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PHLEBOTOMINE SAND FLY CONTROL USING BAIT-FED ADULTS TO CARRY THE LARVICIDE *BACILLUS SPHAERICUS* TO THE LARVAL HABITAT[1]

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ABSTRACT. Sugar meals of plant origin are an important component of the sand fly diet. We show that sugar solution baits have potential as vehicles for phlebotomine sand fly control. In the laboratory, adult *Phlebotomus duboscqi* Neveu-Lemaire and *Sergentomyia schwetzi* (Adler, Theodor, and Parrot) that have consumed an aqueous sucrose solution containing *Bacillus sphaericus* Neide toxins and are subsequently eaten by larvae produce significant larval death ($P < 0.01$).

In the field, when vegetation near animal burrows and eroded termite mounds was sprayed with sucrose solution with or without incorporation of the larval toxicant *B. sphaericus,* 40% of female sand flies fed *in situ.* Dispersing *B. sphaericus*-carrier sand flies caused significant larval mortality ($P < 0.01$) in resting and breeding sites in animal burrows 10–30 m from the sprayed vegetation for 2–12 wk posttreatment. Also, adult sand fly populations breeding and resting inside animal burrows were significantly reduced ($P < 0.01$) following direct application of the sucrose/*B. sphaericus* solution to the burrow entrances. This control effect lasted 4–10 wk post-treatment. The effect was not seen for sand fly populations breeding and resting inside eroded termite mounds. This approach may be useful for the application of biological control agents against phlebotomine sand flies in biotypes where larvae and adults use the same habitats.

INTRODUCTION

Phlebotomine sand flies consume sugars and other plant-derived materials as an essential part of their diet (Schlein 1986). This provides both sexes of adult sand flies with their main carbohydrate needs (Samie et al. 1990) and complements the female's blood meal. Although most sources of natural sugars consumed by sand flies are unknown (Killick-Kendrick 1979), some species may obtain sugar of plant origin directly (Yuval and Schlein 1986) or obtain sugar from aphid or coccid honeydew (Killick-Kendrick and Killick-Kendrick 1987, Moore et al. 1987). Ashford (1974) reported an association between sand flies and plants in Ethiopia when he observed 4 species of sand flies (*Phlebotomus longipes* Parrot and Martin, *P. orientalis* Parrot, *Sergentomyia bedfordi* [Newstead], and *S. magna* Sinton) probing leaves and stems of a variety of plants. Schlein and Warburg (1986) observed that *P. papatasi* Scopoli feed effectively and preferentially on various plant species offered under experimental conditions. In nature, *P. papatasi* is attracted to several species of plants to obtain sugar meals (Schlein and Yuval 1987). In general, sugar meals are important determinants of sand fly ecology, the sand fly–*Leishmania* relationship (Schlein 1986), and hence the transmission of leishmaniasis.

Schlein (1987) suggested the possibility that sprayed, colored sugar baits could be used for both behavioral and control studies. This idea prompted us to investigate the feasibility of this methodology for controlling phlebotomine sand flies. We first monitored sand flies feeding on cotton soaked with a food dye/sugar solution. Then we added *Bacillus sphaericus* Neide, which contains specific antilarval toxins (Novo Nordisk Inc.), to the solution and investigated the effect on sand fly larval populations. The average life span of adult sand flies in nature is short (Killick-Kendrick 1979); therefore, the flies should be able to quickly deliver the toxin to the larval habitat before death. Also, most sand fly species in the study area disperse less than 50 m (Mutinga et al. 1992). Thus, from a population of adult sand flies resting in animal burrows and termite mounds, *B. sphaericus* will likely be introduced into the larval habitat by adult flies either dying or defecating bacterial toxins and viable spores.

MATERIALS AND METHODS

Laboratory experiments: For differential marking of sugar-fed sand flies (Schlein 1987), the untreated sucrose solution contained 10 g blue food dye (Indigotin, C.I., Stern, Natanya, Israel) and 200 g sucrose per liter of water. The treated solution contained 10 g red dye (Carmoisine E 122, Stern, Natanya, Israel); 10 g *B. sphaericus,* strain 2362; and 200 g sucrose per liter of water. Separate groups of teneral female sand flies were allowed to feed *ad libitum* for 5 days on the solutions. Fed

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Table 1. Mortality of 10 phlebotomine sand fly 2nd-instar larvae in containers with 10 whole *B. sphaericus*-gorged adult female sand flies per container.

Species	No. of replicates	Mean larval mortality ± SE per replicate	
		Control	Treatment
Phlebotomus duboscqi	15	0.15 ± 0.02	3.93 ± 0.83a[1]
Sergentomyia schwetzi	15	0.24 ± 0.04	3.57 ± 0.64b

[1] Means within a row followed by different letters are significantly different (ANOVA, $P < 0.01$).

females (indicated by dye in the gut) were anesthetized with CO_2 and killed by pressing their heads. Dead *P. duboscqi* Neveu-Lemaire or *S. schwetzi* (Adler, Theodor, and Parrot) females that had fed on cotton soaked with a solution of sucrose and food dye with or without *B. sphaericus* were introduced into larval containers to assess their effect on larvae. The larval containers were made from plastic specimen cups (5.7 cm diam × 6.5 cm height) with moist plaster covering the bottom (1 cm deep). Larval food (0.1 gm) (Young et al. 1981) was added to the container, then 10 dead females were placed on the food surface. Larval containers were maintained in walk-in incubators at 25°C and 80% relative humidity. After 10 days, 10 2nd-instar larvae were added to each container. The larvae were allowed to feed on the dead adults and larval food, and the numbers of larvae, pupae, and adults were recorded daily. Comparisons of mean larval mortality were made using ANOVA.

Field experiment: The field experiment was conducted May–October 1994, 2 km south of Marigat (0°29′N, 36°0′E), a village located in an arid region of the Rift Valley, Kenya. This study site was characterized by rolling hills covered with various succulents and *Acacia* spp. Numerous termite mounds built by *Macrotermes subhyalinus* Rambur and animal burrows built by the unstriped ground squirrel (*Xerus rutilus* Ehrenburg) were widely distributed in this area. One control site and 2 treatment sites were selected that had similar numbers of termite mounds and animal burrows. The 3 sites were separated by at least 1 km. An aqueous suspension of sugar/red food dye/*B. sphaericus* toxin (as described above) was sprayed on vegetation at one treatment site, and a solution of sugar/blue food dye was sprayed at the control site. The solutions were sprayed on all living vegetation around 3 eroded termite mounds and 3 animal burrows for a diameter of 20 m to bait adult sand flies. Vegetation was sprayed to a height of 3 m using 2 liters of solution for each treatment. At the second treatment site, the openings of 3 animal burrows and 3 termite mounds were sprayed directly with the sugar/food dye/*B. sphaericus* suspension to a depth of 1 m using 2 liters of solution for each treatment. Both areas were sprayed a second time 4 wk after the first spraying.

Adult sand flies were sampled during the night following bait spraying by collection with 24 sticky paper traps at 1, 5, and 10 m from the sprayed area around each animal burrow and termite mound. Two sticky paper traps, sheets of paper (21.5 × 28 cm) manually coated with castor oil, were placed at the points of the compass for a total of 8 traps at each distance. The sand flies caught were immersed in 2% detergent solution, and specimens with and without food dye in the abdomen were sorted under a stereomicroscope.

Adult sand fly populations were monitored using entrance–exit sticky traps (Yuval and Schlein 1986) 3 nights biweekly 3 times (total of 9 nights) before treatment and 8 times biweekly (total of 24 nights) after treatment. These traps were placed in the openings of the animal burrows and termite mounds. Trapping was discontinued 16 wk after the first treatment, when the sand fly population returned to pretreatment levels. Two-way analysis of variance was used to compare numbers of sand flies before and after treatment because the same animal burrows and termite mounds were sampled repeatedly over time (Sokal and Rohlf 1981). This procedure was also used to determine if the sand fly species compositions were significantly different between sites and before and after treatments. The termite mounds and animal burrows were one factor (considered as random and serving as replication) and the time dimension was the 2nd factor, a fixed treatment effect (Statistix II 1987).

RESULTS

Laboratory experiments: Dead adult female sand flies in the containers were eaten by larvae. Adults containing *B. sphaericus* caused significant ($P < 0.01$) larval mortality for both *P. duboscqi* and *S. schwetzi* (Table 1). Ten dead *Bacillus*-carrier adults per container caused an average of 38% larval mortality. Control mortality was below 3% throughout.

Field experiment: A total of 138 sand flies were caught 24 h after the first treatment at treatment site 1 where vegetation was sprayed; 59 (38 males and 21 females) sand flies contained the sugar/food dye/*B. sphaericus* solution in their guts (Table 2). *Phlebotomus martini* Parrot and 5 species of *Sergentomyia* sand flies that fed on the sugar/food dye/*B. sphaericus* solution were collected on sticky paper traps. Similar results were obtained in the sprayed areas following the 2nd treatment. The number of marked sand flies decreased linearly with distance

Table 2. Number of dye-marked phlebotomine sand fly adults collected on sticky traps 24 h after vegetation was sprayed with sugar/food dye/*B. sphaericus* solution, May–October 1994, Rift Valley, Kenya.

Species	Males		Females	
	Number collected	Number marked	Number collected	Number marked
Phlebotomus martini	3	1	5	3
Sergentomyia adleri	1	0	2	1
Sergentomyia antennata	54	22	7	3
Sergentomyia bedfordi	1	1	5	1
Sergentomyia clydei	6	2	8	3
Sergentomyia schwetzi	28	12	17	10
Sergentomyia squamipleuris	0	0	2	0
Total	93	38	46	21
Percent marked		40.9		45.7

from the termite mounds and animal burrows (Table 3). A majority (61.1%) of the marked flies were captured within 1 m of the sprayed areas, while only 28.0 and 12.5% of the flies captured at distances of 5 m and 10 m were marked, respectively.

The treatment caused a significant decrease ($P < 0.01$) in the number of adult sand flies collected from animal burrows at both site 1, where the vegetation around the animal burrows was sprayed (Fig. 1), and site 2, where the entrances of the animal burrows were directly sprayed (Fig. 2). The number of adult sand flies was significantly decreased ($P < 0.01$) 2–12 wk after the first application of *B. sphaericus* at site 1, where the vegetation was sprayed. The number of adult sand flies collected was significantly decreased ($P < 0.01$) 4–10 wk posttreatment at site 2, where the animal burrows were directly sprayed. The treatments did not cause a significant decline in the number of sand fly adults collected from termite mounds. No evidence was found to indicate that *B. sphaericus* recycles at a rate sufficient to produce long-term control in termite mounds or animal burrows.

During pretreatment sampling, the sand fly species compositions in the 3 sites were similar, except that there was a significantly higher ($P < 0.05$) percentage of *S. clydei* and a significantly lower ($P < 0.05$) percentage of *S. schwetzi* at treatment site 2 compared to the control site and treatment site 1 (Table 4). Following the 2 treatments, there was a significant decrease ($P < 0.05$) in the relative abundance of *S. clydei* at both treatment sites. Also, there was a significant increase ($P < 0.01$) in the relative abundance of *S. schwetzi* at treatment site 2.

Table 3. Number of dye-marked phlebotomine sand fly adults collected on sticky traps at various distances away from the sprayed vegetation (sugar/food dye/*B. sphaericus* solution) during the first night, May–October 1994, Rift Valley, Kenya.

Distance from sprayed vegetation	Number of flies collected	Number flies marked	% marked
1 m	72	44	61.1
5 m	43	12	27.9
10 m	24	3	12.5
Totals	139	59	42.5

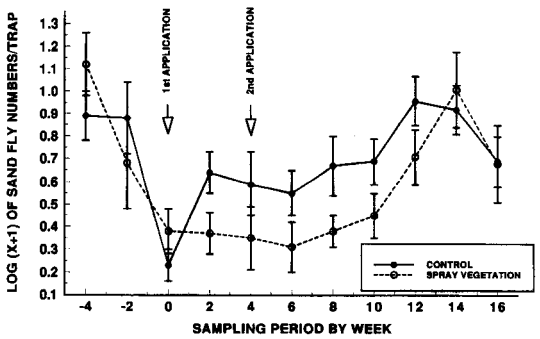

Fig. 1. Mean numbers ($\log_{10}$) of adult phlebotomine sand flies collected during the sampling period from sticky traps in animal burrows at site where vegetation was sprayed with *B. sphaericus*.

DISCUSSION

Dead *Bacillus*-gorged *P. duboscqi* and *S. schwetzi* adults caused larval mortality in the laboratory. This observation is consistent with preliminary work in Israel by Schlein (unpublished data) using *P. papatasi*. Using similar methods, he reported >30% larval mortality in the laboratory.

The evidence provided by these observations supports the initial assumption that phlebotomine sand fly adults can be baited and then act as carriers of *B. sphaericus*. The decreases in adult populations observed in the animal burrows were time-correlated to the preceding treatments. A large proportion of the adult sand flies fed on bait with *B.*

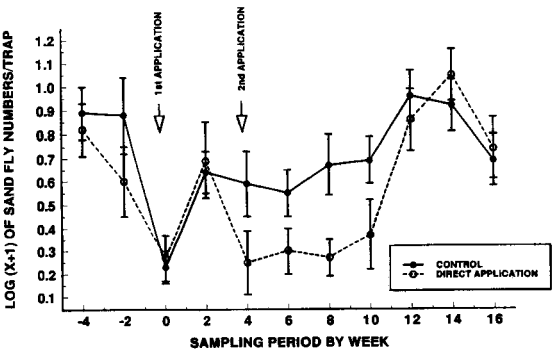

Fig. 2. Mean numbers ($\log_{10}$) of adult phlebotomine sand flies collected during the sampling period from sticky traps in animal burrows where *B. sphaericus* was applied directly.

Table 4. Percentages of phlebotomine sand fly species collected in entry/exit traps at the control site and 2 treatment sites (1, 2) before and after treatment with sugar/food dye/*B. sphaericus* solution, May–October 1994, Rift Valley, Kenya.

Species	Control		Bait-fed adults (1)		Direct application (2)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Phlebotomus martini	10.2	12.7	3.5	9.9	4.6	9.4
Phlebotomus duboscqi	0.9	3.0	1.0	2.1	1.6	2.6
Sergentomyia adleri	0.0	0.1	0.0	0.1	1.1	1.1
Sergentomyia africana	0.9	0.1	6.1	0.4	0.8	0.0
Sergentomyia antennata	5.5	5.5	6.1	13.3	5.5	6.8
Sergentomyia bedfordi	0.9	1.1	2.1	3.0	0.8	1.6
Sergentomyia clydei	31.2	16.4	29.1*	7.7*	68.4*	6.2*
Sergentomyia schwetzi	50.4	60.9	52.1	63.5	17.2*	72.0*
Sergentomyia squamipleuris	0.0	0.2	0.0	0.2	0.0	0.3
Totals	100.0	100.0	100.0	100.0	100.0	100.0

* Values are significantly different ($P < 0.05$).

sphaericus during the first night after spraying. Subsequently, *Bacillus*-gorged adults that dispersed to the larval habitat were collected in sticky traps placed in the entrances of animal burrows and termite mounds. Schlein and Pener (1990) were the first researchers to demonstrate this novel method of vector control against *Culex pipiens* L. mosquitoes in Israel. They found that dispersing *B. sphaericus*-carrier mosquitoes caused larval mortality in breeding sites 60–100 m from the sprayed resting sites.

These observations indicate that the fluctuations in adult populations in animal burrows were due to *B. sphaericus* and that the larvicide was introduced into the larval habitat by dispersing adults from the carbohydrate-seeking population. Treated sand flies were collected from inside termite mounds, but no subsequent larval control was detected. It is hypothesized that sand fly populations were not reduced in the termite mounds due to the physical structure of the mounds. Termite mounds built by *M. subhyalinus* are characterized by vast networks of subterranean chambers (Darlington 1979). Therefore, it is unlikely that *Bacillus*-carrier sand flies would deliver enough toxin to each chamber containing larvae to produce a significant control effect. In contrast, the animal burrows used in this study were typically hollow tubes open at both ends. Thus, sand flies resting and ovipositing in animal burrows use one common chamber, resulting in larvae coming into close contact with *Bacillus* toxin delivered to the chamber.

The data suggest that *B. sphaericus* treatment may influence sand fly species composition. Following treatment, a decrease in the *S. clydei* population at both treatment sites and an increase in the *S. schwetzi* population at treatment site 2 were observed. This suggests a differential susceptibility to *B. sphaericus* among certain sand fly species. This observation needs further investigation and may prove useful in decreasing populations of specific sand fly disease vectors.

In our study, many cecidomyiids and some chironomids also fed on the sprayed sucrose. These insects were collected on sticky papers placed in the openings of animal burrows and termite mounds. It is hypothesized that these insects enter these structures to take advantage of the constant humidity and temperature, which provide protection from extreme daytime temperatures and low humidity. Therefore, it is likely that these *Bacillus* carriers have interspecific effects and that target larvae could be killed by *B. sphaericus* from any infected adult flies sharing their ecological habitat.

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