

## Research Article

# Volatile Chemicals of Adults and Nymphs of the *Eucalyptus* Pest, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae)

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*Thaumastocoris peregrinus* is an introduced “true bug” that is now a severe pest in *Eucalyptus* plantations of various Southern Hemisphere countries. The semiochemicals of thaumastocorids are completely unknown. Therefore, volatile chemicals from *T. peregrinus* nymphs and adults were identified as possible leads for pheromones potentially useful for control. The contents of nymphal exocrine glands, which are shed at molting, were identified from extracts of exuviae. Adults lack functional metathoracic scent glands that are characteristic of most heteropterans; however, both males and females possess a glandular-appearing hold-fast organ that they quickly extrude posteriorly when disturbed. Whole body hexane extracts from males and females were prepared by freezing the insects in a flask so that they extruded the hold-fast organ, and then they were extracted with hexane. Volatiles from nymphal exuviae included benzaldehyde, octanol, (*E*)-2-octenol, octanoic acid, decanal, and hexanoic acid. Adult volatiles included 3-methylbut-2-en-1-yl butyrate and 3-methylbut-3-en-1-yl butyrate.

## 1. Introduction

*Thaumastocoris peregrinus* Carpintero and Dellapé (Heteroptera: Thaumastocoridae) is an introduced pest of nonnative *Eucalyptus* plantations in various countries in Southern Hemisphere (e.g., South Africa, Argentina, Uruguay, and Brazil) [1–3]. In 2005, it was first found in Buenos Aires, Argentina, on *Eucalyptus viminalis*, *E. tereticornis*, and *E. camaldulensis* [1]. In Brazil, *T. peregrinus* was first found in 2008, on a hybrid clone of *E. grandis* × *E. urophylla* in São Francisco de Assis, Rio Grande do Sul, and on *E. camaldulensis* trees in Jaguariaúna, São Paulo [4]. Initial studies on life history of *T. peregrinus* were done in Australia [5]; however, no investigation was performed on semiochemicals from these insects.

Heteropteran nymphs and adults characteristically produce allomones for defense; typically, the defensive secretions

of nymphs are produced in dorsal abdominal glands (DAGs) [6]. The contents of DAGs are shed along with the exuviae each time the nymph molts, and extraction of exuviae is a convenient method to obtain the DAG secretion [7]. Adult heteropterans characteristically possess metathoracic scent glands from which they release irritating secretions [6]. However, examination of *T. peregrinus* adults by one of us (JRA) revealed that the metathoracic glands are vestigial (unpublished data). On the other hand, adults and nymphs of these unusual bugs possess a rectal organ, similar to that described for plant bugs (Miridae) [8] that is everted when the insects are disturbed. The *Thaumastocoris* rectal organ has a glandular appearance and instantly sticks the insects to the substrate when the insects are disturbed and can be quickly released (JRA, personal observation) (Figure 1). Pheromones are known for members of several heteropteran families [9], but the semiochemicals of *T. peregrinus* and

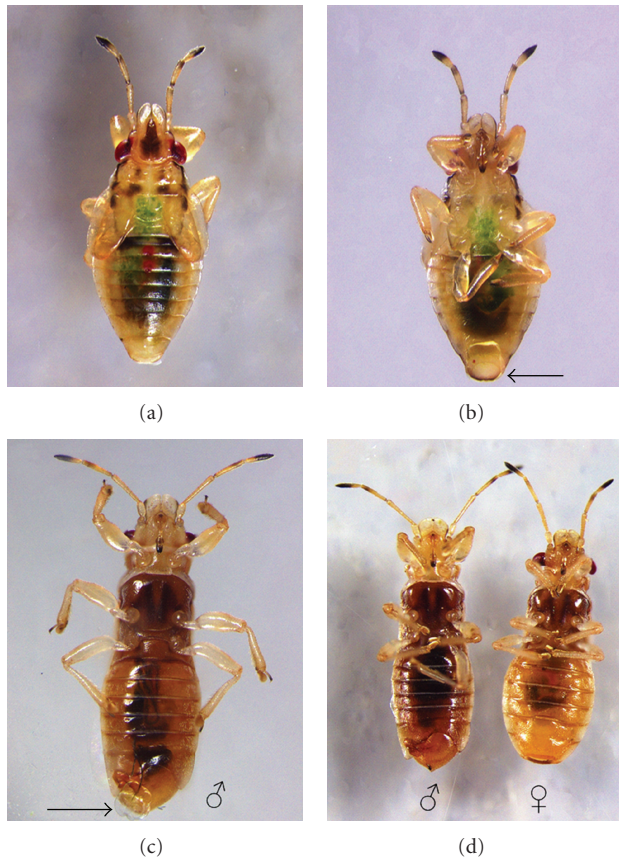


FIGURE 1: *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae) male, female, and nymph. (a) Dorsal view of a 5th-instar nymph; (b) nymphal ventral view showing the rectal organ (arrow); (c) ventral view of the male showing the everted rectal organ (arrow); (d) male and female ventral view with the rectal organ not exposed.

other thaumastocorids are completely unknown. Therefore, the volatile chemical compounds present in the exuviae for the five nymphal instars, and both adult sexes were identified and quantified.

## 2. Materials and Methods

**2.1. Insect Rearing and Extractions.** Insects were obtained from a colony at EMBRAPA Florestas, Colombo, Paraná, maintained in the Laboratório de Semioquímicos, Departamento de Química of the Universidade Federal do Paraná (UFPR) under controlled laboratory conditions of  $25^{\circ}\text{C} \pm 2$  and 12 L: 12 D. Adults and nymphs were reared on branches of *Eucalyptus benthamii* in acrylic boxes (30 cm  $\times$  30 cm  $\times$  30 cm) until their use. To obtain exuviae and virgin adults for the extractions, nymphs of each instar were held individually in small round plastic containers (2.5 cm of diameter) with a gel (Hydroplan—EB/HyC, SNF S.A. Floger) in the bottom of the container for moisture and a leaf disc of *E. benthamii* on the gel. Leaf discs were changed every other day. After nymphs molted, exuviae were collected for extraction, and recently emerged males and females were isolated. Males

or females of the same emergence date were grouped in Petri dishes (5 cm of diameter) containing gel and a leaf disc until the extraction. Fifth instar nymphs were grouped in cages provisioned as above to obtain mated males and females for extraction. Couples were formed within 2 days of emergence, and extractions of adults were performed only after eggs were present, which confirmed the mated status of adults.

**2.2. Extraction of *T. peregrinus* Exuviae (1st–5th Instar).** Exuviae were extracted with 180  $\mu\text{L}$  of hexane for 24 hours. Each extraction was made with the exuviae available in that day, with a minimum of 12 and maximum of 24 exuviae. At least three repetitions were made for each instar, consisting of at least 45 exuviae in total. After extraction, tridecane (ca. 10 ppm) was added to each sample as an internal standard (IS); the final concentration of the IS was calculated for each extract. Extracts were concentrated and analyzed using a gas chromatograph (GC-2010—Shimadzu) and a gas chromatograph coupled with a mass spectrometer (GC-MS-QP 2010 Plus—Shimadzu). The detected compounds were quantified based on the area of the IS. The GC was equipped with a RTX-5 column (30 m  $\times$  0.25 mm i.d. and 0.25 mm film thickness; Restek, Bellefonte, PA, USA). One  $\mu\text{L}$  of extract was injected into the GC using the splitless mode with injector temperature at  $250^{\circ}\text{C}$ . The column oven temperature was maintained at  $50^{\circ}\text{C}$  for 1 min, then raised to  $250^{\circ}\text{C}$  at a rate of  $7^{\circ}\text{C}/\text{min}$ , and maintained in  $250^{\circ}\text{C}$  for 10 min. Helium was used as carrier gas at a column head pressure of 170 kPa. The same parameters were used for all analyses.

**2.3. Extraction of *T. peregrinus* Adults.** Extractions were made with mated *T. peregrinus* males and females of different ages (3–9, 10–21, 22–34 days old), according to availability of insects. Quantified extracts were compared for virgin males and females (3–9 days), virgin and mated males (10–21 and 21–34 days old), and mated males and females (10–20 and 21–34 days old). There were at least two repetitions per treatment, with a minimum of 15 insects extracted in total. In both experiments, insects were separated by sex in glass Erlenmeyer flasks. The flasks with insects were put in a freezer for one hour so that they died with the rectal organ exposed while “glued” to the glass. Thus, the adults were extracted as complete adults with their rectal organ exposed. The extraction was made between 11:00 AM and 16:00 PM using 150  $\mu\text{L}$  of double distilled HPLC-grade hexane for 10 minutes, then 150  $\mu\text{L}$  of a tridecane solution was added as an IS. The samples were concentrated before injection into a GC-2010, a GC-MS-QP 2010 Plus, and a GC-Fourier transform infrared spectroscopy (GC-FTIR) (GC-2010 coupled to a DiscovIR-GC—Shimadzu). In the infrared analysis, the GC was operated in the splitless mode, and equipped with a DB-5 (0.25  $\mu\text{m}$ , 0.25 m  $\times$  30 m) (J&W Scientific, Folsom, CA, EUA) capillary column with helium carrier gas. The column oven was maintained at  $50^{\circ}\text{C}$  for 1 min and then increased to  $250^{\circ}\text{C}$  at  $7^{\circ}\text{C}/\text{min}$  to  $250^{\circ}\text{C}$ . A liquid-nitrogen-cooled photoconductive mercury-cadmium-telluride (MCT) detector was used with FT-IR resolution of  $8\text{ cm}^{-1}$ . As for nymphal extracts, the final concentration of the IS was calculated

for each extract, and extracted compounds were quantified based on the area of the IS.

**2.4. Identification of Chemical Compounds and Synthesis of Esters.** Compound identifications were based on coinjections with synthetic standards, Kovats indices (KI), mass spectra (MS), and GC-FTIR analysis. Benzaldehyde, octanol, octanoic acid, decanal, hexanoic acid were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). (*E*)-2-octenol was purchased from Acros Organics (Geel, Turnhout, Belgium).

Twenty-one esters were synthesized by esterification of propionic acid, isobutyric acid, and butyric acid with the following alcohols: pentanol, 3-methylbutan-1-ol, 3-methylbut-2-en-1-ol, 3-methylbut-3-en-1-ol, (*Z*)-pent-2-en-1-ol, (*E*)-pent-2-en-1-ol, pent-4-en-1-ol (all from Aldrich Chemical Company, Milwaukee, WI, USA). 3-methyl-2-buten-1-ol (34.03 mmol, 3 g) and butyric acid (68.06 mmol, 6 g) were refluxed in a round-bottom flask with *p*-toluenesulfonic acid (TsOH) (1 mol%, 0.35 mmol, 0.06 g) and hydroquinone (5% (w/w) in relation to alcohol, 1.75 mmol, 0.191 g). The reaction medium was heated to 60°C with magnetic stirring for 3 h under argon. The product was purified by addition of aqueous solution NaOH 10% (m/V) until the pH was neutral. Afterwards, the product was extracted with ethyl ether; the combined organic solutions were washed with saturated NaHCO<sub>3</sub> solution and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated by rotary evaporation. The crude product was purified by distillation at reduced pressure and collected from 150–160°C. The same experimental conditions were used in the syntheses of the other saturated esters; however, hydroquinone was not employed with saturated alcohols.

The following esters were coinjected with the natural extracts on three different GC columns (DB-5, DB-Wax and HP-1) for identifications: pentyl propionate (yielding 94%), 3-methylbutyl propionate (yielding 74%), pent-4-en-1-yl propionate (yielding 91%), (*Z*)-pent-2-en-1-yl propionate (yielding 83%), (*E*)-pent-2-en-1-yl propionate (yielding 83%), 3-methylbut-2-en-1-yl propionate (yielding 92%), 3-methylbut-3-en-1-yl propionate (yielding 90%), pentyl 2-methylpropanoate (yielding 90%), 3-methylbutyl 2-methylpropanoate (yielding 87%), pent-4-en-1-yl 2-methylpropanoate (yielding 92%), (*2Z*)-pent-2-en-1-yl 2-methylpropanoate (yielding 61%), (*2E*)-pent-2-en-1-yl 2-methylpropanoate (yielding 71%), 3-methylbut-2-en-1-yl 2-methylpropanoate (yielding 92%), 3-methylbut-3-en-1-yl 2-methylpropanoate (yielding 75%), pentyl butyrate (yielding 90%), 3-methylbutyl butyrate (yielding 92%), pent-4-en-1-yl butyrate (yielding 92%), (*Z*)-pent-2-en-1-yl butyrate (yielding 68%), (*E*)-pent-2-en-1-yl butyrate (yielding 74%), 3-methylbut-2-en-1-yl butyrate (yielding 92%), and 3-methylbut-3-en-1-yl butyrate (yielding 80%).

**2.5. Statistical Analysis.** Statistical analyses were performed using R version 2.13 [10]. To analyze the six main compounds found in the exuviae, the Kruskal-Wallis rank sum test was used followed by a nonparametric multiple comparisons test using the package “pgrimess” in case of

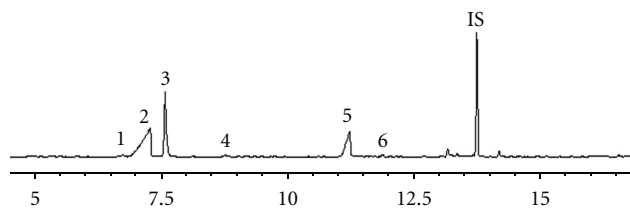


FIGURE 2: Typical gas chromatogram of a *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae) exuvial extract. Numbers (1–6) correspond, respectively, to benzaldehyde, hexanoic acid, octanal, (*E*)-2-octenal, octanoic acid, and decanal. Extracts were analyzed on a Shimadzu GC MS-QP 2010; the internal standard (IS) was tridecane.

significance. Data for the comparison of extracts of virgin and mated adults were tested for normality by the Liliefors and Shapiro-Wilk test. After the normality of the data was confirmed ( $P > 0.05$ ), we performed a GLM (generalized linear model) procedure following Gaussian distribution, considering that mating status was an independent variable. For all analyses,  $P$  values  $>0.05$  were considered not significant.

### 3. Results and Discussion

**3.1. *T. peregrinus* Exuvial Extraction.** Six compounds were present in the exuviae of *T. peregrinus* nymphs, including benzaldehyde, octanol, (*E*)-2-octenol, octanoic acid, decanal, and hexanoic acid (Table 1) (Figure 2). Fourth and fifth instars produced more hexanoic ( $H_4 = 15.9$ ,  $P$  value = 0.003) and octanoic acids ( $H_4 = 15.9$ ,  $P$  value = 0.003) than did first instars. All other compounds did not differ significantly by instar; benzaldehyde ( $H_4 = 7.9$ ,  $P$  value = 0.09), octanol ( $H_4 = 6.1$ ,  $P$  value = 0.19), (*E*)-2-octenol ( $H_4 = 3.2$ ,  $P$  value = 0.52), and decanal ( $H_4 = 3.9$ ,  $P$  value = 0.41) (Table 1).

Some of the compounds present in the exuviae of *T. peregrinus* have been found in other heteropteran species, either as repellents or attractants. For example, benzaldehyde from copulating pairs of *Triatoma infestans* (Klug, 1834) (Reduviidae) was highly attractive to conspecific females at low doses (0.05–0.1 μg) [11]. In the bed bug, *Cimex lectularius* (Linnaeus, 1758) (Cimicidae), decanal, (*E*)-2-octenal, and benzaldehyde are reportedly essential components of the airborne aggregation pheromone [12]. The hexanoic acid is produced in metathoracic scent gland secretions of many bugs (e.g., Scutelleridae: *Eurygaster maura* (Linnaeus, 1758)), along with (*E*)-2-hexanal, (*E*)-2-hexenyl acetate, *n*-tridecane, octadecanoic acid, and *n*-dodecane [13]. The alarm pheromone of *Leptoglossus zonatus* (Dallas, 1852) (Coreidae) adults includes hexyl acetate, hexanol, hexanal, and hexanoic acid [14]. Also, in Japan, a mixture of (*E*)-2-octenyl acetate and 1-octanol attracted the rice bug, *Leptocoris chinensis* Dallas, 1852 (Alydidae) [15]. While the compounds identified here for *T. peregrinus* nymphs are commonly known exocrine compounds of Heteroptera, the combination of these compounds in these thaumastocorid nymphs is unique compared to the secretions of other heteropteran nymphs [6]. Other heteropterans produce some of these compounds (e.g., *Cimex lectularius*) but not



TABLE 1: Identification and quantification (ng) of compounds present in *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae) exuviae. Different letters for each compound indicate significant differences between instars (KI: Kovats Index; SE: standard error). Statistical comparisons: Kruskal-Wallis rank sum test followed by a nonparametric multiple comparisons test ( $P > 0.05$ ).

Chemical compounds	DB-5 column	1st instar	2nd instar	3rd instar	4th instar	5th instar
	KI	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
(1) Benzaldehyde	962	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
(2) Hexanoic acid	988	0.4 ± 0.0 <sup>a</sup>	1.7 ± 0.5 <sup>a</sup>	8.5 ± 3.9 <sup>a</sup>	21.2 ± 7.8 <sup>b</sup>	25.9 ± 6.7 <sup>b</sup>
(3) Octanal	996	2.1 ± 0.6 <sup>a</sup>	5.9 ± 1.2 <sup>a</sup>	5.7 ± 1.7 <sup>a</sup>	6.6 ± 2.6 <sup>a</sup>	3.6 ± 1.1 <sup>a</sup>
(4) ( <i>E</i> )-2-octenal	1062	0.3 ± 0.1 <sup>a</sup>	0.4 ± 0.1 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.2 ± 0.1 <sup>a</sup>
(5) Octanoic acid	1209	0.2 ± 0.0 <sup>a</sup>	0.9 ± 0.3 <sup>a</sup>	2.6 ± 1.1 <sup>a</sup>	7.4 ± 2.9 <sup>b</sup>	7.3 ± 0.8 <sup>b</sup>
(6) Decanal	1239	0.3 ± 0.1 <sup>a</sup>	0.4 ± 0.1 <sup>a</sup>	0.3 ± 0.2 <sup>a</sup>	0.2 ± 0.1 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
Total (ng)		3.4	9.5	17.7	35.7	37.2

all of them combined. The significance of this uniqueness is unknown.

**3.2. Extracts of *T. peregrinus* Adults.** The chromatographic profiles of extracts from *T. peregrinus* males and females revealed the presence of two esters, one minor (**A**) and one major (**B**). Their retention times (Rts) and Kovat's Indices (KIs) on the RTX-5 column were as follows: **A**: Rt = 8.867 min., KI = 1068 and **B**: Rt = 9.558 min, KI = 1103. The MS of **B** showed a base peak at  $m/z$  71, fragments at  $m/z$  68,  $m/z$  85,  $m/z$  128, and a molecular ion of 156 Da (Figure 3). When this spectrum was compared to the NIST library, it was evident that **B** might be a propionic, isobutyric, or butyric ester, with a molecular formula of  $C_9H_{16}O_2$ . The most important signals in the GC-FTIR spectrum of **B** (Figure 3) were a C-H vibration band (2962; 2935; 2871  $cm^{-1}$ ), an ester carbonyl band (1730  $cm^{-1}$ ), and multiple bands of C(CO)O characteristic of esters (1445, 1381, and 1189  $cm^{-1}$ ). These bands associated with a band of C-H stretching vibration of substituted double bond in 3023  $cm^{-1}$ , and the presence of a band in 1674  $cm^{-1}$ , characteristic of trialkyl-substituted alkenes, showed that compound **B** was an unsaturated ester with an internal double bond. In contrast, the molecular ion in MS of compound **A** was not obvious; however, the base peak at  $m/z$  68 and a fragment at  $m/z$  71 suggested **A** was an ester similar to **B**. Although the GC-FTIR spectra of **A** showed the same characteristic bands for esters that were detected for **B**, the presence of a band at 3080  $cm^{-1}$  due to asymmetric stretch of a terminal double bond, demonstrating that **A** was an unsaturated ester with a terminal double bond. To positively identify the natural products **A** and **B**, the twenty-one above-mentioned esters were synthesized. Thus, the major compound **B** was identified as 3-methylbut-2-en-1-yl butyrate by coinjection of this standard with the natural extract on the three GC columns (RTX-5, RTX-WAX, and HP-1). Identification was based on coelution and MS. Additionally, the minor compound **A** was identified as 3-methylbut-3-en-1-yl butyrate by coinjection of this standard with the natural extract on the different GC columns.

Females and males produced the same esters, but their quantities varied by sex and age, particularly for the major compound, 3-methylbut-2-en-1-yl butyrate (Figure 4). Although the concentration of the esters in males increased

with age (Table 2), reaching a maximum of approximately 1  $\mu g$  per insect in 22-day-old mated males, this age difference could not be detected statistically. Only the amount of the major compound (**B**) of mated males was statistically different from that for mated females ( $F_{1,3} = 10.3$ ,  $P$  value = 0.048) (GLM). Ester concentrations of virgin males and females were not statistically different (GLM) for either the minor (**A**) ( $F_{1,4} = 0.6$ ,  $P$  value = 0.47) or major (**B**) ( $F_{1,4} = 3.2$ ,  $P$  value = 0.14) compounds. Likewise, ester concentrations of mated and virgin males (**A**:  $F_{1,3} = 2.4$ ,  $P$  value = 0.21; **B**:  $F_{1,3} = 5.7$ ,  $P$  value = 0.09), and of mated males and females (minor  $F_{1,3} = 4.5$ ,  $P$  value = 0.12) were not statistically different (GLM) (Table 2). The adults of 10–21 days old did not have enough repetitions to be compared. Thus, they were not considered for the concentration analysis.

Butyrates and isobutyrate are pheromone components for other Heteroptera, such as broad-headed bugs (Alydidae) [16], plant bugs (Miridae) [17, 18], and predacious stink bugs (Pentatomidae: Asopinae) [19]. Mirid bugs, particularly species of the genus *Phytocoris*, produce unsaturated butyrate and acetate semiochemicals. *Phytocoris* females attract males with sex pheromones based on butyrate and acetate blends [20–22], while males apparently release high concentrations of certain butyrates as antisex pheromones [17]. In *Alydus eurinus* (Say) (Alydidae), the sex pheromone of females is a blend of 2-methylbutyl butyrate and (*E*)-2-methyl-2-butenyl butyrate [16].

The biological function(s) of 3-methylbut-2-en-1-yl and 3-methylbut-3-en-1-yl butyrates in *T. peregrinus* remain to be elucidated. An aggregation function was attributed to the major compound through olfactometer experiments, in which males attracted only males (Gonzalez et al. 2012 this issue); however, we did field tests using delta traps with different concentrations of the major compound, and they all failed to attract insects in the field and in a greenhouse with a *T. peregrinus* population. Allomones and pheromones known for other heteropterans, such as those described above, undoubtedly originate from the dorsal abdominal glands of nymphs or the metathoracic scent glands that are characteristic of most true bug adults. In *T. peregrinus*, however, the metathoracic scent glands are vestigial. The butyrates from *T. peregrinus* appear to be associated with extrusion of the rectal organ (Figure 1) that has

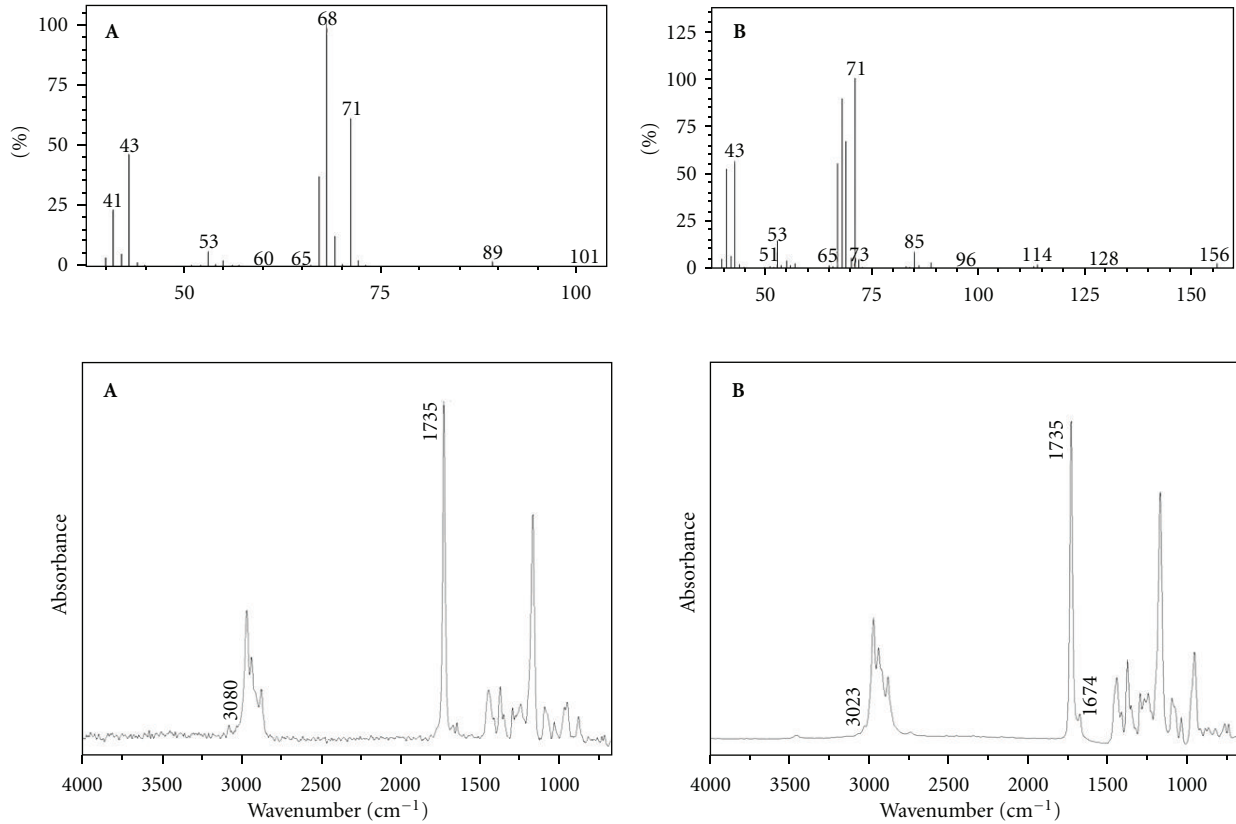


FIGURE 3: Mass and infrared spectra of the minor compound **A** and major compound **B** found in adults of *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae). Extracts were analyzed on a Shimadzu GC MS-QP 2010 and GC-Fourier transform infrared spectroscopy (GC-FTIR) GC-2010 coupled to a DiscovIR-GC—Shimadzu.

TABLE 2: Quantification ( $\mu\text{g}$ ) of esters identified in whole body extracts of *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae) virgin and mated males and females of different ages; 3–9 days (1) and 22–33 days (3). For each ester identified, ns: not statistically different, and \*: statistically different; SE: standard error. Statistical comparisons: GLM (generalized linear model) with Gaussian distribution ( $P > 0.05$ ).

		3-Methylbut-3-en-1-yl butyrate (A)		3-Methylbut-2-en-1-yl butyrate (B)	
		Mean	SE	Mean	SE
Virgin males (1)		0.1	± 0.0		11.2 ± 3.8
Virgin females (1)	ns	0.2	± 0.1	*	0.5 ± 0.0
Virgin males (3)		8.3	± 0.2		191.8 ± 19.1
Mated males (3)	ns	20.7	± 5.4	*	743.5 ± 61.7
Mated males (3)		20.7	± 5.4		743.5 ± 61.7
Mated females (3)	*	0.1	± 0.1	*	0.5 ± 0.3

heretofore only been described within the Heteroptera for plant bugs (Miridae) [8]. Unequivocal verification that the rectal organ tissue is the source of these esters awaits further experimentation. Mated *T. peregrinus* males produce greater quantities of both esters, especially ester B, compared with virgin males and younger mated males. Moreover, these esters are produced by females, suggesting that these compounds are not involved in aggregating the sexes for mating. Speculating the differences of concentration, these esters could be indicators of sex and age recognition by conspecifics.

#### 4. Conclusion

Benzaldehyde, octanol, (*E*)-2-octenol, octanoic acid, decanal, and hexanoic acid were present in the exuviae of *T. peregrinus* nymphs. Volatiles from adult males and females included 3-methylbut-3-en-1-yl butyrate and 3-methylbut-2-en-1-yl butyrate. Compounds identical or similar to those found in *T. peregrinus* exuviae and esters identified in the adults were found in other heteropterans with various functions. The possible pheromonal roles of these volatile blends are being studied.

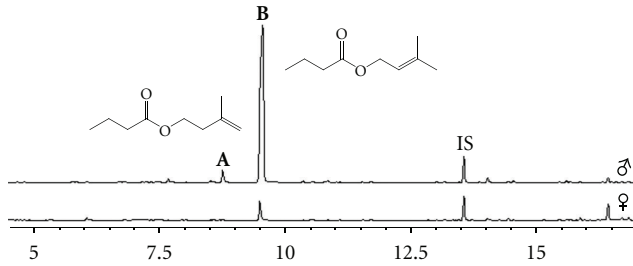


FIGURE 4: Representative gas chromatograms of body extracts of 21-day-old *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae) males and females. Minor and major compounds, 3-methylbut-3-en-1-yl butyrate (A) and 3-methylbut-2-en-1-yl butyrate (B), respectively. The internal standard (IS) was tridecane; extracts shown here were analyzed on GCMS-QP 2010 Plus.

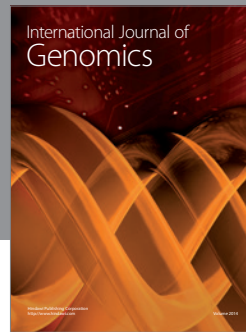
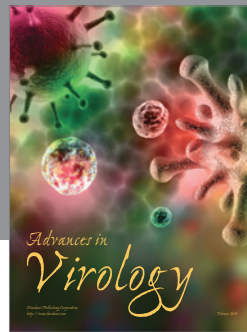
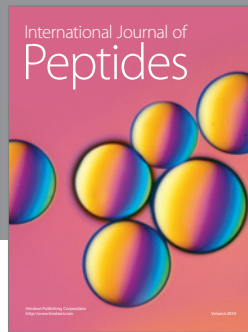
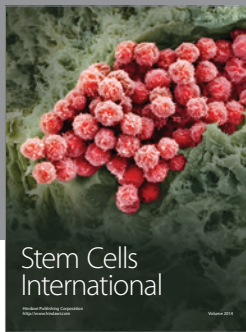
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