Physical Factors Influencing the Oviposition of *Lutzomyia migonei* (Diptera: Psychodidae) in Laboratory Conditions

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To determine the influence of physical factors on oviposition of Lutzomyia migonei *(Diptera: Psychodidae) under laboratory conditions, two sets of experiments were performed. The first test was to determine the influence of the size of pots on oviposition. Gravid flies were placed individually or in groups in different oviposition pots. The number of eggs laid, oviposition time and survival of gravid females were observed. In the second experiment, the influence of irregular surfaces on oviposition was studied. The results suggested that physical space was not an important factor in the oviposition behavior of* L. migonei *and that the flies showed a preference to oviposit on irregular horizontal surfaces in response to thigmotropic behavior.*

Key words: *Lutzomyia migonei* - sandfly - oviposition - physical space - irregular surface - laboratory conditions

Lutzomyia migonei (França 1920) is widely distributed in South America (Young & Duncan 1994). This is an important man-biting sandfly and is considered to be the vector of cutaneous leishmaniasis in Venezuela and Brazil (Pessoa & Pestana 1940, Pifano & Ortiz 1952, Feliciangeli 1991, Queiroz et al. 1994).

One of the main problems that occur during sandfly colonization is the high mortality of gravid females before or during oviposition. This mortality hampers studies of sandfly biology, the productivity of the colony and experimental studies of transmission on *Leishmania* (Killick-Kendrick et al. 1977, Endris et al. 1982, Chaniotis 1986). Oviposition in sandflies is controlled through a combination of complex interactions between environmental, physical and chemical factors. Studies on the physical factors showed that temperature and relative humidity were important factors in the regulation of oviposition behavior (Foster et al. 1970, Chaniotis 1986). Furthermore, it was observed that the oviposition substrate stimulates a thigmotropic response in gravid flies (El Naiem & Ward 1992a). El Naiem and Ward (1990, 1991) discovered the existence of an oviposition pheromone associated with the eggs of *L. longipalpis*. This pheromone has been isolated from female accessory glands and is secreted on to the eggs during oviposition (Dougherty et al. 1992). El Naiem and Ward (1992b) also demonstrated oviposition preferences in *L. longipalpis* for surfaces containing frass, larval rearing medium and rabbit faeces. In our study, we examined the influence of physical factors on oviposition by *L. migonei.* The study was designed to determine if the female sandfly lays more eggs on irregular surfaces than on flat surfaces, and to determine the effect that physical space has on oviposition in the gravid female.

MATERIALS AND METHODS

Sandflies - L. migonei used in the experiments belonged to a colony initiated in 1993 with flies collected in Shannon light traps in a coffee plantation located at 1360 m above sea level at Ejído, Mérida, Venezuela. The methods for establishing and maintaining the colony in the laboratory were those described by Killick-Kendrick et al. (1977). Larvae and adults were reared in an incubator (Eletrolab) at a temperature of 25°C with 95% RH. Larval food was a powder mixture 1:1 of coffee-leaves and the larval diet was as described by Young et al. (1981).

Before use in experiments, females were fed on hamsters anesthetized with a 50 mg/ml solution of sodium pentabarbitone. They were subsequently isolated with equal numbers of males in 15x15x15 cm nylon cages and offered a 50% sucrose solution, in an atmosphere of 25°C, 95% RH, for three days to allow complete oogenesis and defaecation.

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Physical space - The effects of physical space on oviposition were investigated using five types of oviposition pots of different sizes and capacities (Table I). These oviposition pots were constructed in a base of polymethyl pentane, with a layer of plaster of Paris on the bottom and walls of the pots. The pots were tested using 1-3 day-old females individually and in groups.

TABLE I

Size of the pots

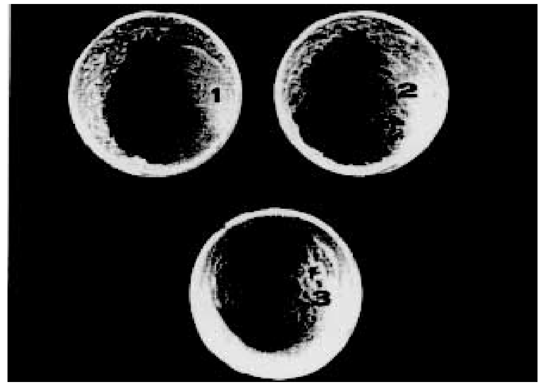
Pot type	Width (cm)	Height (cm)	Volume (ml)[a]
1	3.5	6	60
2	5.5	6	140
3	8.5	10	580
4	11.5	11.5	1020
5	16.5	17.5	3300

a: quoted by manufacturer.

In the group experiment, twenty females were introduced into each of five types of pots. This experiment was replicated six times on different days. Oviposition in each pot was monitored until the females died. The number of females that survived for one day post oviposition were recorded. At the end of the experiment, females were dissected to determine the number of females with complete oviposition, and the number of laid eggs per pot were counted.

In the individual experiments, single females were placed in one of five types of oviposition pots. This was replicated twenty times on different days. Atmosphere conditions were as already described. The oviposition and survival of the female in each type of pot were recorded, and the number of laid and retained eggs were counted. The results were analyzed statistically by generalized linear model and analysis of variance.

Irregular surfaces - To determine if the sandfly females lay more eggs on irregular or flat surfaces, three oviposition pots were constructed using layers of plaster of Paris, as follows: (a) an irregular bottom surface and a flat wall surface, (b) a flat bottom surface and an irregular wall surface, and (c) irregular bottom and wall surfaces (Fig.). As controls, pots with flat bottom and wall surfaces were used. Females of 1 day-old were fed and sixty gravid females were placed singly in each type of pot. The oviposition and survival of the females in the different types of oviposition pots were recorded. The total number of eggs laid and retained per female in each type of pot was counted. For eggs laid, surface of preference was then determined by counting. The results were analyzed statistically using analysis of variance.



Oviposition pots: a flat bottom surface and irregular wall (1), irregular bottom and wall surfaces (2) and irregular bottom surface and a flat wall surface (3).

RESULTS

Oviposition response of *L. migonei* females placed in groups of twenty in oviposition pots of different sizes is shown in Table II. There was considerable variability in the numbers of eggs per pot, as is shown by the standard error. Statistical analysis revealed significant variation between the replicate sets of flies tested in each experiment. However, analysis also showed that the variation in the number of eggs laid in oviposition pots of different sizes can be accounted for by differences between replicates and not by pot parameters (Table III).

The oviposition response of *L. migonei* females placed individually in oviposition pots of different sizes is shown in Table IV. No significant difference was detected between the parameters and physical space of the oviposition pots.

TABLE II

Oviposition response of *Lutzomyia migonei* females placed in groups of twenty in oviposition pots of different sizes[a]

Pot type	Number of eggs laid	Females with complete oviposition	Females surviving first day
	Mean ± SE	%	Mean ± SE
1	724.0 ± 267.0	54.2	7.33 ± 4.13
2	792.7 ± 87.2	67.5	8.67 ± 2.16
3	661.3 ± 157.0	60	6.17 ± 3.06
4	629.8 ± 138.6	48.3	4.50 ± 1.76
5	718.2 ± 228.9	55	5.50 ± 2.43

a: with six replicates per pot type; SE: standard deviation.

TABLE III

Deviance of generalized linear model

Cause of variation	df	"deviance"	p-value
Replicate number	5	39.97	0.000[a]
Width of pot type	1	1.01	0.314
Height of pot type	1	0.59	0.440
Width x height	1	4.61	0.032[a]
COP	1	9.5	0.000[a]
Residual	20	20	0.458
Total	29	75.71	

Width x height: relationship between width and height of pot type; COP: females with complete oviposition; *a*: significant values.

Results of the oviposition response of *L. migonei* females placed individually in oviposition pots with irregular surfaces are summarized in Table V. The response of female *L. migonei* to pots with irregular and/or flat surfaces was not statistically different in relation to number of eggs laid, complete oviposition and days of survival post oviposition. An irregular surface had an inhibitory effect on the oviposition and significantly delayed oviposition time in pots with irregular surface versus the controls and also a significant difference was detected for mean longevity of the females among pots with irregular and flat surfaces. While the total number of eggs in each type of pot was relatively constant, we observed that when presented with a choice between irregular and smooth surfaces in the same pot, more eggs were laid on the horizontal irregular surface than on the flat surfaces ($P<0.05$) (Table VI).

DISCUSSION

The results obtained with *L. migonei* using groups of females or individually isolated females in oviposition pots of different sizes, indicated that physical space is not an important factor controlling oviposition behavior in laboratory conditions. In the sandfly, the act of oviposition is apparently an exhausting process. Under laboratory conditions only a few females survive long enough to complete oviposition and subsequently take a second blood meal (Chaniotis 1967). In nature, where more than one gonotrophic cycle is considered nor-

TABLE IV

Oviposition response of *Lutzomyia migonei* females placed individually in pots of different sizes

Pot type	Number of eggs laid				Females with complete oviposition	Females surviving		Oviposition time			
	Mean	±	SE	(range)	%	%	(days)	Mean	±	SE	(range)
1	41.15	±	18.0	(11-61)	70	10	(1-3)	4.80	±	1.39	(4-6)
2	49.45	±	12.0	(10-69)	80	30	(1-2)	4.35	±	0.48	(4-5)
3	43.00	±	15.1	(14-64)	55	25	(1-1)	4.30	±	0.57	(4-6)
4	48.90	±	13.5	(37-65)	90	30	(1-4)	4.20	±	0.52	(4-6)
5	46.70	±	16.8	(8-73)	55	30	(1-2)	4.55	±	0.75	(4-6)

n: twenty gravid females per pot type; SE: standard deviation.

TABLE V

Oviposition response of *Lutzomyia migonei* females placed individually in oviposition pots with irregular surfaces

Pot type	Number of eggs laid[a]				Complete oviposition	Days of survival post oviposition				Oviposition time				Longevity			
	Mean	±	SE	(range)	%	Mean	±	SE	(range)	Mean	±	SE	(range)	Mean	±	SE	(range)
Control	47.67	±	13.02	(4-68)	81	0.883	±	0.222	(1-4)	5.20	±	1.38	(4-8)	6.08	±	1.47	(4- 9)
Bottom	47.53	±	14.05	(13-70)	83	1.067	±	1.191	(1-6)	5.80	±	1.51	(4-10)	6.86	±	1.67	(4-12)
Wall	47.45	±	15.08	(8-73)	85	0.983	±	0.948	(1-4)	5.48	±	1.50	(4-8)	6.46	±	1.80	(4-11)
All	51.37	±	10.82	(28-69)	83	1.317	±	1.142	(1-5)	5.85	±	1.65	(4-10)	7.16	±	1.69	(5-11)

a: included eggs laid in all the pot; n: sixty gravid females per pot type; Control: all flat; Bottom: irregular bottom surface and flat wall surface; Wall: irregular wall surface and flat bottom surface; All: irregular bottom and wall surfaces; SE: standard deviation.

TABLE VI

Preference of *Lutzomyia migonei* females placed individually in oviposition pots with irregular surfaces

Pot type	Number of eggs laid on[a]					
	Bottom			Wall		
	Mean ±	SE	(Range)	Mean ±	SE	(Range)
Control	12.98 ±	13.70	(0-61)	15.49 ±	13.8	(0-44)
Bottom	18.76 ±	17.84	(0-62)	15.31 ±	14.7	(0-55)
Wall	10.00 ±	9.61	(0-41)	21.20 ±	14.5	(0-45)
All	20.58 ±	14.00	(0-56)	17.89 ±	11.9	(0-50)

a: not included eggs laid in the nylon cover; n: 45 gravid females per pot type; Control: all flat; Bottom: irregular bottom surface and flat wall surface; Wall: irregular wall surface and flat bottom surface; All: irregular bottom and wall surfaces; SE: standard deviation.

mal, it is possible that eggs are laid one by one over a comparatively wide area. However, several reasons can be offered to explain our observation that pot size has no effect: (1) under natural conditions, sandflies may spend most of their time in optimum microhabitats and have a short capacity for flight (Forattini 1973); (2) in the laboratory, gravid females are often routinely individually confined for oviposition in small vials. These vials are able to supply the vital requirements for satisfactory oviposition (Chaniotis 1967); (3) there is evidence that sandflies have short longevity (Endris et al. 1984). Thus, there is evidence to support our finding that physical space is not important in the oviposition behavior of *L. migonei*.

In sandflies, gravid females use a complex set of oviposition stimuli, that are thought to be controlled by complex interactions between environmental and biochemical factors. Difficulties in mimicking natural environmental conditions in the laboratory may result in high mortality rates during oviposition (Killick-Kendrick et al. 1977, Chaniotis 1986, El Naiem & Ward 1992b). The physical nature of the oviposition substrate is known to be an important oviposition stimulus for sandflies (Killick-Kendrick 1987, Dougherty et al. 1992). El Naiem and Ward (1992a) demonstrated thigmotropic behaviour in *L. longipalpis*, showing that gravid females prefer to oviposit in vertical crevices rather than open, flat surfaces. Similarly, our observations showed that *L. migonei* has a preference for laying eggs on irregular horizontal surfaces of oviposition pots rather than on flat surfaces. In addition to preference, oviposition was generally delayed on irregular pots thus stimulating longevity in females. These results support the previous suggestions that natural oviposition of sandflies occurs in cracks and crevices (Young et al. 1926, Lewis & Kirk 1954). Similarly, the results obtained agree with the observations of El Naiem and Ward (1992a) who suggested that under natural conditions, thigmotropic behavior of ovipositing sandflies may have a selective advantage in directing the females to lay eggs in crevices or cracks. This would subsequently provide protection and higher humidity to the emerging larvae, and crevices in natural breeding sites may contain more potential sandfly larval food than flat surfaces.

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