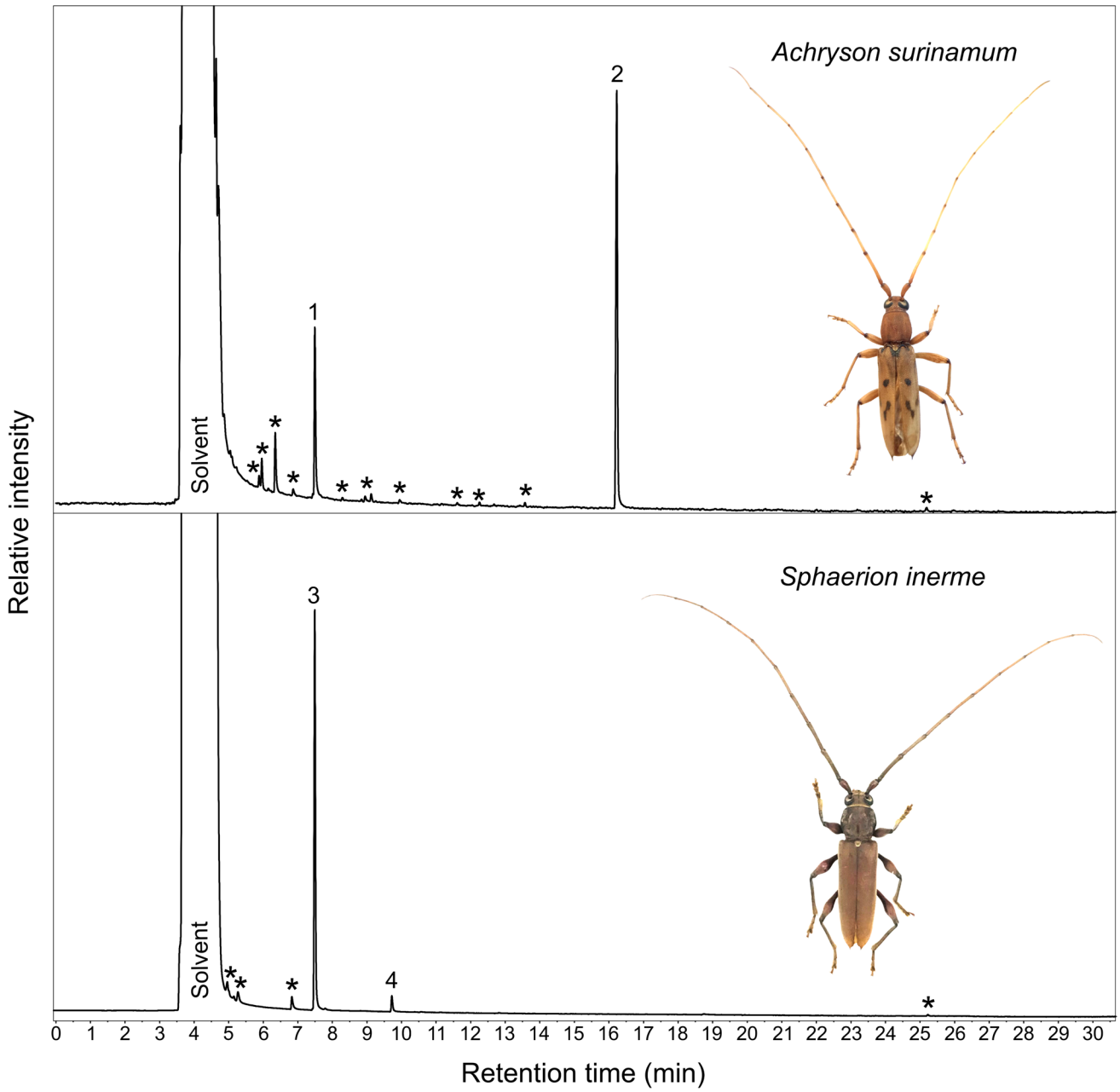

Fig. 1 Representative gas chromatograms of headspace volatiles collected from males of *Achryson surinamum* and *Sphaerion inerme*. Peaks are numbered to denote male-specific compounds: 1 = (*S*)-2-methylbutan-1-ol, 2 = (2*S*,3*R*)-2,3-octanediol, 3 = (*R*)-2-methylbutan-1-ol, and 4 = (*R*)-2-methylpentan-1-ol. Asterisks (*) indicate analytical artifacts or contaminants from the aeration system

cerambycid pheromone component MeBuOH, and identification was confirmed by matches with an authentic standard. Analyses on the Cyclodex B column then determined that the beetles produced the (*S*)-enantiomer.

GC-FID chromatograms of extracts of volatiles emitted by male *S. inerme* revealed two peaks which were not observed in equivalent chromatograms from females or in aeration controls (Fig. 1b). The first peak was readily identified as MeBuOH from its retention time and mass spectrum, as described above for *A. surinamum*. The second peak was tentatively identified as the homologous MePeOH from matching its mass spectrum to the NIST mass spectral database, and identification was confirmed by matching its mass spectrum and retention time with those of an authentic standard. Analysis of an extract on the Cyclodex B column, in conjunction with appropriate standards, determined that males of this species produce *R*-MeBuOH and *R*-MePeOH.

Field bioassays of identified candidate pheromones of *A. surinamum* and *S. inerme*

In bioassay 1, 152 adults of *A. surinamum* (86 females and 66 males) were captured. Among the treatments, *SR*-C8-diol alone or combined with MeBuOH, as well as the racemic *anti*-C8-diol + MeBuOH attracted significantly more beetles than MeBuOH alone or the control (Fig. 2a). Catches with *anti*-C8-diol alone were intermediate (Fig. 2a). During this bioassay, 27 adults of the non-target cerambycid *S. inerme* were also captured, most being in traps baited with *SR*-C8-diol + MeBuOH (Fig. 2b). Moreover, 20 adults of the cerambycid *Andraegoidus rufipes* (F.) (Cerambycinae: Trachyderini) were captured, but catches of this species appeared to be randomly distributed across treatments (means not significantly different; Friedman's $Q_{5,78} = 10.5$, $p > 0.017$).

Seventy-seven adults of *A. surinamum* (50 females and 27 males) were captured during bioassay 2, with beetles showing a significant preference for the *anti*-C8-diol + *S*-MeBuOH blend (Fig. 3). Although nine adults of *A. rufipes* were also captured, catches again appeared random with respect to treatments (means not significantly different; $Q_{4,40} = 2.0$, $p > 0.025$).

In bioassay 3, which tested different ratios of *anti*-C8-diol with *S*-MeBuOH, 894 adults of *A. surinamum* (433 females and 461 males) were captured. Attraction steadily increased with increasing amounts of *S*-MeBuOH in blends (Fig. 4). All blends attracted significantly more beetles than the control, and the 100:100 ratio attracted significantly more beetles than the 100:0, 3.3, and 10 ratios. Catches with the 100:100 blend did not significantly differ from those with the 100:33 blend (Fig. 4). Twenty-four adults of the cerambycine *Ambonus interrogationis* (Blanchard) (tribe Elaphidiini) and 11 of *A. rufipes* were also captured. As in the other bioassays, these captures seemed incidental rather than a response to the treatments because mean captures of both species were not significantly different across all treatments ($Q_{5,84} = 18.2$, $p > 0.017$; $Q_{5,66} = 4.4$, $p > 0.017$, respectively).

During the bioassay that targeted *S. inerme*, 273 adults (149 females and 124 males) were captured. Beetles were more strongly attracted to the MeBuOH + MePeOH blend than to either compound alone, and all treatments were significantly more attractive than the control (Fig. 5). No other cerambycid species was caught in sufficient numbers for statistical analysis.

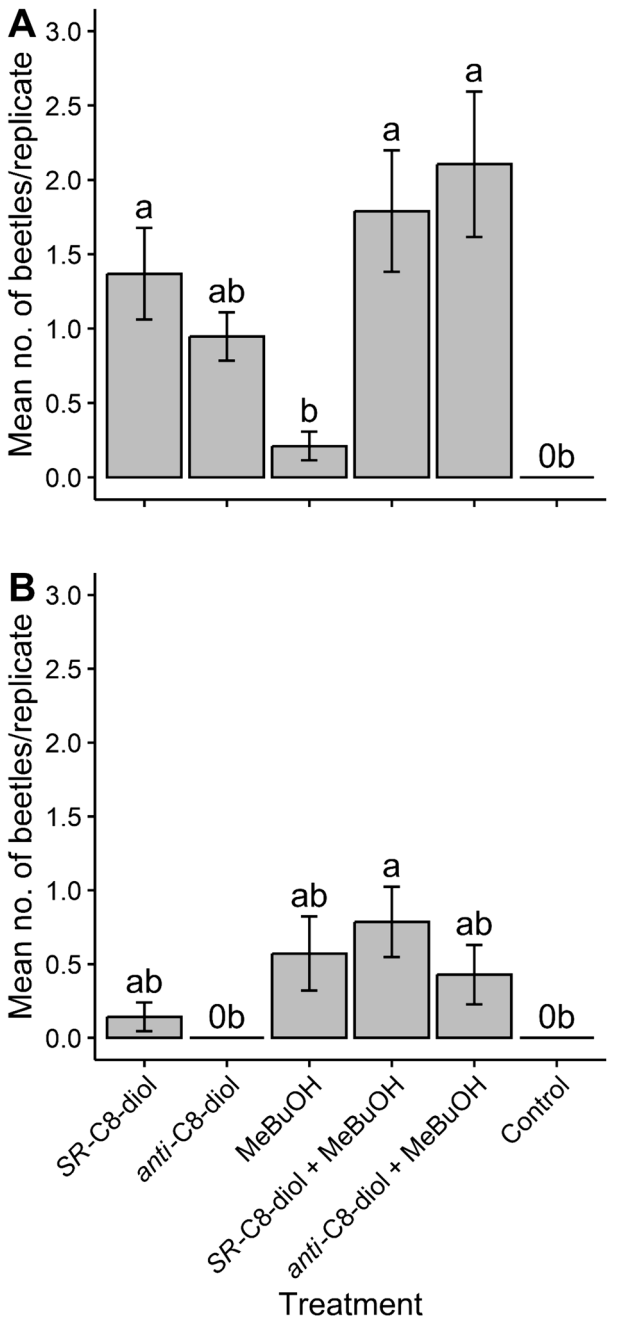

Fig. 2 Mean number (±1 SE) of beetles of both sexes combined, of (**a**) *Achryson surinamum* and (**b**) *Sphaerion inerme* captured during field bioassay 1 in Piracicaba. Compound abbreviations: *SR*-C8-diol = (2*S*,3*R*)-2,3-octanediol, *anti*-C8-diol = racemic *anti*-(2,3)-octanediol, MeBuOH = racemic 2-methylbutan-1-ol, and Control = solvent (neat isopropanol). The effect of treatment type on trap catches was statistically significant for both *A. surinamum* (Friedman's $Q_{5,114} = 40.4$, $p < 0.001$, N = 19 replicates) and *S. inerme* ($Q_{5,84} = 19.4$, $p < 0.001$, N = 14). Differing letters within a species denote statistically significant differences determined by the REGWQ multiple range test ($p < 0.05$)

Discussion

This study showed a sex-specific emission pattern in adult males of *A. surinamum*, characterized by the production of (2*S*,3*R*)-2,3-octanediol and lower amounts of (*S*)-2-methylbutan-1-ol. Through field trapping bioassays employing

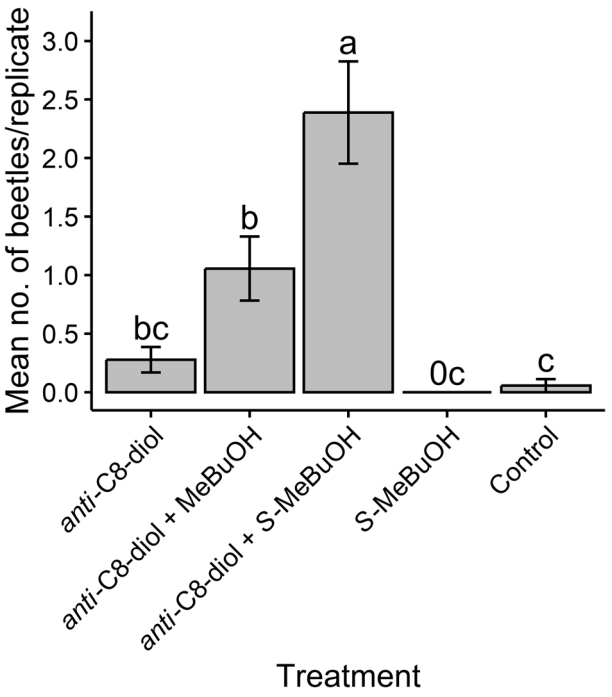

Fig. 3 Mean number (±1 SE) of adult *Achryson surinamum* of both sexes combined, captured during field bioassay 2 in Piracicaba. Compound abbreviations: *anti*-C8-diol = racemic *anti*-(2,3)-octanediol, MeBuOH = racemic 2-methylbutan-1-ol, *S*-MeBuOH = (*S*)-2-methylbutan-1-ol, and Control = solvent (neat isopropanol). The effect of treatment type on trap catches of *A. surinamum* was statistically significant (Friedman's $Q_{4,90} = 48.2$, $p < 0.001$, N = 18 replicates). Differing letters denote statistically significant differences determined by the REGWQ multiple range test ($p < 0.05$)

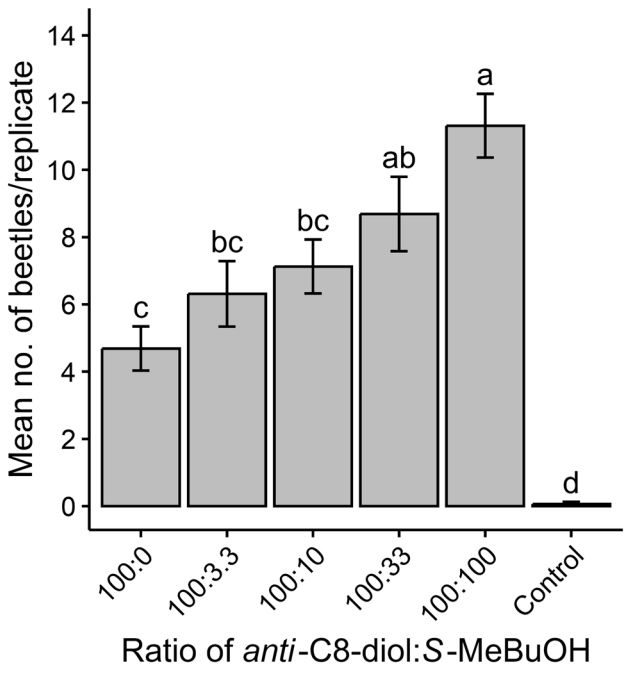

Fig. 4 Mean number (±1 SE) of adult *Achryson surinamum* of both sexes combined, captured during field bioassay 3 in Piracicaba. The numbers in treatments denote different ratios of racemic *anti*-(2,3)-octanediol:(*S*)-2-methylbutan-1-ol. Control = solvent (neat isopropanol). The effect of treatment type on trap catches of *A. surinamum* was statistically significant (Friedman's $Q_{5,96} = 54.2$, $p < 0.001$, N = 16 replicates). Differing letters denote statistically significant differences determined by the REGWQ multiple range test ($p < 0.05$)

synthetic compounds, our findings indicate that blending *SR*- or racemic *anti*-C8-diol with MeBuOH led to significantly increased catch rates compared to individual compounds or controls. The non-natural enantiomer in *anti*-C8-diol did not disrupt attraction of *A. surinamum*. From a practical point of view, this information is useful because the racemate is much cheaper to synthesize than the pure stereoisomer. However, subsequent field tests comparing the effects of racemic MeBuOH and *S*-MeBuOH showed that attraction of *A. surinamum* to *anti*-C8-diol was significantly increased when combined with *S*-MeBuOH, indicating that the non-natural *R*-enantiomer present in racemic MeBuOH was partially disrupting attraction. However, in this case, because *S*-MeBuOH is relatively inexpensive, using the pure enantiomer should not be a hindrance to developing a practical lure for this species. In a final bioassay, testing ratios of the two compounds, the results suggested that ratios containing approximately equal amounts of the two components were most attractive.

To our knowledge, our study is the first confirmation of a 2,3-alkanediol being produced by a South American cerambycid species, although there has been a previous report of attraction of cerambycids to 2,3-hexanediols in field trials in Chile (Curkovic et al. 2022). 2,3-Alkanediols were among the first attractant pheromones identified within the family Cerambycidae (Sakai et al. 1984), and they have been reported from a number of Asian, North American, and European species in the subfamilies Cerambycinae and Prioninae (Millar and Hanks 2017). Thus, this appears to be another structural motif that is widely shared within the family.

Serendipitously, live specimens of both sexes of the sympatric *S. inerme* were captured during the first bioassay targeting *A. surinamum*, with traps baited with MeBuOH combined with *SR*-C8-diol yielding highest captures. Subsequent analysis of extracts of volatiles collected from live beetles confirmed sex-specific production of *R*-MeBuOH and *R*-MePeOH by males. Subsequent field tests employing racemic versions of these compounds demonstrated that both sexes were significantly attracted to both compounds, but more strongly to MeBuOH, and even more strongly to the blend of MeBuOH+MePeOH. The fact that beetles were attracted to the blend of racemic compounds is fortuitous, because the racemic versions of both compounds are cheap and readily available commercially should monitoring lures be required for this species. To our knowledge, this is also

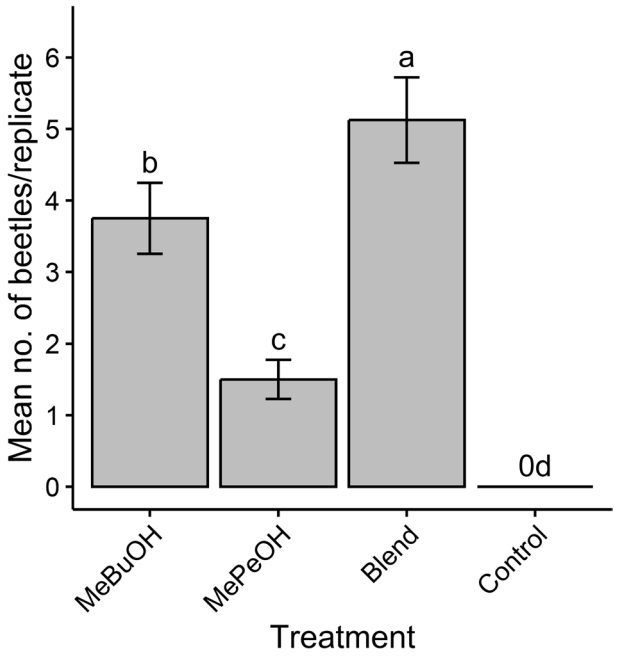

Fig. 5 Mean number (±1 SE) of adult *Sphaerion inerme*, both sexes combined, captured during a field bioassay in Valentim Gentil. Compound abbreviations: MeBuOH = racemic 2-methylbutan-1-ol, MePeOH = racemic 2-methylpentan-1-ol, and Control = solvent (neat isopropanol). The effect of treatment type on trap catches of *S. inerme* was statistically significant (Friedman's $Q_{3,64} = 46$, $p < 0.001$, N = 16 replicates). Differing letters denote statistically significant differences determined by the REGWQ multiple range test ($p < 0.05$)

the first time MePeOH has been identified as a cerambycid pheromone component, expanding the known "chemical space" occupied by pheromones of this family.

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Author contribution All authors contributed to the study's conception and design. W.D.S. collected insects, prepared extracts, performed initial chemical analyses, and conducted field bioassays. J.G.M. identified compounds, and Y.Z. synthesized several of the compounds used in this study. W.D.S. and L.M.H. carried out the statistical analyses. W.D.S. wrote the first draft of the manuscript, J.G.M. added sections related to chemistry, and J.G.M. and L.M.H. reviewed and edited the manuscript. Funding was acquired via grants submitted by L.M.H., J.M.B., and J.G.M. All authors read and approved the final manuscript.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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