Lutzomyia spp. (Diptera: Psychodidae) Response to Olfactory Attractant- and Light Emitting Diode-Modified Mosquito Magnet X (MM-X) Traps

RAJINDER S. MANN,[1] PHILLIP E. KAUFMAN, AND JERRY F. BUTLER

Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, Building 970,
Natural Area Drive, University of Florida, Gainesville, FL 32611–0620

J. Med. Entomol. 46(5): 1052–1061 (2009)

ABSTRACT Mosquito Magnet-X traps were modified for use with blue, green, red, and blue-green-red light-emitting diodes and olfactory attractants to determine the response of *Lutzomyia shannoni* (Dyar) and *Lutzomyia vexator* (Coquillett) (Diptera: Psychodidae) field populations to these attractants. Red and blue-green-red–baited traps captured the highest numbers of *Lu. shannoni* and *Lu. vexator*, respectively, although, there were no significant differences between the colors. Baiting the traps with CO_2 attracted significantly higher numbers of *Lu. shannoni* but showed no effect on *Lu. vexator* capture. In comparison with CO_2 alone, *Lu. shannoni* preferred 1-octen-3-ol and 1-hexen-3-ol (0.05 g per trap) in combination with CO_2.

KEY WORDS sand fly, *Lutzomyia shannoni*, *Lutzomyia vexator*

Phlebotomine sand flies (Diptera: Psychodidae) are of worldwide importance because of their ability to transmit *Leishmania* parasites that affect humans in >80 countries (Desjeux 1996). Sand flies also carry and transmit other zoonotic pathogens such as *Bartonella bacilliformis,* the causative agent of human bartonellosis, and arboviruses causing health problems in humans and other animals. Adult female sand flies usually require vertebrate blood for production of eggs, although autogeny (egg production without a bloodmeals) occurs occasionally but usually only in the first gonotrophic cycle, after which a bloodmeal is required for subsequent gonotrophic cycles (Young and Perkins 1984). The biting activity of sand flies is considered to be crepuscular or nocturnal with few species known to bite during daylight (Killick-Kendrick 1999). *Lutzomyia shannoni* (Dyar) is one of the more thoroughly studied species in the United States because of its proven vectorial capacity for vesicular stomatitis virus and its actual or suspected capability to transmit *Leishmania* species and sand fly fever virus (Lawyer and Young 1987, Tesh 1988, Comer et al. 1991). *Lutzomyia vexator* (Coquillett) feeds on reptiles and has been shown capable of transmitting the lizard malarial parasite *Plasmodium mexicanum* (Klein et al. 1987).

Female sand flies are attracted to several components of host odor. Host odors and sex pheromones, when present together, synergize the attraction, compared with the sex pheromone or host odor alone. Dougherty et al. (1999) identified ketones, alcohols, aldehydes, and carboxylic acids containing kairomone compounds from the fox *Vulpes vulpes* L., which stimulated olfactory organs of *Lutzomyia longipalpis* (Lutz & Neiva). Pheromone and host odor and a combination of both attracted higher numbers of *Lu. longipalpis* females than did the hexane used as control treatment (Bray and Hamilton 2007). Santana et al. (2002) demonstrated the attraction of *Lu. longipalpis* to 1-octen-3-ol (octenol) in air currents. Octenol reportedly serves as a kairomone for several mosquito species and some species of Ceratopogonidae (Kline et al. 1994, 2007).

The role of vision and the use of color vision to locate a host by sand flies have not been ascertained with the exception of a few studies documenting similar spectral sensitivity of *Lu. longipalpis* to that of the tsetse fly, *Glossina morsitans morsitans* Westwood, and the mosquito *Aedes aegypti* (L.) (Mellor et al.1996). The eyes of adult *Lu. longipalpis* respond maximally to light in the UV region and blue-green-yellow region (Mellor et al. 1996). Mellor and Hamilton (2003) provided evidence that *Lu. longipalpis* is more attracted to UV, blue, green, and yellow wavelengths and were able to discriminate between wavelengths independent of intensity. However, *Phlebotomus papatasi* (Scopoli) were attracted to red light produced from light-emitting diodes (LED) more than incandescent blue and green light (Hoel et al. 2007).

Interest in improving trapping efficiencies through host-seeking behavior studies have focused on the Culicidae and Ceratopogonidae because of their widespread distribution and close association with human interests. However, due to recent interests in sand flies

---

[1] Corresponding author, e-mail: mannrs@ufl.edu.

0022-2585/09/1052–1061$04.00/0

and emergent challenges with *Leishmania*, we evaluated the response of *Lu. shannoni* and *Lu. vexator* to light produced from colored LEDs and the attractiveness of olfactory chemicals with an objective to develop a highly effective light trap to collect *Lutzomyia* spp. for rearing purposes.

Materials and Methods

Studies were carried out in San Felasco Hammock Preserve State Park (29° 45′ N, 82° 30′ W), Gainesville, Alachua County, FL. The study site was selected for internal consistency of vegetation, nature of habitat, area, accessibility, and previous reports of *Lutzomyia* spp. abundance (Young and Perkins 1984). The state park covers 2,306 ha of land, with a variety of plant communities, including the largest protected stand of climax mesic hammock in Florida (Dunn 1982). Structurally, these forests have a closed canopy, a diverse understory, and a deep layer of leaf litter (Platt and Schwartz 1990). The area is characterized by moderate winters (15–20°C average temperature) and hot summers (30°C average temperature) with variable rainfall (average 1,370 mm) occurring between June and September. The fauna of this site includes salamanders, frogs and toads, turtles, lizards, snakes, opossums, armadillos, lagomorphs, rodents, wild animals, and many bird species (Florida Department of Environmental Protection 2003, Florida State Parks 2005). Complete descriptions of plant and animal communities and flora of San Felasco Hammock may be found in Dunn (1982) and Florida Department of Environmental Protection (2003).

Counter-flow geometry traps (Mosquito Magnet-X, MM-X model, American Biophysics Corp., Kingstown, RI) were modified with blue (B), red (R), green (G), and blue-green-red (BGR) LEDs (Digi-Key Corporation, Thief River Falls, MN) wired in parallel to motors on the bottom ring of the MM-X trap. Traps with only one color of light (blue, green, or red) had three LEDs of the same color affixed and the tri-color (BGR) traps had nine LEDs affixed to the trap. All diodes had a viewing angle of 30°. Color, part number, wavelength, and millicandela (mcd) chosen for testing were blue (P 466-ND, 470 ± 30 nm, 650 mcd), green (67–1755-ND, 502 ± 25 nm, 1,500 mcd), and red (67–1611-ND, 660 ± 30 nm, 1,800 mcd). Each diode was provided with a 180-Ohm resistor to prevent overdriving of LEDs. Each LED was inserted in the bottom ring of the trap at an angle of 90° to the ground.

Sand fly response to light under field conditions was evaluated for individual light color and combinations of colors with both transmitted and reflected light presentations. In the first series of studies, four MM-X traps containing B, G, R, and BGR LED modifications were set up five times weekly for 108 actual nights and 432 total trap nights (total trap nights = actual nights × number of traps) from September 2006 to May 2007. Traps were positioned at least 200 m apart in such a way that they were not visible from other traps. Trap placement was randomized before each trial and traps were rotated daily between the sites to avoid positional biases. Traps were baited with the attractant Red Mix (RM) obtained from USDA–ARS–CMAVE (Gainesville, FL) at 0.01 g per trap and CO_2 to maximize collections. The RM contained 0.5% by volume each of 1-octen-3-ol and 1-hexen-3-ol in acetone. The blend was developed originally as a mosquito attractant that proved to attract sand flies in the field (U. R. Bernier and D. L. Kline, personal communication).

The RM was placed on a cotton swab within a plastic tube that was suspended below the MM-X trap at the CO_2 outlet (Fig. 1). Carbon dioxide was provided through a 9-kg compressed gas cylinder, delivered to the traps through 2-m-long, 6.4-mm-diameter plastic Tygon tubing taped at the underside of the rain shield. A flow rate of 500 ml/min was achieved using a 15 psi single-stage regulator (Flowset 1, Clarke Mosquito Control, Roselle, IL). Traps were suspended on shepherd's hooks with an odor outlet 15–20 cm above the ground level. Traps were operated with 12-V batteries that were replaced with charged batteries after every 48 h. All traps were activated between 0800 and 0900 hours and run for 24 h before collection and rotation.

The lid at the trap inlet (Fig. 2) was replaced with a 1.2- by 1.2-mm mesh bag to exclude mosquitoes and prevent other large-sized insects from damaging collected sand flies. Each trap was provided with an apple (*Malus* spp.) slice in the traps as a sugar source for captured sand flies. The apple slice was fixed on exterior of the larger inner airflow pipe with a rubber band. Sand flies were collected and counted daily after placing the traps in a −20°C freezer. A cool air was sucked into the traps by operating the traps inside the freezer for 45–60 s. Sand flies were chill immobilized for identification and counting.

Treatments evaluated light combinations, as well as reflected light over transmitted light. The traps were modified for reflected light by placing an aluminum rain shield above the LEDs to redirect the 45° upward-angled LED output off of the aluminum shield (Fig. 2). The treatments comprised of six combinations that included red light + Red Mix + CO_2 (R+RM+CO_2), red light + Red Mix (R+RM), red-blue light fixed at 45° angle + Red Mix + CO_2 (RB45°+ RM+CO_2), red-blue light + Red Mix (RB+RM), red-blue-reflected light fixed at 45° angle + Red Mix + CO_2 (RBRL45°+ RM+CO_2), and CO_2 alone (control). The traps were outfitted with red and blue lights based on the results obtained with single and tricolor trap results. BGR- and green-lighted traps captured the fewest sand fly adults and therefore were not used in further studies. Traps were operated from May 2007 to June 2007 at six sites with set up and data collection methods described previously.

Two independent experiments were conducted to evaluate sand fly response to three olfactory attractants. The attractant mixtures and dosages chosen on the basis of their attraction to mosquitoes (J.F.B., unpublished data) were as follows: urea + ammonium benzoate + Purac powder + lactic acid + ammonium sulfate (AD); urea + ammonium benzoate + Purac powder + lactic acid + glycerin + dibutyl succinate

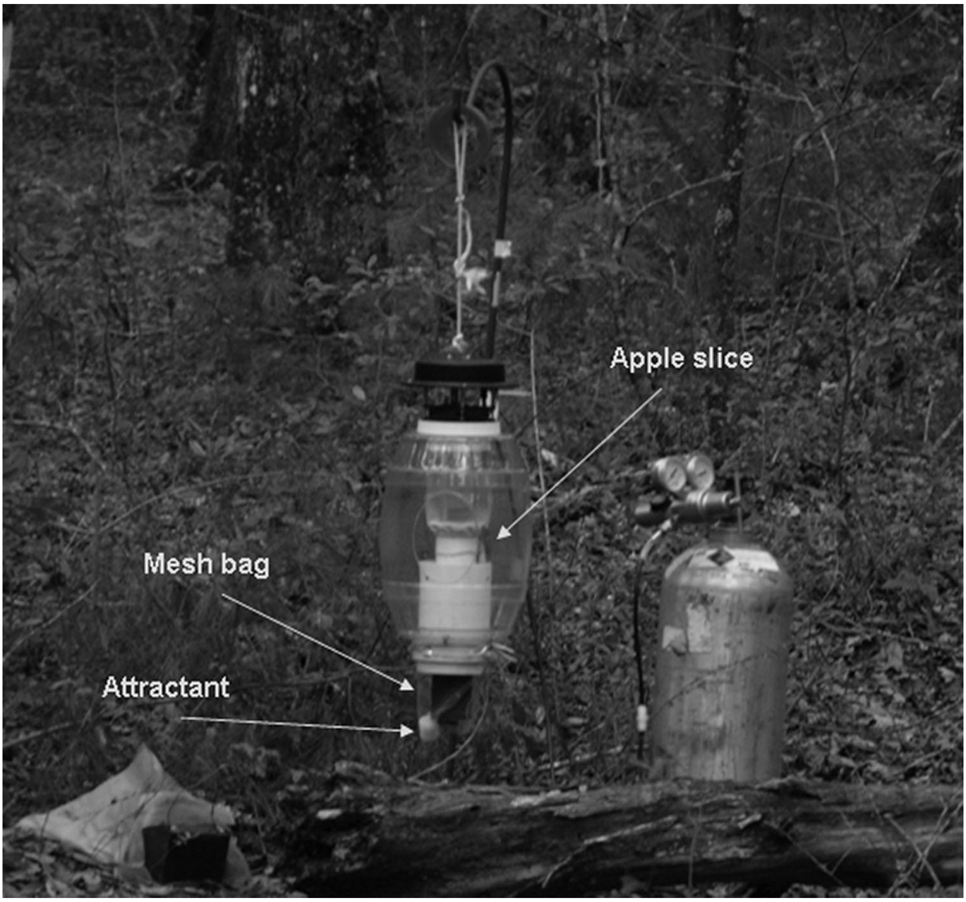

Fig. 1. Light emitting diode-modified Mosquito Magnet-X trap that was used for collection of *Lutzomyia* spp.

(BR); and dibutyl succinate (DS) at two rates, high rate (0.5 g per trap) and low (0.1 g per trap). The mixtures contained equal ratios of each compound by weight. The mixtures were prepared in advance and stored at room temperature (22°C) under dark and dry conditions. The attractants were released in the same way as was the RM. All the traps in this experiment were equipped with red LEDs. Tests were conducted using six treatments, namely, AD+R+CO_2, AD+R, BR+R+CO_2, DS+R+CO_2, RM (0.01 g per trap)+R+CO_2 attractant mixtures, and CO_2+R (control). The experiments were conducted from July 2007 to August 2007 at 0.5 g per trap and September 2007 to October 2007 at 0.1 g per trap by using the methodology described earlier with the exception of the aforementioned RM that was maintained 0.01 g per trap.

Multifactor analysis of variance was performed for each experiment with LED color attractant combination and trap position as factors. If differences among means were present then the means were separated using Tukey's mean separation test with ($\alpha = 0.05$). Sets of independent contrasts also were computed to compare the means between groups. All data were log ($n + 1$) transformed to meet the assumptions of normality. Statistical analysis was carried out using SAS software version 9.1 (SAS Institute 2003).

Results

In total, 4,261 sand flies was collected from 163 actual nights and 762 total trap nights, yielding 5.6 sand flies per trap night. *Lu. shannoni* made up 76.5% of total collection of 3,244 adult specimens from all the trials from September 2006 to October 2007. During trials with LED comparisons, 3,464 sand flies were captured between September 2006 and June 2007 from 127 actual nights and 540 total trap nights, yielding an average of 6.4 sand flies per trap night. *Lu. shannoni* made up 79.5% of the collection, with 2,753 adult specimens with *Lu. vexator* making up the remaining 711 adult specimens. During trials with olfactory attractants, 797 sand flies in total were captured between July and October 2007 from 36 actual nights and 216 total trap nights, yielding an average of 3.7 sand flies per trap night. *Lu. shannoni* made up 64.5% of the collection with 514 adult specimens and *Lu. vexator* made up the remaining 283 adult specimens (35.5%). In general, higher numbers of females were captured

Fig. 2. Mosquito Magnet-X trap modified for reflected light that was used for collection of *Lutzomyia* spp.

than males, although the adults were sexed only during May and June 2007.

Evaluation of LEDs. In total, 2,613 sand flies was captured from traps outfitted with nonreflected B, G, R, and BGR LEDs from 108 actual nights and 432 total trap nights with 6.1 sand flies per trap night in the individual color trials. *Lu. shannoni* made up 77% of the total collection (2,081 adults), whereas *Lu. vexator* made up 23% of the total collection (532 adults). Traps with red-only LEDs captured the highest numbers of *Lu. shannoni* (Table 1), whereas traps with BGR LEDs captured the highest numbers of *Lu. vexator.* Neither species yielded significant differences among the treatments for trap colors or trap positions.

When combinations of colors and reflected light were evaluated, 851 sand flies in total were collected from 19 actual nights and 114 total trap nights, with an average of 7.6 sand flies per trap night. *Lu. shannoni* again was the most abundant species, making up 80% of the total collection (672 adults). Females of this species preferred CO_2-baited-lighted traps to CO_2-unbaited-lighted traps and CO_2 alone ($F = 16.89$; df = 5,103; $P < 0.0001$) (Fig. 3A). The lighted traps without CO_2 were as effective as CO_2 alone. *Lu. shannoni* did not show attraction to light colors, reflected light over transmitted light, or trap positions. The red LED plus CO_2 treatment produced the largest *Lu. shannoni* (15.75) collections per trap night.

Lu. Vexator, making up 23% of the collection (179 adults), preferred RB (45°)-CO_2–baited traps over CO_2 alone ($F = 2.62$; df = 5,103; $P < 0.0285$) (Fig. 3B). There were no differences in *Lu. vexator* collection

Table 1. Collection of *Lu. shannoni* and *Lu. vexator* from MM-X traps outfitted with colored lights produced from light emitting diodes

LED color	Mean ± SEM *Lutzomyia* spp. per trap night	
	Lu. shannoni	*Lu. vexator*
Blue	4.63 ± 0.53	1.44 ± 0.20
Green	4.56 ± 0.76	1.45 ± 0.43
Red	5.81 ± 1.37	1.10 ± 0.24
BGR	4.26 ± 0.68	1.88 ± 0.62

One hundred and eight trap nights per LED color ($P > 0.05$).

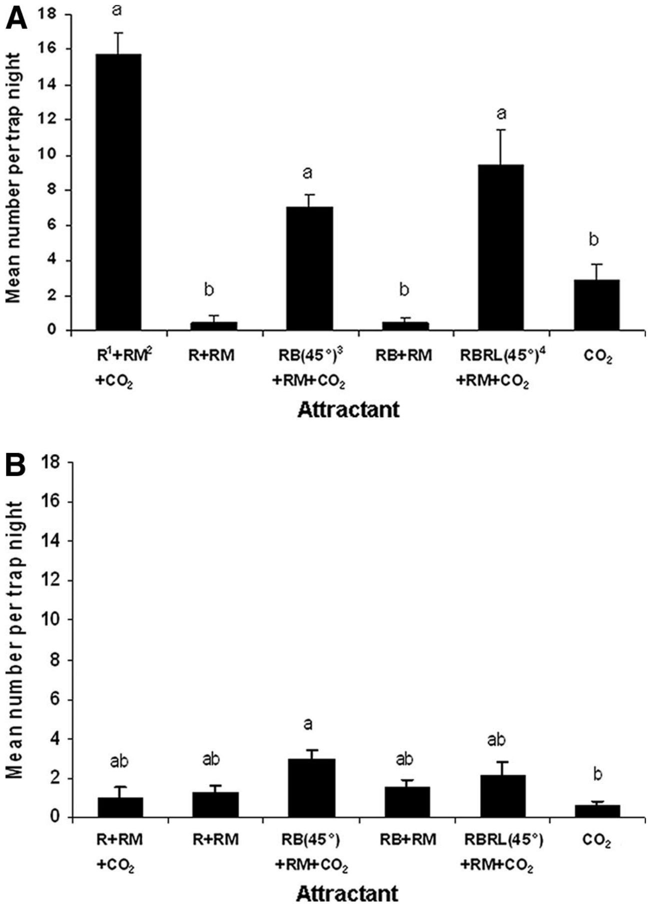

Fig. 3. Mean (SE) *Lu. shannoni* (A) and *Lu. vexator* (B) collected with MM-X traps baited with various visual and/or olfactory attractants. ^{1}Red LED. ^{2}Red-Mix (1-octen-3-ol + 1-hexen-3-ol). ^{3}Red-blue light fixed at 45° angle. ^{4}Red-blue-reflected light fixed at 45° angle. Columns with different letters are significantly different ($P < 0.05$), Tukey's means separation applied to log (n + 1)-transformed data.

with light color or CO_2 baiting; however, the three treatments encompassing RB(45°)+RM+CO_2, RBRL(45°)+RM+CO_2, and RB+RM traps constituted 70% of the *Lu. vexator* collection. The traps baited with RB(45°)+RM+CO_2 had the highest captures of *Lu. vexator* (3.00 per trap night), whereas the traps with CO_2 alone captured the fewest (0.59). However, there were no significant differences between these treatments.

Evaluation of Olfactory Attractants. During trials with olfactory attractants at 0.5 g per trap, 469 sand flies in total were captured from July to August 2007 from 18 actual nights and 108 total trap nights, yielding an average of 4.3 sand flies per trap night. *Lu. shannoni,* making up 55% of the total collection (256 adults), were more attracted to RM (1-octen-3-ol +1-hexen-3-ol) than to other attractants, including CO_2 alone (F = 9.86; df = 5,97; $P < 0.0001$) (Fig. 4A). CO_2-baited traps captured more *Lu. shannoni* than traps without CO_2. There were no significant differences between CO_2-enhanced AD-, BR-, and DS-baited traps, and CO_2 alone. *Lu. vexator* made up 45% of total collection (213 adults). There were no significant differences in *Lu. vexator* collection among the olfactory attractants and no effect of CO_2 baiting (Fig. 4B).

In studies using the low rate of olfactory attractants (0.1 g per trap), 328 sand flies in total were captured from September to October 2007 from 18 actual nights and 108 total trap nights, with 2.8 sand flies per trap night. Low- and high-rate (0.5 g per trap) treatments yielded similar results for *Lu. shannoni.* In these studies, *Lu. shannoni,* making up 79% of total capture (258 adults), was more attracted to CO_2-baited traps than to CO_2-unbaited traps, whereas no differences in capture between AD, BR, DS, and CO_2 alone were observed (F = 5.15, df = 5,97; P = 0.0081) (Fig. 5A). The BR-baited traps captured the highest number of *Lu. shannoni* among the non-Red Mix attractants tested. Seventy (21%) *Lu. vexator* in total were captured, and there were no significant differences among the olfactory attractants (Fig. 5B). Trap position had no effect on either species collection in any of the trials (data not shown).

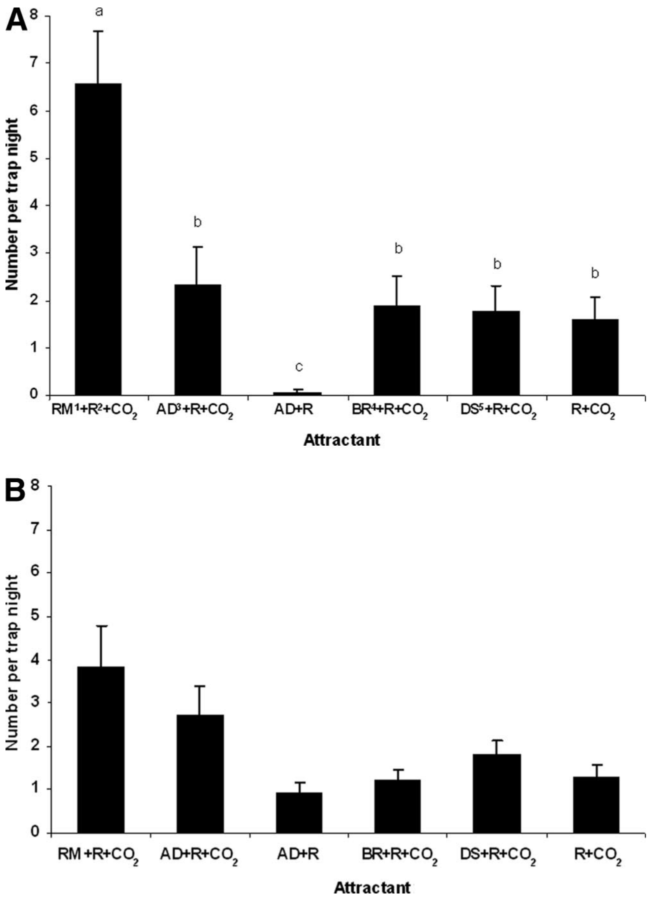

Fig. 4. Mean (SE) *Lu. shannoni* (A) and *Lu. vexator* (B) collected with MM-X traps baited with red LEDs and various olfactory attractants (0.5 g per trap). [1]Red-Mix (1-octen-3-ol + 1-hexen-3-ol). [2]Red LED. [3]Urea + ammonium benzoate + Purac powder + lactic acid + ammonium sulfate. [4]Urea + ammonium benzoate + Purac powder + lactic acid + glycerin + dibutyl succinate. [5]Dibutyl succinate. Columns with different letters are significantly different ($P < 0.05$); Tukey's means separation applied to log (n + 1)-transformed data.

Discussion

Our results reveal that red LED-fitted (660 nm, 1,800 mcd) traps collected the most *Lu. shannoni* in LED evaluation experiments under field conditions, although there were no significant differences among the colors. Our results support the findings of Burkett et al. (2007) who captured the highest numbers of sand flies with red-colored chemical light sticks but also showed no significant differences among blue-, green-, yellow-, orange-, and red-colored lights. The authors did not identify the species in their studies. Hoel et al. (2007) captured significantly higher number of *P. papatasi* with red LEDs followed by blue, green, and incandescent LED-modified light traps. Although *Lu. shannoni* capture in our study followed a similar pattern, we did not observe significant differences between the light colors tested. Attraction of *Lu. shannoni* to red light may be due to its attraction to longer wavelengths over shorter wavelengths.

Sand flies obtain sugar by feeding directly on plant tissues and honey dew secreted by aphids and coccids (MacVicker et al.1990, Wallbanks et al. 1991, Schlein and Jacobson 1994, Schlein and Muller 1995). Attraction of sand flies to longer wavelengths might help in host plant location for sugar feeding. Sand flies might land on plants for honey dew in response to longer wavelengths reflected by plant as suggested by Coombe (1981) and Byrne et al. (1990) for greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), that short wavelengths stimulate flight and inhibit landing, whereas longer wavelengths stimulate landing and inhibit flight. The authors further suggested that whiteflies would orient toward sky at short wavelengths but would tend to land on plants at longer wavelengths (500–600 nm) because plants reflect maximally at longer wavelengths (520–600 nm). This is supported by Affeldt et al. (1983) who captured maximum *T. vaporariorum* on traps reflecting 500–600 nm and observed inhibited landing at 400–490 nm. It is interesting that color is the only factor that influences whitefly landings on plant and nonplant surfaces (Woets and van Lantern 1976, van Lantern and Woets 1977).

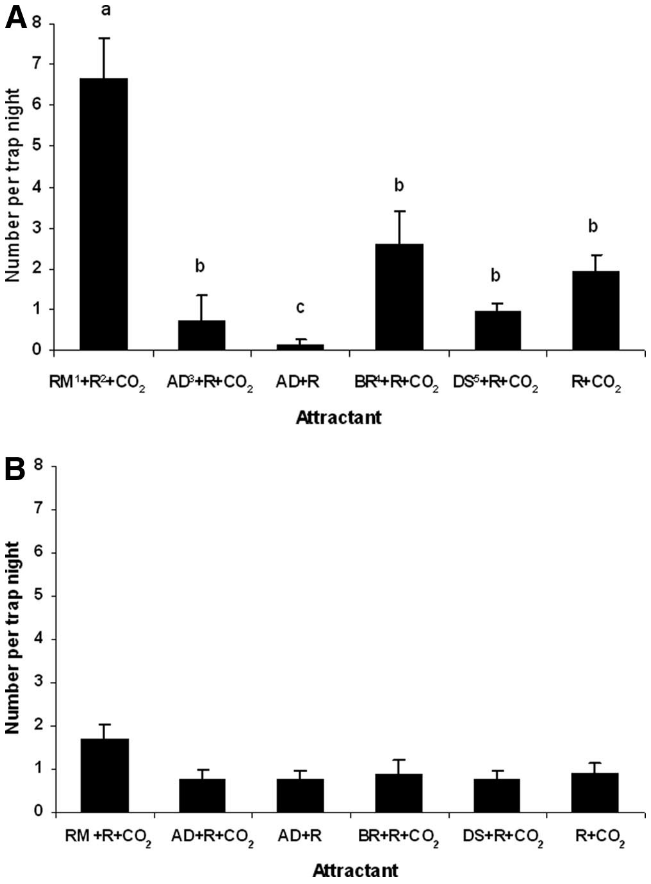

Fig. 5. Mean (SE) *Lutzomyia shannoni* (A) and *Lu. vexator* (B) collected with MM-X traps baited with red LEDs and various olfactory attractants (0.1 g per trap). [1]Red-Mix (1-octen-3-ol + 1-hexen-3-ol). [2]Red LED. [3]Urea + ammonium benzoate + Purac powder + lactic acid + ammonium sulfate. [4]Urea + ammonium benzoate + Purac powder + lactic acid + glycerin + dibutyl succinate. [5]Dibutyl succinate. Columns with different letters are significantly different ($P < 0.05$); Tukey's means separation applied to log $(n + 1)$-transformed data.

In a study conducted by Mellor and Hamilton (2003), a greater percentage of *Lu. longipalpis* was attracted toward 490–546-nm than 400-nm wavelength light at lower, equivalent, and higher light intensities. Greater numbers of males were attracted to 546-nm light, whereas more females were attracted to 490-nm light at lower light intensity. *Lu. longipalpis* discriminated between wavelengths independent of light intensity, suggesting a true color vision involving more than one photoreceptor. The authors further suggested that *Lu. longipalpis* may be able to navigate under dusk, moonlight or starlight conditions using the blue-green-yellow part of spectrum.

To our knowledge, there are no documented studies on sand fly photoreceptor pigments. Most Diptera are known to possess three types of photoreceptors—R1–6, R7, and R8—and lack red photoreceptors (White 1985). However, Green (1984) postulated the presence of a red receptor in addition to the green receptor in *G. m. morsitans* because of its ability to discriminate green and red light. Gibson (1995) showed that *Anopheles gambiae* (Giles) can sense near infrared light but not the far infrared light. A review by Briscoe and Chittka (2001) indicated that some species of bees (*Callonychium petuniae* Cure & Wittman), wasps [*Xiphydria camelus* (L.)], and butterflies [*Lycaena* spp., *Pieris* spp., *Papilio xuthus* (L.), *Spodoptera exempta* (Walker), and *Mamestra brassicae* (L.)] behaviorally respond to wavelengths between 340 and 660 nm, which includes a receptor specific for the red wavelength. These species have no behavioral or ecological correlations. Spectral peaks and color light attraction of above-mentioned species and *Lu. shannoni* in our studies ranged between the same wavelengths. Therefore, our studies, along with those of Burkett et al. (2007) and Hoel et al. (2007) suggest the likely presence of red receptors in sand flies and the need for more definitive laboratory studies. However, behavioral responses do not always prove the existence of true color vision (Menzel 1979). Future studies on the molecular biology of sand fly photoreceptor pigments may serve to provide a better understanding of the physiological basis of vision as it relates to behavior.

The intensity of the LEDs used in our study differed and although it could be argued that this interfered with our observations, it seems that the attraction patterns of *Lu. shannoni* and *Lu. vexator* were not altered by light intensity as lower intensity (650 mcd) green light and higher intensity (1500 mcd) blue light traps captured similar numbers of *Lu. shannoni* (493 and 500) and *Lu. vexator* (157 and 156), respectively. Hoel et al. (2007) documented similar observations for *P. papatasi* attraction to varied light intensity.

The attraction pattern of *Lu. vexator* was the reverse of that observed for *Lu. shannoni*, where red light collected the fewest *Lu. vexator.* In our studies, *Lu. vexator* was captured more often in treatments containing multiple LED colors than with single color-affixed traps. This indicates that combined wavelengths, consisting of broader spectral bands, achieve greater attraction with *Lu. vexator.* Another explanation for these results may be the production of short-wavelength violet light at the mixing point of the blue and red lights in paired treatments compared with the longer wavelength of individual blue and red lights. This would indicate *Lu. vexator* has a preference for shorter wavelengths. However, it is not known what wavelength patterns are produced at the combination point of blue and red LED wavelengths, and we did not measure LED light emission spectra on reflected surfaces; therefore, we can only speculate. Longer wavelength red light (660 nm) captured fewer *Lu. vexator* compared with the shorter wavelength blue (470 nm) and green (502 nm) lights, showing analogous attraction behavior to other dipterans.

Mellor et al. (1996) reported that spectral sensitivity of *Lu. longipalpis* was similar to that of *Ae. aegypti* and *G. m. morsitans.* Comparing *Lutzomyia* spp. attraction with *Ae. aegypti,* and *G. m. morsitans* attraction, our results indicated that *Lu. shannoni* attraction patterns do not correspond to spectral sensitivities of *Aedes aegypti* (L.) and *G. m. morsitans.* However, wavelength attraction patterns of hematophagous insects may not correspond to their spectral sensitivities (Burkett and Butler 2005). Thus, *Lu. shannoni* may possess similar spectral sensitivities but varied behavioral attraction from *Lu. longipalpis, Ae. Aegypti,* and *G. m. morsitans.*

Hoel et al. (2007) suggested that a reflected light triggered *P. papatasi* response to background contrast over the transmitted light. In their study, CDC light traps modified with LEDs directed toward the aluminum rain shield (reflected light) collected more sand flies than traps with the LED directed away from traps (transmitted light). Burkett and Butler (2005) suggested that *Ae. aegypti* used reflected light for resource location over transmitted light. Thus, we expected that broader spectrum-producing lights (red-blue-green) would work better than narrow spectrum lights and the red-blue light on reflected surfaces would result in added wavelength spectrums as the light bands were deflected, yielding a higher capture of sand flies over transmitted light. In converse, it also was expected that narrow and bright beams of light produced by the LEDs would be visible from a further distance and would result in higher trap captures. However, regardless of adaptation, there were no significant differences in the number of sand flies captured between reflected and transmitted light.

Higher numbers of *Lu. shannoni* were captured in the treatments containing CO_2 versus treatments without CO_2, indicating that CO_2 is an effective attractant. Furthermore, CO_2, when combined with other cues improved trap efficacy. Treatments using color LEDs+RM (1-octen-3-ol + 1-hexen-3-ol)+CO_2 captured more *Lu. shannoni* than did CO_2 alone or RM+LEDs without CO_2, indicating an additive action of RM and CO_2. In addition, the additive action of CO_2 with RM was also observed in the olfactory evaluation experiments for both species. CO_2 is the main volatile kairomone released by vertebrates, and it is used by most hematophagous insects to find their hosts (Eiras 2001), whereas octenol is a constituent of human odor (Bernier et al. 2000). Andrade et al. (2008) demonstrated higher *Lutzomyia intermedia* (Lutz & Neiva) attraction to octenol than synthetic human odor emitted by the BG-Mesh Lure (BGML-lactic acid, caporic acid, and ammonia) product. Brinson et al. (1992) collected up to 188 adult *Lu. shannoni* per trap night with supplemental CO_2-baited CDC traps but collected no adults without CO_2 supplementation at Ossabaw Island, Georgia. The combination of octenol and CO_2 captured more *P. papatasi* than octenol alone (Beavers et al. 2004). The combination of light, octanol, and CO_2 captured significantly more *Anopheles farauti* Laveran than CO_2 and light alone (van den Hurk et al. 1997).

L-Lactic acid, a component present in human breath and on human skin, is only slightly effective by itself but acts synergistically with CO_2 and other components from human skin for *Ae. aegypti* attraction (Geier et al. 1996). Using AD in conjunction with CO_2 doubled the capture of *Lu. vexator* over CO_2 alone, whereas CO_2 action was synergistic to AD for *Lu. shannoni* collection, increasing capture by five-fold.

Lu. shannoni and *Lu. vexator* were largely unaffected by other attractants. The mixtures we examined have been successfully used to attract *Ae. aegypti* in olfactometer trails (J.F.B., unpublished data). Surprisingly, these compounds had little or no effect on *Lu. shannoni* and *Lu. vexator.* One explanation for inefficiency of these compounds to *Lutzomyia* spp. can be the lack of dose standardization of constituent compounds. All high-level attractants (0.5 g per trap), in combination with CO_2, captured higher numbers of *Lu. shannoni* compared with CO_2 alone, but the number captured declined when the same attractants were used at a lower level (0.1 g per trap). *Lu. intermida* showed similar dose dependent response to the BG-Mesh Lure blend containing octenol and lactic acid, caproic acid and ammonia (Andrade et al. 2008). Only with BR did the capture of *Lu. shannoni* increase with the lower rate. Higher release rates of some attractant compounds have been shown to repel tsetse flies and face flies, *Musca autumnalis* (De Geer) (Vale and Hall 1985, Birkett et al. 2004). However, there are several other factors, including temperature, wind turbulence, heat, water vapor, sound, CO_2 level, resource density and dispersion, and presence of other species, that can influence host-searching behavior of sand flies in the field (Bell 1990, Lefevre et al. 2006). Therefore, our studies suggest that attraction of *Lutzomyia* spp. to colors and odor attractants is species specific. Colors demonstrating higher attraction, such as red and red-blue, could be combined with odor attractants such as Red Mix and CO_2 to improve trapping systems.

Lu. shannoni was the predominant species captured among the two sand fly species. We did not record any other *Lutzomyia* spp., although two other species, *Lutzomyia cubensis* (Fairchild & Hertig), and *Lutzomyia cruciata* (Coquillett), also are known to occur in Florida (Young and Perkins 1984).

Acknowledgments

We thank Uli Bernier and Daniel Kline for providing the RM attractant and San Felasco Hammock Preserve State Park, Gainesville, FL, for access to the study sites. We also thank R. Hammel, L. Wood, J. Pecora, J. Matta, S. Nunez and E. Fitzgerald for assistance with this study. We thank Sandy Allan for review of this manuscript. This study was funded under U.S. Armed Forces Pest Management Board grant number W9113M-06-S-001 and Florida State Project grant FLA-ENY-04616.

References Cited

Affeldt, H. A., R. W. Thimijan, F. F. Smith, and R. E. Webb. 1983. Response of the greenhouse whitefly and the vegetable leafminer to photospectra. J. Econ. Entomol. 76: 1405–1409.

Andrade, A. J., M. R. Andrade, E. S. Dias, M. C. Pinto, and A. E. Eiras. 2008. Are light traps baited with kairomones effective in the capture of *Lutzomyia longipalpis* and *Lutzomyia intermedia*? An evaluation of synthetic human odor as an attractant for phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae). Mem. Inst. Oswaldo Cruz 103: 337–343.

Beavers, G. M., H. A. Hanafi, and E. A. Dykstra. 2004. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for *Phlebotomus papatasi* (Diptera: Psychodidae) in southern Egypt. J. Am. Mosq. Control Assoc. 20: 130–133.

Bernier, U. R., D. L. Kline, C. E. Schreck, D. R. Barnard, and R. A. Yost. 2000. Chemical analysis of human skin emanations: comparison of volatiles from human that differ in attraction of *Aedes aegypti* (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 18: 186–195.

Bell, W. J. 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35: 447–467.

Birkett, M. A., N. Agelopoulos, K.M.V. Jensen, J. B. Jespersen, J. A. Pickett, H. J. Prijs, G. Thomas, J. J. Trapman, L. J. Wadhams, and C. M. Woodcock. 2004. The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. Med. Vet. Entomol. 18: 313–322.

Bray, D. P., and J.G.C. Hamilton. 2007. Host odor synergizes attraction of virgin female *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Entomol. 44: 779–787.

Briscoe, A. D., and L. Chittka. 2001. The evolution of color vision in insects. Annu. Rev. Entomol. 46: 471–510.

Brinson, F. J., D. V. Hagan, J. A. Comer, and D. A. Strohlein. 1992. Seasonal abundance of *Lutzomyia shannoni* (Diptera: Psychodidae) on Ossabaw Island. Georgia. J. Med. Entomol. 29: 178–182.

Burkett, D. A., and J. F. Butler. 2005. Laboratory evaluation of colored light as an attractant for female *Aedes aegypti, Aedes albopictus, Anopheles quadrimaculatus,* and *Culex nigripalpus.* Fla. Entomol. 88: 383–389.

Burkett, D. A., R. Knight, J. A. Dennett, V. Sherwood, E. Rowton, and R. E. Coleman. 2007. Impact of phlebotomine sand flies on U.S. military operations at Tallil Air Base, Iraq: 3. Evaluation of surveillance devices for the collection of adult sand flies. J. Med. Entomol. 44: 381–384.

Byrne, D. N., T. S. Bellows, and M. P. Parrella. 1990. Whiteflies in agricultural systems, pp. 227–261. *In* D. Gerling [ed.] Whiteflies: their bionomics, pest status and management. Intercept Ltd., Andover, United Kingdom.

Comer, J. A., D.E.J. Stallknecht, L. Corn, and V. F. Nettles. 1991. *Lutzomyia shannoni* (Diptera: Psychodidae): a biological vector of the New Jersey serotype of vesicular stomatitis virus on Ossabaw Island, Georgia. Parassitologia 33 (Suppl. 1): 151–158.

Coombe, P. E. 1981. Wavelength specific behaviour of the whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). J. Comp. Physiol. 144: 83–90.

Desjeux, P. 1996. Leishmaniasis. Public health aspects and control. Clin. Dermatol. 14: 417–423.

Dougherty, M. J., P. M. Guerin, R. D. Ward, and J.G.C. Hamilton. 1999. Behavioral and electrophysiological responses of the phlebotomine sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae) when exposed to canid host odour kairomones. Physiol. Entomol. 24: 241–262.

Dunn, W. J. 1982. Plant communities and vascular flora of San Felasco Hammock, Alachua County, Florida. Ph.D. dissertation, University of Florida, Gainesville.

Eiras, A. E. 2001. Mediadores químicos entre hospedeiros einsetos vetores de doenças médico-veterinárias, pp. 99–122. *In* E. F. Vilela and M.T.D. Lúcia [eds.]. Feromônios de insetos: biologia, química e emprego no manejo de pragas. Editora Holos.

Florida Department of Environmental Protection. 2003. Devil's Millhopper Geological State Park Unit Management Plan 2003. State of Florida Department of Environmental Protection, Division of Recreation and Parks, Tallahassee, Florida. (http://www.dep.state.fl.us/parks/planning/parkplans/DevilsMillhopperGeologicalStatePark.pdf).

Florida State Parks. 2005. Florida State Parks. (http://www.floridastateparks.org/).

Geier, M., H. Sass, and J. Boeckh. 1996. A search for components in human body odor that attract females of *Aedes aegypti,* pp. 71–80. *In* G. R. Bock and G. Cardew [eds.] Olfaction in mosquito-host interactions. Ciba Foundation Symposium 200. Wiley, Chichester, England.

Gibson, G. 1995. A behavioural test of the sensitivity of a nocturnal mosquito, Anopheles gambiae to dim white, red and infra-red light. Physiol. Entomol. 20: 224–228.

Green, C. H. 1984. A comparison of phototactic response to red and green light in *Glossina morsitans morsitans* and *Musca domestica.* Physiol. Entomol. 16: 165–172.

Hoel, D. F., J. F. Butler, E. Y. Fawaz1, N. Watanyl, S. S. El-Hossary, and J. Villinski. 2007. Response of phlebotomine sand flies to light-emitting diode-modified light traps in southern Egypt. J. Vector Ecol. 12: 302–307.

van den Hurk, A. F., N. W. Beebe, and S. A. Ritchie. 1997. Responses of mosquitoes of the *Anopheles farauti* complex to 1-octen-3-ol and light in combination with carbon dioxide in northern Queensland, Australia. Med. Vet. Entomol. 11: 117–180.

Killick-Kendrick, R. 1999. The biology and control of phlebotomine sand flies. Clin. Dermatol. 17: 279–289.

Klein, T. A., D. G. Young, S. R. Young, Jr. Telford, and R. Kimsey. 1987. Experimental transmission of *Plasmodium mexicanum* by bites of infected *Lutzomyia vexator* (Diptera: Psychodidae). J. Am. Mosq. Control Assoc. 3: 154–164.

Kline, D. L., D. V. Hagan, and J. R. Wood. 1994. *Culicoides* responses to 1-octen-3-ol and carbon dioxide in salt marshes near Sea Island, Georgia, U.S.A. Med. Vet. Entomol. 8: 25–30.

Kline, D. L., S. A. Allan, U. R. Bernier, and C. H. Welch. 2007. Evaluation of the enantiomers of 1-octen-3-ol and 1-octyn-3-ol as attractants for mosquitoes associated with a freshwater swamp in Florida, USA. Med. Vet. Entomol. 21: 323–331.

Lawyer, P. G., and D. G. Young. 1987. Experimental transmission of Leishmaniasis to hamsters by bite of phlebotomine sand flies from the USA (Diptera: Psychodidae). J. Med. Entomol. 24: 458–462.

Lefevre, T., J. C. Koella, F. Renaud, H. Hurd, and D. G. Biron. 2006. New prospects for research on manipulation of insect vectors by pathogens. PLoS Pathog. 2: 633–635.

MacVicker, J.A.K., J. S. Moore, D. H. Molyneux, and M. Maroli. 1990. Honeydew sugars in wild-caught Italian phlebotomine sandflies (Diptera: Psychodidae) as detected by high performance liquid chromatography. Bull. Entomol. Res. 80: 339–344.

Mellor, H. E., and J.G.C. Hamilton. 2003. Navigation of *Lutzomyia longipalpis* (Diptera: Psychodidae) under dusk or starlight conditions. Bull. Entomol. Res. 93: 315–322.

Mellor, H. E., J.G.C. Hamilton, and M. Anderson. 1996. Spectral sensitivity in the eyes of male and female *Lutzomyia longipalpis* sand flies. Med. Vet. Entomol. 10: 372–374.

Menzel, R. 1979. Spectral sensitivity and colour vision in invertebrates, pp. 503–580. *In* H. Autrum [ed.]. Handbook of sensory physiology, vol. 7. Springer, Berlin, Germany.

Platt, W. J., and M. W. Schwartz. 1990. Temperate hardwood forests, pp. 194–229. *In* R. L. Myers and J. J. Ewel [eds.]. Ecosystems of Florida. University of Central Florida Press, Orlando, FL.

Santana, A., E. A. Eiras, E. Reginaldo, and R. Cavalcante. 2002. Ecology, behavior and bionomics, Electroantennographic responses of the *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera: Psychodidae) to 1-octen-3-ol. Neotrop. Entomol. 31: 13–17.

SAS Institute. 2003. PROC user's manual, version 9th ed. SAS Institute, Cary, NC.

Schlein, Y., and R. L. Jacobson. 1994. Mortality of *Leishmania major* in *Phlebotomus papatasi* caused by plant feeding of the sandflies. Am. J. Trop. Med. Hyg. 50: 20–27.

Schlein, Y., and G. Muller. 1995. Assessment of plant tissue feeding by sand flies (Diptera: Psychodidae) and mosquitoes (Diptera: Culicidae). J. Med. Entomol. 32: 882–887.

Tesh, R. 1988. The genus *Phlebovirus* and its vectors. Annu. Rev. Entomol. 33: 169–181.

Vale, G. A., and D. R. Hall. 1985. The role of 1-oten-3-ol, acetone, and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae) to ox odor. Bull. Entomol. Res. 5: 209–217.

van Lenteren, J. C., and J. Woets. 1977. Development and establishment of biological control of some glasshouse pests in The Netherlands, pp. 81–87. *In* F. F. Smith and R. E. Webb [eds.], Pest management in protected culture crops. USDA–ARS, AS, ARS-NS-85, Washington, DC.

Wallbanks, K. R., J. S. Moore, L. R. Bennet, R. Soren, D. H. Molyneux, J. M. Carlin, and J. E. Perez. 1991. Aphid-derived sugars in the Neotropical sandfly *Lutzomyia peruensis.* Trop. Med. Parasitol. 42: 60–62.

White, R. H. 1985. Insect visual pigments and color vision, pp. 431–494. *In* G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive insect, physiology, biochemistry and pharmacology. Oxford University Press, New York.

Woets, J., and J. C. Van Lenteren. 1976. The parasite host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *T. vaporariorum* (Homoptera: Aleyrodidae). The influence of the host plant on the greenhouse whitefly and its parasite *E. formosa.* IOBC/WPRS Bull. 4: 151–154.

Young, D. G., and P. V. Perkins. 1984. Phlebotomine sand flies of North America (Diptera: Psychodidae). Mosq. News 44: 263–285.

Received 28 January 2009; accepted 22 May 2009.