

Efficacy of Commercial Mosquito Traps in Capturing Phlebotomine Sand Flies (Diptera: Psychodidae) in Egypt

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ABSTRACT Four types of commercial mosquito control traps, the Mosquito Magnet Pro (MMP), the Sentinel 360 (S360), the BG-Sentinel (BGS), and the Mega-Catch Ultra (MCU), were compared with a standard Centers for Disease Control and Prevention (CDC) light trap for efficacy in collecting phlebotomine sand flies (Diptera: Psychodidae) in a small farming village in the Nile River Valley 10 km north of Aswan, Egypt. Each trap was baited with either carbon dioxide (CO₂) from combustion of butane gas (MMP), dry ice (CDC and BGS traps), light (MCU and S360), or dry ice and light (CDC). Traps were rotated through five sites in a 5 × 5 Latin square design, repeated four times during the height of the sand fly season (June, August, and September 2007) at a site where 94% of sand flies in past collections were *Phlebotomus papatasi* (Scopoli). A total of 6,440 sand flies was collected, of which 6,037 (93.7%) were *P. papatasi*. Of the CO₂-baited traps, the BGS trap collected twice as many *P. papatasi* as the MMP and CDC light traps, and at least three times more *P. papatasi* than the light-only MCU and S360 traps ($P < 0.05$). Mean numbers ($\pm$ SE) of *P. papatasi* captured per trap night were as follows: BGS 142.1 (± 45.8) > MMP 56.8 (± 9.0) > CDC 52.3 (± 6.1) > MCU 38.2 (± 6.4) > S360 12.6 (± 1.8). Results indicate that several types of commercial traps are suitable substitutes for the CDC light trap in sand fly surveillance programs.

KEY WORDS baited trap, carbon dioxide, BG-Sentinel trap, Mosquito Magnet Pro trap, *Phlebotomus papatasi*

Disease-carrying phlebotomine sand flies cause an estimated 1.5 million new cases of human cutaneous leishmaniasis annually (WHO 2000). In the Middle Eastern and North African desert environs, an estimated 335,000 cases of cutaneous leishmaniasis occur annually, and this number is likely an underestimation. Cutaneous leishmaniasis infection rates are increasing through much of this region, which currently represents 12% of the global burden (WHO 2007). In the Middle East, phlebotomine sand fly populations occasionally occur in great numbers, resulting in biting pressures of >1,000 bites per person per night

(Coleman et al. 2006). Besides personal protective measures (skin and clothing repellents, bed nets), ultra low volume (ULV) insecticide application is the most common method used to control host-seeking adult sand flies. Unfortunately, control attempts using ULV at military bases in Iraq with large sand fly populations have proven inadequate with adult sand fly numbers rebounding quickly, sometimes in as little as 24 h (Coleman et al. 2006).

Trap-out programs to reduce biting fly populations to a tolerable level have recently been explored using commercial mosquito traps. Some success has been achieved in Iraq with Mosquito Magnet Pro (MMP) traps (Woodstream, Lititz, PA) placed along the perimeter of an Iraqi military camp to reduce sand fly populations inside and intercept incoming flies from the outside (M. C. Carder, personal communication). At Balad Air Base, Iraq, preventive medicine personnel set 50 MMP traps from April through November 2005 to supplement ULV control efforts. These traps collected over 600,000 sand flies during this season, an extraordinarily high number compared with traditional Centers for Disease Control and Prevention (CDC)-type light traps. Although the impact on adult sand fly-biting activity was not determined (Blow et al. 2007), these preliminary results suggested that com-

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Fig. 1. Traps tested for sand fly capture (left to right): the MMP, the BGS, CDC light trap, the MCU, and the S360.

mercial mosquito traps might be good candidates for inclusion into integrated sand fly control or suppression programs in desert settings. Also, these traps might be employed to provide superior surveillance results in place of the traps commonly used for sand fly surveillance (i.e., the sticky trap or CDC light trap).

Commercial mosquito control traps have been developed for the residential homeowner and have gained wide acceptance for use as tools to reduce backyard mosquito populations (Dennett et al. 2004, Hoel et al. 2007a). These traps are powered by batteries, electricity from main line sources, or combustible gases (propane or butane), and they can be baited with attractants such as colored light-emitting diodes (LEDs), incandescent light, sound, heat, moist air, visually contrasting features, carbon dioxide (CO_2), and odors such as 1-octen-3-ol (octenol) and L-lactic acid (Acree et al. 1968, Hall et al. 1984, Kline 1994, Rueda et al. 2001, Dekker et al. 2002, Bernier et al. 2003, Hoel et al. 2007a).

Preliminary paired tests with MMP traps in the Northern Sinai Desert of Egypt resulted in the MMP trap capturing $>10\times$ more flies than lighted, dry ice-baited CDC light traps (D. E. Szumlas, unpublished data). The purpose of the current study was to assess whether each of four commercial mosquito traps could catch a larger number and greater variety of sand flies than the CDC light trap, which is currently used in routine sand fly surveillance programs (Alexander 2000).

Materials and Methods

Study Area. Testing was conducted in June, August, and September 2007, in Bahrif village, a farming community of 500 people 10 km north of Aswan on the east bank of the Nile River in southern Egypt (Hogsette et al. 2008). Daily temperatures ranged from 24 to 43°C, relative humidity was typically $<20\%$, and there was no rainfall during the 3 mo of the study. Wind speed seldom exceeds 8 km/h in Bahrif, as this village is situated in the Nile River Valley ≈ 20 m below the surrounding desert floor. Researchers from the United States Naval Medical Research Unit No. 3 (NAMRU-3) have used Bahrif as a site for entomological field studies for >20 yr because of its high populations of *Leishmania*-free *Phlebotomus papatasi* and the ease of village access (Beavers et al. 2004, Hoel et al. 2007b).

Trapping began in June to coincide with the initial peak sand fly population in Aswan Governorate (H. A. Hanafi, personal communication), and during the height of *P. papatasi* activity in southern Egypt (El Said et al. 1985). Subsequent collections were made in early and late August and early September. The four commercial traps tested included the Mega-Catch (Ultra) Mosquito (MCU) trap (model MCU-800, Envirosafe Technologies, Auckland, New Zealand), the BG-Sentinel Mosquito (BGS) trap (BioGents AG, Regensburg, Germany), the Sentinel 360 Mosquito (S360) trap (Intermatic, Spring Grove, IL), and the MMP trap (Fig. 1).

Trap Description. A model 512 downdraft CDC light trap (John W. Hock Company, Gainesville, FL) equipped with a standard CM-47 incandescent lamp served as the control trap (Fig. 1). The trap was shielded with a black plastic lid and the trap opening set to ≈ 40 cm above ground level, a height advantageous for sand fly trapping (Burkett et al. 2007). The trap was baited with 2 kg of dry ice on all trap nights.

The MCU (25 cm $\times$ 25 cm $\times$ 40 cm) is a black plastic mosquito trap (3.4 kg) powered with main line electricity or a 12 V battery. Four LEDs mounted on the inside trap ceiling rotate through four colors of light (white, red, blue, and green), believed to be attractive to most biting insects. A low-intensity 40 W black light (ultraviolet [UV]) bulb serves as a second visual attractant. Recommended trap height is 90 cm; however, we hung our trap with the bottom 10–15 cm above ground to accommodate the low-flying habits of desert sand flies (trap opening was 35–40 cm above ground). The octenol strip supplied with the trap was not used because octenol has not been shown to be attractive to *P. papatasi* in Egypt (Beavers et al. 2004). The MCU trap was not baited with dry ice, although it can be used with dry ice or a pulse regulator (variable quantity slow CO₂ gas release system).

The BGS trap was designed to catch *Aedes* (*Stegomyia*) mosquitoes without CO₂ as bait. This ground-mounted trap stands 40 cm high (opening at 40 cm in top center of trap) and 115 cm in circumference, weighs 1.2 kg, and resembles a footstool. The BGS trap was powered with a 12 V battery and was baited with 2 kg of dry ice per trap. This trap does not use light as an attractant.

The S360 trap is a main line-powered mosquito trap that uses incandescent, UV, and colored LEDs as light attractants. Eight LEDs flash two at a time through four colors (blue, green, orange, and violet) at a rate of ≈ 1 s per color. We did not supplement this trap with dry ice because the instruction manual indicates that CO₂ is not needed. The trap is mushroom shaped with a light-bearing head 135 cm in circumference supported by a plastic stand 60 cm in circumference and 105 cm tall; the trap opening is at ≈ 95 cm. It weighs 5.5 kg and is intended to be set in one permanent position, as the manufacturer recommends burying the trap's power cord. This trap was powered with a 12 V battery because of an inconsistent supply of electricity to the village.

Our MMP trap (American Biophysics Corp., East Greenwich, RI) was modified by American Biophysics to use butane gas, which is commonly sold in North Africa (MMPs sold in North America burn propane). The MMP trap is a counter-flow geometry trap that uses opposing air currents to trap flying insects (Kline 1999). It is an updraft trap with the opening ≈ 50 cm above ground. Butane is converted into CO₂, water vapor, electricity, and heat through catalytic combustion (Hoel et al. 2007a). Enough electricity is generated from butane combustion to produce ≈ 500 ml/min CO₂. The MMP is mobile, but heavy (32 kg), and difficult to transport over uneven ground. This trap does not use light as an attractant.

Because sand fly host-seeking behavior in Egypt occurs between sunset and sunrise, when the wind speed is low (≤ 3 km/h), traps were set 30–60 min before sunset and collected the next morning within 30 min of sunrise. Morning trap collections were anesthetized with dry ice, and specimens were stored in 70% ethyl alcohol until identified to species at NAMRU-3 in Cairo using the sand fly keys of Lane (1986).

The four commercial traps and the CO₂-baited CDC light trap were set in a 5 $\times$ 5 Latin square-configured experiment with each trap rotated once through each trapping site for a single night. Traps were set a minimum of 30 m apart, adjacent to a line of village houses where domestic animals were kept. Site 1 was an animal shelter built into the outside wall of a mud brick house, and protected from wind. Site 2 was ≈ 30 m north of site 1, at the base of six date palms (*Phoenix dactylifera*) in a raised (1-m) rock terrace. Test traps were set at the base of the date palms next to the terrace stonewall (ground and low-set traps were hung off the wall at their appropriate heights). Site 3 was located 30 m north of site 2 inside the same courtyard, and traps were set in a manner similar to those at Site 1. Site 4 was north of site 3 in a smaller courtyard constructed of a 2-m tall mud brick wall adjacent to a small vegetable garden. Site 5 was ≈ 100 m to the north of site 4, located between a small irrigated plot of land and an open garbage dump. The entire trap line extended slightly over 200 m.

Data Analysis. Sand fly collections were analyzed for treatment (trap model), site, and trial effects using a three-way analysis of variance (SAS Institute 2001). The Ryan-Einot-Gabriel-Welsh multiple range test was used to separate mean differences and, unless otherwise stated, significant differences are based on $P < 0.05$. Sand fly capture data were transformed with $\log_{10}(n + 1)$ before analysis, but actual numbers are shown in text and tables.

Results

Collectively, the traps captured a total of 6,440 phlebotomine sand flies comprising six species. The majority of these were *P. papatasi* (6,037, 93.7%) and *Sergentomyia schwetzi* (Adler, Theodor & Parrot) (314, 4.9%). The remaining 1.4% were 48 *Sergentomyia palestinensis* (Adler & Theodor), 33 *Phlebotomus sergenti* Parrot, seven *S. tiberiadis* (Adler, Theodor & Lourie), and a single *Sergentomyia clydei* (Sinton), none of which were included in the analysis because of their small numbers.

The main effects model for all flies was highly significant ($F = 9.26$; $df = 11, 99$; $P < 0.0001$), and significant differences were seen between trap types, trap site, and trial periods. The BGS trap collected almost as many sand flies as the other four traps combined. The rank order of total catch size was BGS (2,973, 46.2%) $>$ MMP (1,190, 18.4%) $>$ CDC (1,164, 18%) $>$ MCU (832, 13%) $>$ S360 (281, 4.4%). The main effects model for *P. papatasi*, which consisted of 93.7% of the total catch, mirrored that of the all flies finding

Table 1. Mean numbers (mean $\pm$ SE) of sand fly species captured by five commercial mosquito traps in Bahrif, Aswan Governorate, Egypt, in June, August, and September 2007 ($n = 20$ trap nights)

Sand fly species	BGS trap	MMP trap	CDC light trap	MCU trap	S360 trap
<i>Phlebotomus papatasi</i>	142.0 $\pm$ 45.83a	56.7 $\pm$ 8.97ab	52.3 $\pm$ 6.14ab	38.2 $\pm$ 6.38b	12.6c $\pm$ 1.84c
<i>Sergentomyia schwetzi</i>	4.8 $\pm$ 1.72a	2.1 $\pm$ 0.45ab	5.0 $\pm$ 2.18a	3.1 $\pm$ 0.68a	0.6 $\pm$ 0.25b
<i>P. sergenti</i> ^a	11	6	5	7	2
<i>S. palestinensis</i> ^a	23	4	12	7	2
<i>S. tiberiadis</i> ^a	2	2	1	2	0
<i>S. clydei</i> ^a	0	1	0	0	0

Means within rows having the same letter are not significantly different ($P < 0.05$, Ryan-Einot-Gabriel-Welsh multiple range test [SAS Institute 2001]).

^a Total number of sand flies collected, not large enough for statistical analysis.

($F = 10.55$; $df = 11, 99$; $P < 0.001$). Treatment, site, and trial period were all significant. Order of rank for *P. papatasi* was BGS (2,841, 47.1%) > MMP (1,135, 18.8%) > CDC (1,046, 17.3%) > MCU (763, 12.6%) > S360 (252, 4.2%). Trap means and standard errors ($\pm$ SE) are given in Table 1 for *P. papatasi* and *Sergentomyia schwetzi*, with trap totals for the remaining four species (not analyzed).

As shown by the high standard errors in Table 1, the numbers of sand flies collected between sites and over time were highly variable for each type of trap. Even though the BGS caught 2.5-fold and 2.7-fold more *P. papatasi* than did the MMP and CDC traps, respectively, there were highly significant effects of site ($F = 7.28$; $df = 4, 99$; $P = 0.001$) on trap catches, and therefore, there was no significant difference between these means. The mean numbers of *P. papatasi* captured by the BGS trap were, however, significantly greater than those captured by the MCU and S360 traps (Table 1).

The standard error was extremely large for the BGS trap because of outliers in collection data (601 *P. papatasi* collected the night of 6 August, 711 on 26 August, and 409 on 31 August). These three large collections were made at site 2 at the base of six date palms. In all, >100 flies were captured per night during 10 of the 100 trap nights of the study, with the BGS trap accounting for five nights, the MMP trap three nights, and the CDC light trap two nights.

Site productivity proved significant ($F = 7.28$; $df = 4, 99$; $P = 0.001$) for *P. papatasi*, with the largest number of flies collected from the two palm cluster sites (2,660, 44.1%; 1,328, 22.0%), accounting for two-thirds (66.1%) of all *P. papatasi* collected. The mean number of *P. papatasi* collected at site 2 was significantly greater than all sites except site 3, but it was still numerically twice as many flies as site 3. The fewest number of *P. papatasi* (523) was taken from site 1, the animal shelter, most likely because sand flies were attracted to nearby animals more strongly than to the traps. Eight of 10 large trap collections (sand fly capture >100) were obtained from sites 2 and 3.

Traps caught approximately similar ratios of male and female *P. papatasi*, with all trap collections ranging from 59 to 69% female. Trap total by sex (and percentage female) over 20 test nights per trap were as follows: BGS, 1,689 (female), 1,152 (male) (59.5%); MMP, 690 (female), 445 (male) (60.8%); CDC, 645

(female), 401 (male) (61.7%); MCU, 503 (female), 260 (male) (65.9%); S360, 174 (female), 78 (male) (69.0%).

Trap diversity, or the average number of sand fly species per trap over the 20-night test period, was as follows: BGS, 2.4; MMP, 2.2; CDC, 2.3; MCU, 2.4; S360, 1.6.

Discussion

Human landing collections, the CDC light trap, and the sticky paper trap are the three standard surveillance techniques used to determine adult sand fly densities (Killick-Kendrick 1987, Davies et al. 1995). Although human-landing collections often attract the largest number of sand flies of these three methods (Hanafi et al. 2007), they can also be highly variable, and ethical questions arise as collectors risk *Leishmania* infection. CDC light and sticky paper trap totals were consistently lower than human landing collections over a 3-yr collecting period in the North Sinai (Hanafi et al. 2007). Several years later at this same site, MMP traps were set within 50 m of lighted CDC traps and caught $\approx$ 900 sand flies per night as compared with 80–90 sand flies per night caught in dry ice-baited CDC traps (D. E. Szumlas, unpublished data). The success of the MMP trap relative to the CDC light trap and the large numbers of sand flies captured at a military base in Iraq with MMP traps (Blow et al. 2007) indicate that commercial mosquito traps might serve effectively as an adult population suppression device in conjunction with other sand fly control measures or as a more sensitive surveillance tool than CDC light traps.

Whereas CDC and other types of light traps typically perform well as sand fly surveillance devices, our results demonstrated that traps baited with CO₂ catch higher numbers of sand flies than traps baited with only light; the two unlighted traps baited with CO₂ (BGS and MMP) collected two-thirds of all *P. papatasi* sampled (3,976). The MCU and S360 trap using only light as attractant caught significantly fewer adult *P. papatasi* (1,015) than did the CO₂-baited traps. The S360 trap produced similar types of light (UV, LED) as the MCU trap; however, the S360 trap's 95-cm-high entrance may have been the reason for its smaller capture totals. *P. papatasi* is a weak flyer (<2.5 km/h, Killick-Kendrick et al. 1986) and tends to fly close to

the ground. The MCU was not baited with CO₂ (as recommended by the instruction manual), so that we could compare light-only to CO₂-baited-only effects on capture. Had the MCU trap been baited with CO₂, we suspect our catches would have been much higher because its trap opening was set only 40 cm above the ground.

The BGS trap offers *P. papatasi* a large, visually attractive, unlighted target. An advantage of this trap is that it rests directly on the ground, where sand flies are likely to rest during the day and from where they begin to forage for blood meals during the night. Hogsette et al. (2008) recovered adult male and female *P. papatasi* with a battery-powered backpack aspirator (model 1412, John W. Hock Company, Gainesville, FL) from mud bricks piled 0.5 m deep on the ground in Bahrif. The lack of rodent burrows in Bahrif, which are commonly found in other *P. papatasi*-producing sites in Egypt (especially the Sinai desert), led to a search for adult resting sites in and around mud brick walls, stables, garbage dumps, palm groves, and crop areas throughout the village. Adult sand flies resting in ground debris where humidity levels, shelter, and shade are likely more enticing than the aforementioned sites probably gave the ground-mounted BGS trap an advantage over other trapping systems.

The MMP trap offers several features that appear to enhance sand fly catches: a large visual target (trap head); production of a warm and moist CO₂ plume directed toward the ground (close to sand fly resting sites); an updraft trapping air current, which has been shown to be more effective than similar downdraft traps (Mutero et al. 1991, Burkett et al. 2007); and the use of propane or butane as an energy source, enabling continuous use (24 h/d) for 3 wk, using a standard 9-kg propane tank. The MMP was the only trap we tested that could operate unattended for 3 wk. This capability offers an important logistical advantage in remote field situations versus traps requiring daily battery exchange and charging.

The sex ratio of *P. papatasi* in all traps ranged from 59 to 69% female. The similarity of female *P. papatasi* sex ratios between the CDC light trap (61.7%) and the two best performing commercial traps, the BGS trap (59.5%) and the MMP (60.8%), is encouraging because the latter two commercial traps are not catching a disproportionately larger number of nonfeeding males. The much higher numbers of adult sand flies captured in the BGS trap compared with those of the CDC light trap are of extra value in surveillance programs in which *Leishmania* infection rates or parasite species determination is sought, with no more labor or maintenance (battery recharging, provision of dry ice) than would be necessary for the CDC light trap. Surveyors can reasonably expect to capture similar percentages of host-seeking female *P. papatasi* in BGS and MMP traps as they would with CDC light traps.

Species diversity, or the mean number of sand fly species trapped nightly over 20 trap nights per trap type, was about equal for the BGS (2.4 species), the MMP, (2.2), and the MCU (2.4). This indicates that

commercial traps are as good in attracting the same sand fly species complex as the CDC light trap (2.3) in the Nile River Valley. Similarity of the species complex captured by all four traps ensures that collection of the vector species, if attracted to traps, can be captured and that disease threat assessments will be unbiased regardless of which trap is in use.

The surprisingly high numbers of sand flies caught during our study at the date palm sites may be the result of the abundance of a favored sugar source for carbohydrate meals. Dates begin ripening in late summer, with harvest taking place in September and early October. Sugar feeding in sand flies is important to their survival and is necessary for *Leishmania* transmission (Young et al. 1980, Schlein and Jacobson 1999). Date palms may also offer shade, higher humidity, potential moisture sources in leaf axils, good harborage at their leaf bases around the trunks, as well as by fallen leaves and branches covering the ground.

After completion of studies to assess the correlation between sand fly landing rates and commercial trap catches over a range of sand fly densities, additional studies can be conducted to determine how effectively traps can be used to survey changes in the density of biting sand flies, and the extent to which these traps might effectively decrease sand fly populations through time. The results of this study suggest that BGS and MMP traps are worth using as sand fly surveillance tools and might be useful in an integrated sand fly control program.

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