Comparison of various configurations of CDC-type traps for the collection of *Phlebotomus papatasi* Scopoli in southern Israel

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ABSTRACT: We conducted two experiments to determine the best CDC-trap configuration for catching male and female *Phlebotomus papatasi*. First, visual features were evaluated. Standard CDC traps were modified to have black or white catch bags, black or white lids, or no lids and these were tried in different combinations. Significantly more male sand flies were caught by darker traps; significantly more females were captured by traps with either all black or a combination of black and white features. Attraction may be due to dark color or contrast in colors. CDC traps with suction and the following features were also evaluated: no light; incandescent light; ultraviolet (UV) light; combination of black color, heat and moisture; CO_2 alone, or a combination of black color, heat, moisture, and CO_2 simultaneously, all in upright and inverted positions, with the opening for insect entry always 50 cm above the ground. Significantly more females than males were caught by all traps (standard and inverted) except the control traps with suction only. Traps with CO_2 caught more sand flies than traps without CO_2. Traps with black color, heat and moisture captured significantly more sand flies than the control traps, but with the addition of CO_2, these traps catch significantly more sand flies than the other traps evaluated. Inverting traps increased the catch for like traps by about two times. ***Journal of Vector Ecology*** **36 (Supplement 1): S212-S218. 2011.**

Keyword Index: sand flies, visual targets, UV light, inverted traps, carbon dioxide, heat, color.

INTRODUCTION

Phlebotomine sand flies have a wide distribution, though mainly in the tropics and subtropics (Lane 1993). Towards the north, they reach south west Canada (Young and Perkins 1984), and in the south they are found until latitude 40°S (Killick-Kendrick 1999). Both female and males are dependent on sugar as an energy source (Schlein and Warburg 1986), but females also need additional blood meals for egg production every few days (Killick-Kendrick 1999). This is the reason for frequent contacts between vector and host, and why phlebotomine sand flies are such a nuisance as well as vectors of numerous diseases (Comer and Tesh 1991; Ashford 2001, Birtles 2001).

The dynamics of sand fly attraction to hosts is rather complex and little is known compared to mosquitoes and other biting flies (Gibson and Torr 1999). As for most biting flies, carbon dioxide (CO_2) is the single most potent attractant for sand flies (Pinto et al. 2001), but they are also attracted by host odors alone as shown in several experiments in the laboratory and field (Killick-Kendrick et al. 1986, Morton and Ward 1989, Dougherty et al. 1999. Moreover, temperature and humidity gradients seem to play a role in sand fly attraction (Nigam and Ward 1991) and there is also evidence that optical aspects are important for host detection (Mellor et al. 1996).

Almost every attempt to study natural behavior or applied control of sand flies in the field involves sampling the population in one way or another. For adult sand flies, either small CDC-like traps or sticky traps (sheets of paper or plastic covered with a viscous adhesive such as motor oil) are commonly used (Alexander 2000). Non-attractive traps, like simple sticky papers and unlighted, unbaited CDC traps only catch flies from their immediate area and accordingly, tend to yield relatively low numbers of sand flies. Several productive trapping methods and collecting procedures have been standardized for sampling sand flies. Selection of an appropriate method depends on the objectives and type of study to be performed, species, sex or physiological state of the insects required, and any constraints on preservation and transportation of the specimens (Service 1993, Alexander 2000).

A more active and selective way is to attract biting flies, in most cases females, to all kind of baits including animals and humans (Sharp et al. 1984, Andrade et al. 2008), as well as elements of them like clothes, hair, urine, feces, etc. (Allan et al. 2006, Kline 1998). More commonly, isolated or combined attractive features, like CO_2, visual targets, chemical lures, heat, moisture, and movement, are now used to increase trap catches (Kline 2006, Bernier et al. 2003, Murphy et al. 2001). Light, especially in the long-wave ultraviolet (UV) range, is generally regarded as an attractant. However, it often causes disorientation of nocturnally active flying insects (Nowinszky 2004). With their orientation thus compromised, both male and female sand flies are drawn towards the direction of the light source and are unable to

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14. ABSTRACT
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avoid the capture mechanisms of traps.

A large body of literature exists in which numerous methods and trap designs for the collection of sand flies are discussed (for a review see Alexander 2000). However, little attention has been paid to the fact that a few small changes in the design and presentation of CDC-like traps might increase the catch size and change the sex ratio. This is important if data from different areas need to be compared or if sex ratios are used as an indication of possible breeding sites (Feliciangeli 2004). The purpose of this study was to evaluate the impact of selected modifications of the standard CDC trap on the number and sex ratio of *P. papatasi* captured.

MATERIALS AND METHODS

Study site

The study was carried out in mid-autumn near Jericho, about 10km north of the Dead Sea, at an altitude of about 300 m below sea level. This region is an extreme desert and belongs to the Saharo-Arabian phyto-geographical zone (Danin 1988). The annual precipitation of 50 to 100 mm is restricted to short winter rains and average daily temperature ranges from ca. 20° C between late September and April to >30°C from May through August (Ashbel 1951). The traps were evaluated in a neglected date plantation where *P. papatasi* is the dominant sand fly species and others, like *P. sergenti,* are rare or absent (Faiman et al. 2009, Müller and Schlein 2004, Schlein et al. 2001).

The experiments were conducted in the dry autumn when the annual winter and spring vegetation was already dry. About 20% of the remaining natural vegetation inside the plantation was scattered shrubs and semi-shrubs, including *Suaeda asphaltica* (Boiss.), *S. fruticosa* Forsk., *Atriplex halimus* (L), *A. leucoclada* Boiss. (Chenopodiaceae) and *Prosopis farcta* (Macbride) (Mimosaceae). Along the periphery of the oasis, groups of *Tamarix nilotica* (Ehrenb.) (Tamaricaceae) Bge. trees and shrubs, like *Alhagi graecorum* Boiss. (Papilionaceae) and *Salsola tetranda* (Chenopodiaceae) Forssk., were restricted to small water catchments. No flowering plants, honeydew or honeydew-producing insects of any kind were found in the area at the time of the experiments.

Traps and experimental description

Efficacy of the modified CDC traps was evaluated in two experiments conducted from early September to early October, 2006. The modified traps (based on the CDC trap model 512, John Hock, Gainesville, FL, U.S.A.) were operated simultaneously and continuously, along an unpaved road crossing the plantation, with a distance of 20 m between each trap location. Traps were hung on bamboo tripods so the opening for insect entry was 50 cm above the ground (in both upright and inverted configurations). Traps were rotated clockwise between the trap locations at 17:00 daily to eliminate positional bias. Insects captured in traps during the night were removed at 07:00. Traps were powered by 6 volt motorcycle batteries which were recharged daily with a generator.

Experiment I-Visual Features

Traps were evaluated as visual targets by illuminating them with incandescent bulbs and operating them in the upright (normal) position with variations in lid presence/absence and lid and catch bag color. Catch bags were used either in their original white color or were stained black with a commercial textile dye determined to have no repellent effects. Trap lids were used either in their original black color or were painted white. Stained and painted catch bags and lids, respectively, and unaltered catch bags and lids were submerged in a clear outdoor water pond 1 mo prior to use to eliminate any possible odors from the stain and paint. The list of modified traps is as follows: 1) White catch bag/no lid; 2) White catch bag/white lid; 3) White catch bag/black lid; 4) Black catch bag/no lid; 5) Black catch bag/white lid; 6) Black catch bag/black lid.

Experiment II-Trap Orientation

Traps, either upright or inverted, were evaluated in combination with selected attraction features. For a control, we used suction only with no light. To evaluate the effect of incandescent light, the original CDC light trap configuration was maintained. To evaluate the effect of UV light, the incandescent bulb was replaced with a small portable money checker (Tragbarer Geldschein-Prüfer mit Leuchte, model 751778 – 62, Conrad Electronics, Munich, Germany) equipped with a 4 watt, 6 volt UV tube attached horizontally, 3 cm above the opening for insect entry on the trap body (similar to the CDC Model 1212, John Hock, Gainesville FL, U.S.A). The UV unit was connected to a separate 6 volt motorcycle battery.

Heat was generated by heat film (Westham Innovations LTD., Tel Aviv, Israel) placed beneath a metal jacket of 4 mm iron sheet which fit tightly around the entire trap body. The modified trap bodies were then covered with a heavy non-glossy black paper. The surface temperature of the covered trap bodies, which was set at 41° C, was verified with an infrared thermometer (CEM DT8862 Professional 12:1 IR Infrared Dual Laser Thermometer, Meter Shack) gun. Moisture was supplied from sheets of 80 x 80 cm filter papers folded fan-like, with their tightly folded side inserted in beakers of water (Müller and Schlein 2006). Traps equipped with CO_2 used a bottled supply with a flow rate of 250 ml/min. The CO_2 lines were affixed to the body of the traps so CO_2 was released into the airflow 5 cm above the opening for insect entry.

The combinations of evaluated features were as follows: 1) Upright trap with suction only as control; 2) Inverted trap with suction only as control; 3) Upright trap with UV light; 4) Inverted trap UV light; 5) Upright trap with black body, heat and moisture; 6) Inverted trap with black body, heat and moisture; 7) Upright trap with black body, heat moisture, and CO_2; 8) Inverted trap with black body, heat moisture, and CO_2; 9) Upright trap with CO_2 only; 10) Inverted trap with CO_2 only.

Table 1. Mean *P. papatasi* adults captured on CDC traps modified to have black or white lids and catch bags ($n = 20$).

Trap	Sand flies captured[1]
Bag Black-Lid Black	14.5 ± 1.7a
Bag White-Lid Black	12.1 ± 1.6ab
Bag Black-Lid White	11.3 ± 1.7ab
Bag Black-No Lid	10.7 ± 1.5ab
Bag White-No Lid	9.0 ± 0.8b
Bag White-Lid White	5.5 ± 0.5c

[1]Means followed by the same letter are not significantly different ($P < 0.05$; Ryan-Einot-Gabriel-Welsch Multiple Range Test [SAS Institute 2003]).

Statistical analysis

Data were first normalized by conversion to $\log_{10}$ (n+1) then subjected to ANOVA (SAS 2003) using the following main effects model statements: Total = Treatment Sex Replication, where the dependent variable represented numbers of sand flies captured, Treatment was one of the modified traps, Sex was either male or female, and Replication was an indication of trap location on one of the consecutive trapping days of each study. Means were separated with the Ryan-Einot-Gabriel-Welsch Multiple Range Test (REGWQ), and unless otherwise stated, $P < 0.05$ (SAS 2003). Although $\log_{10}$ (n + 1) values were used for the analyses, actual values are reported in the text, figures and tables.

RESULTS

Experiment I-Visual Features

The main effects model was significant for the dependent variable (F=8.54, d.f.=15,119, P<0.0001). Means for the total numbers of flies captured ranged from 14.5 to 5.5 and overall, traps captured significantly more females than males.

There were no significant differences between the mean numbers of sand flies captured by any of the three traps having black catch bags, and the trap with white catch bag and black lid (Table 1). The all-black trap caught significantly more sand flies than the trap with no lid and a white catch bag, and the all-white trap caught significantly less sand flies than all of the other traps evaluated (Table 1). With trap preference ignored, significantly more females (14.1 ± 0.9) than males (6.9 ± 0.4) were captured overall. There were significantly more females than males captured by all traps except the all-white trap and the white catch bag/no lid trap (Table 2). The all-white trap and the white catch bag/no lid trap captured significantly fewer females than the four other traps, however, significance groupings for males were less clearly defined (Table 2). The all-black trap captured numerically more male sand flies than all other traps evaluated, and significantly more male sand flies than the black catch bag/no lid and all-white traps.

Experiment II-Trap Orientation

The main effects model was significant for the dependent variable (F=71.99, d.f.=19,199, P<0.0001). Means for the total numbers of flies captured ranged from 996.3 to 2.7 and overall, traps captured significantly more females than males.

All traps with CO_2 captured significantly more sand flies than the trap configurations not using CO_2; however, traps with CO_2 plus black bodies, heat and moisture captured significantly more sand flies than the traps with CO_2 alone (Table 3). When CO_2 is added to the black body, heat and moisture combination, the sand fly catch increases significantly, indicating the importance of CO_2 as an attractant. All added features significantly increased the trap catches when compared with the controls, but there were no significant differences between like traps resulting from trap inversion when sex is overlooked (Table 3). With trap preference ignored, significantly more females (435.4 ± 62.6) than males (61.8 ± 9.2) were captured overall.

With the exception of the control traps, all trap

Table 2. Mean numbers (± SE) of female and male *P. papatasi* adults captured on CDC traps modified to have black or white lids and catch bags ($n = 10$).

Trap	Females[1]	Males
Bag Black-Lid Black (BB-LB)	19.4 ± 2.5aX	9.6 ± 1.2bX
Bag White-Lid Black (BW-LB)	17.3 ± 2.1aX	6.9 ± 0.9bXYZ
Bag Black-Lid White (BB-LW)	16.5 ± 2.2aX	6.0 ± 1.0bXYZ
Bag Black-No Lid (BB-NL)	15.7 ± 1.8aX	8.5 ± 1.3aXY
Bag White-No Lid (BW-NL)	9.4 ± 1.1aY	5.6 ± 0.9bYZ
Bag White-Lid White (BW-LW)	6.4 ± 0.8aY	4.5 ± 0.6aZ

[1]Means for females and males for like traps in rows (lower case) and for females or males in columns (upper case) followed by the same letter are not significantly different ($P < 0.05$; Ryan-Einot-Gabriel-Welsch Multiple Range Test [SAS Institute 2003]).

configurations captured significantly more females than males (Table 4). Significantly more females were captured by traps with CO_2 than by those without CO_2, and the inversion of traps resulted in significant increases in catches of female sand fly in like traps, notably the traps with CO_2 alone and the traps with black bodies, heat and moisture, but no CO_2 (Table 4). With males, traps with CO_2 and traps with UV light captured significantly more sand flies than the other traps evaluated (Table 4). In fact, there were no significant differences in males captured between the traps with UV light and those with CO_2 alone. Inversion of traps had no significant effect on male trap catches between like traps (Table 4).

Table 3. Mean numbers (± SE) of *P. papatasi* adults captured on CDC traps with selected modifications (*n* = 20).

Trap	Sand flies captured[1]
CNTRL trap black body, heat, moisture and CO_2 inverted,	996.3 ± 187.4a
CNTRL trap black body heat moisture, and CO_2	628.1 ± 125.6a
CNTRL trap with CO_2 inverted.	473.4 ± 108.5b
CNTRL trap with CO_2	262.4 ± 61.5b
CNTRL with UV inverted	41.8 ± 4.0c
CNTRL trap with UV light	36.2 ± 3.7c
CNTRL trap black body, heat and moisture inverted	26.5 ± 5.3d
CNTRL trap black body, heat and moisture	15.0 ± 2.7d
CNTRL trap inverted	3.4 ± 0.6e
CDC with suction and incandescent light	2.7 ± 0.5e

[1]Means followed by the same letter are not significantly different ($P < 0.05$; Ryan-Einot-Gabriel-Welsch Multiple Range Test [SAS Institute 2003]).

DISCUSSION

The literature is replete with studies indicating that many biting flies are attracted to optical targets (for a review see Allan et al. 1987). In previous studies, *P. papatasi* and *Lutzomyia* spp. sand flies were attracted, if given a choice of colors, mainly to red LEDs (Hoel et al. 2007, Mann et al. 2009). Also, mosquitoes are attracted to different types of LEDs but it appears that colors are species, or at least ecotype, specific (Burkett et al. 1998, Burkett and Butler 2005). Bearing in mind that sand flies have a similar spectral sensitivity as mosquitoes (Muir et al. 1992), it would not be surprising if in the future color preferences for different sand fly species are also found. Because these previously reported studies were conducted with colors of light produced by LEDs and bulbs, while in our study we used colored surfaces, results might vary.

To the best of our knowledge, this is the first study to demonstrate that female sand flies are significantly attracted to black color, a trait which is very wide spread among hematophagous Diptera (Gibson and Torr 1999). However because our traps were not entirely black, this attraction could be due to the contrast between the light and dark surfaces in the traps. An attraction by the females to the traps having contrasting colors rather than to the all-white trap was significant (Table 2), and this could be a general trait among sand flies. Additional knowledge about this factor might lead to improvements in the CDC trap.

Carbon dioxide is a strong long-range attractant for most female biting flies (Gibson and Torr 1999). Therefore it is not surprising that traps with CO_2, regardless of trap

Table 4. Mean numbers (± SE) of female and male *P. papatasi* adults captured on CDC traps with selected modifications (*n* = 10).

Trap	Females[1]	Males
CNTRL trap black body, heat, moisture and CO_2 inverted	1740.8 ± 153.9aU	251.7 ± 38.1bW
CNTRL trap black body heat moisture	1086.8 ± 138.9aUV	169.4 ±24.0bW
CNTRL trap with CO_2 inverted	880.5 ± 112.9aV	66.3 ± 12.1bX
CNTRL trap with CO_2	484.7 ± 70.2aW	40.1 ± 6.0bX
CNTRL trap black body, heat and moisture inverted	46.1 ± 5.6aX	6.9 ± 1.3bY
CNTRL with UV inverted	45.4 ± 5.6aX	38.1 ± 5.7bX
CNTRL trap with UV light	39.4 ± 5.7aXY	33.0 ± 4.8bX
CNTRL trap black body, heat and moisture	23.6 ±3.5aY	6.4 ± 1.1bY
CNTRL trap inverted	3.5 ± 1.0aZ	3.2 ± 0.7aYZ
CDC with suction and incandescent light	3.0 ± 0.8aZ	2.4 ± 0.5aZ

[1]Means for females and males for like traps in rows (lower case) and for females or males in columns (upper case) followed by the same letter are not significantly different ($P < 0.05$; Ryan-Einot-Gabriel-Welsch Multiple Range Test [SAS Institute 2003]).

orientation, captured large numbers of sand flies (Table 3). The black trap body-heat-moisture combination provides about a 10-fold increase over the control traps in female sand flies captured (Table 4), but when CO_2 was added to that combination the female captures increased by 38 to 45 times. Similar almost synergistic effects were produced by combining CO_2 with red LEDs and a 1-octen-3-ol/1-hexen-3-ol lure for *Lutzomyia shannoni* (Mann et al. 2009) and combining CO_2 with octenol for *P. papatasi* (Beavers et al. 2004).

The traps with UV light increased the females captured by ca. 12 times over the control traps (Table 4). While this is not competitive with traps using CO_2, Burkett et al. (2007) reported excellent results with a similar UV-CDC device when evaluated against other non-CO_2 traps. Unfortunately, this device captured considerably more *Sergentomyia* spp. than *P. papatasi*.

In a recent study near our experimental area, Faiman et al. (2009) demonstrated that inverted CDC traps, with openings 10 cm above the ground and baited with dry ice caught an average of 1.6 times more female and 1.7 times more male *P. papatasi* as upright CDC traps, baited with dry ice with the trap opening 40 cm above the ground. In our study, we observed a similar effect for *P. papatasi* if traps were baited with anything but suction alone (control)(Table 4). Burkett et al. (2007) also had favorable results with an updraft CDC trap and it captured a higher percentage of *P. papatasi* than the other traps evaluated.

Faiman et al. (2009) speculated that the higher catches with inverted traps might be related to the proximity of the trap openings to the ground and a more dense and more focused CO_2 plume (Cooperband and Carde, 2006). Our trap openings were 50 cm above the ground regardless of trap orientation but in an additional experiment we observed no significant differences between inverted traps with openings 10 cm and 50 cm above the ground (data not shown). This suggests that the results cannot be explained only by the difference in height. Sand flies are supposedly flying only short distances, very low and moving along the ground often in short jumps (Killick-Kendrick et al. 1986, Doha et al. 1991, Alexander and Young 1992). In previous studies with repellents in southern Israel, we observed that most *P. papatasi* bite the lower extremities mainly below the knee (unpublished data of the authors). If female sand flies are approaching potential hosts in an upward movement, they may be more easily caught if the suction is at the bottom of a trap than at the top. This might also explain why significantly more females, but not males, were caught in inverted traps baited with materials characteristic of potential hosts (black color, heat, moisture, CO_2) compared to upright traps baited similarly. Males are attracted to hosts for the opportunity of mating with host-seeking females. They are probably reacting differently from females and are not trying to find a suitable area for blood feeding (Lane et al. 1990, Memmott 1991, 1992) which might explain the smaller catches.

In summary, when exposed to traps with black and white components, adults of *P. papatasi* are more attracted to darker traps or traps with more contrast. When exposed to traps having some characteristics of a live host, CO_2 plays a strong role in attraction, with smaller degrees of attraction observed from other components. Inversion of traps can result in a 1.5- to 2-fold increase in trap catch.

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