

# Comparison of Black Fly Species (Diptera: Simuliidae) on an Amerindian Reservation with a High Prevalence of Fogo Selvagem to Neighboring Disease-Free Sites in the State of Mato Grosso do Sul, Brazil

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**ABSTRACT** Fogo selvagem is an autoimmune blistering skin disease that principally occurs among rural Brazilians living in geographically clumped disease foci. Exposure to hematophagous black flies possibly is related to the cause of the disease. We compared the occurrence, proportions, and richness of simuliid species immatures and the biting activity of adult females within a recently discovered, high prevalence focus of fogo selvagem, the Limão Verde Terena Reservation, to that of neighboring regions with no reported cases of fogo selvagem. Nine black fly species were collected from 12 stream sites during 5 trips to the fogo selvagem focus. The species showed longitudinal (upstream-downstream) trends in occurrence, proportions, and richness, and the abundance of simuliid immatures was greater at downstream sites. The most prevalent species at the focus, *Simulium nigrimanum* (Macquart), dominated the stream sites with highly abundant simuliid assemblages, and was the most common black fly in human bait collections. This species was absent or in very low numbers in neighboring valleys and villages that did not have cases of fogo selvagem.

**KEY WORDS** *Simulium nigrimanum*, Simuliidae, fogo selvagem, Amerindians, Brazil

ENDEMIC PEMPHIGUS FOLIACEUS, also known as fogo selvagem, is a potentially fatal autoimmune disease with a unique epidemiology (Diaz et al. 1989a, b). Most cases have been reported from Brazil among poor, outdoor laborers in geographically distinct regions or foci. The historical distribution of these foci has shifted over the past 50 yr from southeastern to central and midwestern Brazil (Diaz et al. 1989a). This geographical clustering and temporal shifting of focal locations has led investigators to the hypothesis that an environmental factor(s) associated with the foci is related to the cause(s) of fogo selvagem.

Of the many plausible environmental factors, the presence of anthropophagic black flies near disease foci has been discussed most often in the fogo selvagem literature (Aranha-Campos 1942; Vieira 1940, 1948; Diaz et al. 1989a). During the historical peak of reported fogo selvagem cases in the state of São Paulo, Lane (1950) collected black flies and showed that *Simulium nigrimanum* (Macquart) was abundant near fogo selvagem foci, but absent from the coastal region of the state where fogo selvagem rarely had been reported. More recently, Lombardi et al. (1992) showed that outdoor laborers exposed to black fly bites were more likely to develop fogo selvagem than those not exposed, with an odds ratio of 4.7.

Based on these observations, and as part of the ongoing investigation of the etiology of fogo selvagem, the Cooperative Group on Fogo Selvagem Research began a study of the black flies at fogo selvagem foci in regions of Brazil where the disease was historically or is currently most prevalent. The goal of the study was to examine the distribution and biting activity of species near foci and in regions without the disease, and to determine whether particular species were characteristic of the foci. Here, we present the results of a black fly survey from one recently characterized focus of fogo selvagem with

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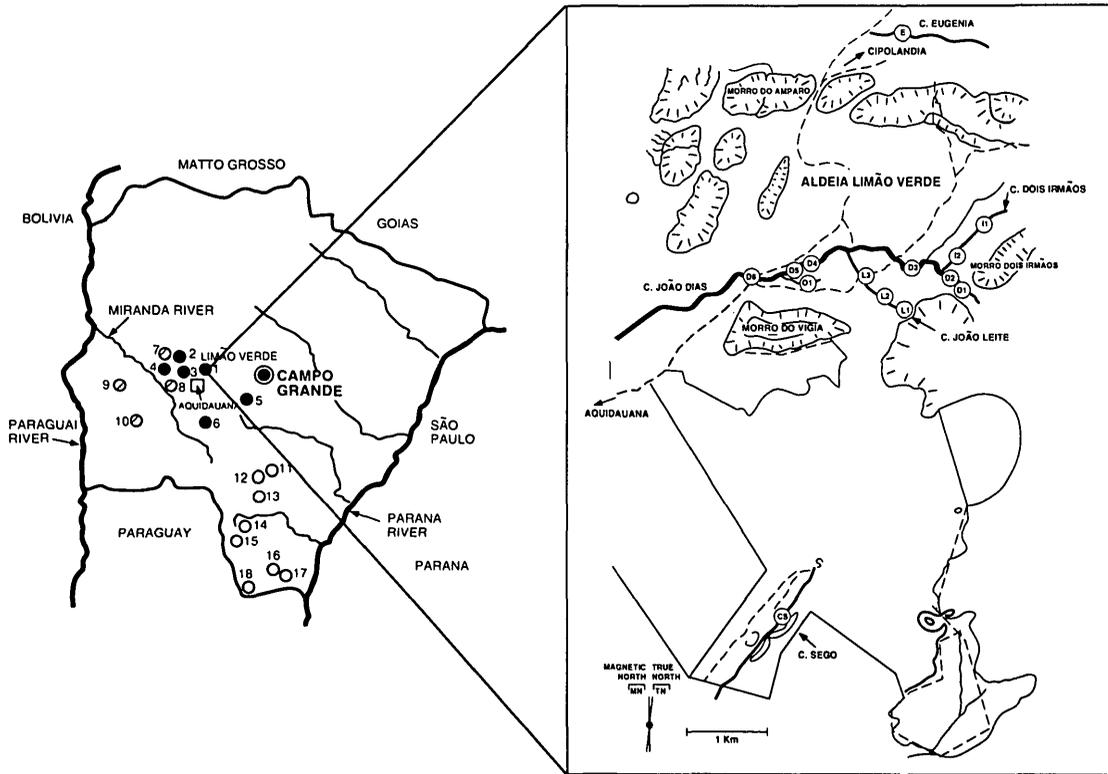


Fig. 1. (Left) The State of Mato Grosso do Sul in Brazil is the home of several Amerindian tribes. Approximately 10,000 Terena tribe members have settled in the reservations numbered from 1 to 6 (closed circles) and other Amerindian groups near 7 to 17 (open circles). The Limão Verde (circle 1) and the Ipegue/Taunay (circle 2) reservations are the sites of the current study. (Right) The valley and streams of the Limão Verde focus of fogo selvagem. For each stream, the sample sites are labeled in ascending order from upstream to downstream. Córrego Seco and Córrego Eugenia are shown in the lower and upper portions of the figure, respectively.

a high prevalence of the disease, 2.6% (Hans-Filho et al. 1996). The fogo selvagem focus comprises the Amerindian Terena Reservation of Limão Verde, which is located in the state of Mato Grosso do Sul, Brazil. Terena societies are closed to immigration and emigration compared with the general population of Brazil (Hans-Filho et al. 1996). Consequently, Limão Verde and its neighboring Amerindian villages provided a unique opportunity to compare environmental factors associated with a population exhibiting a high prevalence of fogo selvagem with those of genetically and geographically related but fogo selvagem-free populations.

The objective of the current study was to characterize the black flies of the Limão Verde focus and compare these findings with those of neighboring regions without reported cases of fogo selvagem. To assess the prevalence of species at the sites, we documented the occurrence, richness, and proportions of species immatures and the abundance of immature assemblages. To assess human exposure to anthropophagic species, we collected black fly females at fogo selvagem focal and nondisease sites.

### Materials and Methods

**Limão Verde Focus of Fogo Selvagem.** The Limão Verde Reservation of the Terena tribe, which was our principal study site, is located in the município of Aquidauana, Mato Grosso do Sul, Brazil (Fig 1; also see figure 1 of Hans-Filho et al. 1996). The geography, a complete census of the human population, a description of the society, and analyses of the geographic, temporal, and familial distributions of fogo selvagem cases in the region were reported by Hans-Filho et al. (1996). All fogo selvagem cases originated within the northern section of the reservation whose natural boundaries encompass the valley of a stream encircled by rocky mesas (Fig. 1). The village in the valley also is called Limão Verde and has a population of 854 inhabitants.

The stream, Córrego João Dias, is a tributary of the Rio Aquidauana which flows north and west to the Rio Miranda shortly before the Miranda enters the Rio Paraguai (Fig. 1). The João Dias enters Limão Verde through a steep gorge and then meanders for 3.3 km through a relatively flat, rural

landscape before exiting the reservation. It has 2 spring-fed, headwater tributaries, Córrego Dois Irmãos and Córrego João Leite, which originate near the bases of steep cliffs. The streams flow for 1.4 and 1.0 km, respectively, before entering the João Dias.

Most native vegetation of Limão Verde, a type of seasonal savannah forest called "cerrado" (Whitmore and Prance 1987), was cleared for agriculture, but gallery forests remain along the margins of the streams. The stream channels in upstream reaches are relatively steep with bedrock and boulder substrates (gradient,  $\approx 0.06$  m/m). The downstream sections consist of slowly flowing channels (gradient,  $\approx 0.02$  m/m) with sand substrates interrupted by bedrock outcrops, which form waterfalls. Water falls are used by the locals for bathing and washing clothes. The width of the João Dias ranges from 1.5 m in its upstream sections to 4.0 m at its downstream exit. Both Córrego Dois Irmãos and Córrego João Leite have widths ranging from 1 to 2 m. Near the downstream section of the João Dias is a slough or oxbow channel running parallel to the stream. During the rainy season, it has a small volume of running water, which is inhabited by black fly immatures.

**Neighboring Study Sites.** The black fly faunas of 2 valleys in close proximity to the fogo selvagem focus and 2 Terena villages on a neighboring reservation also were investigated. The southern section of the Limão Verde Reservation, known as Córrego Seco, has a population of 144 inhabitants, and has no reported cases of fogo selvagem (Hans-Filho et al. 1996). Córrego Seco is located within the valley of a small, seasonal stream of the same name (Fig. 1). This stream is also a tributary of the Rio Aquidauana and flows for 1.8 km within the reservation boundaries. It is very similar, with respect to size and substrate materials, to the headwater tributaries of the João Dias, but the riparian vegetation is more typical of the drier plateau regions of the reservation.

The valley north of the Limão Verde focus of fogo selvagem, which is outside of the reservation, is inhabited by non-Amerindian ranchers. Cases of fogo selvagem have not been reported from this region (unpublished data). The valley and its principle stream, Córrego Eugenia, appear similar to Limão Verde and the João Dias, but less of the land is cultivated (Fig. 1).

Ninety kilometers west of the Limão Verde Reservation, also in the município of the Aquidauana, is the Ipegue/Taunay Reservation of the Terena tribe (Fig. 1, left; also see figure 1 of Hans-Filho et al. 1996), which, until the fall of 1995, had no recorded cases of fogo selvagem. At this time, 1 new case of fogo selvagem was confirmed in the Bananal village of the reservation. The patient was a 16-year-old Terena girl with the classical clinical and immunological features of fogo selvagem.

Although the people of the Ipegue/Taunay Reservations are related genetically to those of Limão Verde, the geography and ecology of the 2 regions

are markedly different. Limão Verde is located on the extreme western edge of the Brazilian plateau, which rims the basin of the Rio Paraguai. This is an area of high relief, with altitudes of 230-500 m. By contrast, the Ipegue/Taunay Reservation is flat and lies within the Paraguai basin below 200 m of altitude. The native vegetation of Ipegue/Taunay is characteristic of the border regions of the Pantanal, a huge wetlands area that encompasses the upper Paraguai basin. In contrast to the streams of Limão Verde, those of Ipegue/Taunay have flat, meandering, mud-lined channels with little current velocity. Many channels have running water only during the wet season.

**Sampling.** Sampling trips to the Terena Reservations were regulated by the federal agency that administers Amerindian regions in Brazil, Fundação Nacional do Índio no Brasil (FUNAI), and were part of a large team effort including the participation of foreign and Brazilian dermatologists, local health agents, tribal officials, an epidemiologist, and a biologist. As a result, trips occurred opportunistically when research permits were obtained and the multiple tasks of the team could be coordinated.

At the Limão Verde focus, immature simuliids were collected at 12 sites, 11 spaced longitudinally along the 3 streams and 1 on the oxbow channel (Fig. 1). Sites were numbered in ascending order from upstream to downstream for each of the streams. Host-seeking females were captured at the stream sites and near the homes of fogo selvagem patients and unaffected controls. Samples were collected during 3 annual rainy season trips in March 1994, 1995, and 1996, and 2 dry season trips in August 1996 and July 1997. Some of the stream sites were sampled during the 3 rainy seasons to document variability.

At Córrego Seco, 2 stream sites were sampled and nearby homes served as bait sites for anthropophilic females during March 1995, 1996, and July 1997 (Fig. 1). One stream site along Córrego Eugenia and 3 within the Ipegue/Taunay Reservation, near the villages of Ipegue and Bananal, were sampled in March 1996 and July 1997 (Fig. 1).

For immature stages, equal-effort sampling (Cox 1976) was used to determine the proportional composition of the species present and the relative abundance of the simuliid assemblages among sites. For each site and date, D.P.E. conducted from 2 to 6 timed searches (3- to 10-min duration) for larvae and pupae. The search pattern was systematic, so that stream substrates would be sampled in roughly the proportion that they were available at the site. Each of the searches served as a replicate sample and was used to calculate the mean and variability of species proportions for a site and date. Samples from all substrate types were used in the calculations. Because proportion data are seldom normally distributed, we calculated 95% CL of the means after arcsine transformation (Sokal and Rohlf 1981).

To compare the relative abundance of immature simuliid assemblages among sites, we reported the

Table 1. Black fly species, genus *Simulium*, collected at the Limão Verde focus of fogo selvagem, município of Aquidauana, Mato Grosso do Sul, Brazil, and known blood hosts

Subgenus, species <sup>a</sup>	Hosts	References
<i>Chirostilbia pertinax</i> Kollar	Humans, dogs	Lutz (1910), obs.
<i>spinibranchium</i> Lutz	Humans	obs.
<i>Hemicnetha rubrithorax</i> Lutz	Horses	Lutz (1910)
<i>Inaequalium inaequale</i> Paterson & Shannon	Humans, mules	Coscarón and Wygodzinsky (1984), obs.
<i>subnigrum</i> Lutz	Horses	Lutz (1910)
<i>Psaroniocompsa incrustatum</i> Lutz	Humans	Vulcano et al. (1975), obs.
	Horses, cows	Lutz (1910), obs.
<i>Psilopelmia perflavum</i> Roubaud	Horses	Lutz (1910)
Unknown sp. E <sup>b</sup>		
<i>Trichodagmia</i>		
<i>nigrimanum</i> Macquart	Humans	Vulcano et al. (1975), obs.
	Horses	Lutz (1910)

obs., Blood hosts observed during our investigations of fogo selvagem foci.

<sup>a</sup> Subgeneric names are from Crosskey (1987).

<sup>b</sup> We tentatively assigned this species to the subgenus, *Psilopelmia*, based on its similarity to *S. perflavum*.

mean plus the standard error of larvae + pupae collected per sample per minute of collection at each site. At one Limão Verde site, we determined the absolute density of larvae + pupae by taking randomly chosen, replicate samples over a known area of stream substrate. Species richness was reported as the number of species at a site and species occurrence as the percentage of fogo selvagem or non-fogo selvagem sites where a species was collected.

To express overall species prevalence for immatures at Limão Verde, we weighted mean species proportions from each site by the relative abundance of the simuliid assemblage, and calculated an average of these weighted proportions over all sites. With this method, mean species proportions from sites with larger simuliid assemblages were weighted more than those from sites with fewer simuliids.

The actual collecting was accomplished by scraping stream substrates to release larvae and pupae into a downstream aquatic net (mesh size, 150  $\mu$ m). We also removed substrates from the stream (e.g., grasses and branches trailing in the water) and collected the specimens with forceps. These were preserved with 5% formalin for later analysis.

Host-seeking females were captured at human bait using aspirators (Service 1987) and preserved in 93% ethanol for later identification (HRRC protocol number 173-88). Three to 6 persons served as bait, and sampling periods were primarily at dusk near stream sites and homes of fogo selvagem patients and unaffected individuals. During each of the annual or seasonal visits to the Limão Verde focus, we sampled for 3–4 consecutive evenings for a 1- to 3-h period and visited 3 or more sample sites per evening. We used similar methods to bait non-fogo selvagem neighboring sites for 1 or 2 d per site during the wet and dry seasons. The biting rates of anthropophagic species were expressed as the number of bites per person-hour.

**Species Identification.** After sorting preimaginal specimens from debris, species of mature larvae and

pupae were identified from descriptions in the literature (e.g., Vulcano 1959) and larval-pupal associations. Except for 1st instars, which could not be distinguished, immature larvae were identified by examining traits that persist throughout the stage and allow comparison to identified mature larvae. We identified adults from species descriptions and pupal-adult associations. To associate larval, pupal, and adult stages, we examined the respiratory histoblasts of mature larvae and reared pupae to adults in individual moist vials.

Voucher specimens of mature larvae, pupae, and adults were confirmed by A.J. Shelley (Department of Entomology, British Museum [Natural History], London). Our determinations were based solely on external morphology. We did not attempt to unravel species complexes, which are common within the family Simuliidae (Crosskey 1990). This analysis will be included in future investigations if a connection between black flies and fogo selvagem is firmly established.

## Results

**Black Flies of the Limão Verde Focus of Fogo Selvagem.** More than 17,000 immatures and adults of 9 species of black flies were collected at the Limão Verde focus of fogo selvagem (Table 1). Five species, *Simulium pertinax* (Kollar), *Simulium spinibranchium* (Lutz), *Simulium inaequale* (Paterson & Shannon), *Simulium incrustatum* (Lutz), and *S. nigrimanum* (Macquart), are known to bite humans (Lutz 1910, Vulcano et al. 1975, Coscarón and Wygodzinsky 1984) or were collected at human bait. The scientific name *Simulium pruinosum* Lutz, which is frequently used in the fogo selvagem literature, is a synonym of the name *Simulium nigrimanum* macquart (Crosskey 1987). Except for *S. spinibranchium*, these species also have been observed taking blood from nonhuman mammals. Three species, *Simulium rubrithorax* (Lutz), *Simulium subnigrum* (Lutz), and *Simulium perflavum* (Roubaud), were observed biting horses by Lutz (1910). Biting fe-

Table 2. Occurrence and number of black fly species at stream sites within the Limão Verde fogo selvagem focus

Streams and sites	<i>S. nigrimanum</i>	<i>S. perflavum</i>	<i>S. inaequale</i>	<i>S. subnigrum</i>	<i>S. incrustatum</i>	<i>S. pertinax</i>	<i>S. spini-branchium</i>	<i>S. rubri-thorax</i>	Unknown sp. E.	No. species
Córrego Dois Irmãos										6
I1		X		X	X				X	4
I2	X	X	X	X	X				X	6
Córrego João Leite										4
L1				X						1
L2				X						1
L3	X		X		X					3
Córrego João Dias										9
D1	X	X		X	X	X	X		X	7
D2	X	X	X	X	X	X	X	X	X	9
D3	X	X	X	X	X	X	X	X	X	9
D4	X	X	X	X		X	X		X	7
D5	X	X		X	X	X		X	X	7
D6	X	X				X	X		X	5
Oxbow										2
O1			X						X	2
Species occurrence, % <sup>a</sup>	67	67	50	75	58	50	42	25	75	

X, Species collected at site.

<sup>a</sup> Percentage of sites where a species was collected.

males of the unidentified species "E" were not collected during our study.

Figure 1 and Table 2 depict the distribution of the sampling sites and species within the Limão Verde focus, species occurrence, and species richness for the streams and individual sites. The species that occurred at the most sites were *S. subnigrum* and species E, followed by *S. nigrimanum* and *S. perflavum*. *S. subnigrum* and *S. nigrimanum* were distributed among all 3 streams, whereas *S. perflavum* and species E did not occur in Córrego João Leite. *S. inaequale* was distributed widely, occurring on all 3 streams and the oxbow channel, but was found at only half of the total sites.

Species richness was greater on the João Dias than on either of its headwater tributaries (Table 2). In addition, there were longitudinal changes in the number of species as the João Dias flowed from D1 to D6 (Table 2; Fig. 1). Richness was greatest in the upper-middle section of the stream, sites D2 and D3. The João Leite and Dois Irmãos showed an upstream to downstream increase in the number of species, and the seasonally fluctuating oxbow channel had only 2 species.

To compare the relative abundance of simuliid assemblages, we estimated the mean number of larvae + pupae per sample per minute of collection at each of the Limão Verde sites (Fig. 2A, fogo selvagem focus sites). Except for the downstream site of Córrego Dois Irmãos, the simuliid immatures generally were more abundant along the João Dias than along the João Leite or Dois Irmãos. In all 3 streams, abundance was greatest in downstream samples. In the narrow, grass-choked channel of the oxbow, immature abundance per sample per minute was greatest among the Limão Verde sites.

Species proportions at Limão Verde varied among streams and sites along each stream (Fig. 3). On Córrego Dois Irmãos, *S. perflavum* was the most abundant species at both the upstream and downstream sites (Fig. 3A). At the upper 2 sites of Córrego João Leite, L1 and L2 (not shown), *S. subnigrum* was the only species collected (Fig. 3B), and at the downstream site, the larvae and pupae were mostly *S. incrustatum* and *S. inaequale*. *S. nigrimanum* made up a small proportion of the simuliid assemblages at the downstream sites on both of these streams, (Fig. 3A and 3B). The upstream site of the João Dias was dominated by *S. subnigrum* and *S. pertinax* (Fig. 3C). The other 5 species, including *S. nigrimanum*, occurred in low proportions. In contrast, *S. nigrimanum* made up a large proportion of the simuliid assemblage at the downstream João Dias site, and *S. subnigrum* and *S. pertinax* were absent or rare. The larvae and pupae of species E dominated the oxbow channel (Fig. 3D).

Longitudinal trends along Córrego João Dias differed for the proportions of the 4 most abundant species (Fig. 4). *S. nigrimanum* showed a dramatic longitudinal increase in mean proportion along the João Dias (Fig. 4A). At the extreme upstream site, D1, it was rare, but 250 m downstream, at D2, its larvae and pupae made up more than half of the simuliid assemblage. Mean proportions were greatest at the downstream sites, D5 and D6, where immature simuliids were relatively abundant (Fig. 2). Density at D6 was estimated to be 170,850 larvae + pupae of *S. nigrimanum* per square meter ( $n = 3$ , 25-cm<sup>2</sup> samples from a bedrock fall, SD = 133,630). The longitudinal trend for *S. nigrimanum* was consistent during our 3 yr of sampling. Annual variability was greatest at the upper-middle sites, D2

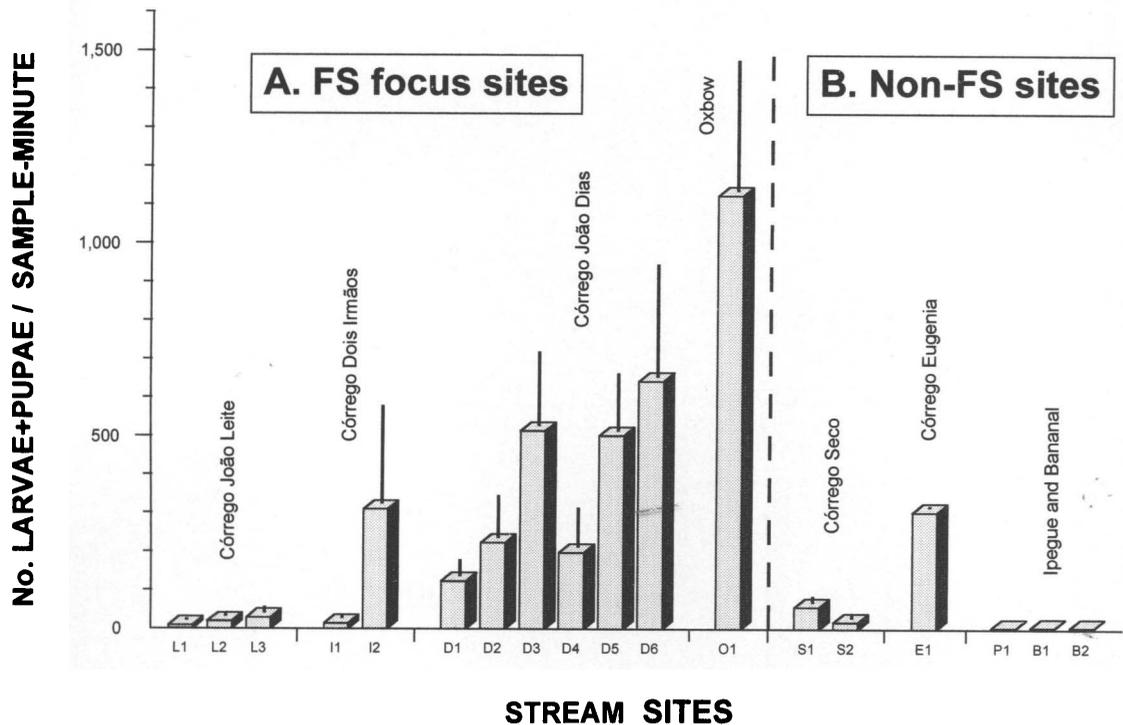


Fig. 2. Relative abundance of immature simuliid assemblages (all species) at Limão Verde fogo selvagem (FS) (A) and neighboring non-fogo selvagem (B) sites. The means plus standard errors of the number of larvae + pupae per sample per minute of collection are shown.

and D3, the reach where species richness was greatest (Table 2).

*Simulium perflavum* showed a unique longitudinal pattern (Fig. 4B). Its relative abundance initially increased through the middle sections of the João Dias, reaching a maximum at site D3, and then it decreased toward the downstream sites. Like *S. nigrimanum*, this trend was consistent for the 3 yr and varied most through the upper-middle sites. *S. subnigrum* and *S. pertinax* showed longitudinal trends that were mirror images of the *S. nigrimanum* pattern (Fig. 4 C and D). These species were relatively abundant only at the upstream site, D1. Variability was highest at D1, where the 2 species annually traded ranks as the most abundant simuliid.

The prevalence of species over all Limão Verde sites, estimated as the average of mean site proportions (e.g., Figs. 3 and 4) weighted by relative site abundances (Fig. 2), is shown in Fig. 5. *S. nigrimanum* was the most prevalent species, followed by *S. perflavum*, *S. subnigrum*, and *S. pertinax*. The other 5 species were relatively rare.

**Comparison of the Limão Verde Focus of Fogo Selvagem with Neighboring Sites.** Of the 6 stream sites investigated in regions without cases of fogo selvagem, only 1, Córrego Eugenia in the valley north of Limão Verde, had a diverse simuliid assemblage (Table 3; Fig. 1). As shown in Fig. 2B (non-fogo selvagem sites), the relative number of

larvae and pupae collected at this site was similar to the medium abundance sites along Córrego João Dias. Fig. 6 compared species proportions at the Córrego Eugenia site with those of site D6, the most similar site on the João Dias with respect to longitudinal channel position and stream size. The sites had 4 of 6 species in common and showed a similar pattern of 1 prevalent and several rare species. However, *S. pertinax*, rather than *S. nigrimanum*, was the dominant species on Córrego Eugenia.

The 2 sites we sampled on Córrego Seco in the southern region of the Limão Verde Reservation had only the larvae and pupae of species E in relatively low numbers (Table 3; Figs. 2 and 6). Like the oxbow channel of Limão Verde, where species E was also common, the water level of Córrego Seco fluctuated seasonally. We did not find immature black flies at stream sites near the villages of Ipegue and Bananal on the Ipegue/Taunay Reservation (Table 3; Fig. 2).

**Biting Activity at the Limão Verde Focus of Fogo Selvagem and Neighboring Sites.** During 3 rainy season visits and 66 person-hours of sampling at Limão Verde, only 2 biting *S. nigrimanum* were captured. At the non-fogo selvagem site, Córrego Eugenia, we captured *S. pertinax* females during the rainy season (30.0 bites per person-hour in 5 person-hours of collecting). Black flies were not collected

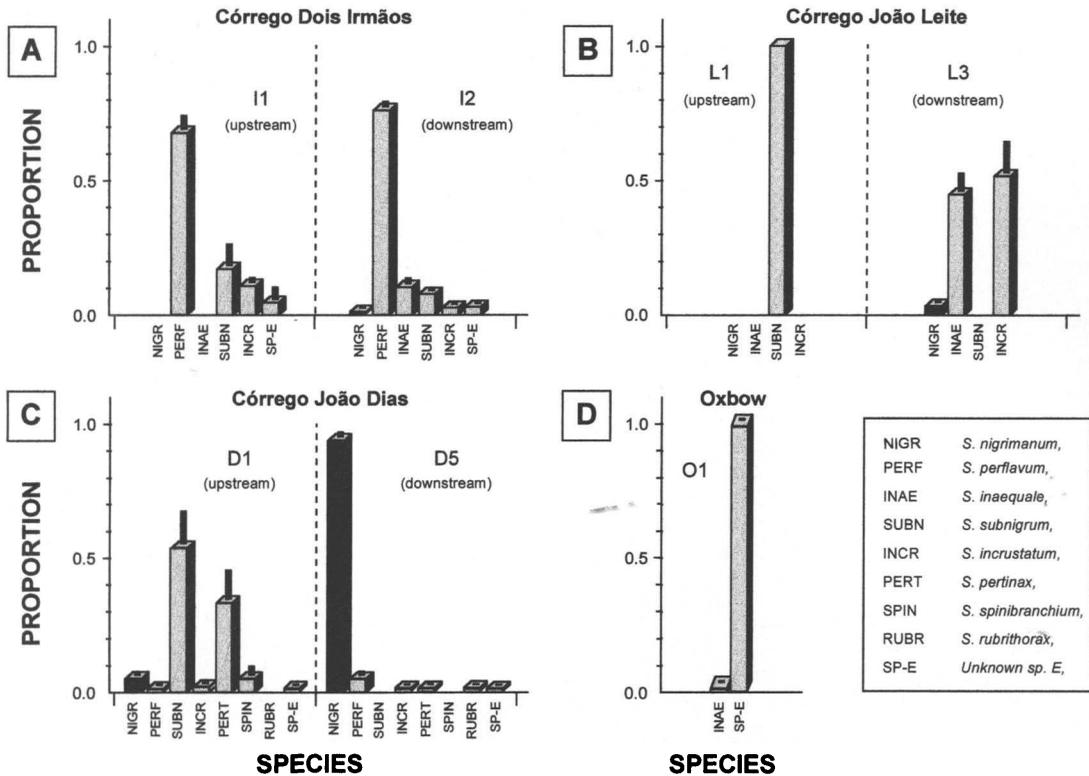


Fig. 3. Proportions of simuliid species (larvae + pupae) at stream sites within the Limão Verde focus of fogo selvagem (A–D). Bars show means plus standard errors of species proportions from each site ( $n = 2-7$ , depending on the site). One upstream and 1 downstream site are shown for the 3 streams; graphs A, B, and C, and 1 site is shown from the oxbow channel, graph D.

during an additional 25 person-hours in the rainy season at 5 other non-fogo selvagem sites.

During two, 4-d, dry season visits to Limão Verde, we captured 732 anthropophagic simuliids during 20 person-hours. Most of these were captured at dusk near stream sites and homes along the downstream section of Córrego João Dias (sites D4, D5, and D6). The mean biting rate of *S. nigrimanum* was greatest, followed by *S. pertinax* + *S. spinibranchium* (rates combined), *S. incrustatum*, and *S. inaequale* (Fig. 7). Biting rates varied substantially for all species and appeared to be related to small daily differences in temperature, wind, and humidity. The females of *S. nigrimanum* were collected exclusively at dusk and were most active on warm ( $>25^{\circ}\text{C}$ ) humid evenings with little wind. The biting activity of the other 4 species also peaked near dusk, but a few females of *S. pertinax* and *S. spinibranchium* also were caught near midday in weather that was relatively hot ( $30^{\circ}\text{C}$ ), windy, and dry.

Only females of *S. pertinax* were captured during 24 person-hours of collecting in the dry season at non-fogo selvagem sites. At Córrego Eugenia, the biting rate was 48.0 bites per person-hour (4 person-hours of baiting). One female of *S. pertinax* was captured at Córrego Seco (0.3 bites per person-

hour, 4 person-hours of baiting), despite the absence of immatures in samples from the site. Biting black flies were not encountered during dry season catches at the Ipegue/Taunay Reservation.

## Discussion

**Prevalence and Distribution of Simuliid Species at the Limão Verde Focus of Fogo Selvagem.** Immature simuliids at the Limão Verde focus of fogo selvagem displayed geographic trends in overall abundance, species proportions, occurrence, and richness. *S. nigrimanum* was the most abundant species. Its pattern of distribution and abundance showed it to be relatively scarce in the headwater tributaries and upper reaches of the João Dias, where simuliids generally occurred in low numbers. However, *S. nigrimanum* was the most prevalent species at large stream sites along the João Dias where the aquatic stages of simuliids were abundant.

*Simulium perflavum* was the 2nd most abundant species at Limão Verde and appeared in highest proportions at sites that were not dominated by the prevalent downstream species, *S. nigrimanum*, or the prevalent upstream species, *S. subnigrum*. These

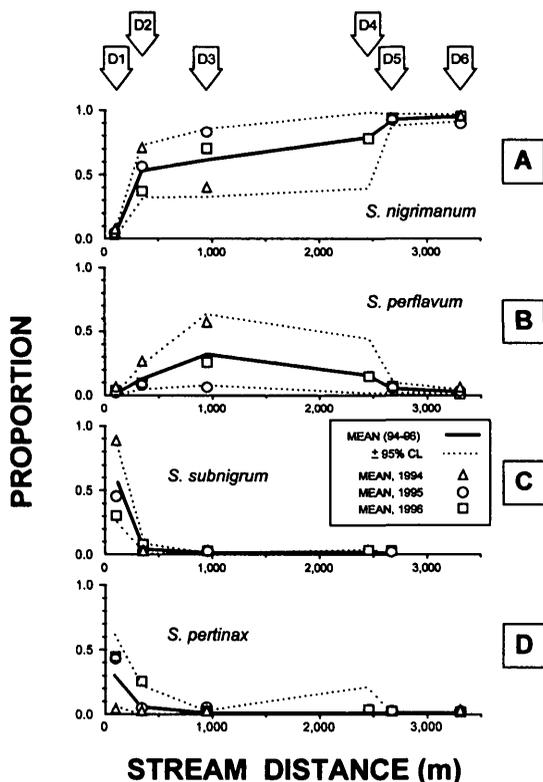


Fig. 4. Longitudinal (upstream-downstream) trends in the proportions of the 4 most common simuliid species (larvae + pupae) at 6 sites, D1–D6, along Córrego João Dias. Solid and dotted lines show the trends of means  $\pm$  95% CL, respectively, for species proportions of all samples collected during 3 yr ( $n = 3-8$ , depending on the site). Annual means from 1994, 1995, and 1996 are also shown.

sites were medium in abundance with respect to the total simuliid assemblage and occurred in the upper-middle reaches of the João Dias and the downstream section of Córrego Dois Irmãos. The larvae and pupae of *S. perflavum* at these sites were collected most commonly from organic substrates, such as trailing grasses and leaves near the stream margins, whereas *S. nigrimanum* was collected in more rapid current from bedrock falls.

*Simulium subnigrum* ranked 3rd in abundance and was clearly associated with headwater regions of all 3 streams. The prevalence of *S. pertinax* was relatively low at Limão Verde, and its distribution was restricted mainly to the upper reaches of the João Dias. This highly anthropophagic species is common along the coast of Brazil (Araújo-Coutinho et al. 1988), where very few cases of fogo selvagem have been reported (Diaz et al. 1989a).

The remaining 5 species were relatively rare at the Limão Verde focus. However, species *E*, which was widely distributed and generally low in abundance, occurred in dense aggregations on the trailing grasses of the oxbow channel. The volume of running water in this channel fluctuated widely with

the seasons. This also was true of Córrego Seco, where species *E* was the only simuliid collected. This unusual distribution indicated that species *E* was able to colonize and thrive in temporary running-water environments that were marginal for other simuliids.

The longitudinal trends observed along the João Dias may have been related to human disturbance. Among sites D1, D2, and D3, dramatic changes in the simuliid assemblages occurred. Interestingly, this section also corresponded with the transition from native vegetation on the upstream slopes to the crops and houses of the lower valley floor. Human-related influences along the downstream sections of the João Dias, where simuliid densities were high, have not been quantified. However, it is likely that a moderate level of nutrient and organic enrichment occurred, favoring species that thrive under enriched conditions. *S. nigrimanum* may be such a species, and this may explain its dominance and high densities in the downstream reaches of the João Dias. In support of this hypothesis, Vulcano (1959) recorded the presence of huge aggregations of *S. nigrimanum* larvae and pupae on the outflow channel of a nutrient and organic-matter enriched reservoir near the city of São Paulo. The species had become a biting pest in the region after the construction of the reservoir.

In contrast to *S. nigrimanum*, *S. subnigrum* appeared to be characteristic of small upstream sites at Limão Verde, where algal productivity was low, simuliid aggregations less dense, and human disturbance not evident. The reach from D2 to D3 with presumably an intermediate level of enrichment, contained the greatest number of species and showed the most variability and change in species proportions. *S. perflavum* made up a large proportion of the immatures in this ecological transition zone.

**Simuliids of Neighboring Valleys and Villages with Few or No Cases of Fogo Selvagem.** The black fly assemblages of neighboring valleys and villages free of reported cases of fogo selvagem were very different from those of the Limão Verde focus. In general, simuliid abundance and richness were lower, and species proportions were different. Córrego Eugenia had a diverse simuliid fauna, but the predominant species was *S. pertinax*. We did not observe obvious ecological differences between Córrego Eugenia and Córrego João Dias which would explain this finding. The presence of only species *E* at Córrego Seco probably was related to seasonal fluctuations of stream discharge. On the Ipegue/Tauney Reservation, where we did not encounter simuliids, the absence of permanent running-water environments with sufficient current for filter-feeding larvae undoubtedly precluded colonization by black flies.

**Biting Activity at the Limão Verde Focus and Neighboring Non-Fogo Selvagem Sites.** In addition to the prevalence of its larvae and pupae at Limão Verde stream sites, *S. nigrimanum* was the most

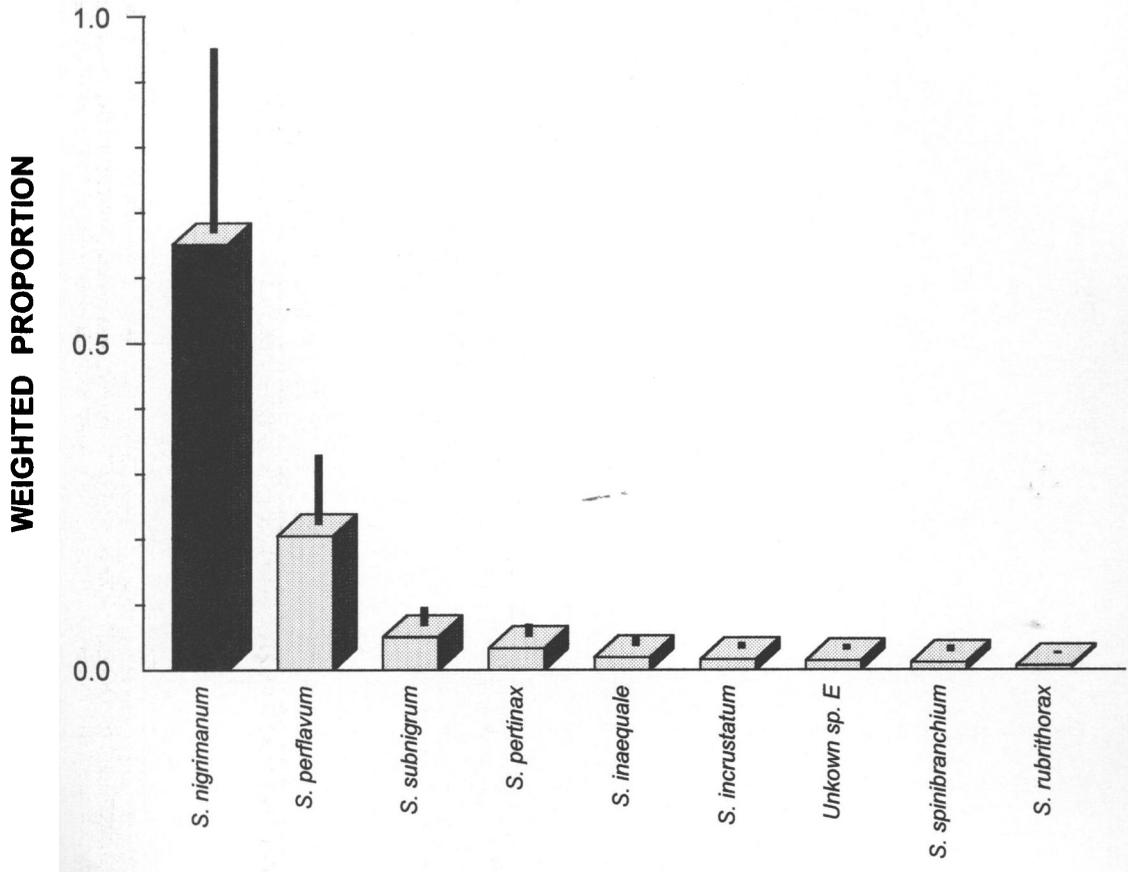


Fig. 5. Prevalence of simuliid species (larvae + pupae) at the Limão Verde focus of fogo selvagem. To express prevalence, the mean proportions of the species at each site (e.g., Figs. 3 and 4) were weighted by the relative abundance of the simuliid assemblage (Fig. 2). The means plus standard errors for the weighted proportions over all Limão Verde sites, except the oxbow channel, are shown ( $n = 11$ ).

common anthropophagic species encountered during dry season catches at human bait. At other fogo selvagem foci in the Brazilian state of Goiás and the Federal District, we also observed late dry season biting by this species (D.P.E., unpublished data). Highly variable seasonal and diel biting peaks were observed at Limão Verde, indicating that human exposure to *S. nigrimanum* may be variable as well. One non-fogo selvagem site, Córrego Eugenia, had a relatively constant level of biting activity by *S. pertinax*, but anthropophagic simuliids were nearly absent from the remaining disease-free sites.

Based on the presence of mature pupae that we collected during our 3 rainy season visits to Limão Verde, we inferred that adults of all species probably were present. Therefore, the near absence of biting females during these periods was puzzling and will require further investigation. This apparent seasonality of biting activity when adults are present throughout the year could indicate the existence of aetogenous generations that do not require a blood

meal or temporally separate anthropophilic and zoophilic sibling species.

**Black Flies and the Epidemiology of Fogo Selvagem at Limão Verde.** The epidemiological conclusions of Hans-Filho et al. (1996) and our results from the Limão Verde focus are consistent with the hypothesis that a black fly species, *S. nigrimanum*, may be related causally to fogo selvagem. In an analysis of the home locations at Limão Verde, Hans-Filho et al (1996) discovered that the homes of tribe members with fogo selvagem were not closer, statistically, to Córrego João Dias than those of unaffected tribe members. Although the aquatic stages of black flies are restricted to running water, the females of many species are strong fliers and able to travel long distances (Dalmat 1952, Garms and Walsh 1987). This is especially true of species that live in seasonal, open environments similar to the "cerrado" region of Brazil (e.g., the plains of western North America or the tropical savannahs of western Africa [Garms and Walsh 1987]). In the tropical forests of Guate-

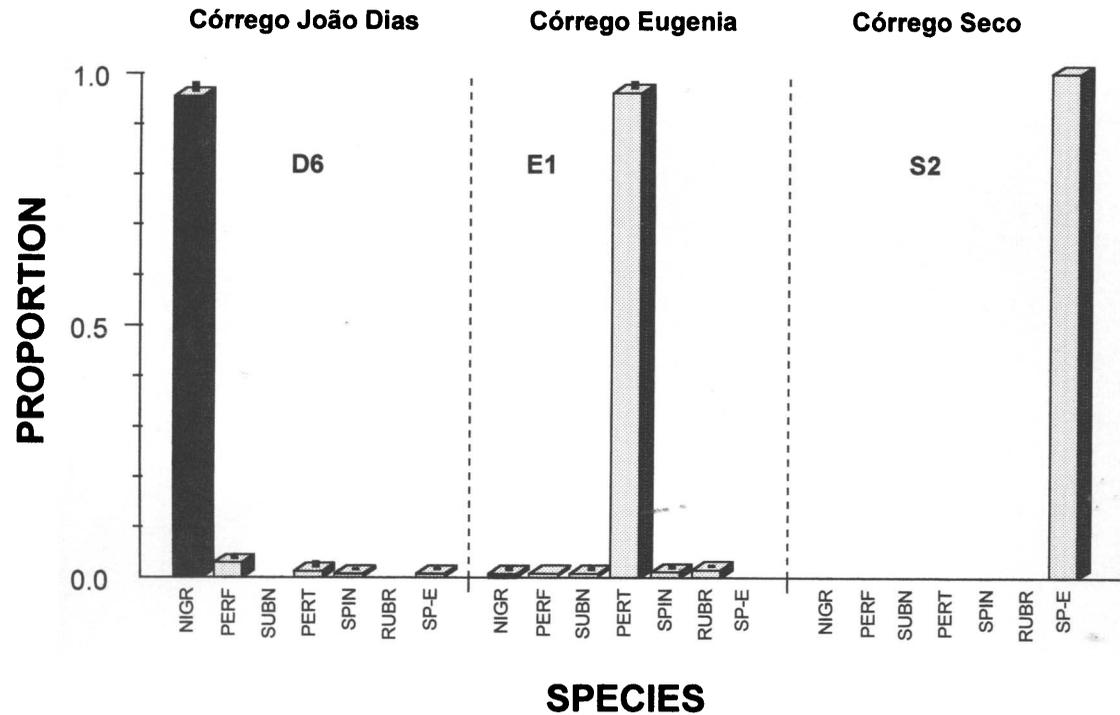


Fig. 6. Comparison of simuliid species proportions (larvae + pupae) from the downstream section of Córrego João Dias, D6, at the Limão Verde focus of fogo selvagem, and 2 stream sites within regions without cases of fogo selvagem; Córrego Eugenia, E1, and Córrego Seco, S2. Means plus standard errors of species proportions are shown ( $n = 8$  for D6,  $n = 3$  for E1, and  $n = 3$  for S2).

mala, simuliids dispersed up to 15 km (Dalmat 1952), and *S. damnosum* of tropical African savannahs dispersed 20 km before obtaining their 1st blood meal (Garms and Walsh 1987). Within the Limão Verde focus, a blood-seeking female would need to fly a maximum distance of 4 km to reach outlying homes. Therefore, home location is probably not a significant factor with respect to exposure to biting black flies.

Hans-Filho et al. (1996) also showed that since the 1 case of fogo selvagem at Limão Verde in 1979, the appearance of new cases has occurred only during certain years. They hypothesized that exposure to the environmental factor associated with fogo selvagem was periodic. This is consistent with our data on biting rates of black flies at Limão Verde, which showed seasonal fluctuations. Pronounced variability between years also may occur as has been

Table 3. Occurrence and number of black fly species at non-fogo selvagem stream sites

Streams and sites	<i>S. nigri-</i> <i>nigrimanum</i>	<i>S. per-</i> <i>flavum</i>	<i>S. ina-</i> <i>equale</i>	<i>S. sub-</i> <i>nigrum</i>	<i>S. inc-</i> <i>rustatum</i>	<i>S. per-</i> <i>tinax</i>	<i>S. spini-</i> <i>branchium</i>	<i>S. rubri-</i> <i>thorax</i>	Unknown <i>sp. E.</i>	No. species
Córrego Seco										1
S1									X	1
S2									X	1
Córrego Eugenia										6
E1	X	X		X		X	X	X		6
Ipegue										0
P1										0
Bananal										0
B1										0
B2										0
Species occurrence, % <sup>a</sup>	17	17	0	17	0	17	17	17	33	

X, Species collected at site.

<sup>a</sup> Percentage of sites where a species was collected.

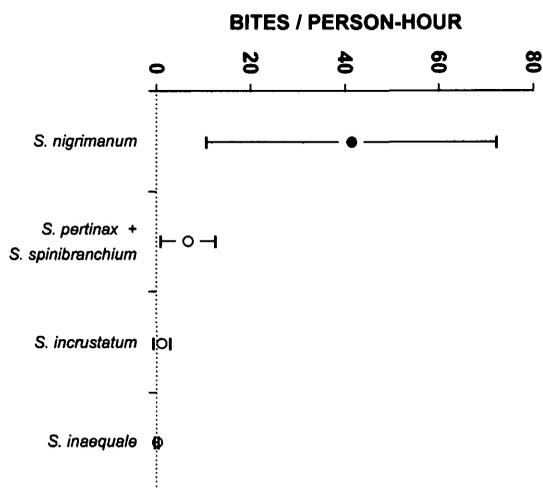


Fig. 7. Dry season biting rates of anthropophagic black flies at the Limão Verde focus of fogo selvagem during 8 evenings and 20 person-hours of baiting, August 1996 and July 1997. Mean rates  $\pm$  95% CL are shown.

shown for populations of simuliids on west African savannahs (Wenk 1981, Kurtak et al. 1987) and other tropical insects in seasonal environments (Wolda 1992).

The most striking finding of Hans-Filho et al. (1996) was that all of the fogo selvagem cases originated within a relatively small geographic region, and that neighboring populations, some living only a few kilometers away, had no reported cases. They concluded that an environmental factor unique to the Limão Verde valley was associated with the fogo selvagem cases they observed. Our observations from this high prevalence focus support the findings from other regions with the disease (Lane 1950; D.P.E., unpublished data), which showed that *S. nigrimanum* was the predominant black fly species encountered near fogo selvagem foci. Our study is unique, however, in showing that neighboring regions, which did not have cases of fogo selvagem among the populations (except for 1 new fogo selvagem case that appeared at the Ipegue/Tauney reservation late in 1995), also had very different black fly faunas. Based on our results, the presence of large populations of *S. nigrimanum* is one of the environmentally unique aspects of Limão Verde, and it should be included in epidemiological and experimental investigations of fogo selvagem etiology.

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