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EVIDENCE FOR SEX-SPECIFIC PHEROMONES IN *ULOMOIDES DERMESTOIDES* (COLEOPTERA, TENEBRIONIDAE)

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Ulomoides dermestoides (Fairmaire, 1893) is an Asiatic insect used for medicinal purposes all over the world. In Brazil, people buy these insects in animal product stores not only to feed birds and fish, but also as alternative medication for asthma and arthritis. The aqueous extract of *U. dermestoides* has been demonstrated to have an anti-inflammatory activity in rats (Santos et al. 2010), which supports popular medicinal usages. Recently, Villaverde et al. (2009) identified organic volatile compounds released by *U. dermestoides,* such as methyl-1,4-benzoquinone, ethyl-1,4-benzoquinone, 1-tridecene, and 1-pentadecene, which represent more than 90% of the volatile blend. The volatile collection was carried out by SPME, and no sex specific compound was detected. We worked with the same species and collected the volatiles produced by groups of 300 males and females, separated by sex, for 96 h in glass aeration chambers (33 cm height by 4.5 cm outside diameter) with 1 broken peanut inside of each chamber. The volatiles emitted were trapped on 0.8 g of Super Q (Alltech, Deerfield, IL) columns as previously reported (Zarbin et al. 2003). Five repetitions were performed. The same methodology was used for a 12-h photo-scotophase aeration collection. Volatiles were eluted from Super Q with 4 mL of distilled hexane and concentrated to 600 µL (1 insect per 2 µL) under an argon stream (Ambrogi et al. 2009), and 1 µL was injected in a GC-MS system (QP-2010 Plus, Shimadzu).

Bioassays for these extracts were based on the protocol of Suzuki & Sugawara (1979). The responses of 3 virgin males or females to aeration extracts from both males and females were tested. Filter papers cut to 2 cm × 1 cm were impregnated with 5 µL of extract (2.5 IE) and placed on each side of a Petri dish (9 cm of diameter) for 20 min. Twenty repetitions were performed for each test, and the presence of insects on the filter paper was considered a positive response.

The statistical analyses were performed by BioStat 3.0 (Ayres et al. 2003) with paired *t*-test and 95% significance. Beetles were purchased in a local store and maintained inside a plastic box with peanuts at room temperature with a photoperiod of 12:12 (L:D). Pupae were sexed and placed in plastic containers, and emerged adults were fed with peanuts.

Behavioral bioassays revealed statistically significant preferences of females and males to male extracts (t = 1.972* and P = 0.032*; t = 3.824** and P = 0.0007**, respectively) versus female extract. The chromatographic profiles of volatiles from male and female *U. dermestoides* are shown in Fig. 1 and clearly indicate the existence of 3 male-specific compounds (**a–c**). GC-MS investigation provided analytical data and a fragmentation pattern that strongly suggested compounds **a-c** to be hydrocarbon-sesquiterpenes ($C_{15}H_{24}$). The CG-MS data with retention time (RT) and Kovat's Index (KI) for these compounds were as follows: (**a**) RT = 16.20 min; KI = 1,422 (DB-5); m/z (%): 204 (M^+; 26.78), 189 (11.46), 161 (7.72), 136 (100), 121 (92.69), 107 (59.68), 93 (68.1); (**b**) RT = 16.24 min; KI = 1,423 (DB-5); m/z (%): 204 (M^+; 13.73), 189 (4.25), 161 (38.21), 147 (5.85), 136 (23.66), 121 (51.1), 119 (100), 105 (96.83); and (c) RT = 16.30 min; KI = 1,426 (DB-5); m/z (%): 204 (M^+; 18.92), 161 (20.59), 147 (10.18), 121 (14.17), 119 (100), 105 (34.58), 93 (36.37). The identification of the other 6 compounds (**1**-**6**) present in both sexes (Fig. 1) was based on the fragmentation pattern of the GC-MS analysis and NIST library suggestion and was in accordance with the components previously reported by Villaverde et al. (2009). The 6 identified compounds were methyl-1,4-benzoquinone **(1)**, limonene **(2)**, ethyl-1,4-benzoquinone **(3)**, 1-tridecene **(4)**, pentadecadiene **(5)**, and 1-pentadecene **(6)**, in a ratio of 5: 9.2: 45.2: 6.2: 1: 123.2, respectively.

Villaverde et al. (2009) agitated the vials where the volatiles were collected and detected ethyl-1,4-benzoquinone as the major compound; however, in our study, pentadecadiene had the highest concentration. Because quinones are common defensive compounds produced by tenebrionid beetles (Brown et al. 1992) when disturbed, the major peak difference is justified.

Our findings provide evidence that the aggregation observed in *U. dermestoides* is mediated by a putative pheromone attracting both sexes and produced by males. The 12-h aeration revealed the continuous production of these 3 putative sesquiterpenes during photo- and scotophase, sup-

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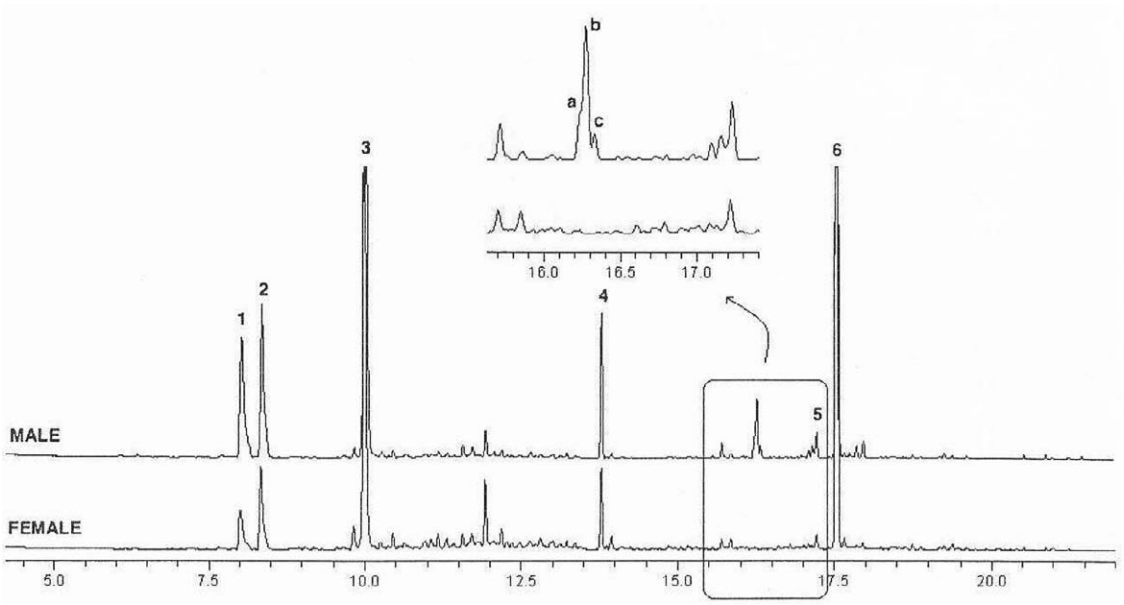


Fig. 1. Comparison of the 96-h aeration extract chromatograms of males and females of *Ulomoides dermestoides*. 1: methyl-1,4-benzoquinone; 2: limonene; 3: ethyl-1,4-benzoquinone; 4: 1-tridecene; 5: pentadecadiene; 6: 1-pentadecene; a-c: male-specific compounds.

porting the permanent aggregation state inside the rearing box. Male-produced aggregation pheromones have been found and identified in other tenebrionid species. *Tribolium castaneum*, *T. confusum*, and *T. freemani* produced 4,8-dimethyldecanal (Suzuki & Mori 1983; Suzuki et al. 1987), and *Alphitobius diaperinus* produced 5 male-specific compounds: (*R*)-(+)-limonene, (*E*)-β-ocimene, (*S*)-(+)-linalool, (*R*)-(+)-daucene, and 2-nonanone (Bartelt et al. 2009). Sex pheromones were found in males (*Z*)-3-dodecenyl acetate) and females (4-methyl-1-nonanol) of *Tenebrio molitor* (Bryning et al. 2005).

Studies are underway to isolate and chemically characterize the male-specific compounds herein described, in order to test the biological activity against males and females on laboratory bioassays.

Summary

Behavioral and chemical evidence support the occurrence of a male-produced aggregation pheromone in *U. dermestoides* that attracts both males and females. Three male-specific compounds were detected and are candidate pheromone compounds for this species.

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