ECOLOGY OF PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) IN A FOCUS OF LEISHMANIA (VIANNIA) BRAZILIENSIS IN NORTHEASTERN COLOMBIA

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The phlebotomine sand fly fauna of two coffee plantations in a Leishmania-endemic area of Norte de Santander, Colombia was studied. Regular insect collections using a variety of methods were made for three and a half years. Information was obtained on diurnal resting sites, host range and seasonal abundance for 17 species, of which five (Lutzomyia spinicrassa, Lu. serrana, Lu. shannonii, Lu. ovallesi and Lu. gomezi) were far more numerous than the others, anthropophilic and present throughout the year. The behaviour of these and the remaining 12 species is discussed in relation to their potential role in transmission of Leishmania (Viannia) braziliensis in the area.

Key words: Leishmania (Viannia) braziliensis – ecology – phlebotomine – sand flies – coffee plantations – Colombia

The phlebotomine sand fly (Diptera: Psychodidae) fauna of Colombia is known to consist of at least 125 species (Young & Morales, 1987), including several proven and suspected vectors of Leishmania. Human leishmaniasis is widespread and has been recorded from each of the 32 Departments of Colombia, with the exception of San Andres and Providencia (Corredor et al., 1990). The distribution and etiology of leishmaniasis in the country has been reviewed by Werner & Barreto (1981) and Corredor et al. (1990).

Phlebotomine sand flies have been recorded from all parts of the country, including the Caribbean island of Providencia (Alexander, unpublished data), and occur in a wide variety of habitats, from very wet tropical rain forest to dry xerophytic scrub. Many species have become adapted to habitats extensively modified by man, including coffee plantations in which large trees are left in place after the original forest has been cleared to shade the crop from the sun. The trunks and buttress roots of these trees provide microhabitats in which sand flies can rest during the day, and their leaf litter, together with that from the coffee bushes, provides additional resting and breeding sites for the insects. Man and his domestic animals provide sources of blood for female sand flies, supplementing or replacing the depauperate mammalian fauna which remains after the original forest is cleared. The insects have therefore all the requirements to complete their life cycle within a relatively small area, and studies of dispersal of Lutzomyia sand flies have revealed their relatively sedentary habits (Chaniotis et al., 1974; Alexander, 1987).

Cutaneous leishmaniasis has apparently long been known from the Department of Norte de Santander, and Werner & Barreto (1981), in their review of leishmaniasis in Colombia, cite four common names for the disease that pertain to the region, i. e., “ulcer de Pamplona”, “ulcer de Chinacota”, “ulcer de Pamplonita” and “ulcer de Cucutilla”, the last of which refers to a village situated 10 km from Arboledas, where the study reported here was carried out.

The purpose of this study was to determine which sand fly species were present in the Arboledas area and to investigate several aspects of their biology. This information was

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used to identify the species likely to be involved in Leishmania transmission on the basis of factors such as their seasonal abundance, diurnal resting sites, degree of anthropophilic behaviour, host range and propensity to enter human habitats.

MATERIALS AND METHODS

Study area – According to the 1985 census, Arboledas (7° 39' N, 72° 48' W) had 10,766 inhabitants, of which 2,030 lived in the town itself and the remainder in outlying “veredas” or rural districts (DANE, 1988). The town is situated approximately 65 km from the Venezuelan border, in the Department of Norte de Santander. Arboledas lies at an altitude of 869 m, in an agricultural region largely devoted to coffee production, with cattle-raising and sugar cane cultivation next in importance. According to the classification of vegetation types devised by Holdridge (1947), the region is in a zone of “very humid subtropical forest” although little of the original forest remains.

The climate of the Arboledas region features two distinct rainy seasons, each of approximately three months’ duration (March-June and October-December), separated by two dry seasons. Average annual rainfall is in the order of 1500 mm, and mean annual temperature is 21°C, although, as in much of the Tropics, temperature fluctuates more in the course of a single day than between the seasons. The causative organism of human cutaneous leishmaniasis in the area has been identified as Le. braziliensis braziliensis (= Le. (Viannia) braziliensis) (Corredor et al., 1990), and Young et al. (1987) isolated promastigotes of this parasite from a single wild-caught female sand fly Lutzomyia spinipennis Morales et al. collected near Arboledas. They also observed unidentified Leishmania-like parasites in five other Lutzomyia species from the same site; however only 13 natural infections were observed in 18,463 dissected sand flies, suggesting that the parasite is maintained at a very low level in the vector population.

Regular sand fly collections and observations were made from May 1984 to December 1987 in two coffee plantations (Finca La Esperanza and Finca La Quinta) in the vereda of Siravita. Both plantations lay at an altitude of approximately 850 m, 3 km from Arboledas and about 1 km apart. In both plantations the coffee crop was shaded by trees which could be grouped into two categories based on height and girth. The first of these consisted of tall, mature trees with large buttress roots and thick trunks reaching heights of up to 30 m. At La Esperanza these trees belonged almost exclusively to two species, i.e., “ceibo” (Erythrina sp.: Papilionaceae) and “curo canelo” (Nectandra sp., Lauraceae), but at La Quinta several other large species were present, including “pardillo” (Cordia alliodora: Boraginaceae), “guacharaco” (Cupania cinerea: Sapindaceae), “tarai” (Simarouba amara: Simaroubaceae) and “caracoli” (Anacardium excelsum: Anacardiaceae). Tree species in the second category were numerically much more important in each of the tree plantations. The trees belonging to this category were fast-growing, rarely exceeded 20 m in height and had relatively smooth, slender trunks without buttress roots. The predominant species of this category in all both plantations was “guamo” (Inga edulis: Mimosaceae), with several other species present in smaller numbers, including “cedro” (Cedro angustifolia: Meliaceae), “majo” (Alchornea sp.: Euphorbiaceae) and “pumorroso” (Eugenia jambos: Myrtaceae). Together with the coffee bushes, these two categories gave a three-tiered covering of vegetation in the plantations, with foliage levels at approximately 2, 20 and 30 m above ground. The floor of both plantations was thickly carpeted with leaf litter, and the dominant plant species in the undergrowth was red-flowered balsam (Impatiens sp.: Balsaminaceae).

The mammalian fauna of the area has also been surveyed (Tesh et al., 1986) and is known to include the opossums Didelphis marsupialis, Caluromys lanatus, Metachirus nudicaudatus and Marmosa sp.; the armadillo Dasypus novemcinctus; the tree porcupine Coendou sp.; the rats Holochilus sp., Orzyomys sp. and Rattus rattus; and the squirrel Sciurus granatensis. Several unidentified species of bat were also present. Domestic animals found in or around the plantations included pigs, horses, mules, dogs and cats. Large numbers of chickens were allowed to forage in the plantations during the day, roosting in shelters near the houses after dark.

The human population of both plantations consisted of one or more large families with several young children living on the edge of the plantation in close proximity to the coffee crop. La Esperanza had three such families
living within its boundaries. In one of these families two of the children (aged 12 and 21) had active Leishmania lesions and were undergoing treatment at the Hospital San Juan de Dios, Arboledas in July 1984. The situation at La Quinta was notable in that all of the children were very young (in 1984 the eldest was 13 years old, and the youngest was born in May of that year) and all but the baby had active lesions or scar tissue from a prior infection with Leishmania.

**Sampling methods – Diurnal collections:** sand flies were collected from diurnal resting sites such as tree trunks, holes and buttress roots and leaf litter using the mouth aspirators and holding containers described in Endris et al. (1982). Sampling of sand flies in leaf litter was done using a wooden frame of triangular cross section covered in fine nylon mesh, similar to the type used in Panama by Chaniotis et al. (1972). This was placed on the ground over a patch of leaf litter, which was agitated with a stick to flush out resting sand flies. These were aspirated as they landed on the walls of the trap. Seasonal abundance of the five most numerous sand fly species was assessed by making 15-minute collections from each of ten selected trees an average of three times per week, and calculating mean numbers per collection per month. **Nocturnal collections:** several trapping methods were employed to capture sand flies within the peak period of biting activity (18.00-21.00). These included protected human bait, in which volunteers wearing long-sleeved clothing and with their exposed skin protected by insect repellent captured sand flies as they landed preparatory to biting; CDC light traps (Sudia & Chamberlain, 1962), illuminated Shannon traps (Shannon, 1939), and Disney traps (Disney, 1966) baited with Proechimys sp. rats. Intradomestic collections were made after dark in houses adjoining the coffee crop, and two flight (Malaise) traps (Gressitt & Gressitt, 1962) were hung in the La Esperanza plantation from 1984-6.

Most sampling was done at or near ground level, but an attempt was also made to sample sand fly populations at higher levels. Samll trees (i.e., those described earlier as belonging to the second category), were scaled using a pair of metal tree steps and a body belt with a safety rope (Ben Meadows Co., Atlanta, GA) and CDC light traps hung at heights of up to 16 m.

**RESULTS AND DISCUSSION**

**Diurnal resting sites** – The species compositions of the sand fly faunas in four diurnal resting site categories are shown in Fig. 1. Sixteen of the 17 sand fly species collected at La Esperanza were found on Nectandra and Erythrina trees, principally on the lower trunks and buttress roots although seven species were also encountered resting in tree holes. The most abundant species were *Lu. spinicrassa* (36.6% of all sand flies collected on tree trunks), *Lu. serrana* (Damascono & Arouck) (20.7%) and *Lu. shannoni* (Dyar) (17.8%). Tree hole collections were dominated by *Lu. shannoni* (39.8%), *Lu. spinicrassa* (21.5%) and *Lu. gomezi* (Nitzulescu) (19.4%).

A few examples of the remaining species, *Lu. dubitans* (Sherlock) were collected in leaf litter. Collections from the latter microhabitat generally contained very few sand flies, although ten of the species from La Esperanza and La Quinta were represented, with *Lu. ovallesi* (Ortiz) predominat at 38.9% of all sand flies collected, followed by *Lu. spinicrassa* (22.6%) and *Lu. serrana* (21.0%).

Small, more slender trees such as *Inga edulis* were apparently not highly favoured as resting sites, although small numbers of eight species were collected from their trunks. Here *Lu. serrana* was most abundant (28.6%), followed by *Lu. ovallesi* (26.1%) and *Lu. gomezi* (19.4%). Most species appeared to be fairly catholic in their choice of diurnal resting sites, and the percentage composition of these species in the sand fly fauna differed little among the categories. Three of the species collected during the present study, i.e., *Lu. shannoni, Lu. ovallesi* and *Lu. gomezi*, were among those featured in studies of diurnal resting sites in Panamanian rain forest by Thatcher & Hertig (1966) and Chaniotis et al. (1972), and the apparent differences between the observations made during these studies and the present one might be explained by dissimilarities in the ecology of the two areas. The variety of microhabitats and plant species available in rain forest is much greater than that in a coffee plantation, as is the number of Lutzomyia species competing for these resting sites.

In most resting site collections males greatly outnumbered females, and this was particularly marked in *Lu. gomezi*, where the ratio was 10/1 in tree hole collections, 6.8/1 on large
Fig. 1: proportions of Lutzomyia species collected in four categories of diurnal resting site (large tree trunks and buttress roots, tree holes, small trees and leaf litter) at Fincas La Esperanza and La Quinta, Arboledas 1984-6.

*Other species collected were: Lu. atroclavata, Lu. davisi, Lu. dubitans, Lu. erwindonaldi, Lu. ferroae, Lu. lichyi, Lu. muñeztovari, Lu. pia, Lu. species near sauroidea and Lu. walkeri; Brumptomyia beauperti and Warileya rotundipennis.

tree trunks, 5.2/1 in leaf litter and 4.4/1 on small trees. Two possible explanations for this finding are that the females were resting in other microhabitats, perhaps higher in the trees, or that the imbalance was the result of differential mortality between the sexes, since only female sand flies have to take a blood meal and face the dangers associated with finding and feeding on a host. The latter would not explain the observation that most females encountered at these levels were older, gravid females, and a combination of factors probably explains this unequal sex ratio.

Perhaps the most interesting observation in terms of possible future control measures is that trees of the second category support much smaller populations of sand flies than the larger ceibos and curos canelo, even when the two categories are compared as a whole and the smaller tree species found to outnumber the larger by more than ten to one. Nevertheless, these smaller trees are of greater importance (by virtue of their larger numbers) in shading the coffee bushes, and removal or treatment with residual insecticides of the “Category 1” group might result in a significant reduction in the populations of certain sand fly species without adversely affecting the coffee crop. The possibility that sand flies displaced from these large trees would simply move to other categories of resting site should also be considered.

Nocturnal trapping – Percentages of each species collected according to the various trapping methods are shown in the Table. Protected human bait collections at La Esperanza and La Quinta included 11 species, with females of Lu. spinicrassa (46.3%), Lu. gomezi (16.6%), Lu. ovallesi (7.6%) and Lu. serrana (5.5%) the most numerous. Males of eight of these anthropophilic species, including the four commonest, were sometimes collected as they
TABLE

Percentages of 14 Lutzomyia species collected by various trapping methods in coffee plantations near Arboledas, Colombia

<table>
<thead>
<tr>
<th>Lutzomyia species</th>
<th>Protected human bait</th>
<th>Intradomiciliar</th>
<th>Day-biting</th>
<th>Shannon trap</th>
<th>Disney trap</th>
<th>Flight trap</th>
<th>CDC light trap 1.5 m</th>
<th>CDC light trap 7.0 m</th>
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<tr>
<td></td>
<td>d</td>
<td>q</td>
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<td>&lt;0.1</td>
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</table>

alit on human volunteers, suggesting that mating in these species may take place on the host. This was particularly true for Lu. gomezi, since 4.5% of all sand flies landing on human volunteers were males of this species, and Lu. serrana, in which the number of males taken (3.7% of all sand flies collected on human bait) was over two-thirds of the number of females. Eight species, again including the four most numerous, were taken in intradomiciliar collections at night, and it is possible that biting in houses is responsible for many cases of leishmaniasis in the area, particularly among young children who do not enter the peridomiciliary area after dark.

Man-biting behaviour has been reported in two of the remaining members of the Arboledas sand fly fauna. Lu. atroclavata (Knab), of which only one specimen was collected during the entire study, has been implicated in the transmission of Le. chagasi on the island of Guadeloupe (Courmes et al., 1966). Fairchild & Hertig (1951) reported Warileya rotundipennis (F. & H.) feeding on man in Panama. A single example of this species was collected in Arboledas in 1984. Little is known of the feeding behaviour of either species however, and their apparent rarity in the Arboledas area it seems unlikely that they are involved in Leishmania transmission to man.

Collections from CDC light traps suspended at heights of approximately 1.5 m included 13 species, predominantly the four major man-bitters (29.5% Lu. spinicrassa, 17.0% Lu. serrana, 18.0% Lu. ovallesi and 8.9% Lu. gomezi), as well as Lu. davisi (Root) (18.8%). Light traps hung at 7-16 m above the ground were dominated by Lu. spinicrassa (51.9%), Lu. serrana (27.5%) and Lu. gomezi (7.3%). Lu. ovallesi was considerably less abundant at the upper level, and its sex ratio at 7 m and above was approximately 1:1, as compared to more than 6:1 near the ground. A similar pattern was seen in Lu. davisi, for which the sex ratio at the lower level was 3:1. In Lu. spinicrassa and Lu. serrana females outnumbered males at both levels in light trap collections, while in Lu. gomezi the ratio was approximately 1:1. Assuming that the female sand flies collected were seeking a blood meal, these results suggest that the preferred hosts of Lu. ovallesi and Lu. davisi were ground-living rather than arboreal mammals, while those of the remaining species occurred at both levels.

Collections at illuminated Shannon traps, which possessed several attractive stimuli (host odour, temperature and CO₂) as well as light, attracted ten species, including all the anthropophilic ones from La Esperanza except Lu. lichyi (Floh & Abonnenc). Females of Lu. spinicrassa dominated these collections, constituting 67.3% of all sand flies caught by this method. Males of this species were taken very rarely in the Shannon trap and accounted for less than 1% of the total.

Disney traps baited with Proechimys were employed on fewer occasions than other trapping methods, and only 38 sand flies were collected in total by this method from May-August 1984. Nevertheless six species were
represented in these collections, including both sexes of *L. gomezi* (47.3%), *L. serrana* (28.9%), *L. spinicrassa* (13.2%) and females of *L. ovalesi* (5.3%), *L. erwindonaldoi* (Ortiz) and *L. lichyi* (both 2.6%).

Flight trap collections included ten species, principally *L. spinicrassa* (25.6%), *L. ovalesi* (19.7%), *L. serrana* (10.8%) and *L. gomezi* (8.2%), the four major anthropophilic sand flies in the area, *L. shannoni*, the other abundant species in resting site collections, constituted only 7.8% of flight trap samples. Two species that were fairly numerous in other types of collection, namely *L. erwindonaldoi* and *L. nuñezovari* (Ortiz), were never found in flight traps, while unexpectedly large numbers of *L. davisi* (13.4%), *L. dubitans* (2.4%) and *L. walkeri* (Newstead) (1.5%) were taken. This being a method of trapping by interception rather than by attraction, it may give a more accurate picture of the relative abundance of species in the area. If so, then the results of resting site collections reflect the true relative abundance of five of the six most important species, with *L. davisi* considerably unrepresented and presumably spending the daylight hours in some microhabitat category that was not investigated.

*Seasonal abundance* – This was determined largely from diurnal resting site collections, supplemented by information obtained through other trapping methods. The most complete information, obtained from La Esperanza between 1984-87, demonstrated that at least eight of the 17 species, i.e., *L. spinicrassa, L. serrana, L. shannoni, L. ovalesi, L. gomezi, L. pia* (Fairchild & Hertig), *L. lichyi* and *L. nuñezovari*, were present throughout the year. *L. davisi* was collected in every month except March, although this species, together with *L. walkeri* (not collected in January, March or April) and *L. erwindonaldoi* (not collected in April or September) is probably also present throughout the year. The numbers of the six remaining species i.e., *L. ferroae Young & Morales, L. sp. near sauroidea* (Osorno et al.), *L. dubitans, L. atroclavata, Brumptomyia beauvertui* (Ortiz) and *Warileya rotundipennis*, were never high at any time of year, either because the habitat was not as favourable to them as to the commoner species or because the sampling methods used were inappropriate, and no meaningful conclusions can be drawn about their apparent absence in certain months of the year.

Population fluctuations for the five species that were by far the most numerous in resting site collections are shown in Fig. 2. Numbers of four species remain fairly constant throughout the year, with an apparent fall in population in July and August and an increase in October, coinciding with the onset of the second rainy season. This pattern was particularly noticeable for *L. spinicrassa*, with two peaks and two troughs during the rainy and dry seasons respectively. Highest numbers of this species at both La Esperanza and La Quinta were recorded in November, and lowest numbers in July.

Figure 2: mean numbers of sand flies of five species per resting site collection (3 man-hours) from 1984-7 at La Esperanza and La Quinta plantations, Siravita, Arboledas. Mean rainfall in area 1983-1985 also shown.

Gillies (1974) suggested that monitoring of the seasonal abundance of *Anopheles* by making collections from daytime resting shelters was too subjective to yield precise measurements. This method may be undesirable because of the difficulty in obtaining consistent replicates of collections and the dangers of depleting the populations in the sampling area, a particular danger in sand flies which appear to be have a fairly short flight range (Alexander, 1987). The accuracy of the estimates made in this study is however supported by the close similarity between the numbers and population changes between collections made at La Esperanza and La Quinta (Fig. 3).

Distribution by month of the 226 cases of cutaneous leishmaniasis reported in Arboledas from 1983-86 are shown in Fig. 3. Since the time between infection and reporting for treatment was very variable, the months in which
most *Leishmania* infections were acquired cannot be determined. In July 1985, 20 cases were reported, the most for any single month during the study, and 105 patients underwent treatment at the Hospital San Juan de Dios during that year, against 37, 54 and 30 for 1983, 1984 and 1986 respectively. Peak months in 1983, 1984 and 1986 were November (9 cases), June (13 cases) and February (7 cases). Of 61 cases recorded from 1984-6, 10 (16.4%) were in children of four years old or less, 24 (39.4%) were in school age children (5-14 years old) and the remaining 27 (44.2%) were in adults of 15 years or older (Elide Paez, personal communication).

![CASES OF HUMAN CUTANEOUS LEISHMANIASIS 1983-1986](image)

Fig. 3: numbers of active cases reported for the first time in the Arboledas area (incorporating records from all districts, including Siravita) from 1983-1986. (Courtesy Hospital San Juan de Dios, Arboledas). Mean numbers per month 1983-6 (histogram) shown with total numbers per month for each of these years.

There is little evidence relating incidence of leishmaniasis to sand fly density, and even in situations where the appearance of cases shows a consistent pattern throughout the year assumptions of a linear relationship between these and the size of vector populations should be made with caution. Dye (1988) developed a mathematical model for canine leishmaniasis epidemiology that takes into account the limited data available relating sand fly density to prevalence of *Leishmania* infection.

Precipitin testing of blood from female sand flies collected at Arboledas revealed that *Lu. spinicrassa* females took blood from man, chickens, opossums, pigs and horses; *Lu. serrana* from man, pigs and chickens; *Lu. shannoni* from man, opossums and chickens; *Lu. ovallesi* from man, chickens, rabbits and pigs; and *Lu. erwindonaldoi* from man and pigs (Dr H. A. Christensen, Gorgas Memorial Laboratory, personal communication).

Whether these animals are the principal hosts of these sand fly species or whether others are of greater importance was not established.

In summary, it appears that the *Leishmania*-endemic focus at Arboledas has a sand fly fauna consisting of 17 species, of which at least 11 are anthropophilic to some extent. Nine species enter human dwellings and all are attracted to light, so that transmission of *Leishmania* could occur in houses on the edge of coffee plantations. Alternatively, local residents could acquire the disease during nocturnal activities such as hunting in the coffee groves or secondary forest that surrounds the Arboledas area. A third method would be to be bitten by sand flies disturbed from their resting sites during the day. However the occurrence of *Leishmania* infections in very young children and the rarity of occasions on which the senior author was bitten during several hundred hours of resting site collections (twice by females of *Lu. spinicrassa* and three times by *Lu. ovallesi*), suggests that the last of these is the least important mechanism of transmission to man in the Arboledas area. Nine species (all of the species captured inside houses except *Lu. lichyi*, as well as *Lu. erwindonaldoi*) were collected by various methods in the town itself on a few occasions. In addition to the above species, *Lu. longipalpis* (Lutz & Neiva) has been collected at Cinera, a vereda of Arboledas not sampled during the present study (Hilda Guzman, personal communication). This is a proven vector of *Le. (Leishmania) chagasi* in other parts of its range but no cases of visceral leishmaniasis have been reported from the Arboledas area.

The relative importance of each species in transmission of *Le. (V.) braziliensis* in the area cannot be fully established based on the findings of the present study. Although the isolation of *Le. braziliensis* made from a female *Lu. spinicrassa*, the highly anthropophilic nature and the year-round abundance of this species suggest that it is the primary vector in Arboledas, several other species may be involved in transmitting the parasite from its reservoir (as yet unidentified) to man, and between non-human hosts. At least three of the other species represented in the phlebo-
tomine fauna of Arboledas, i.e., *Lu. shannoni*, *Lu. gomezii* and *Lu. ovallesi*, have been implicated as vectors of *Le. (V.) panamensis* in other parts of their ranges (Killick-Kendrick, 1990), and natural infections of *Lu. ovallesi* with *Leishmania* species have been recorded from western Venezuela (Felicianelli et al., 1988; Bonfante-Garrido et al., 1991).

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