Trapping sand flies (Diptera: Psychodidae) in the Emilia-Romagna region of northern Italy

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ABSTRACT: The efficiency and practicality of two trapping methods for adult *Phlebotomine* sand flies in two areas of the Emilia-Romagna region (Italy) were evaluated. Suction traps (CO_2) and sticky traps (ST) were used to collect sand flies every two weeks, from June to September, 1999, from 16:00 to 07:00. Two CO_2 traps were activated at the same time for each area (one with light and one without light), whereas 38 (four with light and 34 without lights) and 48 (four with light and 44 without) sticky traps were activated in Borghi and Longiano, respectively. An Index of Apparent Abundance (IAA) was calculated for each trap type and area. A total of 2,253 sand flies was trapped over the four-month period, with 1,765 collected from Borghi and 488 from Longiano. *Phlebotomus perfiliewi* was the most abundant species collected, comprising 99.6% and 84.6% of the total flies trapped in Borghi and Longiano, respectively. Other species were also collected within the two areas (*Phlebotomus perniciosus* and *Phlebotomus mascittii*) but were not considered for further analyses due to low catches. Significantly more specimens were caught using CO_2 than sticky traps and the addition of a light source also improved the catches, however, a significantly greater number of female specimens were collected by a CO_2 trap without a light source. *Phlebotomus perfiliewi* thus appears to show a photophobic reaction in the case of females when trapped using CO_2 /light attractants. ***Journal of Vector Ecology* 32 (2): 313-318. 2007.**

Keyword Index: Sand flies, *Phlebotomus perfiliewi*, CO_2 traps, sticky traps, sex ratio.

INTRODUCTION

There are eight sand fly species currently described in Italy (Boorman et al. 1995), seven of which belong to the genus *Phlebotomus* and one to the genus *Sergentomyia*. The genus *Phlebotomus* includes the vectors of human (visceral and cutaneous) and canine leishmaniasis. Visceral leishmaniasis has been recorded in Italy since the beginning of the last century and the country is considered to be the most important endemic area in the Mediterranean basin (Pampiglione et al. 1974). Following a period of decrease in leishmaniasis, during the past two decades an increase in new cases has been reported (Gradoni et al. 1980), and between 1992-2004, 26 visceral and 15 cutaneous leishmaniasis cases emerged (www.ministerosalute.it/promozione/malattie/datidefcons_malattie.jsp, accessed Jan 15, 2007) .

In addition to leishmaniasis, *Phlebotomus* also transmit several viruses to humans that can cause sand fly fever of the Naples and Sicilian type (Lane 1993). These are mainly transmitted by *Phlebotomus papatasi* Scopoli (1786), *P. perfiliewi* Parrot (1930), and *P. perniciosus* Newstead (1911).

Collection and trapping of sand flies are normally done for monitoring purposes for distribution and the presence or absence of sand flies. Traps include CO_2 traps, sticky traps, and emergence traps (Maroli and Fausto 1986). This paper reports on investigations into the efficiency and practicality of two trapping methods for adult Phlebotomine sand flies in two areas of the Emilia-Romagna region as part of the regional environmental and urban improvement project.

MATERIALS AND METHODS

Trapping of sand flies took place between June and September, 1999, in the municipalities of Borghi and Longiano in the Forlì-Cesena Province. Two main trap types were evaluated, namely, suction traps (CO_2) and sticky traps (ST). The suction traps were the standard CO_2 (dry ice) baited traps used for mosquito monitoring in Italy (Bellini and Veronesi 2001, Petric et al. 1999), and were either used with or without a 12V light source.

The choice of sites to activate the traps was based on the areas with characteristic *Phlebotomus* resting and breeding sites, such as soil rich in organic material, presence of poultry and domesticated animals, animal shelters providing shaded resting sites for adults with a high level of humidity, and dry walls with cracks and crevices where adults tend to rest (Grassi 1907, Theodor 1936, Perfiliew 1968, Pozio 1980, Maroli and Guandalini 1985, Killick-Kendrick 1999). Records of complaints of insect bites from the habitats also helped to define where to activate the traps. Moreover, from 1934 to 1970 in this province, more than 80 human cases of visceral leishmaniasis were recorded. Borghi is a small village on the top of a hill at a maximum altitude of 297 m above sea level (a.s.l.). The traps were positioned in a rural site, in the garden of an isolated farm with poultry and domesticated animals surrounded by few houses and mainly irrigated lands cultivated with sunflowers and vineyards. The second area of trapping, Longiano (179 m a.s.l.), is a semi-urban area and the site were the traps were activated was across the stone wall of an ancient castle, situated in the

middle of the village, that had many cracks, crevices, and drainage holes in the shade of trees. The castle cellar might also be used as a breeding site for sand flies as described in previous studies on the discovery of *P. papatasi* larvae and pupae in cellars in Rome, Italy (Grassi 1907).

For each location, two CO_2 traps were activated at the same time, one with light (CO_2+L) and one without light (CO_2), whereas the number of sticky traps activated were 38 (four with light and 34 without lights) and 48 (four with light and 44 without) for Borghi and Longiano, respectively. In both localities, CO_2 traps were hung on poles or branches at approximately 1 m. Sticky traps were constructed of 20 x 20 cm white cardboard sheets covered with castor oil. Some were operated with light sources (ST+L) consisting of a 12V bulb with luminous intensity of 4000-4300 Lux inserted inside a plastic tube of 5 cm diameter and 9 cm length with an aluminum sheet on the inner surface. The sticky traps placed in Longiano were located near the cracks and crevices of an old boundary stone wall, whereas in Borghi they were across a cement wall, at intervals of 5 m apart for both the two sites. Sticky traps and CO_2 traps with and without light were activated from 16:00 to 07:00 for one day every two weeks. CO_2 traps were deployed at intervals of 15-20 m apart and for each area, trap types were alternated between sites for every collection day.

Catches were interpreted as mean flies caught per trap/ total number of days of monitoring to provide an Index of Apparent Abundance (IAA) for each area. Only undamaged specimens were considered for the IAA.

Collected specimens were preserved dry at 4° C. For identification of species and sex, specimens were slide-mounted in Faure's medium after a clearing process in lactophenol for three days (Maroli and Fausto 1986). Female identification was based on the shape of spermathecae and the disposition of the teeth in the pharyngeal armature, whereas males were identified based on their genitalia (hypopygium), in particular the parameres and aedeagus, the shape, insertion, disposition, and length of the hairs in the coxite, and the spines in the style (Corradetti et al. 1961, Rioux and Golvan 1967, Dolmatova and Demina 1971, Killick-Kendrick et al. 1991, Maroli et al. 1994).

Analyses of species collected among all trap methods used were analyzed with an analysis of variance (ANOVA), and a t-test for dependent samples was used for the analysis of sex ratio obtained with CO_2 traps with and without light.

RESULTS AND DISCUSSION

A total of 2,253 sand flies was collected over the four-month period, with 1,765 from Borghi and 488 from Longiano. A large number of flies collected from the CO_2 traps were destroyed by the blades of the suction fans, comprising 60% from the Borghi area and 27% from the Longiano area. In contrast, the majority of flies collected from the sticky traps were undamaged. Nevertheless, catches from the CO_2 traps were substantially higher than from the sticky traps (Table 1). The most frequently collected species in both areas was *P. perfiliewi* with 99.6%

Table 1. IAA of undamaged sand flies collected in Longiano and Borghi by CO_2 traps and sticky traps.

	LONGIANO								**BORGHI**							
	CO_2 trap (1)* with light		**CO_2 trap (1)* without light**		**ST traps (4)* with light**		**ST traps (44)* without light**		**CO_2 trap (1)* with light**		**CO_2 trap (1)* without light**		**ST traps (4)* with light**		**ST traps (34)* without light**	
	Catch	*IAA*	Catch	*IAA*	Catch	*IAA*	Catch	*IAA*	Catch	*IAA*	Catch	*IAA*	Catch	*IAA*	Catch	*IAA*
P. perniciosus	23	2.87	22	2.75	0	0	9	0.025	0	0	3	0.5	0	0	0	0
P. perfiliewi	193	24.12	74	9.25	0	0	35	0.099	377	62.8	186	31.0	20	0.83	127	0.62
P. mascittii	0	0	0	0	0	0	1	0.003	0	0	0	0	0	0	0	0
Total adults caught	216		96		0		45		377		189		20		127	

*number of traps activated/day.

Table. 2. Sex ratio of adult sand flies collected in Borghi and Longiano by CO_2 traps and sticky traps.

	CO_2 trap with light					CO_2 trap without light					ST traps with light					ST traps without light				
Borghi	Total coll.	♀	♂	♀%	♂%	Total coll.	♀	♂	♀%	♂%	Total coll.	♀	♂	♀%	♂%	Total coll.	♀	♂	♀%	♂%
Pps	0	0	0	0	0	1	1	0	100.0%	0.0%	0	0	0	0.0%	0.0%	0	0	0	0.0%	0.0%
Ppf	251	185	66	73.7%	26.3%	111	103	8	92.8%	7.2%	9	6	3	66.7%	22.2%	50	18	32	33.3%	66.7%
Total	251	185	66			112	104	8			9	6	3			50	18	32		
Longiano	Total coll.	♀	♂	♀%	♂%	Total coll.	♀	♂	♀%	♂%	Total coll.	♀	♂	♀%	♂%	Total coll.	♀	♂	♀%	♂%
Pps	11	6	5	54.5%	45.5%	5	4	1	80.0%	20.0%	0	0	0	0.0%	0.0%	7	2	5	28.6%	71.4%
Ppf	121	62	59	51.2%	48.8%	37	28	9	75.7%	24.3%	0	0	0	0.0%	0.0%	4	2	2	50.0%	50.0%
Pmi	0	0	0	0.0%	0.0%	0	0	0	0.0%	0.0%	0	0	0	0.0%	0.0%	0				
Total	132	68	64			42	32	10			0	0	0			11	4	7		
Total both localities		253	130				136	18				6	3				22	39		
		66.1%	33.9%				88.3%	11.7%				66.7%	33.3%				36.1%	63.9%		

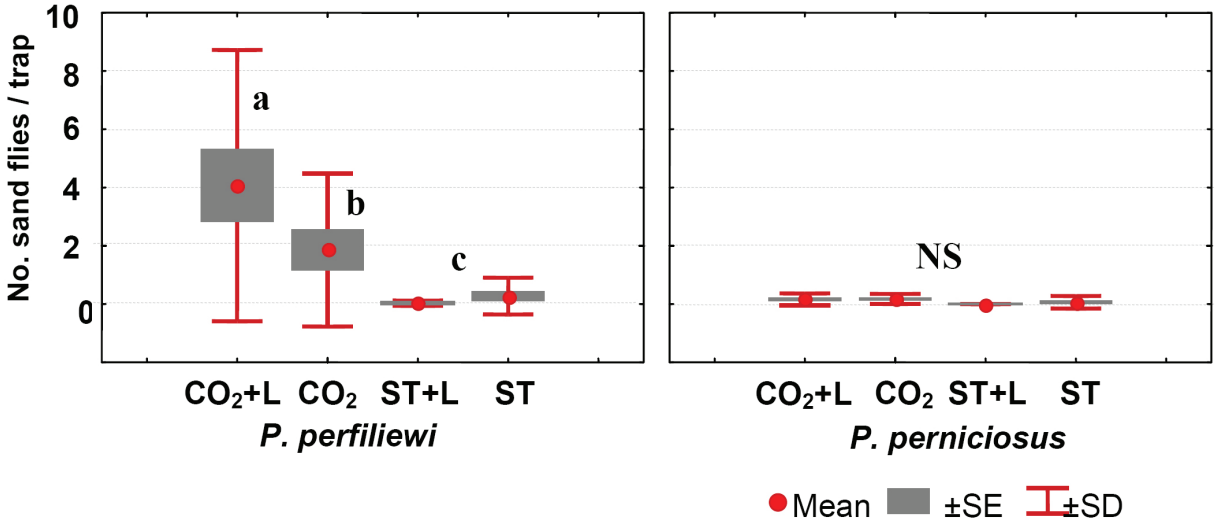

Figure 1. Catch ratios between the traps tested on *P. perfiliewi* and *P. perniciosus.*

and 84.6% in the Borghi and Longiano, respectively. Small collections were made of *P. perniciosus* and *P. mascittii* in both areas (Table 1) and were not considered for further analyses. The IAA of *P. perfiliewi* was 62.8 and 31.0 from the CO_2+L and CO_2 without light, respectively, from the Borghi area. In the Longiano area, the IAA of *P. perfiliewi* was 24.12 and 9.25 from the CO_2+L and CO_2 without light, respectively. The IAA from sticky traps for all species in both areas was very low, and it does not appear to be an appropriate trap for monitoring the abundance of sand flies. Our data suggest that CO_2 traps appear to be more appropriate and the addition of a light source improves catches 2-3 fold (Table 1). However, the use of dry ice and additional light sources and batteries is a cumbersome technique and has disadvantages for field use. The two-way ANOVA illustrated a significant difference among the four sampling methods ($F=17.58$ and $P<0.0001$). There are also significant differences in the efficiency of the traps according with the species caught ($F=6.42$ and $p<0.0001$). *P. perfiliewi* were caught in decreasing order by CO_2+ light > CO_2 > ST = ST+light, showing a strong phototropism in agreement with previous studies (Toprak and Özer 2007, Maroli et al. 1994, Lewis 1971, Gibb et al. 1988) in respect to artificial light (Miščević et al. 1985). Collections of *P. perniciosus* were too low to allow trap comparisons to be made (Figure 1). A positive phototropism for *P. perfiliewi* was already recorded in previous works. Despite the presence of typical breeding sites for *P. papatasi* (Grassi 1907), specifically at the Longiano site, no adults of this species were caught. Maroli and Bettini (1997) reported that *P. papatasi* in Italy seems to be more closely associated with humans and old buildings than other *Phlebotomus* species, moreover, they reported that its larval sites and adult resting places seem to be susceptible to human interference in term of insecticides and hygiene. During an 18-year study on the presence of sand flies in 11 regions of Italy, Maroli et al. (1994) recorded a frequency of *P. papatasi* collection of only 0.3% among five species of *Phlebotomus,* suggesting a high anthrophophilia and endophilia for *P. papatasi.*

This indoor preference of *P. papatasi* may explain why we failed to catch any adults of this species since the traps were all outdoors. In particular for Longiano, the trapping area was surrounded by houses that could have played an important role in preventing *P. papatasi* from moving far from its ideal indoor habitat. Moreover, it seems that *P. papatasi* has a high negative phototropism (reviewed in Lewis 1971). It should be noted that the two devices were installed at different heights: 1 m and 10 cm above the ground for CO_2 traps and ST, respectively. It would be interesting to modify the relative height of the two sampling methods in order to further evaluate their performance. The only light-baited sticky traps that caught adult sand flies were those installed in Borghi, presumably because of their location and the very low light contamination, as the closest house was approximately 200 m from the sampling site. In contrast, at the sampling station of Longiano, the ST+L traps were located in less exposed positions, hidden inside cervices and cracks of the wall. Being in the middle of the urban area it was not easy to avoid public light contamination. The type of light used in the monitoring may also have an effect on the attraction of the sand flies. A previous study by Lane et al. (1988) conducted in Jordan using four different sampling methods (ST+chemical light, ST without light, CDC traps+light, and aspirator) showed that sticky traps with chemical light caught even more individuals and species than the CDC traps with light. Burkett et al. (2007) reported a higher collection of female sand flies in Iraq with UV light, CDC traps rather than standard CDC traps, and sticky traps with chemical light. The 8W UV light (manufactured in Onderstepport, South Africa), normally used for the monitoring of *Culicoides* (Diptera: Ceratopogonidae), has recently been used for sand fly trapping (Ivović et al. 2007). Moreover, the range of attraction to light traps is currently unclear. Killick-

Kendrick et al. (1985) showed a range of attraction for *P. ariasi* Tonnoir to CDC light traps of <2 m, working with a 6V 60 mA spherical bulb giving a nominal lumen at 6V (0.08 cd). A maximum attraction of 6 m was recorded for *Lutzomyia youngi* using a 60 W light bulb (reviewed in Alexander 2000). In previous work, a dispersal pattern of sand flies between 20 to 600 m (Pozio et al. 1980, Maroli and Guandalini 1985, Alexander 1987) up to 1 km (Alexander and Young 1992, Killick-Kendrick 1999) has been reported. Killick-Kendrick et al. (1984) reported a maximum distance of marked *P. ariasi* sand flies of 600 m for male and up to 2,200 m for females, enphasizing that engorged females tend to move farther than unengorged ones.

The distribution of sand flies is very broad, including different habitats and species (Ghosh et al. 1999). There are many factors involved in the choice of habitat such as the environment, the use of the land, nature of the soil, and the presence of domesticated and undomesticated animals. The height of the water table seems to be an important factor for habitat choice as well because it increases the humidity of breeding sites (reviewed in Lewis 1971). The modification of the territory due to irrigation of lands, such as in the Borghi site, may also favor the habitat for rodents that provide good resting sites for the flies within their burrows (Lewis 1971, Alexander 2000).

The sex ratio of *P. perfiliewi* differed between traps and areas (Table 2). Both trap types collected more females, however, contrary to what other authors reported (Killick-Kendrick et al. 1985, Alexander 2000), the majority of females were captured with the CO_2 trap without a light source. In the Borghi area, the female to male ratio was 74:26 for the CO_2+L and 93:7 for the CO_2 without light. In the Longiano area, the ratio was 51:49 for CO_2+L and 76:24 for the CO_2 alone. Comparisons considered catches only from CO_2 traps because the sticky trap catches were too low. Following a t-test for dependent samples, a significant difference was apparent between the sex ratio obtained with light versus without light, with a significantly higher proportion of females from CO_2 than CO_2+L ($t = 4.11$ and $p<0.03$). Females appear to prefer CO_2 without light, and the results indicate that a light source has a deterrent effect on female catches. Our data indicate a strong phototropism for males of *P. perfiliewi*.

Finally, the presence of potential vector species illustrates the importance of maintaining and eventually extending the surveillance to other regions in order to study the distribution and define areas at high risk of transmission. This should involve the overlapping of entomological data with epidemiological surveillance of human and canine cases. It is important to conduct a comparative study on trapping methods for sand flies, using CDC traps (with and without light), sticky traps (with and without chemical lights), CO_2 traps (with and without light), and UV light traps with downdraught suction at the same time. The use of the UV light downdraft traps (manufactured in Onderstepoort, South Africa) might be very useful for sand fly collections, helping to solve the problem of the high percentage of damaged flies due to the rotating blades of the suction fans that we encountered using CDC traps.

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