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Interaction between two aggregation chemical signals in *Triatoma infestans* (Hemiptera: Reduviidae)

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ABSTRACT

The nymphs and adults of *Triatoma infestans* spend much of their time aggregated among themselves within narrow and dark shelters. The search for a suitable shelter depends in part on the recognition of chemical signals coming from the feces and the cuticle of the other individuals who use the refuge. The aim of this study was determine the possible interaction between the chemical signals associated to the feces and to the cuticle of *T. infestans*. The results showed that the insects remained significantly more time on the feces that had contact with legs and the feces plus footprints than feces or footprints alone, demonstrating the interaction between evaluated signals. These results demonstrates also that feces extracted a chemical stimulus from the legs. Understanding the interaction feces-legs as an interaction feces-cuticle of legs, the results suggest that the feces could extract some cuticular compound with activity on the behavior of the insects. This is the first report of the interaction between the two aggregation signals recognized in *T. infestans* and of the increase in the behavioral response of insects exposed to feces that had contact with a cuticular structure.

1. Introduction

Triatoma infestans (Hemiptera: Reduviidae) is a hematophagous insect of the subfamily Triatominae and is the main vector of Chagas's disease in the southern of Southern Cone of South America. Although some studies reported the existence of sylvatic populations (Noireau, 2009), the habitat of this species is mainly the human dwelling in rural zones of the endemic area (Lent and Wygodzinsky, 1979). In dwellings, nymphs and adults of *T. infestans* spend a lot of time aggregated among them within narrow and dark shelters. These refuges temper external climatic variations and protect against predators. In addition, considering the presence of conspecifics, the shelter also facilitate the transfer of symbionts and the meeting of couples (Lazzari et al., 2013). Domiciliary and peridomiciliary areas of the dwellings offer a plethora of possible shelters, but not all them present the optimal environmental characteristics nor are inhabited by conspecifics. The search for a suitable shelter depends in part on the recognition of chemical signals coming from the feces and the cuticle of the other individuals who use the refuge (Lazzari et al. 2013; Barrozo et al., 2017).

The feces of *T. infestans* release aggregation signals and their pattern of distribution around the shelters suggests a role as chemical landmarks that guide insects to their refuges (Lorenzo and Lazzari, 1996; Lorenzo-Figueiras et al., 1994). The aggregation in *T. infestans* also occurs on surfaces previously transited by other bugs who seem to have deposited cuticular compounds through physical contact of the legs or body with the substrate. This signal was described as footprints (Lorenzo-Figueiras and Lazzari, 1998). The two aggregation signals show chemical and physiological differences. The fecal pheromone can be extracted by polar solvents, seems be composed by highly volatile compounds and is detected as an olfactory stimulus (Lorenzo-Figueiras et al., 1994; Lazzari et al., 2013). On the other hand, the cuticular pheromone is extracted by non-polar solvents, seems be composed of epicuticular lipids and is detected by contact chemoreceptors (Lorenzo-Figueiras and Lazzari, 1998; Lorenzo-Figueiras et al., 2009). Both signals also differ in the mechanism of orientation induced: the fecal signal induces a taxis acting as a true attractant factor while the behavior elicited by the cuticular signal would be a kinesis acting as an arresting factor.

Although both pheromones are associated with shelters, both spatially and in their supposed biological meanings, the possible interaction between them has not been studied. A possible interaction scenario has been proposed considering the orientation mechanisms associated with each signal. The olfactory signal released from the feces would attract the insects to the shelter; when they reach at the refuge, the

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insects would decrease their locomotor activity by contacting cuticular signal present on the surface of other insects or deposited on the substrate as footprints (Lazzari et al., 2013). On the other hand, Galvez-Marroquin et al. (2017) identified the chemical composition of feces of *T. dimidiata* that promoted conspecific aggregation and detected that the volatile extract from feces present hydrocarbons that are part of the cuticle of this species as reported by Juárez et al. (2002). Moreover, one of these hydrocarbons (i.e. *n*-tricosane) elicited aggregation when evaluated individually. Galvez-Marroquin et al. (2017) briefly discusses the possible origin of the compounds present in the excreta and focuses mainly on the metabolic activity of symbiotic microorganisms. However, the fact that feces were collected on a filter paper placed under a vessel containing a group of insects (Galvez-Marroquin et al., 2017) allows to speculate that the feces might have acquired epicuticular hydrocarbons when they contacted the cuticle of other insects. In this context, other interaction scenario can be proposed in which the feces could acquire cuticular lipids when they contact the cuticular surface, and then both chemical signals would be deposited together. Once deposited, they could act sequentially as suggested in the first scenario.

The aim of this study was to determine the possible interaction between the chemical signals associated to the aggregation behavior in *T. infestans*. In particular, the hypothesis that feces acquire a greater effect on the behavior of insects when they contact the cuticle was evaluated. For this, the present research determined the behavioral response of *T. infestans* to feces, footprints, feces plus footprints and feces having had contact with cuticle.

2. Materials and methods

2.1. Insects

The insects used were laboratory-reared descendants from field insects collected from 25 de Mayo locality, department of Quitilipi, Chaco, Argentina. The insects were reared and maintained for two generations in the Centro de Referencia de Vectores (CeReVe) (Santa María de Punilla, Córdoba, Argentina) under controlled conditions (26 ± 1 °C, 60 ± 10% RH and 12:12 h L:D) and were fed fortnightly.

2.2. Experimental device

The behavioral assays were performed in a circular glass arena (15 cm diameter) with the floor covered with filter paper and divided in two equal sectors. A piece of filter paper (0.5 × 0.5 cm) impregnated with the stimulus to be tested (experimental paper) or clean (control paper) supported on a coverslip was placed in each sector. The position of the stimulus was reversed in every replicate to control biases due to external asymmetries. The arena was inside an experimental room (28 × 35 × 27 cm) at 23 ± 1 °C and 37 ± 10% RH and kept dark. The behavior of the insects was recorded by means of an infrared videocamera (AHD-318Q-T) connected to a monitor.

2.3. Evaluation of behavioral response

One fifth-instar nymph was gently located in the center of the arena and after 5 min of the acclimation the insect was liberated and could walk freely. The insect was observed for 10 min and the time spent in each zone was registered. Between replicates, the materials were carefully cleaned with ethanol (70%) and the filter papers were replaced to eliminate putative cues deposits by the insects. The assays were conducted using nymphs between 10 and 20 days old and fasted since last molt. Each insect was evaluated in their scotophase and was used only once.

2.4. Experimental series

Four one-way series (i.e. stimulus vs control) were performed: feces vs control, feces with legs vs control, footprints vs control, and feces with footprints vs control. Three two-way series (i.e. stimulus vs stimulus) were performed: feces vs feces with legs, feces vs feces with footprints, and feces with legs vs feces with footprints. A control series (i.e. control vs control) was performed to test whether the arena and surrounding environment were symmetrical. We performed 30 trials per series.

2.5. The stimuli

2.5.1. Feces

Fecal droplets were obtained of fifth-instar nymph by slight compression of the last section of the abdomen with a forceps. Feces from 15 insects were collected in a plastic tube (1.5 ml), maintained with the tube closed and used 24 hr later in a liquid state. This time interval guarantees their aggregating activity (Lorenzo-Figueiras et al., 1994). Any contact between the insect with the plastic tube or the fecal droplets with legs was avoided to exclude other potential cues. The insects were fed between 10 and 20 days after molt and two days before the experiment. The experimental filter papers were impregnated with 2 µl of the collected feces while control papers were impregnated with 2 µl of distilled water.

2.5.2. Feces with legs

Second and third pair of legs obtained from fifth-instar nymph died 24 hs before were immersed for 24 hs in feces obtained following the procedure described above (see Feces). Two microliters of these feces were used to impregnate the experimental paper while 2 µl of distilled water was used to impregnate the control papers.

2.5.3. Footprints

A group of 5 fifth-instar nymph with their anus completely occluded were allowed to walk freely on a piece of filter paper (9 cm diameter) placed inside of a glass container (9 cm diameter). After 24 h, these papers were examined to confirm the absence of fecal spots and were cut into pieces of 0.5 × 0.5 cm for the experiments. Clean filter paper was used as control. The occlusion of the anus was performed with water-based correction fluid (Aqua fluid, Berol, Newell Rubbermaid, Argentina S.A) and verified using a stereomiscroscope (Nikon SMZ745). The insects were between 10 and 20 days old and were fasted since molt.

2.5.4. Footprints with feces

Pieces of filter paper (0.5 x 0.5 cm) with footprints obtained following the procedure described above (see Footprints) were impregnated with 2 µl of feces obtained following the methodology described in the section Feces. The control papers were impregnated with 2 µl of distilled water.

2.6. Statistical analysis

The time that the insects remained in one zone was compared with the theoretical value assuming a random distribution (i.e. 5 min) using the one-sample Wilcoxon test. The differences were considered statistically significant if $P < 0.05$. A Wilcoxon two-sample test was used to compare the treatments that presented significant difference with their control. The Bonferroni correction was applied to adjust the significance level for each of the comparisons made, thus, the differences were considered statistically significant if $P < 0.017$ (overall α level = 0.05). The non-parametric test was used as the data did not follow a normal distribution according to the Shapiro-Wilk test (Sokal and Rohlf, 2009). The data were analyzed with the MINITAB Statistical Software, version 17 (Minitab Inc., PA, U.S.A.).