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(1*R*,2*S*,6*R*)-Papayanal: a new male-specific volatile compound released by the guava weevil *Conotrachelus psidii* (Coleoptera: Curculionidae)

Alicia Romero-Frías[1,†], Yasuhiro Murata[2], José Maurício Simões Bento[3] and Coralia Osorio[1,*,†]

[1]*Departamento de Química, Universidad Nacional de Colombia, Bogotá, Colombia;* [2]*Fuji Flavor Co., Ltd., Tokyo, Japan;* [3]*Departamento de Entomologia, Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Universidade de São Paulo (USP), Piracicaba, Brazil*

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The guava weevil, *Conotrachelus psidii* is an aggressive pest of guava (*Psidium guajava* L.) that causes irreparable damages inside the fruit. The volatile compounds of male and female insects were separately collected by headspace solid-phase microextraction or with dynamic headspace collection on a polymer sorbent, and comparatively analyzed by GC–MS. (1*R*,2*S*,6*R*)-2-Hydroxymethyl-2,6-dimethyl-3-oxabicyclo[4.2.0]octane (papayanol), and (1*R*,2*S*,6*R*)-2,6-dimethyl-3-oxabicyclo[4.2.0]octane-2-carbaldehyde (papayanal) were identified (ratio of 9:1, respectively) as male-specific guava weevil volatiles. Papayanal structure was confirmed by comparison of spectroscopic (EIMS) and chromatographic (retention time) data with those of the synthetic pure compound. The behavioral response of the above-mentioned compounds was studied in a Y-tube olfactometer bioassay, and their role as aggregation pheromone candidate components was suggested in this species.

Key words: 2,6-Dimethyl-3-oxabicyclo[4.2.0]octane-2-carbaldehyde; *Conotrachelus psidii*; aggregation pheromone; Y-tube olfactometer bioassay

Conotrachelus psidii Marshall (Coleoptera: Curculionidae) is one of the most important pests of guava (*P. guajava*, Myrtaceae) crops. The mated females lay eggs in small unripe fruits. During fruit development, the larvae grow and mature larvae abandon the ripe fruits and pupate underground. Larval feeding causes an irreparable damage to the fruit that does not allow its further use. The adults of *C. psidii* are small dark brown weevils (~6 mm long) with crepuscular habits.[1,2] Usually, guava weevils survive to insecticide applications because they do not remain exposed, thus making insecticidal control difficult. Hence, complementary strategies for the management of this pest need to be developed.

Aggregation pheromones have been studied and applied in field to monitor or as support control strategy to control several Curculionidae species.[3–6] These kind of pheromones offers a promising method for direct insect control through mass trapping.[7,8] In addition, previous studies have shown that host plant volatiles are attractive to both sexes of *C. psidii*.[9,10] Recently Romero-Frías et al.[11] identified the terpenes, *β*-caryophyllene, and limonene in both insects (*C. psidii* males and females) and plants (flower bud and fruit setting guava stages). Therefore, these results suggested a possible role of these volatiles as host kairomones for *C. psidii* in the guava reproductive tissues. Later, Palacio-Cortés et al.[12] reported the release of (1*R*,2*S*,6*R*)-papayanol by *C. psidii* males depending on the photoperiod, which in the presence of plant volatiles is highly attractive to both sexes.

As part of our ongoing research on the search for semiochemicals that can be successfully used to monitor and/or control pests in tropical fruis (IPM, Integrated Pest Management), the aim of this work was to study the aggregation pheromone system in *C. psidii*. Thus, the isolation, identification, and synthesis of a new male-specific guava weevil volatile, named papayanal, is reported here.

Materials and methods

Insects. Male and female *C. psidii* adults were collected between January to July 2014 from different managed crops located in Puente Nacional and Vélez (Santander, Colombia) using the beating tray method after shaking branches of the guava plants. Once transferred to the laboratory, individuals were segregated by sex according to the procedure reported by Silva-Filho et al.,[13] and maintained separately in plastic boxes at 20 ± 2 °C, at a relative humidity of 80 ± 10% with pieces of green guava fruits as food and 12-h light regime.[11]

*Corresponding author. Email: cosorior@unal.edu.co
†Facultad de Ciencias, Departamento de Química, Universidad Antonio Nariño

Insect volatile collection. Volatile compound released by *C. psidii* adults were collected using either headspace solid-phase microextraction (HS-SPME) or dynamic headspace (DHS) using polymer-based sorbents. For HS-SPME, the weevils (males or females, separately) were deprived of food, and placed in 100 mL glass flask. The volatile compounds released by each gender were separately collected on a DVB/CAR/PDMS fiber (50/30 µm thickness, Supelco, Bellefonte, PA, USA) for 12 h during the scotophase. The analyses were done by triplicate with different insects each time (10 males or 10 females) upon arrival to the laboratory. Analysis of background volatiles was performed with the SPME fiber in the flask without insects. For DHS collection, groups of 30 males and 30 females were separately held on in all-glass aeration chambers (33 cm high x 4 cm outlet diameter) with three fresh unripe guava fruits (6–8 g each) at 23–24 °C. The released volatiles were continuously collected during 4 days (minimum time required to detect volatile compounds), and trapped on glass columns (10 cm high × 0.5 cm i.d) packed with 0.5 g of Hayesep D 80/100 (DVB, Supelco, Bellefonte, PA). Charcoal-filtered humidified air was pushed through the aeration system (1.0 L/min). The adsorbed volatile compounds were eluted with 500 µL of hexane HPLC grade (Merck, Darmstadt, Germany). The sample was concentrated to about 50 µL under a gentle flow of nitrogen, and then analyzed by GC–MS. Blank experiments were performed under the same conditions, in presence of fresh unripe guava fruits but without insects.

Analytical procedures. The volatile compounds collected by HS-SPME were desorbed into the injection port of a Shimadzu GC17A coupled to a mass selective detector QP5050 (Shimadzu, Tokyo, Japan). Desorption time was set at 5 min. Two capillary columns, RTX-5 and DB-FFAP were used (each 30 m × 0.32 mm i.d., 0.25 µm film thickness; Restek, Bellefonte, PA, USA, and J&W Scientific, Chromatographie-Handel Müller, Fridolfing, Germany, respectively). The column oven was programmed from 50 °C for 1 min, then raised at 7 °C/min to 250 °C for the DB-FFAP and to 300 °C for the RTX-5 column and maintained at those temperatures for 10 min. The injector temperature was fixed at 250 °C for the DB-FFAP and at 300 °C for the RTX-5 column, using helium as carrier gas at 1.5 mL/min. The injection port was used in splitless mode. Linear retention indices were calculated according to the Kovats method using a mixture of *n*-alkanes as external references. Mass spectral identification was completed via comparison with either authentic reference standards or spectra from commercial mass spectral databases (WILEY and EPA/NIH). MS data in the electron ionization (EI) mode were recorded in a mass range of 30–350 µ, with electron energy of 70 eV and processed by Class 5000 v 2.2 MS-Workstation software. A Trace GC Ultra chromatograph connected to a Thermo Scientific ITQ900 ion trap mass spectrometer (Thermo Scientific, Austin, TX, USA) was used for the analysis of volatile samples by chemical ionization (CI-MS) with a RTX-5 column using the same above-described temperature program. Mass spectra in the negative mode were acquired with methane as the reactant gas.

Chiral GC analyses were carried out on an Agilent Technologies 7890A gas chromatograph equipped with an Astec Chiraldex™ B-PH capillary column (30 m × 0.25 mm, 0.12 µm film thickness; Sigma–Aldrich, St.Louis, MO, USA). For (1*R*,2*S*,6*R*)- papayanol and (1*R,*2*S*)-grandisol, the column oven temperature was programmed from 60 to 100 °C at a rate of 1 °C/min, and for (1*R*,2*S*,6*R*)-papayanal, it was programmed from 60 to 100 °C at 0.1 °C/min. Optical rotations were measured with a JASCO Digital polarimeter DIP-370 (Tokyo, Japan). NMR spectra were recorded on a Bruker Biospin AVANCE III spectrometer (400 MHz, $CDCl_3$). The ^1H (δ_H 7.24 ppm) and ^{13}C signals of $CHCl_3$ (δ_C 77.2) were used as references.

Reference compounds. Pure reference standards of *β*-pinene, limonene, 1,8-cineole, decanal, *β*-caryophyllene, *α*-humulene, and (+)-aromadendrene were purchased from Sigma-Aldrich (Taufkirchen, Germany). *α*-Copaene was kindly provided by Dr Ignacio De Alfonso.[14] *n*-Alkane mix (C_8-C_{26}) was acquired from Merck, Darmstadt, Germany.

Synthesis of (1R,2S,6R)-papayanol and (1R,2S,6R)-papayanal. (1*R,*2*S*)-Grandisol was obtained by recrystallization method from (1*R**,2*S**)-grandisol (*cis*-1-methyl-2-isopropenyl-cyclobutane-ethanol, Grandlure I) (Bedoukian Research Inc, Danbury, CT, USA). Briefly, (1*R**,2*S**)-grandisol was acylated with (1*S*)-(−)-camphanic acid chloride (Sigma–Aldrich, St.Louis, MO, USA), the resulting diastereomeric esters were separable by recrystallization using hexane. After alkali hydrolysis, the desired (1*R*,2*S*)-grandisol ($[\alpha]_D^{25}$ = +15.8°(*C* = 1.01, *n*-hexane)) was obtained.[15] The enantiomeric purity of (1*R*,2*S*)-grandisol was estimated GC analysis on a chiral capillary column to be 94% e.e. Then, (1*R*,2*S*,6*R*)-papayanol was obtained by reaction of (1*R,*2*S*)-grandisol with *m*-chloroperbenzoic acid (Tokyo Chemical Industry Co., Tokyo, Japan) as previously described by Zarbin et al.[16] The minor (1*R,*2*R*,6*R*)-papayanol was removed by chromatography over silica gel. To oxidize papayanol to its aldehyde (papayanal), tetrapropylammonium perruthenate (76.0 mg, 0.216 mmol) (Tokyo Chemical Industry Co., Tokyo, Japan) was added in three portions to a mixture of (1*R*,2*S*,6*R*)-papayanol (94% e.e.) (366.5 mg, 2.16 mmol), 4-methylmorpholine N-oxide (380.0 mg, 3.24 mmol) (Wako Pure Chemical Industries, Tokyo, Japan), and powdered 4Å molecular sieves (760 mg) (Wako Pure Chemical Industries, Tokyo, Japan) in dichloromethane (10 ml) at temperature below 30 °C, and with stirring. After 30 min, the reaction mixture was filtered through a short pad of silica gel and eluted with diethyl ether. The solvent was evaporated and the residue purified by column chromatography over silica gel. Elution with hexane/diethyl ether (7:3, *v*/*v*) gave 197.5 mg (54.4% yield) of (1*R*,2*S*,6*R*)-papayanal (94% e.e) as a colorless oil. Because (1*R*,2*S*)-grandisol (94% e.e.) contained (1*S*,2*R*)-grandisol in a small proportion,

the synthesized (1*R*,2*S*,6*R*)-papayanal also contained (1*S*,2*R*,6*S*)-papayanal (Fig. 1).

Spectroscopic data. (1*R*,2*S*,6*R*)-*Papayanol*. ^{1}H NMR (400 MHz, $CDCl_3$) of the synthetic compound: δ [ppm] = 1.12 (s, 3H, CH_3 C-11); 1.16 (s, 3H, CH_3 C-10); 1.32 (ddd, 1H, ^{2}J = 14.0 Hz, ^{3}J = 2.0/2.0 Hz, He C-5); 1.43–1.50 (m, 1H, H_{endo} C-7); 1.55–1.62 (m, 1H, H_{exo} C-7); 1.57–1.65 (m, 1H, H_{exo} C-8); 1.73–1.79 (m, 1H, CH C-1); 1.90 (ddd, 1H, ^{2}J = 14.0 Hz, ^{3}J = 13.3/5.1 Hz, Ha C-5); 1.94–2.04 (m, 1H, H_{endo} C-8); 3.23 (d, 1H, ^{2}J = 10.9 Hz, CH_2OH); 3.42 (d, 1H, ^{2}J = 10.9 Hz, CH_2OH); 3.53 (ddd, 1H, ^{2}J = 12.0 Hz, ^{3}J = 13.3/2.2 Hz, Ha C-4); 3.63 (ddd, 1H, ^{2}J = 12.0 Hz, ^{3}J = 5.2/2.1 Hz, He C-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ [ppm] = 18.0 (C-8); 20.2 (C-10); 28.1 (C-11); 33.6 (C-7); 34.1(C-5); 35.8 (C-6); 45.1 (C-1); 57.5 (C-4); 68.9 (C-9); 72.9 (C-2). $[\alpha]_D^{25}$ = −34.4°(C = 1.00, *n*-hexane). GC/MS (EI, 70 eV) *m/z* (%) = 41 (90), 43 (100), 53 (7), 54 (2), 55 (12), 67 (13), 68 (6), 69 (50), 70 (3), 71 (23), 77 (3), 81 (23), 83 (3), 93 (4), 95 (7), 107 (5), 109 (6), 111 (26), 121 (5), 139 (100), 140 (10), 155 (3), 156 (0.3).

(1*R*,2*S*,6*R*)-*Papayanal*. ^{1}H NMR (400 MHz, $CDCl_3$) of the synthetic compound: δ [ppm] = 1.12 (s, 3H, CH_3C-11); 1.21 (s, 3H, CH_3C-10); 1.37 (ddd, 1H, ^{2}J = 14.0 Hz,^{3}J = 2.3/2.3 Hz, H_e C-5); 1.47–1.55 (m, 1H, H_{endo} C-7); 1.58–1.66 (m, 1H, H_{exo} C-7); 1.59–1.67 (m, 1H, H_{exo} C-8); 1.87–1.93 (m, 1H, CH C-1); 1.88–1.96 (m, 1H, H_a C-5); 1.90–2.00 (m, 1H, H_{endo} C-8); 3.59 (ddd, 1H, ^{2}J = 12.2 Hz, ^{3}J = 12.2/2.2 Hz, H_a C-4); 3.77 (ddd, 1H, ^{2}J = 12.2 Hz, ^{3}J = 4.9/ 2.3 Hz, H_e C-4); 9.44 (s, 1H, CHO C-9). ^{13}C NMR (100 MHz, $CDCl_3$): δ [ppm] = 17.7 (C-8); 19.2 (C-10); 28.1 (C-11); 33.6 (C-7); 34.0 (C-5); 36.2 (C-6); 43.3 (C-1); 57.6 (C-4); 78.7 (C-2); 204 (C-9). $[\alpha]_D^{25}$ = −201°(C = 1.01, *n*-hexane). GC/MS (EI, 70 eV) *m/z* (%) = 41 (90), 43 (100), 53 (7), 55 (12), 67 (11), 69 (60), 70 (3), 71 (28), 77 (3), 79 (6), 81 (17), 83 (3), 93 (4), 95 (7), 109 (4), 111(17), 121 (5), 139 (60), 140 (10), 153 (0.1).

Olfactometer assays. The behavioral response of male and female *C. psidii* individuals was studied in a Y-tube olfactometer bioassay by testing the following samples: DHS extract of males + host plant (fruit

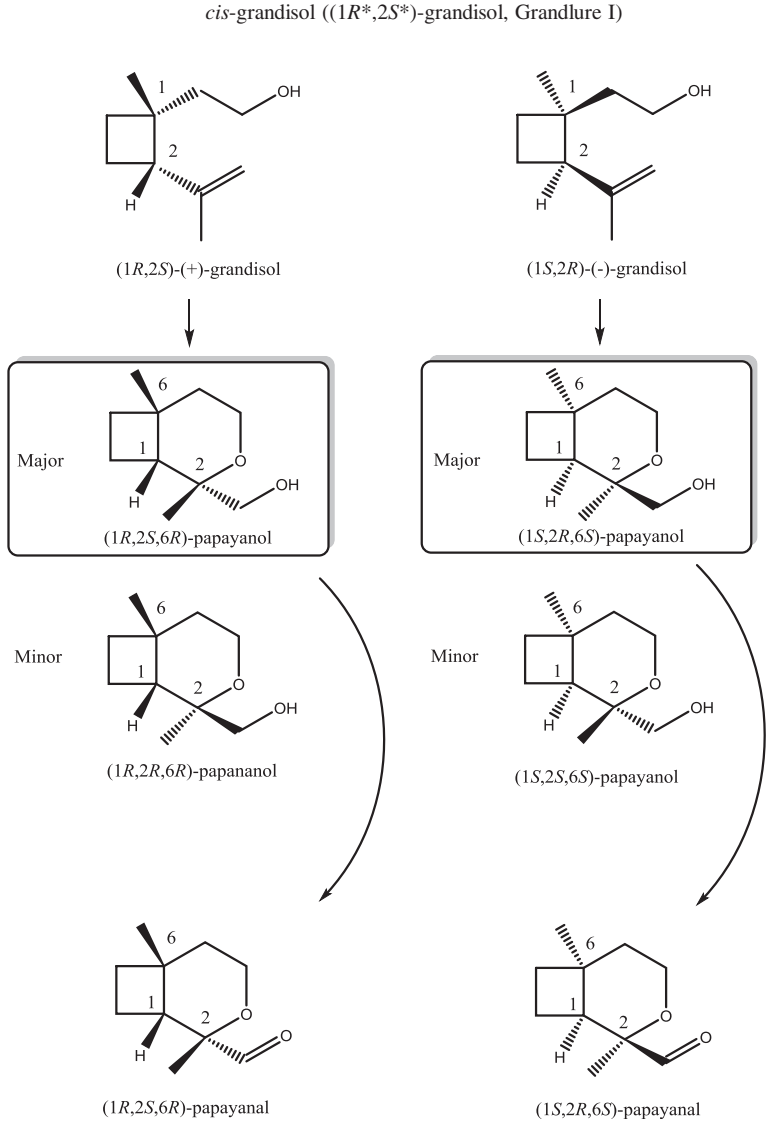

Fig. 1. Scheme of the synthesis of the stereochemically defined papayanol and papayanal stereoisomers.

setting), (1*R*,2*S*,6*R*)-papayanol at a concentration of 1 µg/mL, 10 µg/mL, 100 µg/mL, and a mixture of (1*R*,2*S*,6*R*)-papayanol and (1*R*,2*S*,6*R*)-papayanal in a ratio of 9:1 (10 µg/mL). The dual choice Y-tube olfactometer was used with a pre-humidified and charcoal-filtered airflow at a flow of 1 L/min. Odor propagation simulation tests were performed to visualize the plume distribution inside the system. The olfactometer consisted of a Y-shaped glass tube of 3.5 cm diameter, 20 cm in length, 10 cm of arms, and a 90° Y angle. All the treatments were performed during the scotophase with a red light to avoid any interference of the white light, at 18 ± 2 °C and a relative humidity of 80 ± 10%. One week before each assay, *C. psidii* adults (males and females, with a number varying between 18 to 40 individuals) were collected, placed in plastic flasks, and deprived of food in a room free from odorants for 24 h before the olfatometric bioassay. In all cases, a different insect was used for each test. Age and mating status of the insects were not determined. The insects (male or female) were introduced one by one into the base tube of the olfactometer, and its behavior was observed for 5 min; after that, the next individual was evaluated until the entire test finished. A positive choice for the odor source in one of the two arms was considered when an insect crossed the choice line, went all over 5 cm into the arm, and remained there for at least 2 min. Individuals that did not make any choice during this time were excluded from the statistical analysis. The relative position of olfactometer with respect to the light was rotated 180º after each replicate to avoid positional bias. For each replicate, a new piece of braided cotton roll (0.5 × 0.5 cm; Richmond Dental, Charlotte, NC, USA) loaded with the solutions of tested compounds was used. The evaporation time before starting the tests was 10 s. Sample solutions (2 µL) were tested against hexane HPLC grade (Merck, Darmstadt, Germany) as solvent control. After each test, all components of the olfactometer were cleaned with a detergent solution (Extran alkaline, Merck, KGaA, Darmstadt, Germany), then rinsed with water and acetone, and finally dried at 100 °C for 30 min to avoid from cross contamination.

Statistical analyses. Results of all olfactometer bioassays were analyzed with a binomial test. The null hypothesis was considered 50:50 distribution at a significance level of $p < 0.05$. Data analyses were performed using WinSTAT® for Excel 2007 (V. 2012.1.0.84).

Results

Identification of male-specific volatile compounds released by C. psidii

Two methodologies (HS-SPME and DHS) were evaluated for the analysis of volatile compounds released by adult *C. psidii* (Table 1). Using DHS technique, a major number of substances were detected in male insects, with being papayanol (**6**) the greater constituent. The terpenes limonene and *β*-caryophyllene were detected from male and female guava weevils both with (DHS) and without (HS-SPME) the presence of host plant fruits. These compounds were present in all of the guava reproductive tissues, being quantitatively relevant in flower buds and fruit setting, the two guava stages where *C. psidii* is found.[11] Other terpenes, such as *α*-copaene, *α*-humulene and aromadendrene, were detected only by DHS and mainly on male insects (except for *α*-humulene).

Two male-specific volatile compounds (compounds **4** and **6**, $IK_{RTX\text{-}5} = 1192$ and 1280, $IK_{FFAp} = 1713$ and 1856, respectively) released by the guava weevil (*C. psidii*) were detected in a ratio of 1:9, respectively, during GC-MS analysis (Fig. 2). These results were obtained by collecting Colombian insects in different periods of year. EI-MS and CI-MS spectra of both compounds are shown in Fig. 3.

The major compound (**6**) was identified as (1*R*,2*S*,6*R*)-papayanol, by GC-MS co-injection with the synthetic enantiomeric pure sample (Fig. 4(A)), in agreement with those results reported by for Brazilian *C. psidii*.[12]

The minor male-specific compound **4**, showed a fragmentation pattern very similar to that of (1*R*,2*S*,6*R*)-papayanol by mass spectrometry (Fig. 3(B) and (D)), suggesting in the first instance the presence

Table 1. Volatile compounds released by male and female guava weevils, *C. psidii*.

				Volatile compounds released by *C. psidii*[c] (source and method)			
		RI [b]		Males insects		Females insects	
No	Compound[a]	RTX-5	FFAP	HS-SPME[d]	DHS[e]	HS-SPME[d]	DHS[e]
1	*β*-Pinene	994	1107	−	+	−	−
2	Limonene	1034	<1200	+	+	+	+
3	1,8-cineole	1038	1190	−	+	−	−
4	Papayanal	1192	1713	+	+	−	−
5	Decanal	1208	1480	+	+	−	−
6	Papayanol	1280	1856	++++	++++	−	−
7	*α*-Copaene	1387	−	−	+	−	−
8	*β*-Caryophyllene	1433	1594	+	+	++	++
9	*α*-Humulene	1468	1656	−	+	−	+
10	(+)-Aromadendrene	1476	1701	−	+	−	−

Notes: [a]Compound identification was based on retention index and mass spectra comparison with reference compounds.
[b]RI = retention index.
[c]Volatile relative peak area (%) as mean of three determinations:+ <5, ++ 5–10, +++ 10–15, ++++ >15%, − = not detected.
[d]Without food, 12 h.
[e]Feeding them by four days.

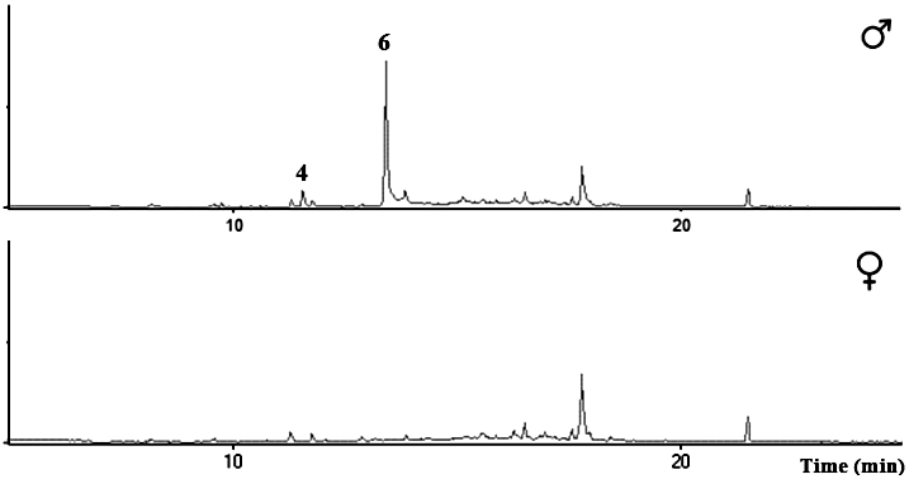

Fig. 2. GC/MS analysis of male-specific volatiles compounds in *C. psidii.*

Notes: Comparison of total ion chromatograms (RTX-5 column) of volatile compounds obtained from male and female *C. psidii* insects by HS-SPME, showing the two male-specific compounds (1*R*,2*S*,6*R*)-papayanal (**4**), and (1*R*,2*S*,6*R*)-papayanol (**6**) (numbering corresponds to Table 1).

of a diasteroisomeric compound. However, the ion fragment at *m/z* 167 $[M\text{-}H]^-$ in the CI negative mode mass spectrum (Fig. 3(A)) clearly evidenced that the molecular weight of this compound was 168 µ. Thus, the structure for compound **4** was suggested to be the 2,6-dimethyl-3-oxa-bicyclo[4.2.0]octane-2-carbaldehyde, an analog aldehyde derived from papayanol and therefore denominated as papayanal. The signals at *m/z* 139 $[M\text{-}29]^+$ and *m/z* 111 $[M\text{-}57]^+$ in the EI mass spectrum (Fig. 3(B)) correspond to the loss of formyl and acrolein (CH_2=CH–CHO) radicals from the molecular ion, respectively. The chemical structure of this compound was confirmed by GC/MS comparison with the synthetic enantiomerically pure standard (Fig. 4(B)).

Y-tube olfactometer bioassays

To evaluate the role of papayanol (**6**) and papayanal (**4**) as aggregation pheromones in *C. psidii*, behavioral responses of males and females to distinct treatments were performed in a Y-tube olfactometer (Fig. 5). In the first treatment, the response of insects to the mixture of male HSD extract and fruit setting guava extract showed significant differences in attractiveness to both sexes compared to the control treatment, with attractiveness being similar for males and females ($p < 0.05$). The other treatments showed that (1*R,*2*S,*6*R*)-papayanol significantly attracts both males and females ($p < 0.05$) at a concentration of 100 µg/mL and 10 µg/mL in hexane, but at 1 µg/mL only males were significantly attracted ($p < 0.05$). The last bioassay consisted of the

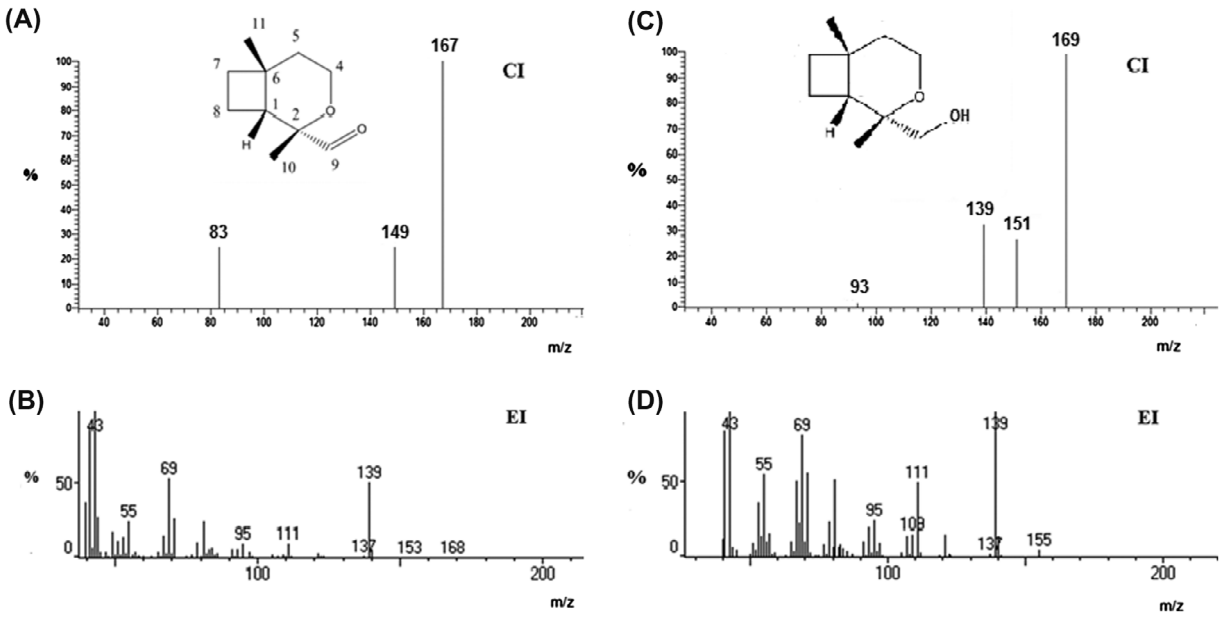

Fig. 3. Mass spectra and chemical structures of male-specific volatiles compounds in *C. psidii*.

Notes: Comparison of (A) CI-MS, and (B) EI-MS of (1*R*,2*S*,6*R*)-papayanal (**4**) with those of (C) CI-MS, and (D) EI-MS of (1*R*,2*S*,6*R*)-papayanol (**6**). CI-MS were recorded in negative mode.

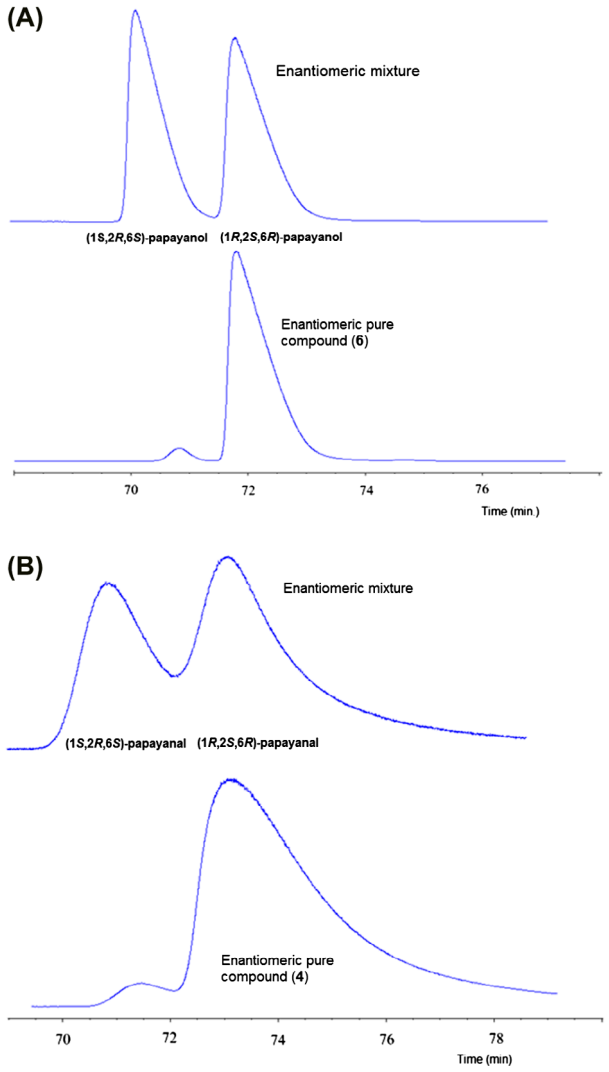

Fig. 4. Enantioselective GC analyses of synthetic (1*R*,2*S*,6*R*)-papayanol (A), and (1*R*,2*S*,6*R*)-papayanal (B), and their enantiomeric mixtures using a Astec Chiraldex™ B-PH column.

evaluation response to the synthetic mixture of (1*R*,2*S*,6*R*)-papayanol and (1*R*,2*S*,6*R*)-papayanal in the natural found ratio of 9:1 at a concentration of 10 µg/ml. Significant differences in attractiveness to both sexes when compared with the control treatment ($p < 0.05$) were found.

Discussion

Among two methodologies used to collect the volatile compounds released by *C. psidii* individuals, DHS (continuous extraction) allowed getting a major number of compounds and the extracts needed for olfactometer bioassays. The fact that male-specific volatiles, papayanol (**6**) and papayanal (**4**), were found by different methodologies confirmed that they are not artifacts from isolation procedure. The fact that limonene and *β*-caryophyllene were found in male as well female *C. psidii* addults, suggests the role of these compounds as kairomones with guava plant. These compounds are released by the guava fruits and also by *C. psidii* insects, thus mediating the communication among two species. In Coleoptera, sequestration of host compounds for later use as pheromones in their unmodified form appears to be rare.[17] Since pheromone production is often associated with feeding, it is experimentally difficult to distinguish between host compounds that are released by masticated food or feces, and those that are sequestered by the weevil and released later. However, kairomones coming from host plant (*P. guajava*) could help the *C. psidii* adults to establish the availability of a place to feed and lay their eggs.

As it was mentioned above, the major male-specific volatile of *C. psidii* (compound **6**) was identified as (1*R*,2*S*,6*R*)-papayanol and the minor compound (**4**) was suggested to be the corresponding aldehyde (1*R*,2*S*,6*R*)-papayanal. However, the synthesis of (1*R*,2*S*,6*R*)-papayanal was performed because this compound had not been reported in nature before. For this

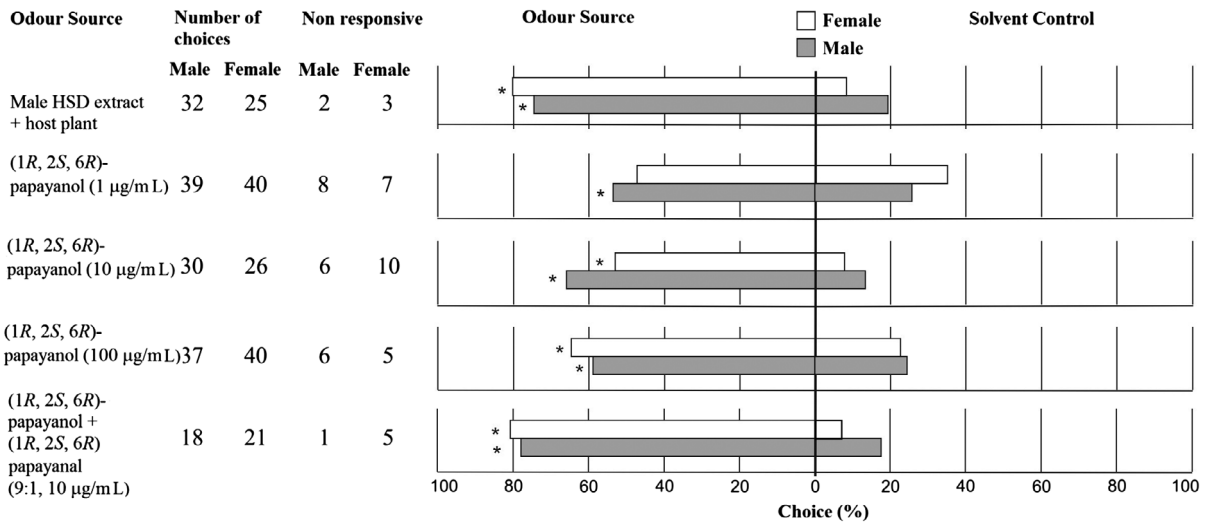

Fig. 5. Y-tube olfactometer responses of male and female *C. psidii* adults to different odour sources.

Notes: The asterisk (*) indicates a statistically significant preference for the binomial test (chi-square test, $p < 0.05$). Number of replicates is the sum of number of responsive and non-responsive choices of both, male and female individuals.

purpose, the use of ruthenium catalyst tetrapropylammonium perruthenate, in conjunction with N-methylmorpholine-N′-oxide as a mild oxidant agent[18] for the sensitive alcohol group in papayanol showed to be an efficient strategy. The ^{1}H and ^{13}C NMR data of papayanal resembled those of papayanol, except for the presence of carbonyl group (δ_C = 204 ppm; δ_H = 9.44 ppm) and the slight deprotection of adjacent protons H-1 and H_{exo} C-8). NOESY spectrum showed the correlation of CH_3C-10/H-1 (δ_H 1.21 and 1.87–1.93 ppm) and CH_3C11/H-1 (δ_H 1.12 and 1.87–1.93 ppm) indicating that both methyl groups are positioned on the same side of papayanal. The correlation of CH_3C-10/H_{ax}-4 (δ_H 1.21 and 3.59 ppm) and CH_3C-11/H_{ax}-4 (δ_H 1.12 and 3.59 ppm) also indicated a 1,3-diaxial relation of both methyl groups with the H-4 that is in axial position. The correlation of H_{eq}-4 with H_{eq}-5 (δ_H 3.77 and 1.37 ppm), and H_{endo} C-7 with H_{endo} C-8 (δ_H 1.47–1.55 and 1.90–2.00 ppm) confirmed the relative position of these protons. To determine the absolute configuration of papayanal, chiral gas chromatography was used. Comparison of the retention time of the synthetic sample with the racemic mixture revealed the natural product to be the enantiomerically pure (1*R*,2*S*,6*R*)-isomer (Fig. 4(B)).

The enantioselective biosynthesis of pheromones appears to be significant for the behavior and specific discrimination of insects.[19,20] Thus, despite the analysis of DHS extract of male *C. psidii* was not performed in the chiral column, the presence of enantiomeric pure papayanal is reinforced by biogenetic reasons. In two other Curculionidae species, papaya weevil (*Pseudopiazurus obesus*),[21] and banded pine weevil (*Pissodes castaneus*),[22] the mixture of (1*R*,2*S*)-(+)-grandisal and (1*R*,2*S*)-(+)-grandisol, aldehyde and alcohol, were identified as aggregation pheromones.

To confirm the role of (1*R*,2*S*,6*R*)-papayanol and (1*R*,2*S*,6*R*)-papayanal, Y-tube olfactometer bioassays were performed (Fig. 5). These results showed that both sexes of the guava weevil are attracted to male-produced volatiles, such suggesting the presence of male-produced aggregation pheromones. After identifying (1*R*,2*S*,6*R*)-papayanol and (1*R*,2*S*,6*R*)-papayanal as *C. psidii* male-produced volatiles, different concentrations of synthetic (1*R*,2*S*,6*R*)-papayanol were tested in the bioassay, as well as the mixture of synthetic (1*R*,2*S*,6*R*)-papayanol and (1*R*,2*S*,6*R*)-papayanal in the found natural ratio (9:1). For the case of (1*R*,2*S*,6*R*)-papayanol, the lowest concentration (1 µg/ml) tested showed a male-specificity in the attraction; in contrast, at the other concentrations (10 and 100 µg/ml) there were no preference for any sex, thus the role of this compound as aggregation pheromone in *C. psidii* was clearly confirmed. To this regard, it has been reported that the response of an insect to a specific volatile compound is significantly influenced by its concentration.[23] The study carried out by Palacio-Cortés et al.[12] with Brazilian population of *C. psidii* showed that synthetic papayanol (100 µg/ml) did not attract insects without host plant volatiles. However, the authors did not use papayanal in their treatments, contrasting with the results here presented for Colombian population. It is important to emphasize the possible synergistic effect of (1*R*,2*S*,6*R*)-papayanal over bioactivity of (1*R*,2*S*,6*R*)-papayanol to attract males (78.3%) and females (80.8%) of *C. psidii* without host plant volatiles (Fig. 5).

This is the first time that (1*R*,2*S*,6*R*)-papayanal is suggested to have a putative role as aggregation pheromone component in guava weevil, however more experimentation is needed to confirm this statement. Field studies to evaluate the effect of these male-specific *C. psidii* volatiles are also needed to better understand the potential of the male-produced volatile compounds, here reported, as monitoring strategy of this pest in guava.

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ORCID

Coralia Osorio http://orcid.org/0000-0001-6222-0138

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