JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Zingiberenol, (1*S*,4*R*,1′*S*)-4-(1′,5′-Dimethylhex-4′-enyl)-1-methylcyclohex-2-en-1-ol, Identified as the Sex Pheromone Produced by Males of the Rice Stink Bug *Oebalus poecilus* (Heteroptera: Pentatomidae)

Márcio Wandré Morais de Oliveira,[†,‡] Miguel Borges,[†] Carlos Kleber Zago Andrade,[‡] Raul Alberto Laumann,[†] José Alexandre Freitas Barrigossi,[§] and Maria Carolina Blassioli-Moraes*[,†]

[†]Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica − W5 Norte, CEP 70770-900, Brasília, DF, Brazil

[‡]Laboratório de Química Orgânica Sintética, Instituto de Química, Universidade de Brasília, Asa Norte-CEP 70910-000, Brasília, DF, Brazil

[§]Embrapa Arroz e Feijão, Rodovia GO-462, km 12 Zona Rural, CEP 75375-000, Santo Antônio de Goiás, GO, Brazil

ABSTRACT: Bioassays using an olfactometer showed that *Oebalus poecilus* males produce the sexual pheromone, and the chemical analysis demonstrated that this compound is zingiberenol. Two groups of isomers, each containing four diastereoisomers, (1*RS*,4*RS*,1′*S*)- and (1*RS*,4*RS*,1′*R*)-zingiberenol, were prepared. These diastereoisomers were not separated on a chiral GC column. Therefore, to determine the absolute configuration of the carbon 1, 4, and 1′ of zingiberenol produced by males, the following strategies were conducted. The extract containing males volatiles was submitted to dehydration microchemistry to produce zingiberene, in which the isomers are separated by chiral GC analysis, and by comparison with the natural zingiberene from ginger oil, the absolute stereochemistry of the carbons 4 and 1′ was determined to be *R* and *S*, respectively, and the carbon 1 was determined as *R* from the ^{13}C NMR spectra of quercivorol. Finally, the bioassays showed that *O. poecilus* females responded to racemic mixture and to (1*RS*,4*RS*,1′*S*)-zingiberenol.

KEYWORDS: *semiochemical, chiral sesquiterpene, olfactometer bioassay, sexual behavior*

INTRODUCTION

The primary stink bugs in Brazilian rice crops are *Oebalus poecilus* (Dallas, 1851), *Oebalus ypsilongriseus* (De Geer, 1773), and *Tibraca limbativentris* (Stal, 1860).[1] However, few studies have been conducted on the chemical ecology of these insects. The sex pheromone of the rice stalk stink bug *T. limbativentris* was identified as zingiberenol, and laboratory bioassays showed that both racemic zingiberenol mixture and groups containing its four diastereoisomers (1*RS*,4*RS*,1′*R*)-zingiberenol (**6**) or (1*RS*,4*RS*,1′*S*)-zingiberenol (**7**) were attractive to females of *T. limbativentris* during Y-olfactometer bioassays.[2] However, there are currently no data available regarding the mating behavior or chemical ecology of the Brazilian *Oebalus* spp. The rice stink bug, *Oebalus poecilus*, is an important rice pest (*Oryza sativa* L.) in Brazil that is found primarily in lowland agroecosystems.[3−5] Both the nymphs and the adults can feed on any part of the rice plant; however, they prefer spikelets in the milky phase and cause injuries that result in reduced yield and quality of the seed.[5,6]

These insects take refuge as adults between growing seasons in diverse locations around the fields where they can feed on various host plants.[5] Infestation of a rice field occurs when the crop begins to flower. Control is accomplished using insecticides without accounting for economic viability and environmental problems.[7] Despite the development of a sampling procedure for estimating population densities to help farmers make decisions about insecticide application,[5] control attempts are still made on a calendar basis or occasionally at the edge of the field. This practice is justified because walking through an irrigated field is difficult and time-consuming. Therefore, it is necessary to develop new alternatives and contribute to innovation in decision making for rice pest management.

Because an *O. poecilus* infestation only occurs during the flowering stage, one option for improving rice stink bug monitoring would be to use sex pheromones to detect the moment when field colonization begins and determine when treatment should be implemented. Therefore, this work aims to evaluate whether the males of *O. poecilus* are the sex pheromone producer, as is the case for other stink bugs,[8] and, if so, to identify the chemical structure of the biologically active compound.

MATERIALS AND METHODS

Chemicals. Decanal, nonanal, 6-methyl-5-hepten-2-one, (*E*)-2-octenal, tridecane, tetradecane, pentadecane, hexadecane, and silica gel (80−100 mesh) were purchased from Sigma Aldrich. *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) and triethylamine were purchased from Fluka (Steinheim, Germany). (*R*)-Citronellal was purchased from TCI-America (Portland, OR), and (*S*)-citronellal was purchased from TCI-Japan (Tokyo, Japan). Hydrocarbons from

Received: April 6, 2013
Revised: July 20, 2013
Accepted: July 24, 2013
Published: July 24, 2013

dx.doi.org/10.1021/jf402765b | *J. Agric. Food Chem.* 2013, 61, 7777−7785

heptane to tetracosane were purchased from Sigma Aldrich (Steinheim, Germany).

Insects. *Oebalus poecilus* adults were obtained from a stock colony in the entomology laboratory of Embrapa Rice and Beans, Santo Antônio de Goiás, GO (16°4′ S, 49°14′ W). The insects were reared in 8 L containers with rice panicles (*Oryza sativa* L.), beans (*Phaseolus vulgaris* L.), and peanuts (*Arachis hypogaea* L.) in an acclimatized room with a temperature of 26 ± 1 °C, a relative humidity of 65 ± 10%, and a 14:10 h light:dark photoperiod. The food was replaced three times per week.[9]

O. poecilus females laid their egg masses on rice plants, which were transferred to new plastic containers (5 L) three times a week where the nymphs were reared using the same protocol that was used for the adults. To avoid interactions between the genders, all males were separated from the females after their final moult. Sexually mature adults (>8 days old) were used for all of the experiments.

Volatile Collection. Volatiles were collected from groups of 15−50 males or females of *O. poecilus* via air entrainment (N = 40 volatile collections from each gender). To minimize the emission of defensive compounds, the insects were carefully introduced into 1 L glass containers.[10] Air (1 L/min) was then drawn sequentially through a bed of 4−12 mesh activated charcoal (Fisher Scientific, Pittsburgh, PA), the glass container, and one trap (10 cm × 0.5 cm i.d.) containing Super Q (100 mg each) (Alltech, Deerfield, IL) using a negative pressure air flow. The adsorbent traps were eluted with hexane (2 × 0.5 mL per sample), and each sample was concentrated to approximately 1 μL/insect under a gentle stream of nitrogen and stored at −20 °C for the posterior analysis and bioassays. During the collection of volatiles, the insects were fed with fresh green beans (*Phaseolus vulgaris*) three times a week. The insects were aerated continuously for 15 days, and the adsorbents were exchanged daily. Dead insects were removed and replaced with new ones. Green beans were used because they release a very low quantity of volatiles when damaged by sucking insects, and their chemical profile has been identified in a previous study.[11]

Chemical Analysis. The air-entrainment extracts were quantitated using a model 17A gas chromatograph coupled to a flame ionization detector (GC-FID) (Shimadzu, Tokyo, Japan). The column used was a 300 mm × 0.25 mm i.d., 0.25 μm, DB-5 (J&W Scientific, USA). The temperature program used was the initial temperature at 50 °C for 1 min, ramped at 5 °C/min to 180 °C and held for 0.1 min, and finally ramped at 10 °C/min to 250 °C, which was maintained for 20 min. Aliquots of 1 μL of the air-entrainment extracts were injected in the splitless mode using helium as the carrier gas, and the flame ionization detector was set to 270 °C. To evaluate the average amount of compounds released per 24 h per insect, 15 collections of volatiles from each gender were preconcentrated to 100 μL and quantitated using the internal standard method by adding 2.0 μL of 16-hexadecanolide (0.1 mg/mL) to each extract. The amount of each compound identified was estimated by comparing the area of the internal standard to the area for the compounds. The response factor for all of the compounds was assumed to be 1.0.

For qualitative analysis, 1 μL of the selected air-entrainment extracts was analyzed with a model 7890A GC coupled to a 5975C inert XL EI/CI MSD (Agilent-Wilmington, DE). The column used was a 300 mm × 0.25 mm i.d., 0.25 μm, DB-5-MS (Agilent, USA). The oven temperature program maintained the temperature at 50 °C for 2 min, ramped at 5 °C/min to 180 °C and held for 0.1 min, and finally ramped at 10 °C/min to 250 °C and held for 20 min. Helium was used as the carrier gas. Ionization was accomplished via electron impact (70 eV, source temperature of 270 °C). The data were collected and analyzed using ChemStation Software (V.E.02.00). The compounds were tentatively identified by comparing their spectra to database libraries (NIST 2005) and confirmed using authentic standards to determine the retention index. The retention index was calculated using a sequence of *n*-alkanes from heptane to tetracosane.

To confirm the presence of a hydroxyl group in the male-specific compound, microsilylation was performed using *N*-methyl-(trimethylsilyl)-trifluoroacetamide (MSTFA) in a 2 mL glass vial. A 50 μL aliquot was taken from a male air-entrainment extract (containing 50 males), concentrated under a stream of nitrogen until almost dry, and then redissolved in 100 μL of MSTFA.[2] The sample was heated to 60 °C for 1 h and analyzed by GC-MS using the conditions described above. The same reaction was performed using standard solutions of **6** and **7**.[2]

Absolute Configuration of C4 and C1 of Zingiberenol Produced by *O. poecilus*. To identify the absolute configuration of C4 in zingiberenol produced by males of *O. poecilus*, the male air-entrainment extract was submitted to dehydration microchemistry to form zingiberene (Figure 1), which was separated in a chiral column,

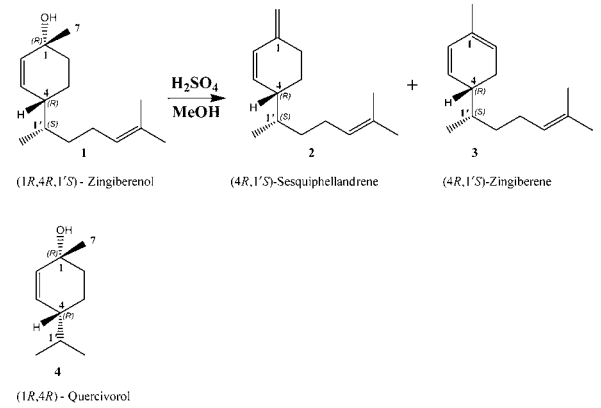

Figure 1. Structures of (1*R*,4*R*,1′*S*)-zingiberenol and the products from its dehydration, (4*R*,1′*S*)-sesquiphellandrene and (4*R*,1′*S*)-zingiberene.

and its absolute configuration was compared to the natural zingiberene from ginger oil. A synthetic solution of **7** (0.1 mg/mL in hexane, 100 μL) was gently preconcentrated under N_2 to dryness, and then 300 μL of an aqueous solution of H_2SO_4 was added (0.1 mol/L) and 50 μL of methanol. The mixture was stirred for 3 h at 45 °C, and after that 500 μL of hexane was added. The water was removed passing the mixture through a small column of $MgSO_4$, and the extract obtained was concentrated to 100 μL. The same procedure was used with **6** (0.1 mg/mL in hexane, 100 μL) and to a volatile collection from males of *O. poecilus* (equivalent to 34 bugs/day). The synthetic standards of (4*RS*,1′*S*)-zingiberene and (4*RS*,1′*R*)-zingiberene obtained via dehydration of **7**, **6**, as well as zingiberenol presented in the *O. poecilus* extract and the natural zingiberene from ginger oil were analyzed by enantioselective gas chromatography using a chiral column 300 mm × 0.25 mm i.d., 0.25 μm, β-DEX 325 matrix nonbonded with 25% 2,3-di-*O*-acetyl-6-*O*-TBDMS-β-cyclodextrin in SPB-20 poly (20% phenyl/80% dimethylsiloxane phase) (Supelco, USA). The oven temperature was programmed as follows: 50 °C/(2 min) then rising at 2 °C/min until 210 °C (10 min). Injections were made in splitless mode with helium as the carrier gas (1.5 mL/min), injector temperature at 250 °C, and detector temperature at 270 °C. To identify the absolute configuration at C1, the ^{13}C NMR spectra of (1*R*,4*R*)-4-isopropyl-1-methylcyclohex-2-en-1-ol (quercivorol) (**4**), obtained from the literature,[12,13] were compared to the information from ^{13}C NMR spectra of **7** obtained in this work.

Synthesis. The synthesis of the eight zingiberenol isomers was performed according to the method of Hagiwara et al.[14] The procedure started from (*R*)-citronellal (**5b**) and resulted in a mixture of the four diastereoisomers of (1*RS*,4*RS*,1′*R*)-4-(1′,5′-dimethylhex-4′-enyl)-1-methylcyclohex-2-en-1-ol (**6**), which was called (1*RS*,4*RS*,1′*R*)-zingiberenol (**6**) (Figure 2). The other four diastereoisomers of (1*RS*,4*RS*,1′*S*)-4-(1′,5′dimethylhex-4′-enyl)-1-methylcyclohex-2-en-l-ol (**7**) were synthesized from (*S*)-citronellal (**5a**) and were called (1*RS*,4*RS*,1′*S*)-zingiberenol (**7**) (Figure 2). The synthesis provided 1.2 g of **6** (75% yield) and 1.3 g of **7** (81% yield).

The products were purified by flash chromatography on silica gel (70−230 mesh) using ethyl acetate/hexane in the appropriate concentration as the eluente (1−15% ethyl acetate/hexane). The compound identities were confirmed by comparing the ^1H NMR

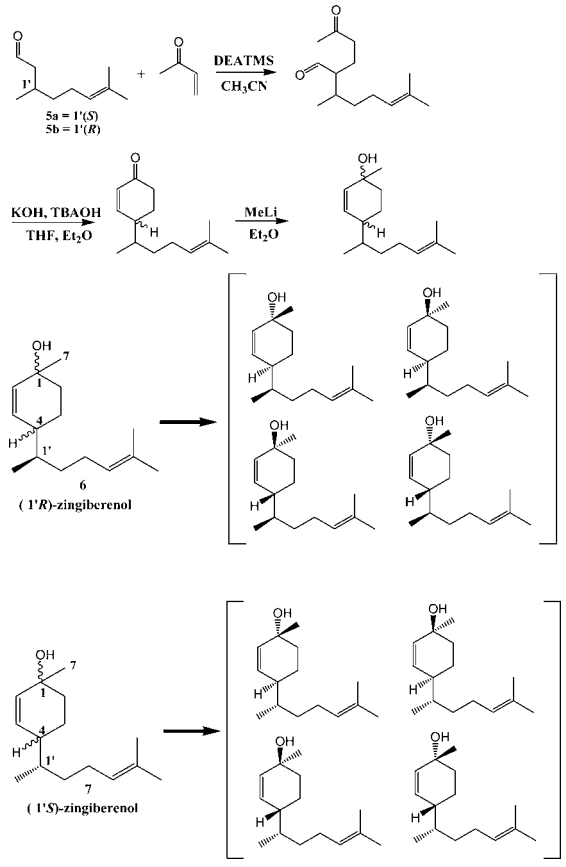

Figure 2. Synthetic route to zingiberenol and structures for the four (1*RS*,4*RS*,1′*R*)-zingiberenol isomers and the four (1*RS*,4*RS*,1′*S*)-zingiberenol isomers.

(Varian spectrometer 300 MHz) and MS data of the samples to those from the literature.[2] The purity of the synthetic standards of **6** and **7** was analyzed by chiral gas chromatography using a chiral column (*β*-DEX 325, 300 mm × 0.25 mm i.d., d_f 0.25 *μ*m, matrix nonbonded; 25% 2,3-di-*O*-acetyl-6-*O*-TBDMS-*β*-cyclodextrin in SPB-20 poly (20% phenyl/80% dimethylsiloxane) phase; Supelco, USA). The oven temperature was programmed as follows: 40 °C/(2 min) then rising at 0.7 °C/min until 210 °C (10 min). Injections were made in splitless mode with helium as the carrier gas (1 mL/min), injector temperature at 250 °C, and detector temperature at 270 °C. (1*RS*,4*RS*,1′*S*)-zingiberenol (**7**) provided four peaks well separated at 154.76, 155.01, 159.26, and 159.71 min, and (1*RS*,4*RS*,1′*R*)-zingiberenol (**6**) was separated in four peaks at retention times 154.83, 159.21, 159.69, and 160.18 min.

Fractionation of Crude Products. To avoid protonation of the OH group, the acidic silica gel (70−230 mesh) was neutralized by suspending approximately 60 g in hexane containing 1% of triethylamine and then pouring the silica gel into a flash chromatography column (35.0 cm ×2.8 cm i.d.). The column was conditioned using 40 mL of hexane followed by 40 mL of 4% ethyl acetate in hexane, both containing 0.1% triethylamine. The synthetic standard **7** (2.3 g of crude material) was loaded onto the column and sequentially eluted with 6 × 40 mL of 4% ethyl acetate in hexane/0.1% triethylamine, 6 × 5% ethyl acetate in hexane/0.1% triethylamine, and then increasing the polarity in 1% increments to 12% ethyl acetate in hexane/0.1% triethylamine, collecting six fractions of 40 mL each. After these elutions, the polarity was increased in increments of 2% to obtain 20% ethyl acetate in hexane/0.1% triethylamine. The less polar diastereoisomers eluted in fraction 6 (240 mL of the eluent with 4% ethyl acetate), the fraction containing the four diastereoisomers eluted immediately thereafter in fraction 7 (240 mL of the eluent with 4% ethyl acetate), and the most polar diastereoisomers eluted as pure compounds using 10% ethyl acetate. The fractionation provided 0.28 g of the least polar isomers of **7**, 0.42 g of **7**, and 0.50 g of the most polar isomers of **7**. The same procedure was used to purify **6** and provided 0.30 g of the least polar diastereoisomers of **6**, 0.47 g of **6**, and 0.53 g of the most polar diastereoisomers of **6**.

Less polar diastereoisomers of **6** and **7**, MS (70 eV): *m*/*z* 222(5), 207(15), 204(13), 189(7), 179(2), 161(14), 137(19), 119(62), 109(28), 93(41), 77(19), 69(100), 55(30), 41(50). ^{1}H NMR (300 MHz): *δ* 0.85 (d, 1.5H, *J* 6.7 Hz) total 3H, 0.89 (d, 1.5H, *J* 6.6 Hz, 3H), 1.26 (s, 3H), 1.60 (s, 3H), 1.7 (s, 3H), 1.11−2.12 (m, 11H), 5.10 (m, 1H), 5.67 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 134.1 (s); 133.3 (s); 133.0 (s); 124.7 (s); 124.6 (s); 67.4 (s); 67.3 (s); 41.0 (s); 40.4 (s); 37.4 (d); 37.2 (d); 36.2 (d); 34.1 (s); 33.5 (s); 29.6 (d); 25.8 (d); 22.1 (s); 20.0 (s); 17.6 (s); 16.3 (s); 15.7 (s).

More polar diastereoisomers of **6** and **7**, MS (70 eV): *m*/*z* 222(4), 207(20), 204(28), 189(6), 179(4), 161(25), 137(28), 119(93), 109(31), 93(68), 77(28), 69(100), 55(29), 41(50). ^{1}H NMR (300 MHz): *δ* 0.87 (d, 1.5H, *J* 6.6 Hz, 3H), 0.83 (d, 1.5H, *J* 6.8 Hz) total 3H, 1.27 (s, 3H), 1.61 (s, 3H), 1.69 (s, 3H), 1.21−2.22 (m, 11H), 5.12 (m, 1H), 5.67 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 134.8 (s); 134.5 (s); 132.0 (s); 131.3 (s); 130.9 (s); 124.6 (d); 69.8 (s); 40.6 (s); 40.0 (s); 38.3 (d); 36.2 (d); 34.1 (s); 33.6 (s); 28.4 (d); 25.9 (s); 25.6 (s); 24.1 (d); 22.2 (s); 17.6 (s); 16.3 (s); 15.6 (s).

Bioassays. *General Bioassay Procedures.* The behavioral responses of both male and female *O. poecilus* to live insects, air-entrainment volatiles, and odor from synthetic zingiberenol solutions were investigated in a Y-tube olfactometer. A square acrylic block (26.0 × 23.0 cm) with a Y-shaped cavity (1.5 cm thickness) sandwiched between two glass plates was used as the bioassay arena. The trunk of the apparatus was 12.0 cm long, and each arm was 10.5 cm long.[15] Charcoal-filtered (4−12 mesh) and humidified air was pushed into the system at rate of 0.4 L/min and pulled out at rate of 0.3 L/min. This "push−pull" system prevents the entry of contaminating volatiles from the exterior. The olfactometer was horizontally positioned, and the bioassays were conducted in a controlled environment at 25 ± 1 °C and 60 ± 10% RH on a white bench under artificial lighting (514 lx). The olfactometer was rotated between replicate runs to avoid any positional bias. To conduct the bioassays using live insects, a single male was gently placed into a 5 mL glass syringe (Arti Glass, Piemonte, Italy), and a single female was placed in another syringe (5 mL). The two syringes were then connected to the two arms of the Y olfactometer. To begin an assay, a single male or female *O. poecilus* was gently placed at the base of the trunk of the Y-tube and allowed to acclimatize for 3 min after beginning the air flow. Bug bioassays were recorded by an observer for 15 min/replicate. The positions of the control and treatment arms were alternated between replicates to avoid any positional bias. The apparatus was cleaned every five replicates with a fragrance-free liquid soap, rinsed thoroughly with water, and dried in a convection oven at 80 °C. The first choice of each individual tested was registered and was considered to have been made when the stink bug moved 3.0 cm into an arm and remained there for at least 30 s.

To determine whether male or female *O. poecilus* are the sex pheromone producers, bioassays were conducted using insects of both genders to test the insects response to odors from live insect sources. The male responses to odors from live females versus odors from live males (N = 57) and the female responses to odors from live females versus odors from live males were also tested (N = 57). To determine whether *O. poecilus* adults have a daily period of maximum activity, bioassays were conducted using females to test the response to male odor versus air at different times of day. The following periods were studied: morning (M = 08:00 to 13:00) (N = 39), afternoon (M = 13:00 to 16:00) (N = 39), and evening (M = 16:00 to 20:00) (N = 39).

Because males did not respond to the odor of live males, the bioassays using both volatile collections and synthetic solutions were only conducted using females. The volatile collections obtained from male *O. poecilus* were concentrated to obtain a solution containing 1 bug-equivalent/*μ*L of hexane. For all bioassays, 5 *μ*L of the volatile collection was applied to a filter paper (1.5 × 0.5 cm). Bioassays using

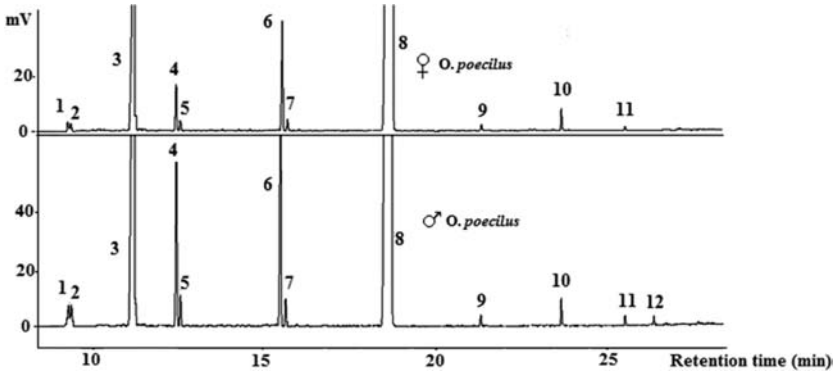

Figure 3. Gas chromatogram of the volatile chemicals collected from live *O. poecilus* males (bottom) and females (top). 6-Methyl-5-hepten-2-one, **1**; octanal, **2**; (*E*)-2-octenal, **3**; undecane, **4**; nonanal, **5**; dodecane, **6**; decanal, **7**; tridecane, **8**; tetradecane, **9**; pentadecane, **10**; and hexadecane, **11**. An isomer of zingiberenol, **12**, was the male-specific compound.

Table 1. Quantity of Defensive Compounds, in ng/bug/24 h (Average ± Standard Deviation (sd)) Identified in the Aeration Extracts of Both Males and Females

		average ± sd (ng/bug/24 h)	
compound	retention index[a]	males	females
6-methyl-5-hepten-2-one	989	12.70 ± 7.06	1.33 ± 1.41
octanal	1005	12.16 ± 11.47	3.40 ± 4.59
(*E*)-2-octenal	1063	912.07 ± 526.15	106.74 ± 122.71
undecane	1100	96.95 ± 60.39	17.30 ± 15.21
nonanal	1107	22.54 ± 23.53	1.88 ± 2.46
dodecane	1200	131.17 ± 96.57	66.06 ± 56.05
decanal	1206	18.94 ± 9.11	1.06 ± 1.33
tridecane	1300	4863.91 ± 2508.48	4739.92 ± 2704.15
tetradecane	1400	7.97 ± 4.32	6.76 ± 5.67
pentadecane	1500	25.35 ± 14.91	18.24 ± 22.63
hexadecane	1600	3.87 ± 3.09	1.71 ± 2.24
zingiberenol	1626	1.19 ± 1.12	0

[a]The retention index was calculated using a DB-5 column.

the volatile collections from male *O. poecilus* were performed with female *O. poecilus* (N = 57). The filter paper was replaced every five bioassays. The control consisted of filter paper treated with 5 μL of hexane.

Synthetic zingiberenol was prepared from (*R*)-citronellal and (*S*)-citronellal to produce two products containing four diastereisomers each: (1*RS*,4*RS*,1′*R*)-zingiberenol (**6**) and (1*RS*,4*RS*,1′*S*)-zingiberenol (**7**), respectively (Figure 2).

Therefore, the bioassays were conducted using synthetic solutions containing the following: the racemic mixture containing all eight isomers, **6**, and **7** versus hexane; this solution was evaluated in two different concentrations 0.01 mg/mL (N = 53) and 0.1 mg/mL (N = 65); the synthetic **7** at 0.01 mg/mL (N = 58) and 0.1 mg/mL (N = 63) versus hexane; and the synthetic **6** at 0.01 mg/mL (N = 93) and 0.1 mg/mL (N = 59) versus hexane. After fractionating, **6** and **7** provided four samples of two isomers each. The bioassays were then conducted using a 0.1 mg/mL synthetic solution with the two least polar isomers of **7** versus hexane (N = 57), a 0.1 mg/mL synthetic solution containing the two least polar isomers of **6** versus hexane (N = 57), and finally **6** was contrasted with **7** using the two different concentrations 0.1 mg/mL (N = 59) and 0.01 mg/mL (N = 59). To each bioassay, using synthetic solutions, 10 μL of each tested solution was applied on filters papers; therefore, from solutions of 0.1 and 0.01 mg/mL, the amount of zingiberenol used in the bioassays was approximately 1 or 0.1 μg, respectively. All of these bioassays were performed as described for male extracts.

Statistical Analysis. Data on the responses of males and females to different treatments (live insects, air entrainment extracts, and fractions thereof, and synthetic standards, versus controls) were analyzed using chi square tests to test the hypotheses of nonpreference (probability of choice each arm of the olfactometer = 0.5). When the number of insects responding to each treatment in each set of bioassays was lower than 5 (<20% of insect choosing an arm of the olfactometer), the statistical analysis was not applied, and the insects were considered as not responding to the treatment tested.

RESULTS AND DISCUSSION

The chemical analysis of the air-entrainment extracts obtained from both genders of *O. poecilus* indicated that the males produce a specific compound, **12** (RT 26.24 min, RI = 1626), that is not present in the volatile collections from females (Figure 3). In addition, a number of volatile compounds common to both sexes were identified, that is, 6-methyl-5-hepten-2-one, **1**; octanal, **2**; (*E*)-2-octenal, **3**; undecane, **4**; nonanal, **5**; dodecane, **6**; decanal, **7**; tridecane, **8**; tetradecane, **9**; pentadecane, **10**; and hexadecane, **11** (Figure 3, Table 1). On the basis of the quantitative GC analysis, mature virgin *O. poecilus* males released the male specific compound at a rate of (mean ± sd) 1.19 ± 1.12 ng/bug/24 h (N = 15).

The mass spectrometric analysis showed that the male-specific compound **12** had a fragmentation pattern consistent with a sesquiterpene structure, that is, m/z 69 corresponding to $C_5H_9^+$, m/z 93 corresponding to $C_7H_9^+$, and m/z 119 corresponding to $C_9H_{11}^+$. The molecular ion at m/z 222 and the loss of 18 ($M^+ - H_2O$) to produce a fragment with m/z 204 indicated that the compound may be an alcohol, which was confirmed by derivatization with MSTFA when the peak at a

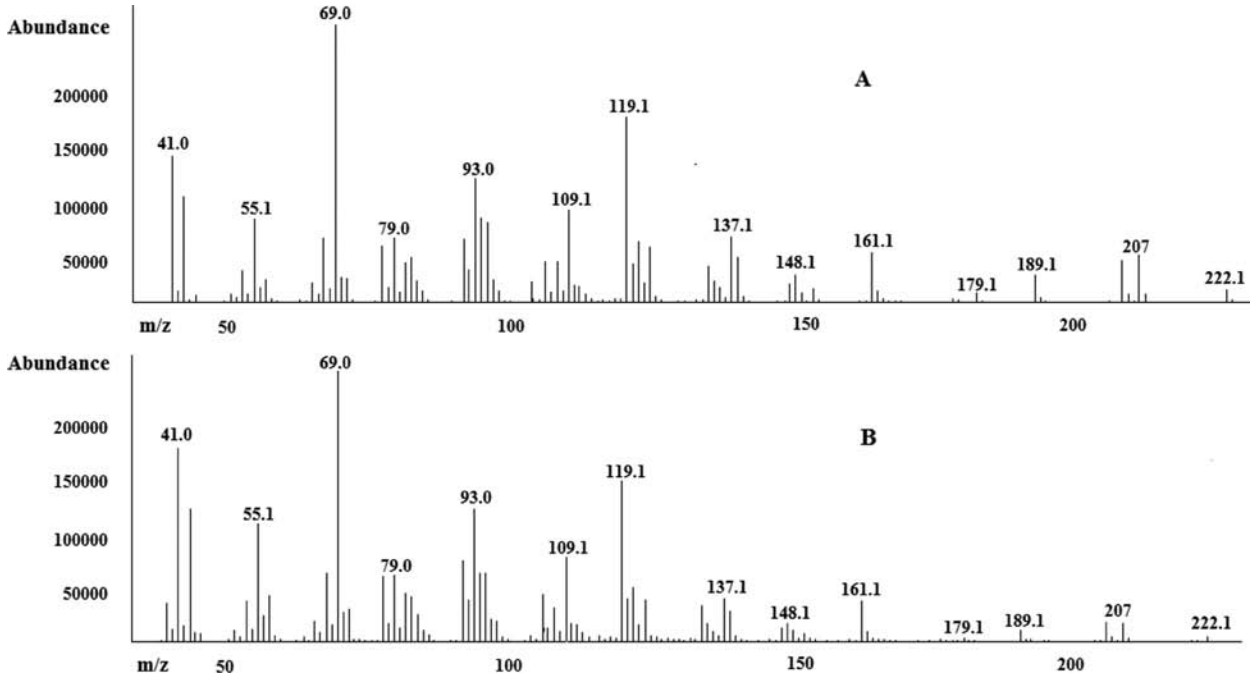

Figure 4. (A) Mass spectra of the male-specific compound and (B) synthetic zingiberenol.

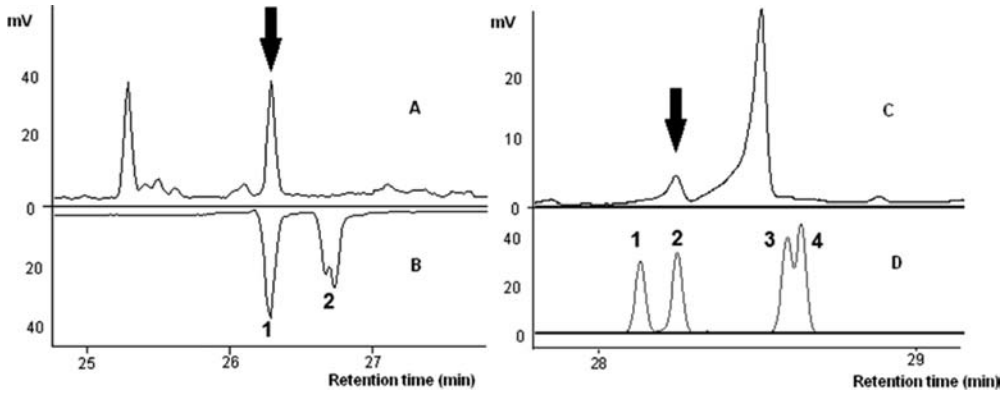

Figure 5. Gas chromatogram profile of (A) male volatiles and (B) synthetic zingiberenol (peak **1** is the least polar diastereoisomers, and peak **2** is the most polar diastereoisomers). (C) Silylated male volatiles and (D) the four silylated diastereoisomers of the synthetic zingiberenol (peaks **1** and **2** are the least polar silylated diastereoisomers, and peaks **3** and **4** are the most polar silylated diastereoisomers).

retention time of 26.24 min disappeared and a new peak at 28.34 min appeared with a molecular ion at m/z 294 (M − H + $Si(CH_3)_3$). The fragmentation pattern (Figure 4) and retention index RI = 1626 (DB-5 column) of this sesquiterpene matched those of zingiberenol. Injecting the synthetic **6** and **7** solutions into a DB5 column made it possible to observe a group of two distinct peaks at RT = 26.58 min and RT = 26.69 min as well as another group that was unresolved at RT = 26.24 min, which coincided with the specific compounds from *O. poecilus* that eluted at RT = 26.24 min (Figure 5A,B). To better separate the four diastereoisomers of zingiberenol, a derivatization of the synthetic (1′*S*)-zingiberenol solution was conducted using MSTFA. After derivatization, the four diastereoisomers were resolved in a DB-5 column and eluted at RT = 28.17, 28.34, 28.60, and 28.71 min. The male specific compound, which also derivatized with MSTFA, coeluted with isomer 2 of zingiberenol at RT = 28.34 min (Figure 5C,D).

A silica gel column was prepared, and the isomers were separated into four groups of two isomers each; the fractions were derivatized with MSTFA, and injecting these fractions into a DB-5 column corroborated that the male-specific compound coeluted with the less polar (1′*S*) and (1′*R*) zingiberenol isomers at RT = 28.34 min.

The fragmentation patterns of the compounds generated from the silylation extracts of male *O. poecilus* and synthetic **7** were very similar. Ions with m/z 294, 295, and 296 corresponded to the molecular ion $M^+ - H + Si^{28}(CH_3)_3$ and their isotopomers $M^+ - H + Si^{29}(CH_3)_3$ and $M^+ - H + Si^{30}(CH_3)_3$ with a ratio typical of the isotopic ratio for compounds containing silicon atoms (7/2/1). The ion at m/z 279 corresponded to $M^+ - H + Si(CH_3)_2$ (loss of $-CH_3$).

To determine the absolute configuration of zingiberenol produced by *O. poecilus*, an extract from volatile collections of males of *O. poecilus* was submitted to dehydration using H_2SO_4 yielding zingiberene **3** and sesquiphellandrene **2**. The same procedure was carried out to synthesize solutions of **6** and **7**. In a chiral column (β-DEX 325), the zingiberenes formed from **6** were not well separated and did not match with the zingiberene

dx.doi.org/10.1021/jf402765b | *J. Agric. Food Chem.* 2013, 61, 7777−7785

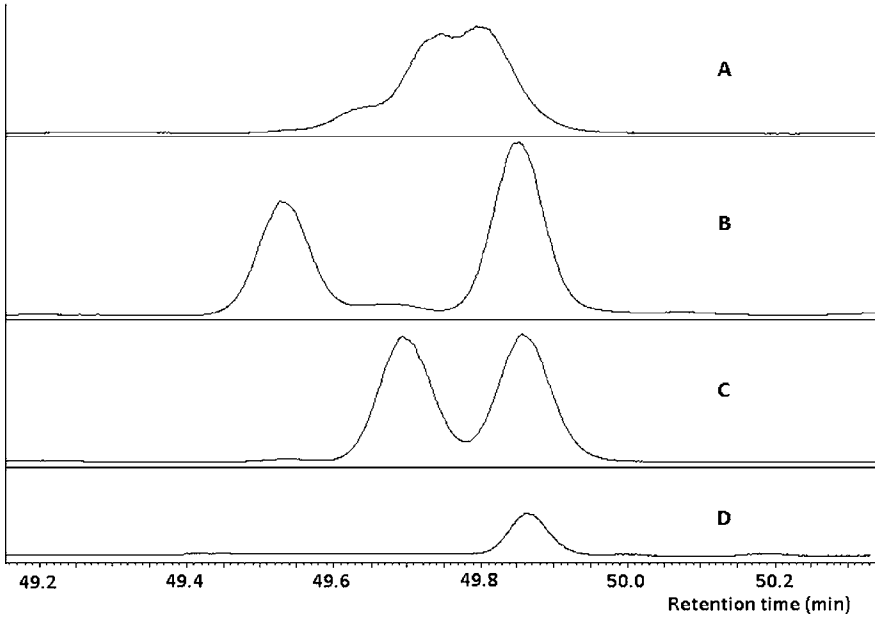

Figure 6. Gas chromatogram profile from GC chiral column. (A) Zingiberenes generated from (*R*)-zingiberenol, (B) natural zingiberene from ginger oil (peak at right is zingiberene, and peak at left is curcumene present in the sample). (C) Zingiberenes generated from (*S*)-zingiberenol and (D) zingiberene obtained from dehydration of the extract of male volatile collection.

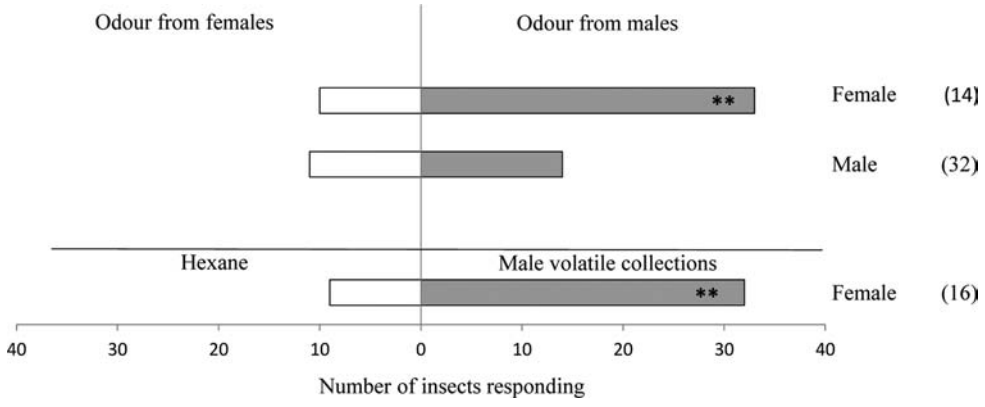

Figure 7. Number of insects of *O. poecilus* females and males (first choice) responding to volatiles from live insects and from male air-entrainment extracts. *O. poecilus* females preferred the odor from conspecific males and the odor from male volatile collections versus hexane. Males did not prefer odor from conspecific males or females. Numbers on the right indicate the number of insects that did not make a choice (*$P < 0.05$, **$0.05 < P < 0.001$, and ***$P < 0.001$, χ^2 test).

produced from the dehydration of insects extracts (Figure 6A,D). Yet, the two isomers of zingiberene provided from dehydration of **7** were well separated at 49.71 and 49.86 min, and the latter peak coeluted with the zingiberene obtained from dehydration of male volatile extract (Figure 7C,D). We compared the retention time of the isomer of zingiberene obtained from dehydration of the extract of male volatile collection (Figure 6D) with the natural zingiberene from ginger oil (Figure 6B), which has its absolute configuration already determined as (4*R*,1′*S*) **3**,[16] and they coeluted at 49.86 min; therefore, the carbon 4 and 1′ of zingiberenol produced by *O. poecilus* males was determined to be *R* and *S*, respectively.

Both compounds, (1*S*,4*R*)-4-isopropyl-1-methylcyclohex-2-en-1-ol, known as quercivorol **4**, and (1*RS*,4*RS*,1′*R*)-4-(1′,5′-dimethylhex-4′-enyl)-1-methylcyclohex-2-en-1-ol, zingiberenol **6**, have a similar chemical structure presenting the same substituents at C1 and C7 (Figure 1), and therefore the same shifts. Analyzing the ^{13}C NMR spectra of quercivorol **4**,[12,13] C1 and C7 shifts are δ 67.4 and δ 29.6, respectively, the same values of C1 and C7 shifts of **7** and different from **6**, which has values of C1 δ 69.7 and C7 δ 28.3, whereas (1*S*,4*R*)-quercivorol presents values of C1 δ 69.8 and C7 δ 28.4. From this information, the configuration of carbon 1 was determined to be *R*. Therefore, the zingiberenol produced by males of *O. poecilus* has the following configuration: (1*R*,4*R*,1′*S*). In addition, the bioassays showed attraction of *O. poecilus* females only to **7** corroborating the (1′*S*) configuration.

The bioassays in the Y-shaped olfactometer indicated that *O. poecilus* females preferred the odor of live males ($\chi^2 = 12.90$, $P < 0.001$) but not that of females (Figure 7). Males did not prefer the odors of either females or males (Figure 7). *O. poecilus* females responded selectively to the male volatile collections as compared to hexane ($\chi^2 = 12.30$, $P < 0.001$) (Figure 7).

Females responded to live males only during the morning (08:00−12:00) ($\chi^2 = 12.45$, $P < 0.001$) and not during the

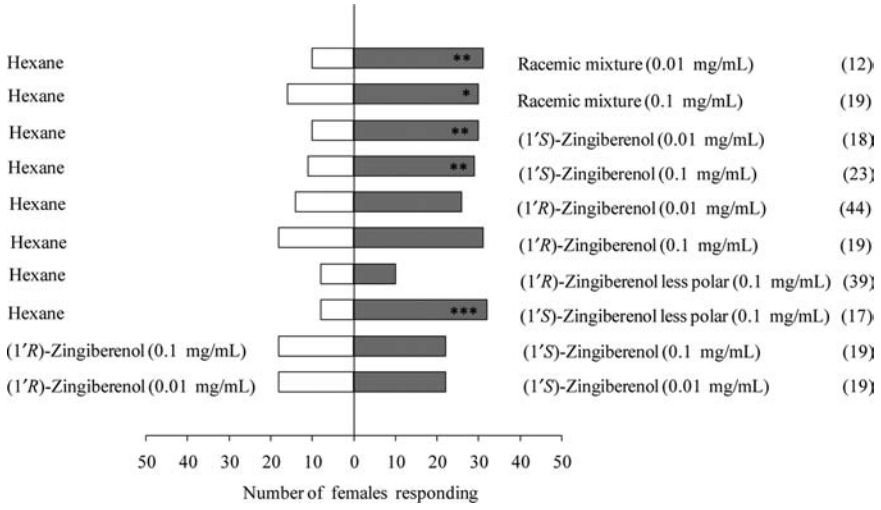

Figure 8. Response of *O. poecilus* females (first choice) to the racemic synthetic mixture of zingiberenol versus hexane, the four synthetic isomers of (1*RS*,4*RS*,1′*R*)-zingiberenol versus hexane, the four synthetic isomers of (1*RS*,4*RS*,1′*S*)-zingiberenol versus hexane, and a combination of less polar synthetic isomers of (1′*S*)-zingiberenol versus hexane and the less polar synthetic isomers of (1′*R*)-zingiberenol versus hexane. The numbers on the right indicate the number of insects that did not make a choice (*$P < 0.05$, **$0.05 < P < 0.001$, and ***$P < 0.001$, χ^2 test).

other evaluated periods, that is, the afternoon (12:00−16:00) and evening (16:00−20:00).

Oebalus poecilus females chose selectively the racemic mixture of zingiberenol at 0.01 mg/mL ($\chi^2 = 10.7$, $P = 0.001$) and 0.1 mg/mL ($\chi^2 = 4.3$, $P = 0.04$) as compared to hexane, the synthetic solutions of **7** in different concentrations 0.1 mg/mL ($\chi^2 = 8.1$, $P = 0.004$) and 0.01 mg/mL ($\chi^2 = 10.0$, $P = 0.002$) as compared to hexane (Figure 8), and also responded to synthetic solution containing the two less polar diastereoisomers of **7** as compared to hexane at the two different concentrations tested ($\chi^2 = 14.4$, $P < 0.001$) (Figure 8). When females of *O. poecilus* were tested with a synthetic solution of **6** at 0.1 mg/mL ($\chi^2 = 3.6$, $P = 0.06$) and 0.01 mg/mL ($\chi^2 = 3.4$, $P = 0.06$), the response to these solutions was marginal. *O. poecilus* females did not respond when the synthetic solution of **7** was contrasted with **6** at the two different concentrations tested, 0.1 mg/mL ($\chi^2 = 0.4$, $P = 0.5$) and 0.01 mg/mL ($\chi^2 = 0.4$, $P = 0.6$) (Figure 8), and also did not respond when the synthetic solution 0.1 mg/mL containing the least polar diastereoisomers of **6** was contrasted with hexane ($\chi^2 = 0.4$, $P = 0.6$) (Figure 8).

The chemical analysis of the air-entrainment extracts of male *O. poecilus* demonstrated that males release a specific compound that was identified as (1*R*,4*R*,1′*S*)-zingiberenol, and Y-olfactometer bioassays demonstrated the sex pheromone role of this compound to females.

Tibraca limbativentris (Stal), another important rice pest in Brazil, also uses zingiberenol as sex pheromone; however, its sex pheromone blend is composed of three zingiberenol isomers, one of which also has the 1′ carbon in the pendant group in the (*S*) configuration, but its absolute configuration was still not elucidated[2] and the zingiberenol isomer produced by *O. poecilus* elutes at the same retention time as does the major zingiberenol isomer produced by *T. limbativentris*. In this work, the absolute configuration of zingiberenol produced by *O. poecilus* was elucidated as (1*R*,4*R*,1′*S*). *O. poecilus* females responded to (1′*S*)-zingiberenol isomers and to racemic mixture, but showed a marginal response to (1*RS*,4*RS*,1′*R*)-zingiberenol and did not respond to the two least polar (1′*R*)-zingiberenol isomers; these results might suggest that the two least polar (1′*R*)-zingiberenol isomers are not recognized or have a deleterious effect in the attraction of *O. poecilus* females, whereas *T. limbativentris* females respond to (1*RS*,4*RS*,1′*S*)- and (1*RS*,4*RS*,1′*R*)-zingiberenol in Y-olfactometer bioassays.[2] Therefore, although these species produce the same compound as sex pheromone, the total blend is different, guaranteeing the species-specificity of the sex pheromone. In addition, these species are found during different periods of the rice season because *T. limbativentris* generally attacks the plant in the vegetative stage, while *O. poecilus* is present during the reproductive stage.[3]

There are several examples in the literature showing how insects use stereochemistry to guarantee specificity in their communications.[8,10,15,17−20] *Nezara* spp. and *Chinavia* spp. (Hemiptera: Pentatomidae: Pentatominae) are species of the *Nezara* group (Tribu Nezarini),[21] and these phylogenetic proximal species share the same sex pheromone compounds, a blend containing both *cis*- and *trans*-epoxy-bisabolene with specificity guaranteed by the ratio between the cis and trans isomers.[17−19,22−24] In addition, species that are not as phylogenetically related, such as *Thyanta perditor* and *Euschistus heros* (Pentatominae) and *Edessa meditabunda* (Edessinae), also produce sex pheromones with analogous structures that contain the same chemical functional groups and long carbon chain. *T. perditor* produces the compound methyl (2*E*,4*Z*,6*Z*)-2,4,6-decatrienoate[15] as a sex pheromone, *E. heros* produces methyl (2*S*,6*R*,10*S*)-2,6,10-trimethyltridecanoate,[25,26] and *E. meditabunda* produces a blend of the two compounds, methyl 4,8,12-trimethylpentadecanoate and methyl 4,8,12-trimethyltetradecanoate;[27] the authors did not identify the absolute configuration. Previous studies conducted on the Pentatomidae group have shown that males produce sex pheromones with similar chemical structures when the species show close phylogenetic relationships.[8,27,28] In addition, at short distances, other communication modalities, such as vibrations[29,30] or chemicals from the cuticle,[31,32] might be important for the final recognition.

This is the first report of a sex pheromone on the genus *Oebalus*; previously only two defensive compounds, (*E*)-2-heptenal and tridecane, were identified for the Neartic species

O. pugnax.[33] Interestingly, for *O. poecilus*, the compounds (*E*)-2-octenal and tridecane were also the primary volatile compounds identified in the air-entrainment extracts from males and females. Both saturated and unsaturated aldehydes (from C4 to C14) and saturated hydrocarbons (C11 to C14) are quite common and are the major compounds released by stink bugs, and these compounds are stored in the metathoracic gland of males and females; however, their role in behavior remains to be evaluated.[8,34]

In summary, the biological activity of zingiberenol was confirmed in laboratory bioassays, which indicated that one of the two least polar (1′*S*)-zingiberenol isomers is produced by the *O. poecilus* males because *O. poecilus* females did not respond to (1′*R*)-zingiberenol isomers. The absolute configuration of the zingiberenol produced by *O. poecilus* was determined from the dehydration product over a chiral stationary phase and from the ^{13}C NMR analysis of the least polar zingiberenol and quercivorol. Therefore, zingiberenol produced by males of *O. poecilus* was determined to be (1*R*,4*R*,1′*S*). The role and biological activity of (1*S*,4*R*,1′*S*)-4-(1′,5′-dimethylhex-4′-enyl)-1-methylcyclohex-2-en-1-ol, as a mediator of *O. poecilus* reproductive behavior under field conditions, still remain to be determined. It may be possible to develop pheromone-based monitoring systems for this insect, as such monitoring systems, when used in conjunction with strategies such as trap cropping, may provide an integrated pest management solution for control of this pest bug species.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +55 61 3448-4932. Fax: +55 61 3448-4762. E-mail: carolina.blassioli@embrapa.br.

Funding

This work was funded by the International Foundation for Science (IFS), the National Council for Scientific and Technological Development (CNPq), the Distrito Federal Research Foundation (FAPDF), the Brazilian Agricultural Research Corp. (Embrapa), and University of Brasília – Chemistry Institute (UnB).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Amanda Rodrigues Viana and Samantha da Silveira for helping with the bioassays and Edmar Cardoso de Moura and José Francisco Arruda e Silva for rearing the insects.

REFERENCES

(1) Panizzi, R. A.; Mcpherson, J. E.; James, D. E.; Javahery, M.; Mcpherson, R. M. Stink bugs (Pentatomidae). In *Heteroptera of Economic Importance*; Schaffer, C. W., Panizzi, A. R., Eds.; CRC Press: Boca Raton, FL, London, New York, WA, 2000; pp 321–336.

(2) Borges, M.; Birkett, M.; Aldrich, J. R.; Oliver, J. E.; Chiba, M.; Murata, Y.; Laumann, R. A.; Barrigossi, J. A.; Pickett, J. A.; Blassioli-Moraes, M. C. Sex attractant pheromone from the Rice Stalk Stink Bug, *Tibraca limbativentris* Stal. *J. Chem. Ecol.* **2006**, *32*, 2749–2761.

(3) Ferreira, E.; Martins, J. F.; Da, S. Insetos prejudiciais ao arroz e seu controle. Goiânia; Embrapa Arroz e Feijão. *Circular Técnica* **1984**, *67*, 1–12.

(4) Ferreira, E.; Barrigossi, J. A. F.; Vieira, N. R.; De, A. Percevejos das panículas do arroz: fauna heteroptera associada ao arroz. Goiânia: Embrapa Arroz e Feijão. *Circular Técnica* **2001**, *43*, 1–52.

(5) Ferreira, E.; Barrigossi, J. A. F. Produção e qualidade do grão do arroz irrigado infestado por adultos de percevejo-das-panículas. *Pesqui. Agropecu. Bras.* **2006**, *41*, 1083–1091.

(6) Ferreira, E.; Vieira, N. R. A.; Rangel, P. H. N. Avaliação dos danos de *Oebalus* spp. em genótipos de arroz irrigado. *Pesqui. Agropecu. Bras.* **2002**, *37*, 763–768.

(7) Martins, J. F.; da, S.; Barrigossi, J. A. F.; Oliveira, V. J.; Cunha, S. U. Situação do manejo integrado de insetos-praga na cultura do arroz no Brasil. Embrapa Clima Temperado. *Série Documentos* **2009**, *290*, 1–40.

(8) Moraes, M. C. B.; Pareja, M.; Laumann, R. A.; Borges, M. The chemical volatiles (Semiochemicals) produced by neotropical stink bugs (Hemiptera: Pentatomidae). *Neotrop. Entomol.* **2008**, *37*, 489–505.

(9) Silva, C. C. A.; Cordeiro, D. M.; Laumann, R. A.; Moraes, M. C. B; Barrigossi, J. A.; Borges, M. Ciclo de vida e metodologia de criação de *Tibraca limbativentris* Stal. 1860 (Heteroptera: Pentatomidae) para estudos de ecologia química. Brasília: Embrapa, Centro Nacional de Recursos Genéticos e Biotecnologia. *Bol. Pesqui.* **2004**, *78*, 1–18.

(10) Zhang, A. J.; Borges, M.; Aldrich, J. R.; Camp, M. Stimulatory male volatiles for the neotropical brown stink bug, *Euschistus heros* (F.) (Heteroptera: Pentatomidae). *Neotrop. Entomol.* **2003**, *32*, 713–717.

(11) Borges, M.; Millar, J. G.; Laumann, R. A.; Moraes, M. C. B. A male-produced sex pheromone from the neotropical redbanded stink bug, *Piezodorus guildinii* (W.). *J. Chem. Ecol.* **2007**, *33*, 1235–1248.

(12) Kashiwagi, T.; Nakashima, T.; Tebayashi, S.; Kim, C. Determination of the absolute configuration of quercivorol (1*S*,4*R*)-p-menth-2-en-1-ol, an aggregation pheromone. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 2544–2546.

(13) Mori, K. Synthesis of (1*S*,4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, the aggregation pheromone of the ambrosia beetle *Platypus quercivorus*, its racemate, (1*R*,4*R*)- and (1*S*,4*S*)-isomers. *Tetrahedron: Asymmetry* **2006**, *17*, 2133–2142.

(14) Hagiwara, H.; Okabe, T.; Ono, H.; Kamat, V. P.; Hoshi, T.; Suzuki, T.; Ando, M. Total synthesis of bisabolane sesquiterpenoids, α-bisabol, curcumene, curcuphenol, and elvirol: utility of catalytic enamine reaction in cyclohexenone synthesis. *J. Chem. Soc., Perkin Trans. 1* **2002**, 895–900.

(15) Moraes, M. C. B.; Millar, J. G.; Laumann, R. A.; Sujii, E. R.; Pires, C. P. S.; Borges, M. Sex attractant pheromone from the neotropical red-shouldered stink bug, *Thyanta perditor* (F.). *J. Chem. Ecol.* **2005**, *31*, 1405–1417.

(16) Kreiser, W.; Körner, F. Stereospecific synthesis of (+)-β-sesquiphellandrene. *Helv. Chim. Acta* **1999**, *82*, 1427–1432.

(17) Borges, M. Attractant compounds of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). *An. Soc. Entomol. Bras.* **1995**, *24*, 215–225.

(18) McBrien, H. L.; Millar, J. G.; Gottlieb, L.; Chen, X.; Rice, R. E. Male-produced sex attractant pheromone of the green stink bug, *Acrosternum hilare* (Say). *J. Chem. Ecol.* **2001**, *27*, 1821–1839.

(19) McBrien, H. L.; Millar, J. G.; Rice, R. E.; McElfresh, J. S.; Cullen, E.; Zalom, F. G. Sex attractant pheromone of the red-shouldered stink bug *Thyanta pallidovirens*: a pheromone blend with multiple redundant components. *J. Chem. Ecol.* **2002**, *28*, 1797–1818.

(20) Mori, K. Significance of chirality in pheromone science. *Bioorg. Med. Chem.* **2007**, *15*, 7505–7523.

(21) Schwertner, C. F.; Grazia, J. O gênero *Chinavia Orian* (Hemiptera, Pentatomidae) no Brasil, com chave pictórica para os adultos. *Rev. Bras. Entomol.* **2007**, *51*, 416–435.

(22) Baker, R.; Borges, M.; Cooke, N. G.; Herbert, R. H. Identification and synthesis of (Z)-(1′*S*,3′*R*,4′*S*)(-)-2-(3′,4′-epoxy-4′-methylcyclohexyl)-6-methylhepta-2,5-diene, the sex pheromone of the southern green stink bug, *Nezara viridula* (L.). *Chem. Commun.* **1987**, *6*, 414–416.

(23) Borges, M.; Jepson, P. C.; Howse, P. E. Long-range mate location and close-range courtship behaviour of the green stink bug, *Nezara viridula* and its mediation by sex pheromones. *Entomol. Exp. Appl.* **1987**, *44*, 205–212.

(24) Blassioli-Moraes, M. C.; Laumann, R. A.; Oliveira, M. W. M.; Woodcock, C. M.; Mayon, P.; Hooper, A.; Pickett, J. A.; Birkett, M.; Borges, M. Sex pheromone communication in two sympatric Neotropical stink bugs *Chinavia ubica* and *Chinavia impicticornis*. *J. Chem. Ecol.* **2012**, *38*, 836−845.
(25) Borges, M.; Aldrich, J. R. Attractant pheromone for nearctic stink bug, *Euschistus obscurus* (Heteroptera: Pentatomidae): insight into a neotropical relative. *J. Chem. Ecol.* **1994**, *5*, 1095−1102.
(26) Borges, M.; Moraes, M. C. B.; Peixoto, M. F.; Pires, C. S. S.; Sujii, E. R.; Laumann, R. A. Monitoring the neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with pheromone-baited traps in soybean fields. *J. Appl. Entomol.* **2011**, *135*, 68−80.
(27) Zarbin, P. H. G.; Fávaro, C. F.; Vidal, D. M.; Rodrigues, M. A. C. M. Male-produced sex pheromone of the stink bug *Edessa meditabunda*. *J. Chem. Ecol.* **2012**, *38*, 825−835.
(28) Millar, J. G. Pheromone of true bugs. In *The Chemistry of Pheromones and Other Semichemicals II-Topics in Current Chemistry*, 1st ed.; Schulz, S., Ed.; Springer-Verlag: Germany, 2004; pp 37−84.
(29) Moraes, M. C. B.; Laumann, R. A.; Cokl, A.; Borges, M. Vibratory signals of four neotropical stink bug species. *Physiol. Entomol.* **2005**, *30*, 175−188.
(30) Silva, C. C. A.; Laumann, R. A.; Ferreira, J. B. C.; Blassioli-Moraes, M. C.; Borges, M.; Cokl, A. Reproductive Biology, mating behaviour and vibratory communication of the brown-winged stink bug, *Edessa meditabunda* (Fabr.). (Hemiptera:Pentatomidade). *Psyche* **2012**, 1−9.
(31) Borges, M.; Colazza, S.; Ramirez-Lucas, P.; Chauhan, K. R.; Aldrich, J. R.; Moraes, M. C. B. Kairomonal effect of walking traces from *Euschistus heros* (Heteroptera: Pentatomidae) on two strains of *Telenomus podisi* (Hymenoptera: Scelionidae). *Physiol. Entomol.* **2003**, *28*, 349−355.
(32) Colazza, S.; Aquila, G.; De Pasquale, C.; Peri, E.; Millar, J. G. The egg parasitoid *Trissolcus basalis* uses n-nonadecane, a cuticular hydrocarbon from its stink bug host *Nezara viridula*, to discriminate between female and male hosts. *J. Chem. Ecol.* **2007**, *33*, 1420−1425.
(33) Blum, M. S.; Traynham, J. G.; Chidester, J. B.; Boggus, J. D. *n*-Tridecane and *trans*-2-heptenal in scent gland of the rice stink bug, *Oebalus pugnax* (F.). *Science* **1960**, *132*, 1480−1481.
(34) Pareja, M.; Borges, M.; Laumann, R. A.; Moraes, M. C. B. Inter- and intraspecific variation in defensive compounds produced by five neotropical stink bugs species (Hemiptera: Pentatomidae). *J. Insect Physiol.* **2007**, *53*, 639−648.