SCIENTIFIC NOTE

Presence of the Fire Ant *Solenopsis invicta* (Westwood) (Hymenoptera: Formicidae) Stimulates Burrowing Behavior by Larvae of the Sandfly *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae)

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ABSTRACT - The sandfly *Lutzomyia longipalpis* (Lutz & Neiva) vectors leishmaniasis in the neotropics. Although much is known about the biology of adult flies, little is known about interactions with its natural enemies. Here, we examined behavior of larvae of L4 *L. longipalpis* on a soil substrate when exposed to the fire ant *Solenopsis invicata* (Westwood). When ants were absent, most larvae tended to remain at or close to the soil surface, but when ants were present the larvae burrowed into the soil. Sandflies seek refuges in the presence of generalist predators, thus rendering them immune to attack from many potential enemies.

KEY WORDS: Defensive behaviour, refuge

The sandfly *Lutzomyia longipalpis* (Lutz & Neiva) vectors leishmaniasis and is abundant in many dry regions of Latin America. Although considerable information is known about certain aspects of the biology of adult flies (Lane 1993, Feliciangeli 2004, Hamilton 2008, Maignon *et al* 2008), the biology, ecology and natural enemies of immature stages has been little studied. Elnaiem & Ward (1992) and Ferro *et al* (1997) showed that adult sandflies prefer to oviposit in soil crevaces that are rich in organic matter. Pener & Wilamowski (1996) found that younger larvae of an African sandfly species, *Phlebotomus papatasi* Scopoli, are vulnerable to attack from the pathogen *Bacillus sphaericus*. Still, few studies have explored the interactions between *L. longipalpis* or other sandfly species and potential insect natural enemies which occur in the same habitats.

When one considers the immense diversity of natural enemies in tropical ecosystems, thus implying that predation is a strong selective force over evolutionary time (Lima & Dill 1989), the abundance of adult flies at various times of the year suggests that larval and pupal stages of sandflies possess effective anti-predatorial strategies. Dougherty & Hamilton (1996) revealed that allomones secreted by fourth instar (L4) and prepupal sandflies repelled both conspecific larvae and workers of the European garden ant, *Lasius niger* (Fabricius). Based on these results, a simple experiment was set up to determine if the behavior of L4 *L. longipalpis* varies in response to the presence of the South American fire ant, *Solenopsis invicta* (Westwood) workers on a soil substrate. In culture, sandflies larvae are frequently observed foraging on the soil surface, and in this investigation we examined if the presence of fire ants stimulates burrowing (avoidance) behavior by *L. longipalpis*.

Lutzomyia longipalpis larvae were obtained from a laboratory colony originally collected in Bahia State, Brazil. The flies were colonized according to the protocol described in Dougherty *et al* (1993). Female flies were blood fed on hamsters anaesthetized with 12 ml of sodium pentobarbitone, and both male and female flies had constant access to 50% sucrose solution. Larval food consisted of a modified food source consisting of equal proportions (dry weight) of *Daphnia* sp., rabbit feces, fine sand and potting compost. Early L4 larvae used in this investigation were removed from rearing pots using soft forceps. The colony of *S. invicta* was obtained from a culture maintained at the University of Gainesville, Florida U.S.A. They were fed on a diet consisting of an equal mixture (dry weight) of ground powdered liver and sponge cake. Ants had a constant access to pure honey and water, supplemented with house crickets and larvae of both mealworms and fruit flies.

Twenty plastic Petri dishes (8 cm dia) were filled to within 2 mm of the rim with evenly spread and moistened potting compost. Twenty-five L4 sandfly larvae were taken from rearing pots using soft forceps, and placed onto the soil surface in each Petri dish, all of which were covered with lids for 1h. Then, the lids were removed, and the number of larvae clearly visible on the surface in all 20 Petri dishes were counted and recorded; 10 dishes were again covered with lids for a further hour (= controls). In each of the other 10 dishes, five workers of *S. invicta* were introduced, and the dishes were covered with lids for a further hour. At the end of the second hour, the lids were again removed, the ants were returned to the colony from the 10 experimental dishes, and the number of sandfly larvae visible on the surface in all of the dishes was counted and recorded.

The number of larvae varied significantly with treatments (ants or no ants, General Linear Model: $F_{1,114}$ = 223.92, P < 0.0001) and with time ($F_{2,114}$ = 959.23, P < 0.0001). Most importantly, there was a significant interactive effect between these parameters on sandfly distribution ($F_{2,114}$ = 244.43, P < 0.0001). After 1h, an average of 17.4 and 17.9 larvae were recorded on the soil surface of the control and experimental arenas respectively; after a further hour, the number of larvae recorded from the controls remained relatively constant (17.4), but the number of larvae recorded on the soil surface in experimental arenas into which ants had been added decreased significantly to an average of 4.9 (Fig 1).

The presence of *S. invicta* stimulated the escape behavior (through burrowing) of *L. longipalpis*. Most observed contacts between ants and sandfly larvae were not actual attacks, but incidental contact when ants walked over the larvae. In the absence of ants, the number of sandfly larvae on the soil surface after 2h remained constant, but significantly decreased in arenas containing ants. Similar avoidance behavior has been recorded in several other studies with arthropods (e.g. Heads 1985, Soluk & Collins 1988, Skutelsky 1996). In the case of *L. longipalpis*, mechanical disturbance (with forceps) did not influence the decision to burrow, but the presence of *S. invicta* did, indicating that specific stimuli are required for the expression of different escape responses.

Preliminary experiments revealed that mechanical and chemical defenses are ineffective in isolation against attack from fire ants, thus *L. longipalpis* compensates by employing a defensive strategy that minimizes the risk of encounters with predators on the soil surface. The importance of refuges in predator-prey relationships is that they provide ‘enemy-free space’ and thus prevent over-exploitation of prey populations through predation (Berdegue *et al* 1996). Moreover, the production of allomones in defense may secondarily act as kairomones that attract specialist natural enemies (Corbet1968, De Baur & Yeargan 1994). Therefore, allomones secreted by *L. longipalpis* may function more as dispersal pheromones, thus reducing the risk of cannibalism, than in deterring predators.

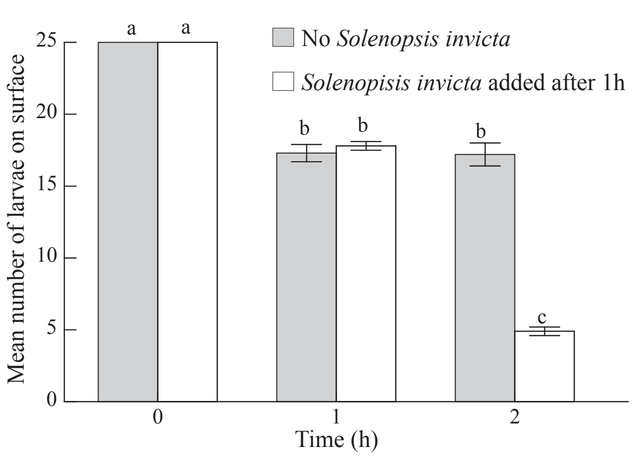

Fig 1 Number of *Lutzomyia longipalpis* larvae observed on the soil surface in arenas after 0, 1and 2h. Shaded bars = controls, hatched bars = arenas in which five *Solenopsis invicta* worker ants were added after 1h. Line bars represent standard error of the mean. Sample size = 25 larvae for each treatment, with 10 replications.

Our results suggest that further research is necessary to evaluate the effectiveness of various defensive strategies employed by immature *L. longipalpis*, particularly in the field. It would be useful to identify more specialized and potentially better adapted natural enemies of sandflies (e.g. parasitoid wasps and flies), and to explore what mechanisms sandfly larvae employ to protect themselves against attack from these natural enemies.

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