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 x E. urophylla in São Francisco de Assis, Rio Grande do Sul, and on E. camaldulensis trees in Jaguariaíva, 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 as quickly be released (JRA, personal observation) (Figure 1). Pheromones are known for members of several heteropteran families [9], but the semiochemicals of T. peregrinus and
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 μL 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 × 0.25 mm i.d. and 0.25 mm film thickness; Restek, Bellefonte, PA, USA). One μL of extract was injected into the GC using the splitless mode with injector temperature at 250°C. The column oven temperature was maintained at 50°C for 1 min, then raised to 250°C at a rate of 7°C/min, and maintained in 250°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 and virgin 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 μL of double distilled HPLC-grade hexane for 10 minutes, then 150 μL 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 DiscoVIR-GC—Shimadzu). In the infrared analysis, the GC was operated in the splitless mode, and equipped with a DB-5 (0.25 μm, 0.25 m × 30 m) (J&W Scientific, Folsom, CA, EUA) capillary column with helium carrier gas. The column oven was maintained at 50°C for 1 min and then increased to 250°C at 7°C/min to 250°C. A liquid-nitrogen-cooled photoconductive mercury-cadmium-telluride (MCT) detector was used with FT-IR resolution of 8 cm⁻¹. As for nymphal extracts, the final concentration of the IS was calculated...

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**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.
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-yl butyrate (yielding 90%), 3-methylbut-2-en-1-yl propionate (yielding 90%), 3-methylbutyl propionate (yielding 74%), 3-methylbut-3-en-1-yl propionate (yielding 83%), 3-methylbut-2-en-1-yl propionate (yielding 92%), 3-methylbutyl propionate (yielding 74%), 3-methylbut-3-en-1-yl butyrate (yielding 80%), 3-methylbut-2-en-1-yl butyrate (yielding 92%), and benzaldehyde (yielding 94%).

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-octenol, 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 mauro (Linnaeus, 1758)), along with (E)-2-hexanal, (E)-2-hexyl 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, Leptocorisa 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 significance. Data for the comparison of extracts of virgin and mated adults were tested for normality by the Lilliefors 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).

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Although the concentration of the esters in males increased with age (Table 2), reaching a maximum of approximately 1 μg 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 (F1,3 = 10.3, P value = 0.048) (GLM). Ester concentrations of virgin males and females were not statistically different (GLM) for either the minor (A) (F1,4 = 0.6, P value = 0.49) or major (B) (F1,4 = 3.2, P value = 0.14) compounds. Likewise, ester concentrations of mated and virgin males (A: F1,6 = 2.4, P value = 0.21; B: F1,6 = 5.7, P value = 0.09), and of mated males and females (minor F1,3 = 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 iso butyrates 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 *Phyto coris*, produce unsaturated butyrate and acetate semiochemicals. *Phyto coris* females attract males with sex pheromones based on butyrate and acetate semiochemicals. 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 μg 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 (F1,3 = 10.3, P value = 0.048) (GLM). Ester concentrations of virgin males and females were not statistically different (GLM) for either the minor (A) (F1,4 = 0.6, P value = 0.49) or major (B) (F1,4 = 3.2, P value = 0.14) compounds. Likewise, ester concentrations of mated and virgin males (A: F1,6 = 2.4, P value = 0.21; B: F1,6 = 5.7, P value = 0.09), and of mated males and females (minor F1,3 = 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.

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| 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 ± 0.0a| 0.1 ± 0.0a| 0.2 ± 0.0a| 0.1 ± 0.0a| 0.1 ± 0.0a|
| (2) Hexanoic acid  | 988         | 0.4 ± 0.0a| 1.7 ± 0.5b| 8.5 ± 3.9b| 21.2 ± 7.8b| 25.9 ± 6.7b|
| (3) Octanal        | 996         | 2.1 ± 0.6a| 5.9 ± 1.2a| 5.7 ± 1.7a| 6.6 ± 2.6a| 3.6 ± 1.1a|
| (4) (E)-2-octenal  | 1062        | 0.3 ± 0.1a| 0.4 ± 0.1a| 0.3 ± 0.0a| 0.3 ± 0.0a| 0.2 ± 0.1a|
| (5) Octanoic acid  | 1209        | 0.2 ± 0.0a| 0.9 ± 0.3a| 2.6 ± 1.1a| 7.4 ± 2.9b| 7.3 ± 0.8b|
| (6) Decanal        | 1239        | 0.3 ± 0.1a| 0.4 ± 0.1a| 0.3 ± 0.2a| 0.2 ± 0.1a| 0.1 ± 0.0a|
| Total (ng)         |             | 3.4       | 9.5       | 17.7      | 35.7      | 37.2       |
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 (µg) 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) | Mean SE | 3-Methylbut-2-en-1-yl butyrate (B) | Mean SE |
|---------------------------|-----------------------------------|---------|-----------------------------------|---------|
| Virgin males (1)          | ns                                | 0.1 ± 0.0 | ∗                                  | 11.2 ± 3.8 |
| Virgin females (1)        |                                   | 0.2 ± 0.1 |                                   | 0.5 ± 0.0 |
| Virgin males (3)          | ns                                | 8.3 ± 0.2 | ∗                                  | 191.8 ± 19.1 |
| Mated males (3)           | ∗                                 | 20.7 ± 5.4 | 743.5 ± 61.7                      |
| Mated females (3)         | ∗                                 | 0.1 ± 0.1 | 743.5 ± 61.7                      |

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.
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