Isopentyl Butanoate: Aggregation Pheromone of the Brown Spiny Bug, Clavigralla tomentosicollis *(Hemiptera: Coreidae), and Kairomone for the Egg Parasitoid* Gryon *sp. (Hymenoptera: Scelionidae)*

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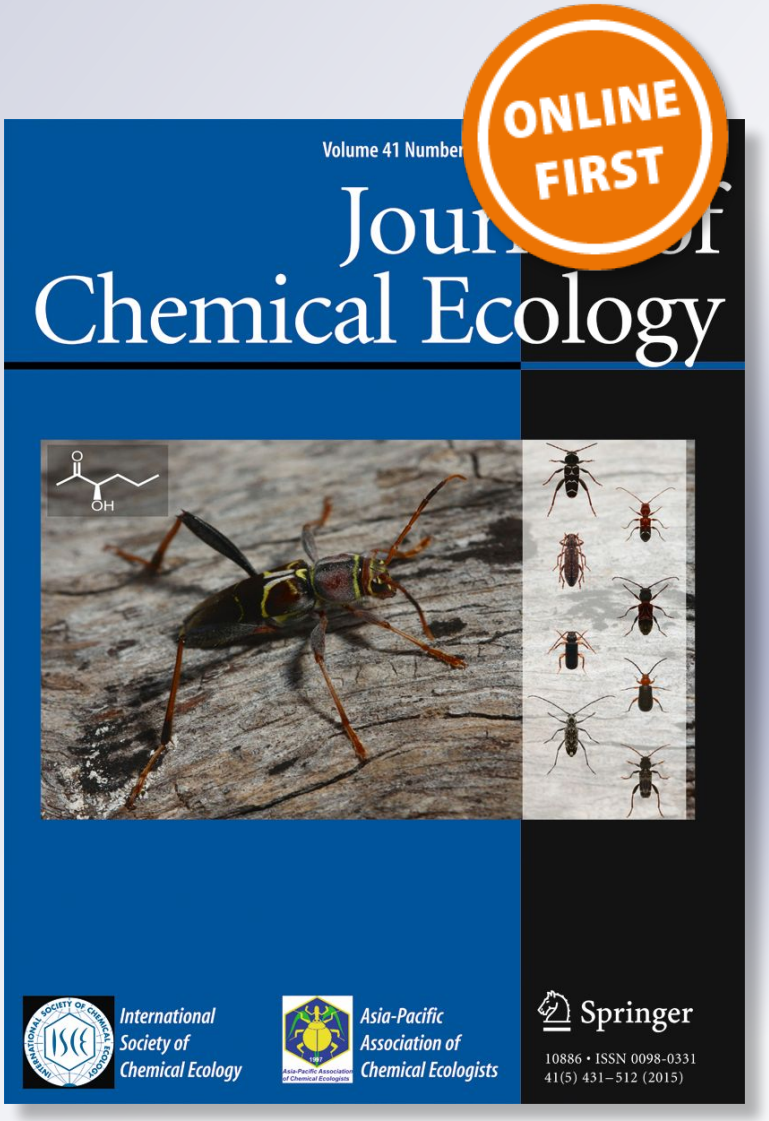

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Isopentyl Butanoate: Aggregation Pheromone of the Brown Spiny Bug, *Clavigralla tomentosicollis* (Hemiptera: Coreidae), and Kairomone for the Egg Parasitoid *Gryon* sp. (Hymenoptera: Scelionidae)

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Abstract

The brown spiny bug, *Clavigralla tomentosicollis* Stål (Hemiptera: Coreidae) is a key pest of leguminous crops in many countries in Africa, causing significant yield losses especially in cowpea, pigeon pea and common beans. Although *C. tomentosicollis* uses olfaction to aggregate, little is known about the identity of the aggregation pheromone. This study aimed to identify the aggregation pheromone of *C. tomentosicollis* and to test its potential role in the behavior of its egg parasitoid, *Gryon* sp. In Y-tube olfactometer bioassays, only male volatiles strongly attracted both sexes of *C. tomentosicollis*. Coupled gas chromatography/electroantennographic detection (GC/EAD) and GC/mass spectrometry were used to identify antennally-active compounds from male volatiles. Antennae of both sexes detected identical components including a male-specific component, identified as isopentyl butanoate, which was also detected by antenna of the egg parasitoid. In olfactometer bioassays, both sexes of *C. tomentosicollis* and the egg parasitoid responded to isopentyl butanoate. These results suggest that isopentyl butanoate serves as an aggregation pheromone for both sexes of *C. tomentosicollis* and a useful kairomone to attract the parasitoid in the management of *C. tomentosicollis*.

Keywords Aggregation pheromone · Biological control · *Clavigralla tomentosicollis* · *Gryon* sp. · Kairomone

Introduction

The brown spiny bugs (*Clavigralla* spp.) are key pests of leguminous crops including cowpea, pigeon pea and common bean in Africa, causing significant damage and yield losses of between 44 and 100% in these crops (Aliyu et al. 2007; Dabire et al. 2005; Dialoke et al. 2010). The high level of damage caused by a key species, *C. tomentosicollis,* is largely attributed to its aggregation behavior on host crops (Dabire et al. 2005; Egwuatu and Taylor 1976, 1977). Both nymphs and adults of *C. tomentosicollis* aggregate, with aggregation indices varying between 1.61 and 2.30, depending upon the insect stage (Dzemo and Asiwe 2010). This behavior suggests that olfaction may play a role in the aggregation of this bug, which if identified may lead to its semiochemical management. Like most spiny bugs, the life cycle of *C. tomentosicollis* involves eggs (2 to 99, laid on pods or leaves), which hatch (7–10 days) into nymphs (five nymphal instars), with a total developmental time of 20–21 days before emerging into adults. In the laboratory, adults can live up to 161 days (Dzemo and Asiwe 2010).

Previous studies have shown that *C. tomentosicollis* can be managed using the egg parasitoids *Gryon gnidus* (Nixon), *G. clavigrallae* (Mineo), and *G. fulviventris* Crawford (all Hymenoptera: Scelionidae) (Asante et al. 2000; Dreyer and Baumgärtner 1996; Taylor 1975). In addition, it has been demonstrated in olfactometer assays that the male-produced pheromone of *C. tomentosicollis* attracts *G. fulviventris* (Sanou et al. 2019). However, in

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this study the male volatiles eliciting attraction in the parasitoid were not identified. These observations suggest that semiochemicals may also be involved in parasitoid location of prey, which if identified could potentially be exploited to enhance parasitoid performance in the management of *C. tomentosicollis*.

The aim of this study was to investigate the olfactory basis of aggregation in *C. tomentosicollis* and to identify the aggregation pheromone. Additionally, we evaluated the responses of the egg parasitoid, *Gryon* sp. to the aggregation pheromone. To achieve this, we used behavioral, electrophysiological and chemical analyses.

Materials and Methods

Insects

Eggs, nymphs and adults, of *C. tomentosicollis* were collected on French beans and pigeon pea from six counties in Kenya: Makueni (01°52.621′ S, 037°42.793′ E), Machakos (1° 10.060′ S, 37° 27.287′ E), Embu (0° 40.532′ S, 37° 39.187′ E; 0° 44.847′ S, 37° 36.151′ E), Kitui (1° 18.155′ S, 38° 02.019′ E), Nakuru (0° 18.345′ S, 35° 59.224′ E; 0° 16.413′ S, 36° 7.172′ E) and Kisumu (0° 5.066′ S, 34° 52.478′ E), put into separate containers and then transferred to the laboratories of the International Centre of Insect Physiology and Ecology (*icipe*), Duduville campus, Nairobi, Kenya (1° 13.42′ S, 36° 53.82′ E; 1600 m a.s.l.). They were reared on young healthy pods of French bean (*Phaseolus vulgaris*, Fabaceae) in cylindrical clear plastic cages (18 cm diameter × 6.5 cm high) (Foodmate 2 l, Kenpoly, Nairobi, Kenya) with a ventilated lid. The cages were lined with a paper towel to absorb excess moisture as well as bug excretions. Each cage contained five healthy fresh pods of French bean. Thirty to forty adults were introduced into cages using a fine brush and an aspirator. Batches of 50 eggs that were laid on the absorbent paper were transferred to new cages of the same dimensions every 48 h, whereas any dead insects were discarded.

Egg batches collected from different localities in Kenya were incubated separately in sterile clear plastic cages (9.0 cm diameter × 4.5 cm height) (Foodmate 0.5 l, Kenpoly, Nairobi, Kenya) with ventilated lids. Emerged parasitoids, identified by molecular techniques to genus level as *Gryon* sp. (Khamis, pers. comm) were collected by means of an aspirator and introduced into a cage containing *C. tomentosicollis* eggs that were less than 48 h old. Parasitoids were fed on droplets of a 10% honey solution. All the rearing was conducted at 25 ± 1 °C and 60–70% RH with a photoperiod of 12:12 h (Light: Dark).

Olfactometer Assays

A Y-tube olfactometer (stem, 10 cm; arms, 23 cm each at 60° angle; internal diameter, 2.3 cm) was used to investigate the responses of adult males and females to volatiles released by conspecifics in a laboratory maintained at 25 ± 1 °C and 60–70% RH with a photoperiod of 12:12 h (Light: Dark). A battery-powered portable vacuum pump (assembled at the USDA/ARS-CMAVE, Gainesville, FL) was used to draw charcoal-purified clean air over odor sources before it entered the Y-tube. Airflow through each of the olfactometer arms was set at 174 ml min^{-1} (combined flow 348 ml min^{-1}).

The following odor treatment combinations were tested: (1) blank vs. blank, (2) male vs. blank, (3) female vs. blank, and (4) male vs. female. To account for the possible effects that volatile sources could have on insect response, all tests were conducted using a group of 5, 10 and 20 adult individuals of the same sex, respectively as odor sources. Thirty 7–8-day old adults (27–28 days old after hatching) (30 replicates) of each sex were tested and the odor source was changed after 10 replicates. The position of the arms containing treatment and control odors was changed after every five tested individuals to avoid any positional bias. The Y-tube was cleaned with liquid soap and water, rinsed with acetone and then with distilled water and dried in an oven at 100 °C for 3 h after every five replicates.

One male or female was introduced into the Y-tube via the entrance of the stem, and the choice made by the individual recorded after 10 min. When an individual entered one of the arms, its response was recorded. When the insect moved further than 5 cm into one of the arms within a period of 10 min and spent at least 30 s in the arm of the olfactometer, it was considered a valid choice. However, if the insect exited the arm before moving 5 cm into the selected arm and then moved to the other arm and spent at least 30 s there, the latter was considered as its choice. All the experiments were conducted between 09:00 and 16:00, which corresponds to the period when adults are most active in the field (Dreyer et al. 1994).

Collection of Volatiles

The headspace volatile collection system used for collecting volatiles from mature green berries was the same as that described by Njihia et al. (2017). Three quick-fit glass jars (250 ml each) (Sigma Scientific, Gainesville, FL, USA), containing either sexually mature males (20; 7–8 days old), females of similar age or no insects (control) were used. No food was provided inside the containers. At a flow rate of 260 ml min^{-1} charcoal-purified air was passed over the jar containing the insects and through a previously cleaned Porapak Q filter (30 mg, mesh size 80–100, Supelco, Bellefonte, PA) for 24 h. Volatiles adsorbed on the Porapak Q filters were each eluted with 200 μl dichloromethane

(Analytical grade, Sigma Aldrich, St. Louis, MO) and then stored at −80 °C until use.

Chemical Analyses

Samples were analysed by coupled gas chromatography/electroantennographic detection (GC/EAD) analysis using antennae of *C. tomentosicollis* males and females (7–8 days old adult) as well as those of *Gryon* sp. The GC/EAD used a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with a HP-5 MSI column (30 m × 0.25 mm diameter × 0.25 μm film thickness; Agilent, Technologies, lnc., California, USA), with nitrogen as the carrier gas at a flow rate of 1.2 ml min^{-1}. Volatiles were analysed in the splitless mode at an injector temperature of 280 °C and a split valve delay of 3 min. The oven temperature was held at 35 °C for 3 min, programmed at 10 °C min^{-1} to 280 °C and maintained at this temperature for 10 min. The column effluent was split 1:1 for simultaneous detection by a flame ionization detector (FID) and EAD. The antennal preparation was made by filling in two sharpened glass capillaries with Ringer saline solution (Kugel 1997). One of the capillaries was inserted into the excised head/pro-thorax. The distal end of the antenna was then placed in a saline filled electrode. The antennal signal was detected through an amplifier (Syntech, Hilversum, The Netherlands), which was acquired and processed by an IDAC-4 data acquisition controller (Syntech, Hilversum, The Netherlands) and later analysed with EAG 2000, GC/EAD software (Syntech) to generate simultaneous FID and EAD signals on a computer. Aliquots (3 μl) of volatile samples and commercially purchased synthetic EAD-active compounds dissolved in dichloromethane were analysed. EAD responses were considered positive when three or more positive responses to the same sample were recorded.

Male, female and control volatiles (1 μl each), were analyzed by coupled gas chromatography/mass spectrometry (GC/MS) on an Agilent Technologies lnc. Series A 7890 GC coupled to a 5977A MS (inert XL/EI/CI MSD) triple axis mass detector, equipped with a HP-5 low bleed capillary column (30 m × 0.250 mm i.d., 0.25 μm) (J&W, Folsom, CA, USA) in the electron impact mode at 70 eV. The GC oven temperature was 35 °C for 5 min with a rise of 10 °C min^{-1} to 280 °C for 10.5 min, then 5 °C min^{-1} to 285 °C and held at this temperature for 9 min. Identification of compounds was done by comparison of mass spectral data with library data; Adams2, Chemecol and NIST11. In addition, the identities of several compounds were confirmed using mass spectral data and retention times of synthetic compounds where available. Quantification was based on calibration curves (peak area vs. concentration) generated from authentic standards of identified compounds. The experiments were replicated three times.

Olfactometer Assays with Male-Specific Compound

Y-tube olfactometer assays described earlier were used to evaluate EAD-active compounds. The responses of both sexes of *C. tomentosicollis* and the parasitoid *Gryon* sp. were evaluated in the assays. In the bioassays with the parasitoid, airflow through each of the olfactometer arms was maintained at 85 ml min^{-1}. The female parasitoids used in bioassays were 1–2 days old. The tests conducted were: (1) solvent vs. solvent, (2) isopentyl butanoate vs. solvent. The naturally-occurring dose of isopentyl butanoate, 152 ng μl^{-1}, was tested and then higher and lower concentrations of 304 ng μl^{-1} (obtained by doubling the natural dose) and 76 ng μl^{-1} (half the natural dose) were used to test the response of females and males of *C. tomentosicollis*, and that of *Gryon* sp. The natural release rate of isopentyl butanoate by one insect in an hour is 63.3 ng h^{-1}. Ten microliters (10 μl) of sample equivalent to each concentration were applied onto 2 cm × 2 cm pieces of filter paper (Whatman filter N°1) by means of a syringe and air dried for 30 s. Controls consisting of dichloromethane (10 μl) were prepared similarly. Control and treated filter papers were then placed separately into the olfactometer arms. The treated filter paper was changed after every five replicates.

Chemicals

2-methylbutanoic acid, isopentyl butanoate, 2-methyl-2-methylbutyl butanoate, 3-methyl-2-methylbutyl butanoate were all purchased from Sigma Aldrich, Germany, with purity ≥98%. Dichloromethane, 2-methylpropanoic acid, 6-methyl-5-hepten-2-one, limonene, acetophenone were all purchased from Sigma Aldrich, Germany (purity ≥95%).

Data Analyses

All statistical analyses were performed in R software version 3.1.2 (R Core Team 2012) at 5% significance level. Chi-square (χ^2) analyses were used to determine (1) the significant difference of *C. tomentosicollis* male and female choices for conspecific volatiles against the respective controls and between volatiles of the different sexes, (2) the comparative response of male and female of *C. tomentosicollis* and egg parasitoid *Gryon* sp. to the three different doses of isopentyl butanoate against the respective controls. The total number of replicates used in these bioassays was 30 per experiment, but only the respondents (n) were considered in the analysis.

Results

Olfactometer Assays

The responses of males and females to the control treatments in the Y-tube were not significantly different ($P > 0.05$). Both males and females were significantly attracted to volatiles of the group of 20 males compared to the control ($P < 0.05$) (Fig. 1c). In paired assays, both sexes were more attracted to odors released from the group of 20 males than to the group of 20 females ($P < 0.05$) (Fig. 1c). There were no significant differences between male and female responses when tested against odors from the group of 20 females and the control ($P > 0.05$). Likewise, no preference was observed in these experiments (except a response of female) when males and females were provided with a choice between the odors from a group of five or ten males or females ($P > 0.05$) (Figs.1a and b). Nonetheless, females were strongly attracted to the volatiles from the group of ten males compared to the control treatment ($P < 0.05$) (Table 1).

Chemical Analyses

Chemical analysis of the headspace volatiles of *C. tomentosicollis* males and females showed similar profiles (Fig. 2). However, differences were both quantitative and qualitative, with the identification of a male-specific component identified as isopentyl butanoate and confirmed with an authentic standard (Fig. 2). Common components identified in male and female volatiles are described in the Table 2. The identities of seven of these components; 6-methyl-5-hepten-2-one, acetophenone, limonene, isopentyl butanoate, 2-methyl-

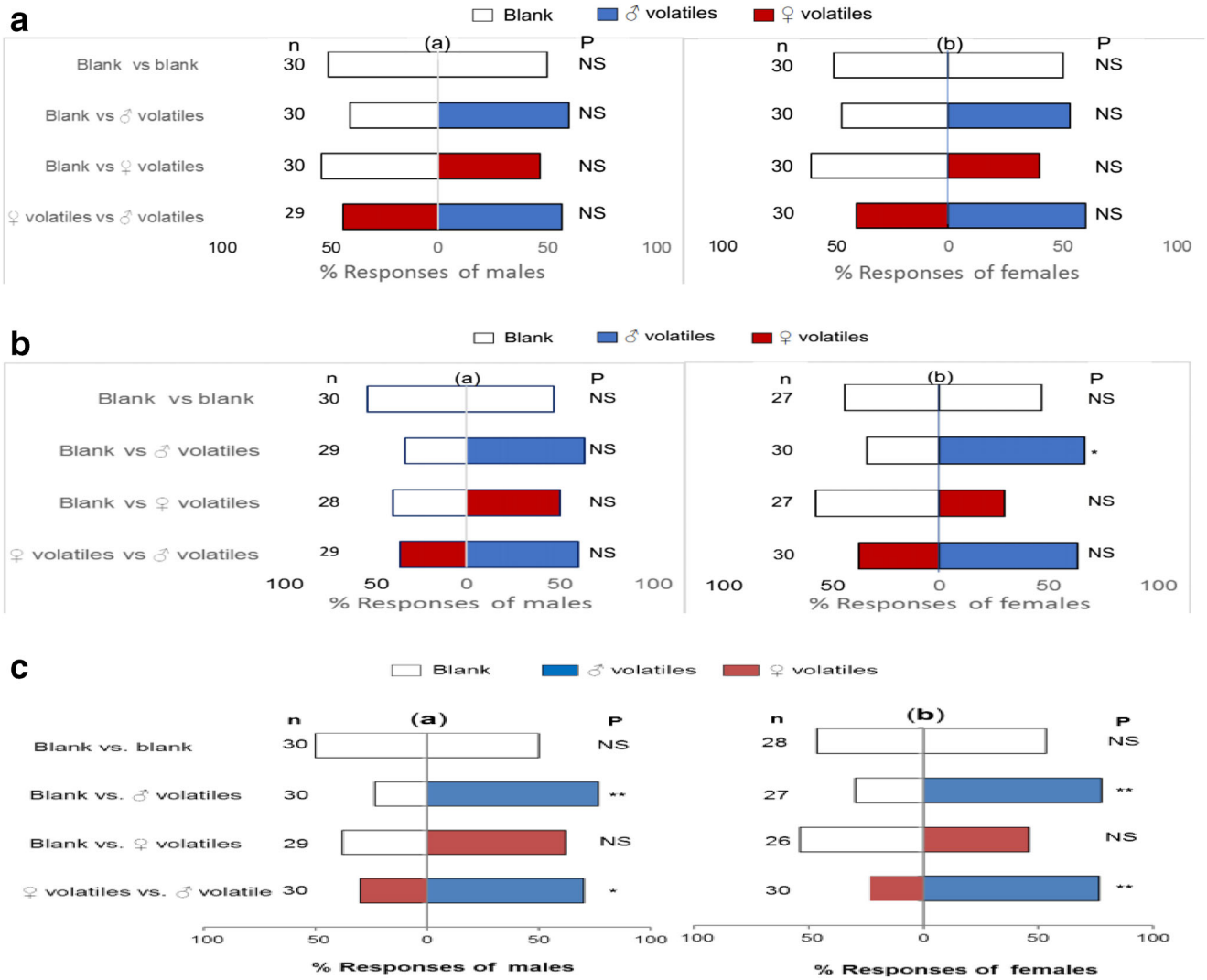

Fig. 1 Olfactometer responses of *Clavigralla tomentosicollis* males and females to their conspecific volatiles. **a** group of 5 individuals, **b** group of 10 individuals, **c** group of 20 individuals. (**a**) = responses of males, (**b**) = responses of females. Thirty adult males/females (7–8 days old) were tested individually for choice between blank and odors from different groups of conspecific males or females. Asterisks indicate significant difference levels: $*P < 0.05$, $**P < 0.01$. n = number of choices, P = probability, NS = non-significant

Table 1 Behavioral responses of *Clavigralla tomentosicollis* males and females subjected to conspecific volatiles of three groups of different sizes

		Male choice			Female choice		
Different groups	Tests	χ^2	*df*	*P* value	χ^2	*df*	*P* value
	Blank vs. Blank	0	1	1	0.1333	1	0.715
Group of 5 individuals	Males vs. Blank	1.6897	1	0.193	0.0344	1	0.852
	Female vs. Blank	0.3103	1	0.577	1.2	1	0.273
	Male vs. Female	0.5333	1	0.465	0.5714	1	0.449
Group of 10 individuals	Male vs. Blank	1.96	1	0.161	3.8462	1	0.049
	Female vs. Blank	0.1666	1	0.683	2.4615	1	0.116
	Male vs. Female	0.6666	1	0.414	1.8148	1	0.177
Group of 20 individuals	Male vs. Blank	8.5333	1	0.003	4.4815	1	0.034
	Female vs. Blank	1.6897	1	0.193	0.1538	1	0.694
	Male vs. Female	4.8	1	0.028	8.5333	1	0.003

χ^2 = Chi-square, *df* = Degrees of freedom

2-methylbutyl butanoate, 3-methyl-2-methylbutyl butanoate, 2-methylbutanoic acid, were confirmed using authentic standards, with the remaining identified from mass spectral library data only. The retention times and mass spectra of compounds identified, and authentic standards used for confirmation were exact matches.

In GC/EAD assays, the male-specific compound and its authentic standard, isopentyl butanoate, elicited antennal detection in both sexes of *C. tomentosicollis* and females of the parasitoid *Gryon* sp. (Fig. 3).

Olfactometer Assays with Male-Specific Compound

The olfactory responses of *C. tomentosicollis* males and females and the parasitoid *Gryon* sp. to the solvent controls were not significantly different ($P > 0.05$) (Fig. 4a, b and c). Dose-response assays using the male-specific compound isopentyl butanoate showed that males and females were significantly attracted to similar concentrations ($P < 0.05$) (Fig. 4a and b). Furthermore, only females and the parasitoid were significantly attracted to isopentyl butanoate at the lower dose of 76 ng μl^{-1} (equivalent to half the naturally-occurring concentration) ($P < 0.05$) (Fig. 4b). The parasitoid *Gryon* sp. was attracted to the naturally-occurring concentration of isopentyl butanoate (Fig. 4c), but not to the higher concentration (304 ng μl^{-1}) ($P > 0.05$) (Table 3).

Discussion

Our results show that *C. tomentosicollis* males and females are strongly attracted to the volatiles released by males, indicating that males release olfactory cues that act as an aggregation pheromone for both sexes. These results agree with previous studies on certain Hemipterans, for example, *Plautia stali* Scott (Hemiptera: Pentatomidae) and *Triatoma infestans*, Klug (Hemiptera: Reduviidae), which showed differential attraction of both sexes to male odors (Guerenstein and Guerin

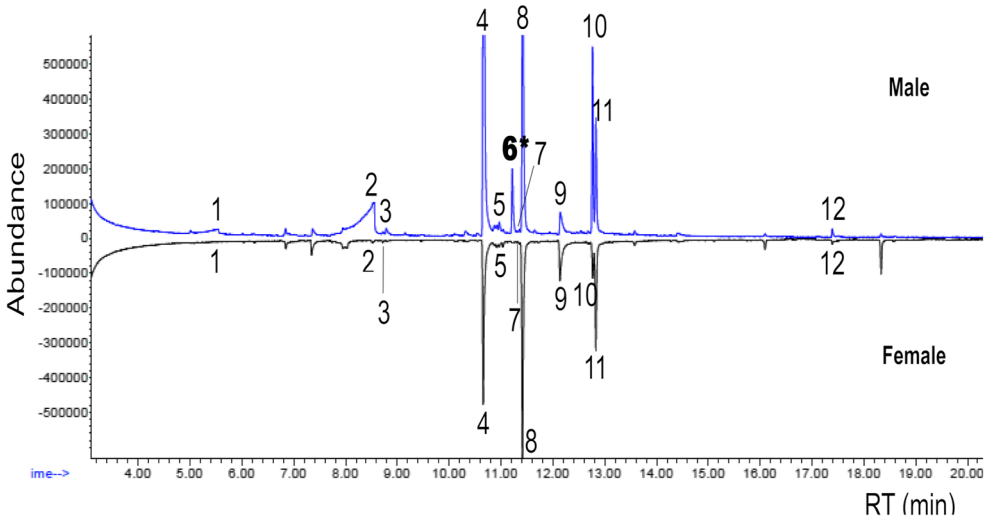

Fig. 2 Chemical profiles of *Clavigralla tomentosicollis* male and female volatiles with the male-specific compound, isopentyl butanoate* highlighted in bold, RT = retention time

Table 2 Compounds detected by means of GC-MS analysis of headspace volatiles of *Clavigralla tomentosicollis* males and females

Peak #	Retention time (min)	Compounds	Category	Retention index	Male	Female
1	5.52	2-Methyl propanoic acid	Aliphatic acid	768	+	trace
2	8.41	2-Methyl butanoic acid	Aliphatic acid	882	+	+
3	8.86	Nonane	Hydrocarbon	900	+	+
4	10.66	6-Methyl-5-Hepten-2-one	Ketone	980	+	+
5	10.97	2-Methylpropyl 2-methylbutanoate	Ester	993	+	trace
6*	**11.22**	**Isopentyl butanoate***	**Ester**	**1012**	+	–
7	11.35	*p*-Cymene	Monoterpene	1013	+	trace
8	11.42	Limonene	Monoterpene	1017	+	+
9	12.14	Acetophenone	Ketone	1057	+	+
10	12.76	2-Methyl-2-methylbutyl butanoate	Ester	1090	+	+
11	12.83	3-Methyl-2-methylbutyl butanoate	Ester	1097	+	+
12	17.40	α-Cedrene	Monoterpene	1409	+	+

A group of 20 *C. tomentosicollis* males/females (7 to 8 days old adults) was used for volatile collection. (+) = present = (−) absent, (**6***) = male-specific compound identified, and which elicited electrophysiological responses in males and females of *C. tomentosicollis* and *Gryon* sp.

2004; Jang et al. 2011). Moreover, a study on *Pristhesancus plagipennis* Walker (Hemiptera: Reduviidae) showed that both sexes are attracted to volatiles released from the male dorsal abdominal glands (James et al. 1994). Although it is less common in hemipterans for females to produce the aggregation pheromone, in *Neomegalotomus parvus* Westwood (Hemiptera: Coreidae) females were found to produce an aggregation pheromone which attracted both sexes (Laumann et al. 2012). These results indicate that the production and release of aggregation pheromones in hemipterans is both species- and sex-dependent.

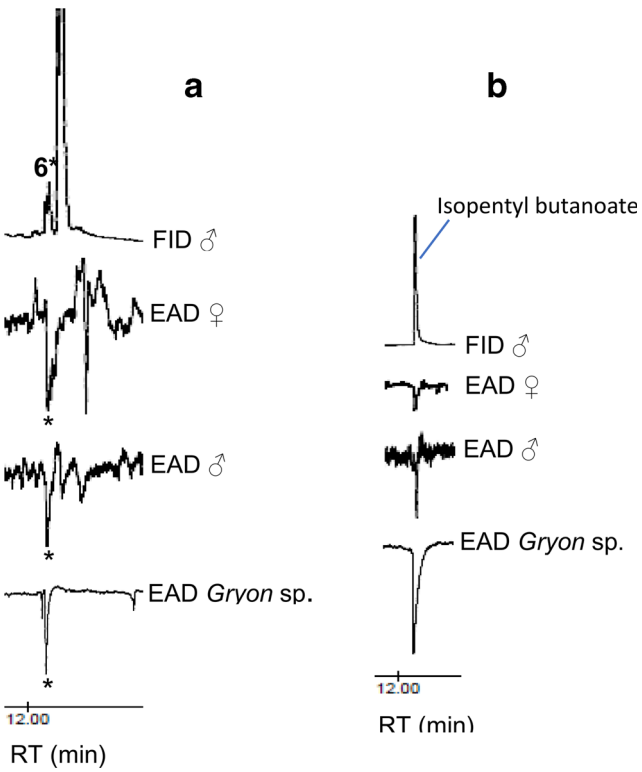

Fig. 3 Electroantennograms showing the male-specific compound, isopentyl butanoate (**6***) detected by *Clavigralla tomentosicollis* males and females, and parasitoid in the male crude volatiles. (**a**) and synthetic standard identified from male adult volatiles (**b**). RT = retention time

Our results also demonstrate that aggregation pheromones are only effective when a critical number of individuals are present. In the present study we found that the volatiles released by 20 males acted as an aggregation pheromone for both sexes. This result corroborates a previous study which showed that the aggregation response of both sexes of the southern chinch bug, *Blissus insuhris* Barber (Hemiptera: Lygaeidae) to conspecific volatiles increased with increasing number of the bugs (Addesso et al. 2012).

On the other hand, we found that females responded more strongly to male volatiles when provided with a choice of ten males and control. This suggests that male odors may also serve as a potential sex pheromone for females depending upon the dose of the pheromone. Previous studies showed that females were attracted to male volatiles of *Pellaea stictica* Dallas (Hemiptera: Pentatomidae) (Fávaro et al. 2015) and *Edessa meditabunda* Fabricius (Hemiptera: Pentatomidae) (Zarbin et al. 2012).

Chemical analysis showed both quantitative and qualitative differences in the emission profiles of male and female volatiles. The major compounds identified included ketones, monoterpenes, esters and aliphatic acids. These classes of compounds are commonly associated with many insect orders including the Hemiptera (Aldrich 1975; Audino et al. 2007; Guerenstein and Guerin 2004; James et al. 1994; Yusufoglu et al. 2018). A previous study reported the presence of isopentyl butanoate in the volatiles of male *Triatoma infestans* Klug (Hemiptera: Reduviidae), but its role in the behavior in this insect was not established (Audino et al. 2007). In our

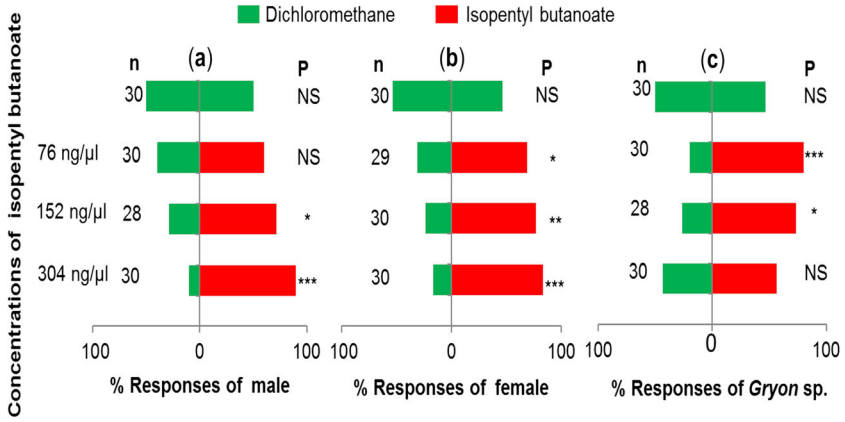

Fig. 4 Olfactometer responses of male and female *Clavigralla tomentosicollis* and *Gryon* sp. to different doses of the synthetic compound isopentyl butanoate. **a** responses of males, **b** responses of females and **c** responses of *Gryon* sp. Thirty *C. tomentosicollis* adult males/females (7–8 days old) and 30 females *Gryon* sp. (1–3 days old) were tested individually for choice between three doses of isopentyl butanoate solution and dichloromethane. Asterisks indicate significant differences: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. n = number of choices, P = probability, NS = non-significant

GC/EAD analysis of male volatiles, antennae of both sexes of *C. tomentosicollis* detected similar components, including the male-specific compound isopentyl butanoate. Interestingly, isopentyl butanoate was also detected by the egg parasitoid *Gryon* sp. The fact that both sexes of *C. tomentosicollis* and the egg parasitoid detect isopentyl butanoate suggests that it may play a role in the aggregation/sex communication observed with the living insects and host location by the parasitoid. Other components detected in the male volatiles may play a role, for example, in enhancing intra-and inter-specific communication when combined with or without isopentyl butanoate, which would require additional research.

In the olfactometer studies with isopentyl butanoate, as found with the odors of living insects, male and female responses were dose dependent, with both sexes responding to the naturally-occurring concentration of the compound released by males in their emission profile. The fact that only females responded to half the naturally-occurring concentration of isopentyl butanoate, agrees with our previous results with odors of living insects, and confirms the dual role that male volatiles play in the behavior of both sexes of *C. tomentosicollis*; as an aggregation pheromone for both sexes and sex pheromone for females depending upon the concentration. It is also noteworthy that at higher emission profiles with living insects and concentration of isopentyl butanoate, both sexes of *C. tomentosicollis* were attracted, which suggests that in the behavioral context, isopentyl butanoate could be used as an attractant/aggregation cue in both sexes and mate recognition for females of this species. Interestingly, isopentyl butanoate has been identified as a component of some plant volatiles. For example, it has been demonstrated that mashed banana volatiles containing isopentyl butanoate attracted both males and females of the scarab beetle *Pachnoda interrupta* Olivier (Coleoptera: Scarabaeidae; Wolde-Hawariat 2008). Future studies should explore whether host plants of *C. tomentosicollis* also contain isopentyl butanoate.

In our study, the parasitoid *Gryon* sp. responded to isopentyl butanoate in a dose-dependent manner; responding strongly to the naturally-occurring concentration and half of

Table 3 *Gryon* sp. and *Clavigralla tomentosicollis* male and female behavioral responses to different doses of isopentyl butanoate in olfactometer bioassays

Doses assays	Male choice			Female choice			*Gryon* sp. choice		
	χ^2	*df*	*P* value	χ^2	*df*	*P* value	χ^2	*df*	*P* value
Dichloromethane vs. Dichloromethane	0.1333	1	0.715	0	1	1	0.0344	1	0.852
Concentration 1 vs. Dichloromethane	1.2	1	0.273	4.1724	1	0.041	10.8	1	0.001
Concentration 2 vs. Dichloromethane	5.1429	1	0.023	8.5333	1	0.003	6.5333	1	0.010
Concentration 3 vs. Dichloromethane	19.2	1	<0.001	13.333	1	<0.001	0.5333	1	0.465

χ^2 = Chi-square, *df* = Degree of freedom. Concentration 1, 2 and 3 are the different concentrations used (concentration 1 = 76 ng μl^{-1}, concentration 2 = 152 ng μl^{-1}, concentration 3 = 304 ng μl^{-1})

the concentration of isopentyl butanoate, but not at higher concentrations, as observed for females of *C. tomentosicollis*. These results suggest that responses shown by both the parasitoid and females are ecologically relevant. At low doses, whereas females may utilize this compound for sex attraction, the parasitoid on the other hand may associate detection of the compound with both the presence of females and their eggs. It is possible that egg-associated chemicals (volatiles and cuticular components) may contribute to parasitoid location of eggs, which would require further studies. Nonetheless, our results also suggest that the olfactory systems of females of *C. tomentosicollis* and the parasitoid are fine-tuned to detect isopentyl butanoate. Indeed, a recent study showed that the volatiles from a group of *C. tomentosicollis* males were attractive to *G. fulviventris* females when they were given a choice between male volatiles and control/host plant (cowpea) volatiles (Sanou et al. 2019). Our results indicate that isopentyl butanoate could be the male-produced aggregation pheromone eliciting attraction in *G. fulviventris* females. It would be interesting to investigate whether different species of *Gryon* utilize the same or different pheromones to locate *C. tomentosicollis* and other related hemipterans. This finding lends support to previous studies that report the use of prey volatiles by hymenopterans to locate their hosts (Aldrich and Zhang 2002; Fatouros et al. 2008; Maruthadurai et al. 2011; Yasuda 1998). For example, in field evaluation studies of male and female volatiles of *Leptoglossus australis* Fabricius (Heteroptera: Coreidae), only traps baited with male volatiles attracted the parasitoid *Gryon pennsylvanicum* Ashmead (Hymenoptera: Scelionidae; Yasuda 1998). In similar field studies, the parasitoid *Telenomus calvus* Johnson (Hymenoptera: Platygastridae) showed a clear preference for male volatiles of *Podisus maculiventris* Say (Hemiptera: Pentatomidae; Fatouros et al. 2008). Furthermore, the egg parasitoid *Trissolcus brochymenae* Ashmead (Hymenoptera: Platygastridae) was found to exploit male volatiles of *Murgantia histrionica* Hahn (Hemiptera: Pentatomidae) as an attractant to locate females and to find their eggs (Conti et al. 2003).

In summary, we show that olfaction plays a key role in the aggregation of *C. tomentosicollis*. We identified several classes of compounds in both sexes of *C. tomentosicollis*, with the ester isopentyl butanoate as specific to male volatiles. Furthermore, we identified isopentyl butanoate as an aggregation pheromone for both sexes of *C. tomentosicollis* and a kairomone for the egg parasitoid *Gryon* sp. These results, therefore, provide the fundamental baseline information required for field evaluations of isopentyl butanoate in the management of *C. tomentosicollis*.

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Author Contributions HK, JVB, FK, MT, and BT conceived and designed the research. HK conducted experiments and analysed data. HK, JVB, FK, MT, and BT wrote the manuscript. EJT gave us support in the morphological identification of *Gryon* sp. All authors edited the manuscript and approved the final version.

Compliance with Ethical Standards

Conflict of Interest Authors declare no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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