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Oviposition response of *Lutzomyia* (*Lutzomyia*) *renei* (Martins, Falcão & Silva) (Diptera: Psychodidae) to extracts of conspecific eggs in laboratory bioassays

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Abstract

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In this study bioassays were carried out to evaluate the possible attractive and/or stimulant effect of extracts of the eggs of gravid female *Lutzomyia renei*. The response to the oviposition pheromone by *L. renei* was not as marked as that found for *L. longipalpis* and *Phlebotomus papatasi*. Extracts equivalent to 100 or 200 eggs did not produce a perceptible attraction and/or stimulation to oviposit, although extracts of 1000 eggs did produce a slight attractive response. Chemical analysis of *L. renei* egg extracts revealed the presence of various fatty acids and complementary bioassay experiments are needed to prove a possible stimulant effect.

Additional key words: Biology, pheromone.

Resumo

MENEZES A JC, HAMILTON C JG, PEÇANHA B R. 2003. Resposta à oviposição de *Lutzomyia* (*Lutzomyia*) *renei* (Martins, Falcão & Silva) (Diptera: Psychodidae) aos extratos de ovos coespecificos em bioensaios de laboratório. Entomotropica 18(2):121-126.

Neste estudo foram realizados bioensaios para avaliar o possível efeito atrativo e/ou estimulante de extratos de ovos de *Lutzomyia renei*. A resposta ao feromônio de oviposição pela *L. renei* não foi detectada como para *L. longipalpis* and *Phlebotomus papatasi*. Extratos equivalentes a 100 ou 200 ovos não produziram atração perceptível e/ou qualquer estimulo à oviposição, embora extratos de 1000 ovos produziram um leve estimulo atrativo. Análise química dos extratos de ovos de *L. renei* revelaram a presença de vários ácidos graxos e experimentos complementario são necessários para provar o possível efeito estimulante.

Palavras chaves adicionais: Biologia, feromônio.

Introduction

The hematophagous Diptera are non-social insects that, with few exceptions, do not show protective maternal behavior. Selection of the oviposition site is therefore crucial to maximize survival of the progeny. It has been suggested that semiochemical attractants (apneumones and oviposition pheromones) are used by many insect species to help the female orientate towards appropriate oviposition sites (McCall & Cameron 1995).

Oviposition pheromones have been demonstrated in several species of *Culex,* (Starratt & Osgood 1972, 1973; Bruno & Laurence 1979; Laurence & Pickett 1982,1985) *Simulium* (Coupland 1992; McCall et al. 1994; McCall 1995), and *Aedes* (Allan & Kline 1998) as well as other hematophagous insects (Barton Browne et al. 1969).

Among phlebotomine sand flies however, the chemical identification of the oviposition pheromone has been restricted to a single species, *Lutzomyia longipalpis* (Lutz Neiva). The oviposition pheromone was demonstrated in several laboratory experiments where eggs, or hexane extracts of eggs, showed an attractant and/or stimulant effect on gravid conspecific females (Elnaiem & Ward 1990, 1991 Elnaiem et al. 1991). The same effect was also observed when extracts of gravid female accessory glands were used in a similar bioassay and suggested that these glands probably constituted the site of production of the pheromone and further

TABLE 1. Attraction of gravid females of *L. renei* to extracts of 100 and 200 conspecific eggs in 100µl hexane in bioassays carried out in acrylic cages.

Bioassays	Females of *L. renei* captured in Petri dishes. Mean ± standard deviation. (No. females captured /%) Test	Control	*P*
100 eggs/100µl hexane [a]	8.33±4.20 (100/41.7%)	7.08±3.57 (85/35.4%)	0.441[NS]
200 eggs/100µl hexane [b]	7.50±3.02 (60/37.5%)	5.50±3.25 (44/27.5%)	0.223[NS]

NS: differences not significant (t-test, $P > 0.05$). **P:** *probability level.* **%:** Percentage of females captured. **[a]:** 12 replicates: total 240 females. **[b]** 8 replicas: total 160 females.

Company, USA). Two sets of apparatus (test and control) were prepared. Each filter paper disk was then treated with either the test extract (100 or 200 eggs/100µl) or pure solvent control (100µl). The test and control dishes were marked and positioned in diagonally opposite corners of the interior of the cage, separated by a distance of 30 cm. After application of the test extract and the solvent control (100µl), 20 gravid *L. renei* females were introduced to the cage. Honey:water was provided in the upper central part of the cage. The experiment was carried out in the sand fly insectary (25ºC-26ºC; 80%-90% RH). The cage was covered with a black cloth left in place from 17:30-08:30. After this period the females found trapped in the Tanglefoot of the test and control dishes were counted. Twelve replicates of experiments using extracts of 100 eggs/100µl and eight of those using 200 eggs/100µl were done. The relative positions of the test and control dishes were changed among the four corners of the cage for each replicate to avoid positional bias. After each experiment the cage was cleaned with 70% alcohol and the black cloth washed with neutral soap.

Olfactometer attraction bioassay: In this bioassay an olfactometer was used to test the attraction of *L. renei* females to egg extracts. The olfactometer consisted of three rearing pots (11.0 cm diameter x 7.5 cm height) (Nalgene®, UK) linked by two plastic tubes (9.5 cm long x 3.5 cm diameter (Nunc International®, USA). The bases of the pots (designated as test and control chambers) were perforated and filled with plaster of Paris. Discs of filter paper (2.5 cm diameter) were secured by pins to the centers of the bases of the two lateral pots . Egg extracts (100, 200 or 1000 eggs/100µl hexane) were applied to the test discs and 100µl of hexane added to the control discs. Ten minutes after application of the extracts, 20 gravid females were introduced into the center pot and the entire assembly was placed inside a plastic box (56.4 X 38.5 X 20.1 cm, San Remo®, São Paulo), that was maintained in darkness inside an incubator (25-26ºC and 90% RH). After 24h the numbers of females and eggs found in the test and control chambers were counted. Twelve replicates of the 100 egg extract, 14 replicates of the 200 egg extract and six replicates of the 1000 egg extract were done. After each replicate was completed, the plaster bases of the pots were changed and the entire apparatus was washed in boiling water and 70% alcohol, rinsed six times in water and dried with paper towels and clean cotton.

Chemical analysis: Extracts of 100 1-2 day-old eggs/100µl hexane of *L. renei* and *L. longipalpis* from Lapinha Caves and their respective controls were analyzed by gas chromatography coupled mass spectrometry (GC/MS) (Dougherty and Hamilton, 1997). Peak area was used to compare the amounts of fatty acid found in *L. renei* with *L. longipalpis* eggs.

Analysis of results: The Kolmogorov-Smirnov statistical test was used to test for a normal distribution of each set of results. Those results that were normally distributed were compared by Student's t-test, the Wilcoxon and Kruskal-Wallis tests were used for those that did not. The 0.05 significance level was used.

Results

Attraction and/or oviposition stimulation bioassays: There was no significant difference between the number of eggs laid on the test and control sites when extracts of either 100 or 200 eggs were applied to test filter paper. When extracts of 100 eggs were applied the number of eggs laid on the test filter paper was 75.83±46.16 and on the control, 70.16±31.05. When extracts of 200-eggs were used, the mean number of eggs laid on the test filter paper was 50.33±39.35 and on the control was 45.25±33.36.

Cage attraction bioassay: The results of the attraction bioassays are shown in Table 1. In both bioassays (with 100 and 200 egg extracts) there was no significant difference between the number of females caught in the test and control dishes.

Olfactometer attraction bioassay: The results of olfactometer attraction bioassays are presented in Table 2. When 1000 egg extracts were placed in the test side of the olfactometer significantly more gravid females were attracted on average to the test side (7.66±3.07) than the control (3.66±2.73) (P=0.039). Also the mean number of eggs laid in the test chamber (139.33±62.48) was significantly higher than in the control chamber (68.16±41.71) (P = 0.043). When we tested 100 and 200 egg extracts, no significant difference

type of bioassay used in the present study. Since no experiments were carried out utilizing extracts equivalent to more than 200 and less than 1000 eggs, we cannot determine the precise value of the response threshold to oviposition pheromone for gravid females of *L. renei.* Nor can we reject the possibility that gravid females of *L. renei* responded positively only to the extracts with large number of eggs because the oviposition pheromone was produced in abnormally low quantities by the insects in our study. This low production may to be related to inadequacy of the bloodmeal source utilized, it being believed that the precursors of oviposition pheromone are derived from the blood of the vertebrate host (Dougherty & Hamilton 1997).

Only the attractive effect of 1000-egg extracts in the olfactometer bioassays was demonstrated during the present study. Although the females in the test chamber laid a significantly higher number greater of eggs than those in the control, we cannot affirm that a stimulant effect was present, since the insects were not examined individually and it was not possible to determine how many oviposited, the number of eggs laid / female or the post-oviposition survival.

It was not possible to determine which of the fatty acids encountered on the surface of the eggs of *L. renei* were involved in semiochemical activity. This would require further studies using electroantennography and olfatometry to evaluate the activity of each substance encountered separately. The unsaturated fatty acids hexadecenoic and octadecenoic acid are more unstable than the saturated compounds and are therefore less likely to act as pheromones (JGC Hamilton, personal communication.). Among the saturated compounds, dodecanoic acid (the oviposition pheromone of *L. longipalpis*) and tetradecanoic acid were encountered in smaller quantities on the surfaces of the eggs of *L. renei* than on those of *L. longipalpis.* Although hexadecanoic acid was 1.5 times more abundant, it is important to emphasize that the compound present in greatest quantity is not always that responsible for semiochemical activity. It is also possible that the oviposition pheromone of *L. renei* consists of a mixture of compounds rather than a single substance as in *L. longipalpis* (Dougherty & Hamilton 1997). In the future new bioassays should be realized using *L. renei* from a close colony and not with sand flies collected in the field.

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