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Male-specific volatiles released by *Homalinotus validus* (Coleoptera: Curculionidae) include (1*R*,2*S*)-grandisyl acetate, a new natural product

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ABSTRACT

Homalinotus validus is an important pest of babassu palm (*Attalea speciosa*) cultures in North Brazil. Investigations on the chemical ecology of this species are essential for the development of an integrated pest management system, since attempts to control the pest by conventional insecticides failed. GC/MS and GC/FT-IR analyses of headspace volatiles obtained from males and females revealed the presence of two male specific compounds, which were identified to be (1*R*,2*S*)-grandisol and the new (1*R*,2*S*)-grandisyl acetate. Both compounds presumably act as an aggregation pheromone of the species.

Homalinotus validus (Coleoptera: Curculionidae) occurs in tropical areas of Latin America including the Brazilian northern region, where it causes serious losses in babassu (*Attalea speciosa*) production. The weevil also feeds on other palm species like *A. maripa* and *A. butyracea*. Detection and control of the beetles are labor-intensive and expensive, predominantly due to the height of the babassu palms. Insecticides are not effective for the control of this pest, and infestations are controlled by manual collection of adults or by removing infested branches. Unfortunately, there is very little information about biological and ecological aspects of the genus *Homalinotus* that might help develop efficient integrated pest management systems.[1] Therefore, the objective of our study was to identify beetle-produced semiochemicals that could be related to the aggregation behavior of *H. validus*.

Volatiles from live insects[2] were obtained by dynamic headspace collection.[3] Chemical analyses started by comparison of GC profiles of samples from males and females, revealing the existence of two male-specific compounds in a ratio of 96:4[4] (Fig. 1). On a DB-5 capillary column, the retention indices of these compounds were found to be 1212 for compound **1**, and 1415 for **2**.[5]

The MS and FT-IR spectra along with retention index datasets suggested the major compound **1** to be grandisol, *cis*-2-(1-methyl-2-prop-1-en-2yl)cyclobutylethanol[6], which was confirmed by co-injection with an analytical standard.[7] The mass spectrum of the minor compound **2** showed a fragmentation pattern closely related to that of grandisol, again with *m*/*z* 68 as the base peak. An abundant fragment of *m*/*z* 43 and a small but diagnostic signal of *m*/*z* 61 (Fig. 2a) indicated an acetate substructure, which was confirmed by its FT-IR spectrum, showing strong carboxyl bands at 1737 cm^{-1} (C=O) and 1243 cm^{-1} ($H_3CC(=O)$—O stretch). The

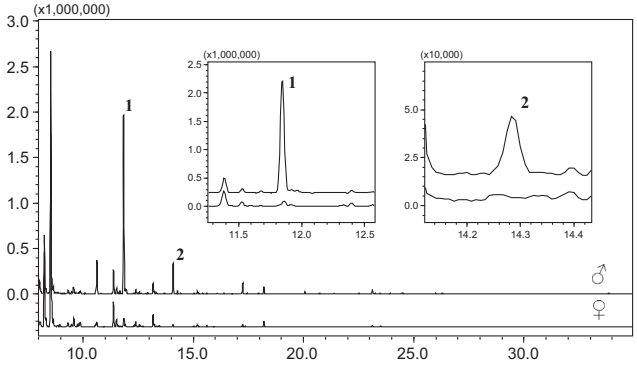

Fig. 1. Comparison of GC profiles from male and female aeration samples revealing two male specific compounds, **1** and **2**.

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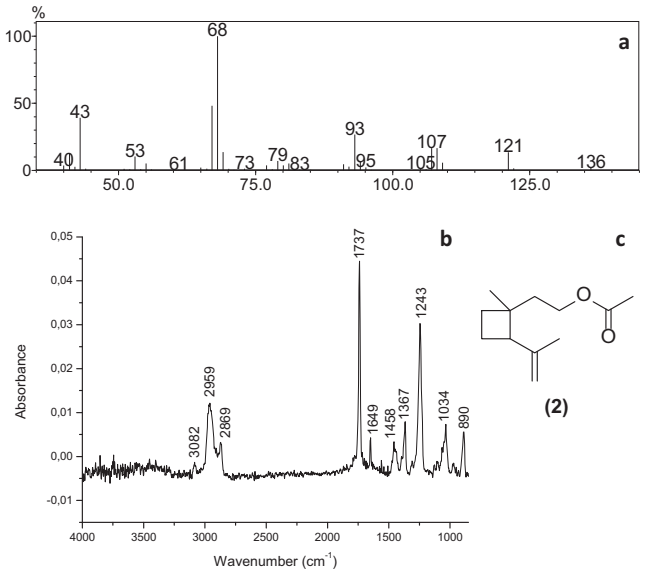

Fig. 2. Mass (**a**) and infrared (**b**) spectra and the proposed structure of the natural compound **2** (**c**).

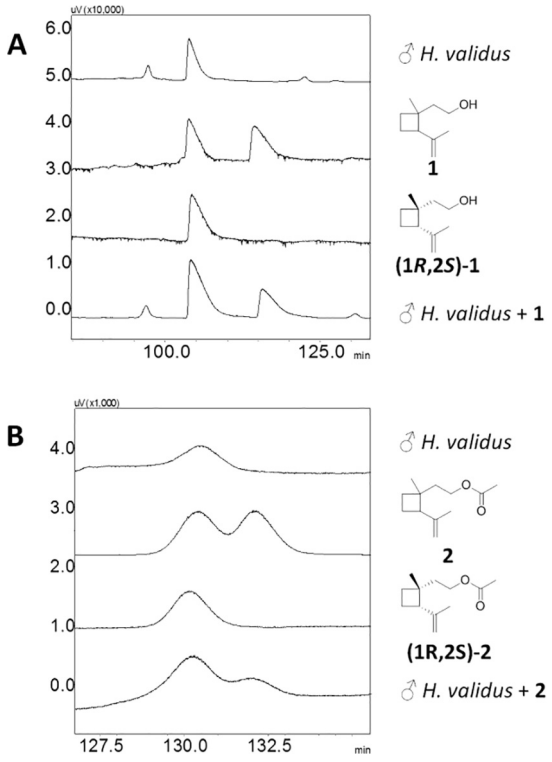

Fig. 3. Determination of the absolute configuration of grandisol (A) and grandisyl acetate (B) released by males of *H. validus*.

presence of a terminal double bond in **2** was obvious because of the band at 3082 cm^{-1} (out-of-phase C=CH_2 stretch) (Fig. 2b). Consequently, **2** was suggested to be the acetate of grandisol (**1**) (Fig. 2c). This was supported by an increase of 200 units in the retention index of **2** as compared to grandisol (1). A reference sample was synthesized by acetylation of grandisol,[8,9] and the assignment of grandisyl acetate for compound **2** was confirmed by comparison of the spectral data of natural and synthetic products, as well as by co-injection.

The absolute configuration of the natural compounds was determined by enantioselective gas chromatography, using a modified β-cyclodextrin as the stationary phase,[10] whereas racemic **1** and enantiopure **(1*R*,2*S*)-1**, and the respective acetates (**2** and **(1*R*,2*S*)-2**) served as references. Fig. 3A shows that grandisol (**1**) is produced as a pure enantiomer, since its retention time coincides with that of the synthetic (1*R*,2*S*)-enantiomer.[11] The absolute configuration of grandisol in *H. validus* proved to be the same as in several other weevil species, where it acts as an aggregation pheromone.[12] As expected, GC analyses showed that the acetate **2** is produced with the same (1*R*,2*S*)-configuration as grandisol (**1**) (Fig. 3B).[13]

To the best of our knowledge, this is the first time that grandisyl acetate has been identified as a natural product, while its *trans*-stereoisomer (fragranyl acetate) has been described as a component of the essential oils in plants of the genus *Achillea* (Asterales: Asteraceae).[14]

Bioassays for both compounds are currently in progress in the laboratory and in the field. However, according to general experience on the chemical ecology of Curculionidae,[12] it can be predicted that they will act as aggregation pheromone of *H. validus*.

Acknowledgments

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2. *H. validus* were collected in Ouro Preto do Oeste, Rondonia - Brazil (10°43'54.6"S 62°15'07.5"W) and maintained at 28 °C and a 12:12 h (L:D) photoperiod in the Laboratory of Semiochemicals at the Federal University of Paraná (UFPR).
3. Three insects of each sex were placed in aeration chambers (37 × 4 cm i.d.), containing banana as food source, with a continuous flow of 1 L/min of humidified, charcoal-filtered air. Volatiles released by banana were collected for comparison of GC profiles. Volatiles were trapped in glass traps (11 × 0.5 cm i.d.) containing 20 mg of HayeSep-D adsorbent polymer (Analytical Research systems, Inc., Gainesville, FL, USA). The adsorbed volatiles were eluted with doubly distilled hexane (300 µL), and the resulting extracts were stored at 20 °C until analyses and bioassays. Extractions were performed every 24 h.
4. GC/MS: QP2010 Plus (Shimadzu) coupled to a GC2010 (Shimadzu). GC/FTIR: DiscovIR-GC (DANI Instruments) coupled to a GC2010 (Shimadzu). DB-5 (Agilent Technologies, 30 m × 0.25 mm id × 0.25 µm film thickness), used in both instruments. Conditions: splitless, 1 min at 50 °C, then increased to 250 at 7 °C/min and kept at this temperature for 10 min.
5. Retention indices were calculated based on the retention times of the target compounds and a series of n-alkanes (C10–C26) in a GC2010 – FID (Shimadzu) equipped with a DB-5 column (Agilent Technologies, 30 m × 0.25 mm id × 0.25 µm film thickness). Conditions: splitless, starting at 50 °C, then increased to 280 °C at 3 °C/min and kept at this temperature for 10 min.
6. NIST 2008 MS Library: NIST MS Search 2.0 f, 9th ed. Wiley.
7. Bedoukian Research, Danbury, CT, US.
8. An excess of acetic anhydride (125 µL, 1.25 mmol) and pyridine (125 µL) were added to grandisol (154 mg, 1.0 mmol) in CH_2Cl_2 (4 mL). The solution was stirred for 12 h, diluted with CH_2Cl_2 (20 mL), washed with aqueous HCl (10%) and with saturated $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, and concentrated. After flash chromatography (hexane/ethyl acetate: 9/1), grandisyl acetate was obtained in 92% yield (180 mg). ^1H NMR (200 MHz, $CDCl_3$): δ 1.19 (s, 3H), 1.32–2.30 (m, 12H), 2.46–2.67 (m, 1H), 3.97–4.19 (m, 2H), 4.60–4.68 (m, 1H), 4.81–4.88 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.0, 20.8, 20.9, 23.1, 28.0, 28.9, 32.3, 41.1, 52.3, 61.9, 109.8, 144.8, 171.1.
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10. Enantioselective GC: GC2010 – FID (Shimadzu) equipped with a β-DEX 325 (Supelco Analytical, 30 m × 0.25 mm id × 0.25 µm film thickness) column. Conditions: splitless, 135 min at 70 °C, then increased to 230 °C at 7 °C/min. Also see: Leal WS, Oehlschlager AC, Zarbin PHG, et al. *J. Chem. Ecol.*. 2003;29:15.
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13. Synthetic (1*R*,2*S*)-grandisyl acetate was obtained starting from (1*R*,2*S*)-**1** in a μg scale following a similar approach used to obtain the racemate**.** An excess of acetic anhydride (10 μL) and pyridine (10 μL) were added to a solution of (1*R*,2*S*)-**1** (100 μL; 1000 ng/μL in hexane) in dichloromethane. The resulting solution was stirred for 12 h at 25 °C, diluted with hexane (400 μL), washed with aqueous HCl (10%), and with saturated $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4 in a tip of a Pasteur pipette, and used for GC analyses without further purification.
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