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An Evaluation of Light-Emitting Diode (LED) Traps at Capturing Phlebotomine Sand Flies (Diptera: Psychodidae) in a Livestock Area in Brazil

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Abstract

A study to evaluate the use of light-emitting diodes (LEDs) as an attractant for phlebotomine sand flies at two animal pens in a livestock area in Brazil was performed. Light-suction traps were operated overnight with the following light sources: green, blue, and incandescent (control) lights. In total, 22 individual collections were made at each site and 44 with each trap type. In total, 2,542 specimens belonging to 14 phlebotomine species were collected. The most abundant species in the light traps were *Nyssomyia whitmani*, *Evandromyia evandroi*, *Micropygomyia goiana*, *Lutzomyia longipalpis*, and *Bichromomyia flaviscutellata*. Taking the two sites together, the green-LED light was the most attractive, followed by the blue and incandescent lights, and the difference between the green-LED and the control was statistically significant. Most species were green-biased at both sites, but some species-specific differences were observed. However, even with these differences, the standard incandescent light was outcompeted by LEDs. The green-LED-biased response observed in the present study, together with numerous advantages in favor of LEDs, suggests that the green-LED light source can be used as an effective substitute for the currently used incandescent bulb in monitoring traps for phlebotomine sand flies in Brazil.

Key words: light source, light trap, sand fly, vector control

Phlebotomine sand flies are primitive Diptera within the family Psychodidae (Maroli et al. 2013, Ready 2013). These small and nocturnally active insects are of great importance in the transmission of *Leishmania* species (causative agents of leishmaniasis) in Brazil and worldwide (Sharma and Singh 2008). *Leishmania* transmission takes place via the bite of infected female sand flies to a range of vertebrates, including man. About 7% of all phlebotomine species are regarded as proven or suspected vectors of *Leishmania* parasites in the New World (Maroli et al. 2013).

Phlebotomine are poor flyers and primarily associated with forests, but some species have the ability to adapt well to human-modified habitats such as peridomestic areas and agricultural settings (Rangel and Lainson 2009). In this context, animal pens are one of the major factors determining the degree of aggregation of sand flies in the peridomiciliary environments (Ximenes et al. 1999, Moreira et al. 2003). Aggregations of sand flies are largely maintained by the suitable conditions found in animal pens, such as a rich organic matter supply, a community of hosts serving as blood-feeding sources and mating location, as well as a range of micro-habitats.

Phlebotomine control designed to prevent leishmaniasis has been carried on for decades (Alexander and Maroli 2003, Warburg and Faiman 2011). In this context, the use of light traps (Sudia and Chamberlain 1962) for sampling and surveying sand flies in forested and nonforested sites has been contributing for establishing strategies to control leishmaniasis. Certainly, researchers for a long time have focused on the factors affecting efficiency of light traps. Some of these factors are the trap design, the height of the trap, the wavelength of light emitted by traps, and the effect of lunar phase on the catch (Service 1970, Burket et al. 2007, Faiman et al. 2009, Mann et al. 2009, Jeraldo et al. 2012, Gaglio et al. 2014, Fernandez et al. 2015). In this regard, light-emitting diode (LED) technology as a light source for attracting insect vectors has shown to be a very suitable tool to improve light-trap efficiency (Bishop et al. 2004, 2006; Hoel et al. 2007; Jenkins and Young 2010; Silva et al. 2015a, b). In addition, light traps need to be energy-efficient, and the integration of LEDs into light traps can help to reduce the energy consumption (Cohnstaedt et al. 2008).

Silva et al. (2015b) conducted a light-trap survey in northeastern Brazil to evaluate the response of phlebotomine sand flies to LEDs in forested sites. These authors found that the green-LED was the most efficient wavelength of light attracting sand flies, followed by blue and incandescent lights. Unlike forested sites, peridomestic areas in rural northeastern Brazil usually have livestock-keeping activities, and the trapping success in these sites is often higher than in forested areas. Furthermore, peridomestic environments have a number of specific risk factors (i.e., animal ownership) that contribute to leishmaniasis transmission (Campbell-Lendrum et al. 2000, Moreira et al. 2003, Teodoro et al. 2004, Silva et al. 2010) as well as a variety of sand fly vectors well-adapted to these anthropic areas (Ferro et al. 1995). Therefore, the main goal of this research was to evaluate the use of LEDs as an attractant for phlebotomine sand flies in a rural nonforested environment, particularly represented by a livestock area.

Materials and Methods

Study Area

The 22-night experiment was conducted thrice a week between July and September 2015 at a farm in northeastern Maranhão, Brazil (3° 38′47″ S and 43° 35′46″ W). Two areas, 110 m apart, were chosen for sand fly sampling. One area (Site A) represented by a pig pen and a cattle corral, and the other area (Site B) represented only by a pig pen. Chickens were frequently found roosting at Site A in the pig pen. Site A was characterized by an open area represented by a typical livestock environment. Site B was a more shadowed area than Site A, including a forested area located just behind the pig pen. At both sites, there were palm trees (*Attalea phalerata* Mart.) inside and outside the animal pens. In the region, the climate is tropical and semi-humid, with a maximum altitude of 100 m. The annual average temperature ranges from 28 to 30°C. Average annual rainfall is between 1,600 and 2,000 mm, with a 6-month rainy season between January and June.

Sand Flies Collection

Adult phlebotomine sand flies were sampled with six Hoover Pugedo (HP) light traps (Pugedo et al. 2005), four of which were modified according to Silva et al. (2015b), and the other two unmodified traps were used as control. HP traps are similar to CDC (Centers for Disease Control and Prevention) battery-powered light traps used routinely in insect inventories, with minor differences, including the fact that the light propagated from the incandescent lamp is directed downward, toward the white net collecting bag. The only modification consisted of replacing the incandescent lamps by LEDs (one LED for each trap). LEDs have been fitted into the incandescent lamp socket without welding or other permanent means of attachment. It is worth pointing out that, based on the LED lights used in the present study, any other further processing step to the correct working of the LEDs was not applied (e.g., by using a resistor).

Two different 5-mm high-intensity LED bulbs (Mollesmell Technology CO, Ltd, China) were used in this survey, as done by Silva et al. (2015b). One LED in the green (520 nm, 15,000 mCD, 20-30°, 20 mA, 72 mW – MLL5G30-1416) and the other in the blue (470 nm, 6,000 mCD, 20-30°, 20 mA, 72 mW – MLL5B30-0608) region of the visible spectrum range. These two colors were the most attractive light sources for sand fly in a previous pilot study (data not shown) that included four more LEDs (red, yellow, ultraviolet, and white). Incandescent lamps (150 mA, 3 V) were used as control.

In total, four LED traps (two green-LED and two blue-LED traps) and two controls (incandescent light) were used. In each of the two areas selected for sand fly sampling, three light traps (green, blue, and control) were simultaneously set from 18:00 to 06:00, at about 1.5 m above soil and distant 8-10 m from each other in a triangular arrangement. This triangular arrangement was set in the two pig pens (Site A and B). Field work was discontinued after every 3 d and then resumed after a 1-d rest period. On the first day of the 3-d sampling period, light trap positioning was randomized before testing.

After captured, sand flies were killed with ethyl acetate vapor and transferred into labeled glass vials containing 70% alcohol. The insects were brought into the Laboratory of Medical Entomology for identification and counting. The specimens were identified based on morphological characters to species level according to Galati (2003) and deposited in the entomological collection of the Federal University of Maranhão.

Statistical Analysis

The Kruskal–Wallis ranking test was performed to compare the abundance of individuals in the light traps and the Mann–Whitney for assessing the pair-wise comparisons. Statistical significance was found when $P < 0.05$. The Kolmogorov–Smirnov test was carried out for assessing for the normality of data distribution. When a criterion of normality was not met, data were $\log_{10}$-transformed prior to analysis. The independent Student *t*-test was applied in case of normally distributed data. Chi-square test was used when needed. All tests were performed using the software Prism (GraphPad, San Diego, CA).

Results

In total, 22 individual collections were made at each site and 44 with each trap type. In total, 2,542 specimens belonging to 14 phlebotomine species were collected (Table 1). *Nyssomyia whitmani* was the prevalent species (40.8%), followed by *Evandromyia evandroi* (24.7%), *Micropygomyia goiana* (12.7%), *Lutzomyia longipalpis* (12.0%), and *Bichromomyia flaviscutellata* (5.7%). The nine remaining species accounted for 4.0% of the total. The distribution of species per site is shown in Table 1.

Taking the two sites together, the sex ratio was male-biased (female/male = 1:1.5), and the difference between males (60.3%) and females (39.6%) was statistically significant ($U = 685$, $P = 0.039$), as was the difference between males (87.0%) and females (13.0%) of *N. whitmani* ($U = 282.5$, $P = 0.000$) and males (33.0%) and females (67.0%) of *M. goiana* ($U = 404.5$, $P = 0.001$). There was no statistically significant difference between males and females of the other species. When the two sites are compared, Site A was more male-biased (50.4 ± 7.5/mean ± SE) than Site B (20.3 ± 2.1/mean ± SE) and this difference was statistically significant ($t = 4.8$, df = 41, $P = 0.000$).

The green-LED light source attracted 39.4% of all phlebotomine sand flies collected, with a mean and standard error of 23.3 ± 3.7, followed by the blue-LED (35.5%; 20.7 ± 3.8) and incandescent (25.1%; 15.1 ± 2.3) lights. Combining the numbers collected at the two sites showed statistically significant between the green-LED and incandescent lights ($U = 681.5$, $P = 0.036$), but not between the green-LED and blue-LED ($U = 790.5$, $P = 0.249$) and blue-LED and

Table 1. Collection of phlebotomine sand flies with modified light traps by the color of light source in a livestock area of northeastern Brazil

Site	Species	Green		Blue		Control		Total (%)
		N	F:M	*N*	F:M	*N*	F:M	
A	*Nyssomyia whitmani* (Antunes & Coutinho, 1939)	256	1:16.1	400	1:24	163	1:12.5	819 (52.1)
	Evandromyia evandroi (Costa Lima & Antunes, 1936)	118	1.1:1	94	1:1	64	1:1.2	276 (17.5)
	Lutzomyia longipalpis (Lutz & Neiva, 1912)	113	1.6:1	75	1:1.5	74	1.1:1	262 (16.7)
	Micropygomyia goiana (Martins, Falcão & Silva, 1962)	86	2.4:1	35	1.3:1	34	2.1:1	155 (9.9)
	Evandromyia termitophila (Martins, Falcão & Silva, 1964)	11	10:1	9	1.2:1	7	1.3:1	27 (1.7)
	Bichromomyia flaviscutellata (Mangabeira, 1942)	–	–	11	1.2:1	2	1:1	13 (0.8)
	Sciopemyia sordellii (Shannon & Del Ponte, 1927)	4	1:1	6	2:1	3	2:1	13 (0.8)
	Psathyromyia shannoni (Dyar, 1929)	1	1:0	1	1:0	–	–	2 (0.1)
	Micropygomyia quinquefer (Dyar, 1929)	1	1:0	1	0:1	–	–	2 (0.1)
	Evandromyia lenti (Mangabeira, 1928)	1	0:1	1	0:1	–	–	2 (0.1)
	Micropygomyia echinatopharynx Andrade Filho, Galati, Andrade & Falcão, 2004	1	0:1	–	–	–	–	1 (0.1)
	Barrettomyia teratodes (Martins, Falcão & Silva, 1964)	–	–	–	–	1	1:0	1 (0.1)
	Total	592	1:1.7	634	1:3.9	347	1:2.1	1573 (100)
	%	37.6%		40.3%		22.1%		100%
B	*Evandromyia evandroi*	162	1:1.1	99	1.1:1	92	1.1:1	353 (36.4)
	Nyssomyia whitmani	76	1:1.1	49	1:1.1	93	1:1.8	218 (22.5)
	Micropygomyia goiana	71	6.3:1	55	2.2:1	43	2.3:1	169 (17.4)
	Bichromomyia flaviscutellata	50	1.5:1	48	2.2:1	33	1.2:1	131 (13.5)
	Lutzomyia longipalpis	23	1.3:1	5	1.5:1	16	1.8:1	44 (4.5)
	Evandromyia termitophila	17	3.2:1	7	1.3:1	7	1:1.3	31 (3.2)
	Sciopemyia sordellii	5	4:1	2	2:0	2	1:1	9 (0.9)
	Psathyromyia shannoni	3	3:0	2	2:0	2	2:0	7 (0.7)
	Micropygomyia quinquefer	2	1:1			1	0:1	3 (0.3)
	Barrettomyia teratodes	–		1	1:0	–	–	1 (0.1)
	Micropygomyia echinatopharynx	–	–	–	–	1	0:1	1 (0.1)
	Evandromyia saulensis (Floch & Abonnenc, 1944)	1	0:1	–	–	–	–	1 (0.1)
	Sciopemyia servulolimai (Damasceno & Causey, 1945)	–	–	1	0:1	–	–	1 (0.1)
	Total	410	1.4:1	269	1.4:1	290	1.1:1	969 (100)
	%	42.3%		27.8%		29.9%		100%

In total, 22 individual collections were made at each site and 44 with each trap type. *N* = number of individuals; F:M = female/male sex ratio. Control = incandescent light.

incandescent ($U = 822.0$, $P = 0.378$) lights. In this context, results were not statistically significant for the two sites (A and B) separately.

Regarding the sex ratio (female/male) per light source, males outnumbered females (Table 1), although without statistical significance for all light sources (green: $U = 909.0$, $P = 0.897$; blue: $U = 781.5$, $P = 0.217$; control: $U = 792.5$, $P = 0.332$). Females were green-biased, but the only statistical significance was the highest number of females attracted to the green-LED trap (10.7 ± 1.5) as compared with the number of females attracted to the control trap (6.0 ± 0.9; $U = 640.5$, $P = 0.014$). The highest number of males observed in the blue-LED trap was due to a high frequency of *N. whitmani* males caught at one of the sites (Site A; Table 1). The sex ratio of 1 female (4.0 ± 0.9/mean ± SE) to 6.7 males (23.1 ± 4.7/mean ± SE) for this species showed a statistically significant difference ($U = 282.5$, $P = 0.000$).

In total, 1,573 (23.8 ± 3.4/mean ± SE) sand flies representing 12 species were sampled at Site A and 969 (15.4 ± 1.7/mean ± SE) representing 13 species at Site B (Table 1), showing a statistically significant difference in the number of individuals ($\chi^2 = 54.37$, df = 2, $P < 0.000$). The green-LED attractiveness and the female green-biased response observed for each of the sites were superior to that of the control, although with no statistically significant difference (Table 1). It is worth pointing out the higher number of *L. longipalpis* at Site A and of *B. flaviscutellata* at Site B and both differences are statistically significant ($U = 35.0$, $P = 0.000$, $U = 22.5$., $P = 0.011$, respectively). Except for the two above-mentioned species and for *N. whitmani*, the others were fairly equally distributed between the two sites.

All of the sand fly species were LED-biased and almost all the species, except *N. whitmani* and some of the rare ones, were green-biased (Table 1). Based on the five most frequently collected species, the LED-biased response was statistically significant only for *E. evandroi* and *M. goiana* (Table 2).

Discussion

All sand flies collected in this study have previously been recorded in the northeast region of Maranhão State, Brazil (Silva et al. 2010, 2012, 2015b), with the exception of *Barretomyia teratodes*, found in other parts of the state (Rebêlo et al. 2010). *Micropygomyia goiana* was misidentified as *Micropygomyia trinidadensis* (Silva et al. 2010, 2012, 2015) and is commonly found in other phytogeographic regions of Maranhão state (Rebêlo et al. 2010). Three of the most frequent species in the present study have been implicated in the transmission of leishmaniasis in Brazil: *N. whitmani*, *L. longipalpis*, and *B. flaviscutellata* (Maroli et al. 2013, Ready 2013, Bates et al. 2015).

Light traps caught more sand flies at Site A than at Site B. These differences may be partially attributed to the higher availability of hosts for sand flies at Site A. Hosts provide suitable aggregation sites for both males and females and blood sources for females. Higher concentrations of hosts produce more odor than a small number of

Table 2. Most frequent phlebotomine sand flies (means ± SE) captured in modified light traps in a livestock area of northeastern Brazil

LEDs	*N. whitmani*	*E. evandroi*	*M. goiana*	*L. longipalpis*	*B. flaviscutellata*
Green	7.72 ± 2.98a	6.51 ± 0.92a	3.67 ± 0.55a	3.16 ± 0.71a	1.16 ± 0.53a
Blue	11.84 ± 3.55a	4.48 ± 0.65ab	2.09 ± 0.29ab	1.86 ± 0.43a	1.37 ± 0.77a
Control	4.55 ± 1.11a	3.62 ± 0.60b	1.79 ± 0.30b	2.09 ± 0.45a	0.81 ± 0.30a

Means followed by the same letter are not statistically different (Mann–Whitney *U*-test). Control = incandescent light.

hosts, and this stimulus is potentially important for the aggregation of sand flies. Aggregating male sand flies, on or near hosts, release sex pheromones to attract females, as well as other males (Kelly and Dye 1997, Campbell-Lendrum et al. 2000, Spiegel et al. 2005). High numbers of available hosts create a male-biased sex ratio (Morrison et al. 1995), as observed at Site A.

The relative high abundance of *L. longipalpis* at Site A and *B. flaviscutellata* at Site B can be explained as follows. *Lutzomyia longipalpis* is more abundant in open and disturbed areas, as Site A. This species is commonly found associated with domestic animals, including cattle (Morrison et al. 1993, Alexander 2000, Andrade Filho and Brazil 2009, Silva et al. 2012). *Bichromomyia flaviscutellata* is very frequently associated with wetter conditions (Shaw and Lainson 1972, Campos et al. 2013), as what was found at Site B, because of the forested area close to the pig pen.

In general, light trapping of sand flies in livestock areas was more efficient when green-LED traps were used instead of the incandescent light, as observed by researchers working with other medically important insects (Bishop et al. 2004, 2006; Silva et al. 2015a). Data on LED attractiveness reported here are comparable with results obtained by Silva et al. (2015b), who trapped phlebotomine sand flies in LED-baited suction traps in a forested environment. These authors found that the green-LED trap attracted the highest number of sand flies (38.3%), followed by the blue (34.2%) and the incandescent (27.5%) lights. In the present study, the percentage numbers for each of the three lights for the sites combined were similar (39.4, 35.4, and 25.1%, respectively). When the two sites (A and B) are analyzed separately, some species-specific differences are found (Table 1). For instance, *N. whitmani* was blue-biased at Site A, control-biased at Site B, and green-biased in the work by Silva et al. (2015b). *Lutzomyia longipalpis* was green-biased at both sites in the present study and control-biased in the forested areas studied by Silva et al. (2015b). Such differences may not be solely due to the two different LED intensities used in these studies, but rather may be related to site characteristics or, more generally, habitat heterogeneity. However, even with these differences, the standard incandescent light was outcompeted by LEDs.

The fact that the blue-LED trap collected more males than females at Site A (more open site) and that males usually outnumber females early in the evening (Morrison et al. 1995) may indicate that the attractiveness of blue-LED traps may be higher during twilight. Based on these findings, hourly LED trap catches are under way to assess the effectiveness of blue-LED traps in sampling sand flies at crepuscular periods.

In studies conducted by Hoel et al. (2007) and Mann et al. (2009), *Phlebotomus papatasi* and *Psathyromyia shannonni*, respectively, preferred red light to other colors used, including green and blue. In a previous pilot study carried out to assess the most attractive light sources for sand flies, red color was the least attractive (2%; Francinaldo S. Silva, unpublished data). It is known that most nocturnal insects have a trichromatic vision based on ultraviolet, blue, and green photoreceptors (Briscoe land Chittka 2001), and the green–blue preference observed in the present study is in agreement with this physiological criterion. Further studies approaching the red light response in a variety of sand fly species and environmental conditions are needed.

LEDs are energy-efficient and the superiority of green LEDs over the incandescent light as shown here is important for developing new devices for sampling medically important arthropods (unpublished data, patent application submitted). The substitution of the incandescent lamp for LEDs is offset by a number of advantages in favor of LEDs, as previously described (Hoel et al. 2007, Cohnstaedt et al. 2008). Thus, the green LEDs have already been suggested to be adopted for monitoring and surveying insects of medical interest (Bishop et al. 2006; Silva et al. 2015a, b), and the observed LED-biased response reported herein and by Silva et al. (2015b) suggests that LEDs can actually be used as an effective substitute for the currently used incandescent bulbs in monitoring traps for phlebotomine sand flies in Brazil.

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