



Article/Artigo

Sandfly frequency in a clean and well-organized rural environment in the State of Paraná, Brazil

Frequência de flebotomíneos em ambiente rural com boas condições de limpeza e organização, no Estado do Paraná, Brasil

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ABSTRACT

Introduction: Sandflies caught in Santa Juliana Farm in Sarandi, State of Paraná, Brazil, were assessed in terms of their fauna, seasonality, and frequency in the homes and in shelters of domestic animals around the homes, as well as in the nearby forest. **Methods:** In Santa Juliana Farm, there are no records of cases of ACL, differing from other relatively clean and organized areas where surveys of sandflies have been conducted in Paraná. Samples were collected with Falcão light traps, fortnightly from 22:00 to 02:00 hours, from November 2007 to November 2008. **Results:** A total of 4,506 sandflies were captured, representing 13 species, predominantly *Nyssomyia whitmani* (71.8%). More sandflies were collected in the forest (52.6%) than outside the forest (residences and pigsty) (47.4%). However, *Ny. whitmani* was collected in greater numbers outside (38.3%) than inside the forest (33.5%). Most sandflies were collected in the warmer months and during periods with regular rainfall. **Conclusions:** The results suggest that cleaning and organization around the houses could reduce sandfly population in peridomicile. Constructing shelters for animal at a distance of approximately 100m from domiciles is recommended to prevent the invasion of sandflies, as this farm has an area of preserved forest, with wild animals and sandflies present to maintain the enzootic cycle of *Leishmania*.

Keywords: Phlebotominae. Vectors ecology. Control of leishmaniasis.

RESUMO

Introdução: Relatam-se os resultados de coletas de flebotomíneos na Fazenda Santa Juliana, município de Sarandi, Estado do Paraná, Brasil, onde se verificou a fauna, a sazonalidade e a frequência desses insetos nos ambientes florestal e extraflorestal. **Métodos:** O trabalho foi realizado nesta fazenda pela inexistência de registro de casos de LTA e por diferir de outras áreas onde foram realizadas pesquisas com flebotomíneos, no Paraná, e pela limpeza e organização do peridomicílio. As coletas foram realizadas com sete armadilhas luminosas de Falcão, quinzenalmente das 22 às 2 horas, de novembro de 2007 a novembro de 2008. **Resultados:** Foram capturados 4.506 flebotomíneos de 13 espécies, com predomínio de *Nyssomyia whitmani* (71,8%). Coletou-se maior proporção de flebotomíneos no ambiente florestal (52,6%) do que no extraflorestal (residências e chiqueiro) (47,4%), entretanto a proporção de *Ny. whitmani* coletada foi maior no ambiente extraflorestal (38,3%) do que no florestal (33,5%). A maioria dos flebotomíneos foi coletada nos meses mais quentes e com quedas regulares de chuva. **Conclusões:** Os resultados sugerem que a limpeza e a organização do peridomicílio podem auxiliar a reduzir a população de flebotomíneos no domicílio e no peridomicílio, recomendando-se que a construção de abrigos de animais guarde a distância aproximada de 100m das residências, para evitar a invasão desses insetos, uma vez que nesta fazenda existe uma área de mata de preservação, com a presença de animais silvestres e flebotomíneos, que mantem o ciclo enzoótico de *Leishmania*.

Palavras-chaves: Flebotomíneos. Ecologia de vetores. Controle de leishmanioses.

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INTRODUCTION

Leishmaniasis are among the six tropical diseases of major public-health significance. They are caused by protozoans of the genus *Leishmania*, which exploit wild mammals (reservoirs) and sandflies (vectors) in their natural life cycles^{1,2}. In Latin America, where the number of cases of American cutaneous leishmaniasis (ACL) has remained relatively high, Brazil accounts for most cases, with 527,976 persons affected from 1990 to 2008³. Of these, 12,115 were recorded in Southern Brazil - 95.4% of them in the State of Paraná³.

The large number and wide distribution of ACL cases in Brazil underscore the importance of studies on the control of sandflies. Traditionally, sandfly control has been accomplished with the use of chemical insecticides⁴⁻⁷. However, these measures have been proven ineffective for use during interrupted and/or inappropriate periods⁸⁻¹⁰.

In Paraná¹¹⁻¹⁴ and in other states of Brazil^{7,10,15-17}, research on the ecology and control of sandflies in areas where ACL is endemic shows that human dwellings are usually located close to areas of vegetation. In endemic areas, lack of sanitation and peridomestic occupation can attract sandflies and create breeding sites for these insects^{12,15,18-21}, hence, the importance of studying the ecology, behavior, and development of alternative ways to control sandflies. The objectives of this study were to investigate the fauna; seasonality; and frequency of sandflies in houses, peridomestic animal shelters, and in the forest. No ACL has been recorded in the area of study, and the area is clean, well-kept, and maintained.

METHODS

The study was carried out in Santa Juliana Farm (Figure 1), located at 23° 30'21" S and 51° 54'14" W, at an altitude of 535m, and with an area of 169.4 hectares in the municipality of Sarandi in North-Central Paraná.

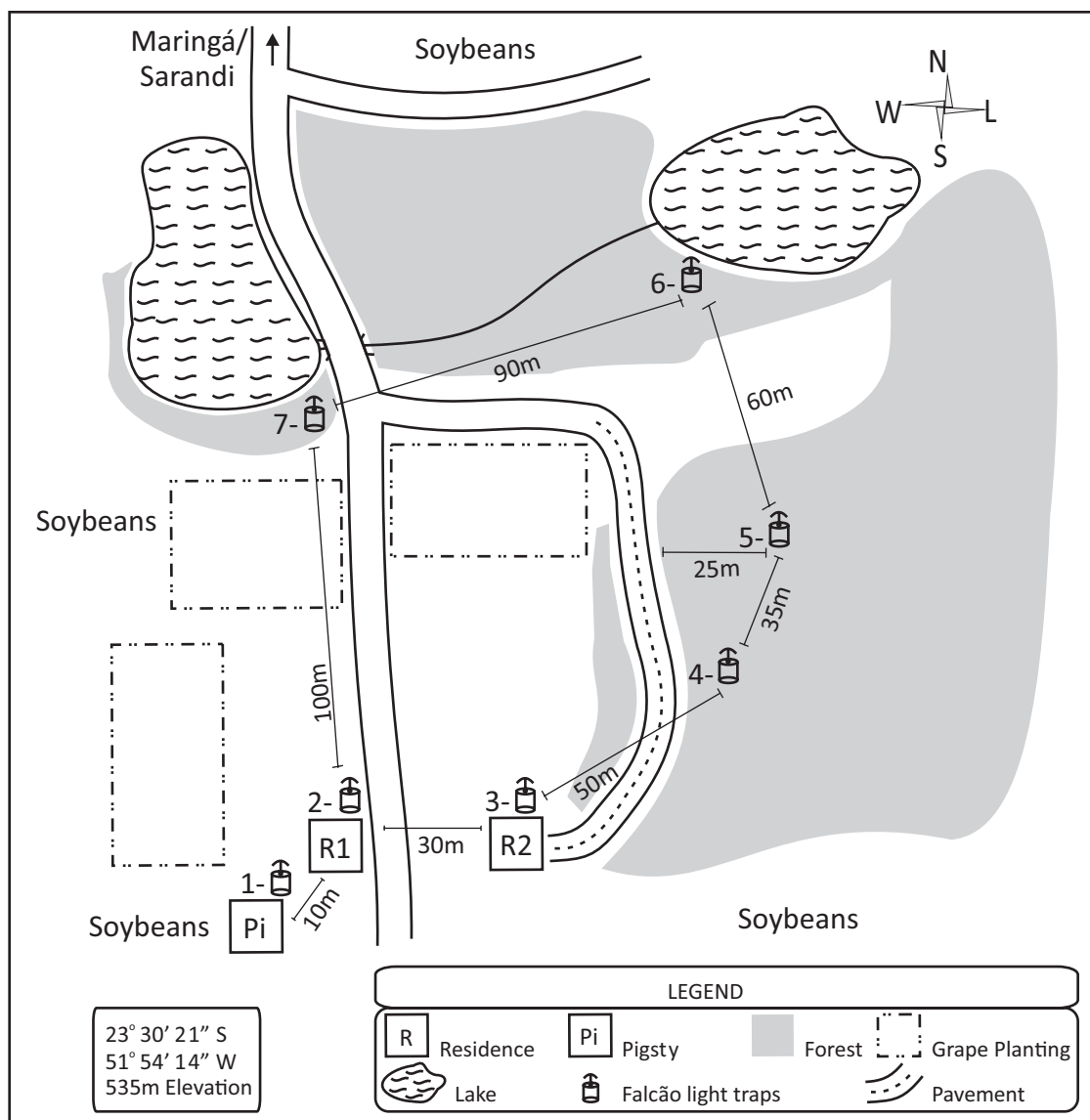


FIGURE 1 - Distribution of Falcão traps in the different ecotypes in Santa Juliana Farm, municipality of Sarandi, State of Paraná, Brazil, from November 2007 to November 2008.

According to the 2000 Census, Sarandi has a population of 71,422 inhabitants - 1,929 of these are residing in rural areas and 69,493 in the urban area²².

The predominant climate in the region, according to the Köppen classification, is Cfa (humid subtropical) in which the mean temperature of the coldest month is below 18°C and the mean annual temperatures are above 20°C.

The mean temperature in the collection period from November 2007 to November 2008 was 23.5°C. The lowest temperature recorded in the period was in 2008 (13.1°C), and the highest was in 2007 (30.9°C). The mean monthly precipitation during this same period was 130mm; the lowest rainfall for the period was 8mm in July 2008, and the highest was 235mm in December 2007. The rainfall data were provided by the Climatological Station of the *Universidade Estadual de Maringá* (UEM).

The region has a basaltic lithology, with a high proportion of clay in its constitution, and the soils are red oxisols (ferric) and alfisols (ferric)^{23,24}. The vegetation includes remnants of native semideciduous submontane forest²³.

Santa Juliana Farm contains 50.8ha of forest, of which 34.3ha is in legal reserves, and a permanent preserve of 16.5ha, where there is still a mammal fauna remaining (rodents, capybaras, wild cats, etc.) The farm has a small population of domestic animals (dogs, pigs, chickens) around the homes and cattle in the vicinity.

The study was conducted in this farm because there is no record of cases of ACL in this area. The organization of peridomicile distinguishes it from other locations in the state of Paraná where surveys of phlebotomines have been carried out^{12,25,26} and the cleanliness and good organization of the farm are evident.

Sandflies were collected with Falcão light traps, twice a month from 22:00 to 02:00 hours, from November 2007 to November 2008. This resulted in a total of 104h per trap collection and 728h in total.

The traps were installed in the following ecotypes: *E1* - a pigsty (Pi) with a roof and beaten earth floor, cleaned regularly, and housing two pigs; *E2* - on the porch of the farm keeper's residence (R1), with a beaten earth floor, where several bags containing food were stored and 2-5 dogs rested at night, as well as several nearby trees where chickens roosted at night; *E3* - on the porch of the residence (R2)

of the owner of the farm, which was frequently cleaned, the grass trimmed, and the peridomestic area always dry; E4 - on the edge of the forest beside a country road; E5 - in dense forest, 25m from the edge; E6 - on the shore of a lake, with grass and fruit trees, where the residents sometimes fish; and E7 - a grassy space between trees on the shore of another lake (Figure 1).

In the *extra-forest* environment, traps were installed in domiciliary areas (E1, E2, and E3). The *forest environment* refers to the traps installed in the forest and the lakeside (E4, E5, E6, and E7).

The sandflies collected were killed with chloroform and placed in small cardboard boxes impregnated with naphthalene. The specimens were prepared and subsequently identified in the Laboratory of Parasitology, Department of Basic Health Sciences of UEM. The nomenclature used here follows Galati²⁷.

Statistical analysis was performed with data for *Nyssomyia whitmani*, which was collected in the largest numbers and because it is the most important species in the epidemiology of leishmaniasis in Paraná^{11,28}. The Chi-square test was done at a significance level of 5%, using the application Statistica 7.1.

RESULTS

A total of 4,506 sandflies were collected, belonging to the species *Brumptomyia brumpti* (Larrousse), *Brumptomyia cunhai* (Mangabeira), *Evandromyia correalimai* (Martins, Coutinho, & Luz), *Evandromyia cortelezzii* (Brèthes), *Expapillata firmatoi* (Barreto, Martins, & Pellegrino), *Micropygomyia ferreirana* (Barreto, Martins, & Pellegrino), *Migonemyia migonei* (France), *Nyssomyia neivai* (Pinto),

Ny. whitmani (Antunes & Coutinho), *Pintomyia fischeri* (Pinto), *Pintomyia monticola* (Costa Lima), *Pintomyia pessoai* (Coutinho & Barreto), and *Psathyromyia shannoni* (Dyar). Of the total number of sandflies, 71.8% were *Ny. whitmani*, 9% were *Pi. pessoai*, 5.3% were *Mi. migonei*, 4.4% were *Pi. fischeri*, and 4.1% were *Pi. monticola* (Table 1). Altogether, these species represented 97.7% of the sandflies collected.

We collected 2,765 females and 1,741 males. The number of female *Ny. whitmani* was significantly higher than the number of males of the same species ($p < 0.0001$).

Table 1 shows that 13% of the sandflies were collected in peridomestic ecotope E1 (the pigsty). At home, in ecotopes E2 and E3 (porch), 27.4% and 7%, respectively, were collected. In the forest environment, E4 and E5 in ecotopes (forest), 25.5% and 25.9%, respectively, were collected, while in ecotopes E6 and E7 (the shores of the two lakes) only 0.8% and 0.4% of the sandflies were collected.

We collected a larger number of sandflies in the forest (52.6%) than in the extra-forest environments (47.4%). However, the number of specimens of *Ny. whitmani* was higher ($p < 0.0001$) in the extra-forest (38.3%) compared with the forest (33.5%).

The largest numbers of sandflies in E1 (79.9%) were collected in January, March, September, and November. In E2, 74.1% of the sandflies were collected in February, May, and September. In E3, 73% of the sandflies were collected in February, March, May, and September. In E4, 77.8% of the sandflies were collected in November 2007 and January, May, July, September, and November 2008. In E5, 71.9% of the sandflies were collected in January, May, and September (Table 2). Note that in all the ecotopes together, in January, February, March, May, September, and November 2008, 82.1% of the sandflies were collected (Table 2 and Figure 2).

TABLE 1 - Sandfly species collected in different ecotypes in Santa Juliana Farm, municipality of Sarandi, State of Paraná, Brazil, from November 2007 to November 2008.

Species	Ecotypes							Total
	E1	E2	E3	E4	E5	E6	E7	
<i>Nyssomyia whitmani</i>	445	1,014	245	662	847	10	12	3,235
<i>Pintomyia pessoai</i>	53	78	30	171	71	3	-	406
<i>Migonemyia migonei</i>	32	63	24	59	61	-	-	239
<i>Pintomyia fischeri</i>	19	25	4	86	68	1	-	203
<i>Pintomyia monticola</i>	5	3	3	107	66	-	-	184
<i>Nyssomyia neivai</i>	27	52	7	20	10	17	3	136
<i>Expapillata firmatoi</i>	1	2	-	39	34	1	-	77
<i>Brumptomyia cunhai</i>	-	-	-	1	1	5	2	9
<i>Psathyromyia shannoni</i>	-	-	1	1	3	-	-	5
<i>Brumptomyia brumpti</i>	-	-	1	-	4	-	-	5
<i>Evandromyia cortelezzii</i>	-	-	-	1	2	-	-	3
<i>Micropygomyia ferreirana</i>	-	-	-	1	1	-	-	2
<i>Evandromyia correalimai</i>	-	-	-	2	-	-	-	2
Total	582	1,237	315	1,150	1,168	37	17	4,506
Percentage	13	27.4	7	25.5	25.9	0.8	0.4	100

Location of traps: E1: pigsty; E2: porch of the farm keeper's residence; E3: porch of the owner's residence; E4: edge of forest; E5: in the forest; E6: beside a lake; E7: beside a lake.

TABLE 2 - Seasonality of sandflies collected in different ecotypes in Santa Juliana Farm, municipality of Sarandi, State of Paraná, Brazil, from November 2007 to November 2008.

Ecotype/Month	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
E1	10	11	100	17	212	14	33	-	-	15	75	17	78	582
E2	5	16	9	322	90	34	251	1	2	22	344	47	94	1,237
E3	5	22	13	34	47	4	68	-	3	5	81	3	30	315
E4	122	41	147	44	97	9	195	10	140	28	152	26	139	1,150
E5	54	13	167	23	78	-	211	4	56	34	462	21	45	1,168
E6	3	-	9	3	1	4	1	-	-	7	6	-	3	37
E7	1	-	1	1	2	3	1	-	-	4	0	2	2	17
Total	200	103	446	444	527	68	760	15	201	115	1,120	116	391	4,506
Percentage	4.4	2.3	9.9	9.9	11.6	1.5	17	0.3	4.4	2.5	25	2.5	8.7	100

Location of traps: E1: pigsty; E2: porch of the farm keeper's residence; E3: porch of the owner's residence; E4: edge of forest; E5: in the forest; E6: beside a lake; E7: beside a lake.

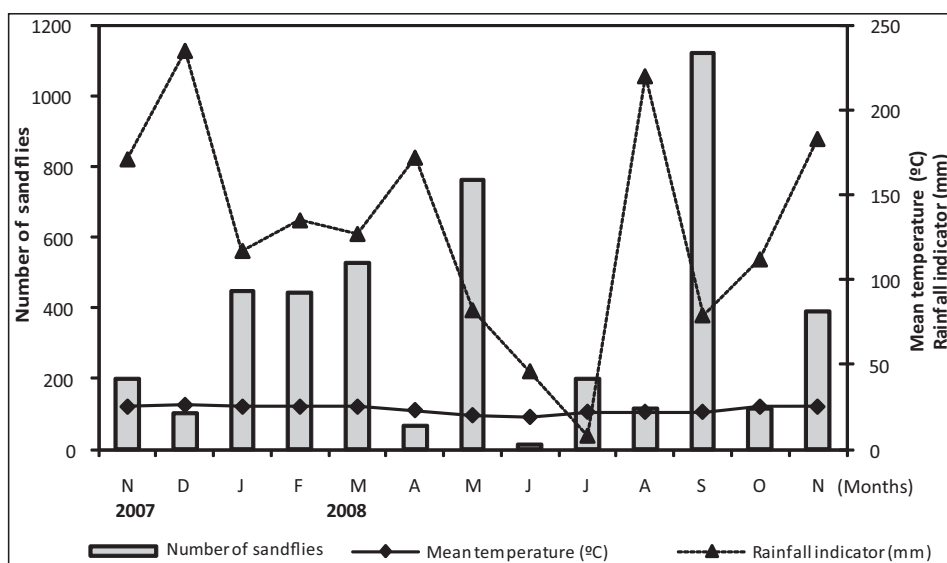


FIGURE 2 - Seasonality of sandflies in all the traps in different ecotypes in Santa Juliana Farm, mean temperatures, and monthly rainfall in the municipality of Sarandi, Paraná, Brazil, from November 2007 to November 2008.

DISCUSSION

The 13 species collected have been recorded in several locations in the State of Paraná^{11,29,30}. We note particularly the species *Ny. whitmani*, *Ny. neivai*, *Pi. pessoai*, and *Mi. migonei* because of their high frequency in domiciliar and peridomiciliar situations and because they have been found naturally infected with *Leishmania* in this state^{28,31} and in other Brazilian states^{17,30,32,33}.

The slightly higher number of sandflies collected in the forest was probably due to environmental conditions - i.e., humidity and organic matter necessary for the development of the immature stages of sandflies - and an available blood supply for the adult females - i.e., birds and mammals. The regular distribution of rainfall throughout the year helped to maintain soil moisture. The small number of sandflies collected in the lakeshores, despite the proximity of the forest, may have been due to the installation of traps in areas with low vegetation (grass). *Ny. whitmani* was collected in greater numbers outside the forest. In this environment, the high proportion of sandflies collected in ecotope E2 can be explained by the presence of several dogs that rested there and some chickens that roosted in the nearby trees at night. This confirms the attractiveness of domestic animals to phlebotomines^{18,19,21}. *Ny. whitmani* is anthropophilic³⁴ and is important in the epidemiology of ACL.

The forest preserve, the presence of wild mammals, as well as the cleanliness and organization and the small supply of blood available in the peridomicile (domestic animals) are factors that may explain the collection of similar numbers of sandflies in the forest and extra-forest areas in Santa Juliana Farm. These insects have often been collected in larger numbers in peridomiciliar and extra-forest environments^{12,35,36}. In Palmital Farm in the municipality of Terra Boa, Paraná, after deactivation of a colony of houses and the absence of residents and domestic animals (dogs, chickens, pigs, and others), which had served as sources of blood for the sandflies, the sandfly population decreased sevenfold³⁷. In Santa Juliana Farm, we also observed the absence of garbage and other waste in the vicinity of the houses and in the edge of the forest³⁷.

During the months of collection, the lowest mean temperatures occurred in May and June, and the lowest rainfall rates were observed in June and July. However, rainfall was well distributed throughout the collection period, and the mean, minimum, and maximum temperatures remained roughly constant. Most (65.1%) sandflies were collected in January, February, March, September, and November 2008, months that were warmer and with regular rainfall. In May, 17% of the specimens were collected, at a time when the temperature is usually milder. The finding of the highest densities of sandflies in the warmer months and with regular rainfall concurs with observations in several previous surveys of these insects^{36,38-42}.

This study demonstrates the importance of maintaining the household and peridomestic environment clean and organized to limit the growth of the sandfly population in these environments. Having collected a large number of sandflies on the porch of one of the residences, due to the presence of domestic animals (dogs and chickens), it is recommended that separate shelters for the animals be constructed at a distance of approximately 100m from domiciles²⁵, to prevent the invasion of sandflies. Cases of leishmaniasis have been reported in locations where environmental characteristics allow the formation of the enzootic cycle of protozoans of the genus *Leishmania*, involving insect vectors (sandflies) and mammals (reservoirs)^{11,12}. In such places, sandflies have been collected in large numbers around houses and in houses that are located in close proximity to forest remnants and domestic animal shelters (chickens, pigpens, etc.), where hygiene conditions are poor, organic matter accumulates (leaves and fallen fruit, pet droppings, and leftover food offered to them), soil moisture is high, and phlebotomine populations are larger^{11,12,13,18}. Studies on sandfly control in northern Paraná showed that after the reorganization and cleaning of household and peridomicile environment, most sandflies were attracted by the domestic animal shelters, while the number of these insects decreased in domiciles^{19,21,25}.

In the present study, thirteen species of sandflies were collected, with a predominance of *Ny. whitmani*. Most sandflies were collected in the forest; however, *Ny. whitmani* was most often collected in the extra-forest. In this environment, the most number of sandflies were collected in a pigsty and on the porch of a residence. The results suggest that cleaning and organization around the houses could reduce sandfly population in peridomicile. Shelters for animals must be constructed at a distance of approximately 100m from domiciles. The study farm has an area of preserved forest, with wild animals and sandflies present, hosting a combination of factors that maintain the enzootic cycle of *Leishmania*. Thus, vector control for *Leishmania* can be achieved through basic housekeeping and maintenance of an orderly environment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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