EFFECT OF LURES AND TRAP PLACEMENT ON SAND FLY AND MOSQUITO TRAPS

[1]ULRICH R. BERNIER, [2]DAVID F. HOEL, [1]JEROME A. HOGSETTE, JR., [2]HANAFI A. HANAFI, AND [1]DANIEL L. KLINE

[1]USDA-ARS-Center for Medical, Agricultural, and Veterinary Entomology,
Gainesville, Florida, USA
[2]U.S. Navy Medical Research Unit 3, Cairo, EGYPT
e-mail: uli.bernier@ars.usda.gov

Abstract Catches of mosquitoes and sand flies in CO_2 traps baited with three different lures and an unbaited control were compared. The lures examined were carbon dioxide, carbon dioxide plus 1-octen-3-ol, and carbon dioxide plus human hair in ethanol. Studies using a 4 x 4 Latin square design, with 3 sets of 4 consecutive trap nights, were conducted between August 6 and September 10, 2007. The study site was the Bahrif Village, Aswan, Egypt. This location had high percentage of *Phlebotomus papatasi* in sand fly populations, and the low incidence of human cases of leishmaniasis. There were 2,152 sand flies caught over the 12 total nights (48 trap-nights) of this study. Mean numbers of sand flies captured in traps baited with any lure were significantly greater than sand those caught in the unbaited control trap. The mean numbers of sand flies trapped using each lure did not differ significantly.
Key Words *Phlebotomus papatasi*, surveillance, monitoring, attractants

INTRODUCTION

Phlebotomine sand flies, especially *Phlebotomus papatasi* (Scopoli), in the Middle East are responsible for transmission of cutaneous leishmaniasis to humans. These insects are sensitive to environmental conditions that will impact their host-seeking behaviour, such as wind speed and location. They are nocturnal in their biting activities (e.g. bimodal) that may vary from region to region and among species (Hoogstraal et al., 1962; Killick-Kendrick, 1999; Guernaoui et al., 2006). One of the complicating issues with this vector is that common control methods have not been as effective in preventing disease transmission in recent years (Maroli and Khoury, 2006; Orshan et al., 2006). Accurate surveillance of these disease vectors is therefore essential to evaluate local populations for calculating disease risk in a local area and in the assessment of control measures (Alexander and Maroli, 2003).

The Centers for Disease Control (CDC) light trap is used often in trapping studies of sand flies (Burkett et al., 2007; Hanafi et al., 2007; Hoel et al., 2007). We used American Biophysics Mosquito Magnet-X (MMX) (Woodstream, Lititz, PA; obtained originally from American Biophysics, East Greenwich, RI) traps in Bahrif, Aswan, Egypt, with and without carbon dioxide, and in combination with additional chemical lures to determine if these combinations enhanced trap collections. Previous studies indicate that carbon dioxide can enhance trap collections (Alexander, 2000; Veronesi et al., 2007). The compound 1-octen-3-ol is known to augment trap collections with mosquito species (Takken and Kline, 1989), and *Lutzomyia* spp. sand flies were attracted to the combination of human odors and carbon dioxide in laboratory (Oshaghi et al., 1994; Rebollar-Tellez et al., 1999) and field studies (Pinto et al., 2001). Bahrif was selected as a site for field experimentation because sand fly population history in the area is known, the predominant sand fly species in this area (> 93%) is *Phlebotomus papatasi*, and the incidence of leishmaniasis in the local sand fly population is practically negligible (Hoel et al., 2007), similar to reasons for studies of these flies in the Sinai (Hanafi et al., 2007).

MATERIALS AND METHODS

Trap Sites
Trapping experiments were conducted using 3 sets of a Latin square design with four treatments rotated through four locations / sites over four nights of trapping. The treatment-trap combinations consisted of an unbaited trap with no CO_2, one with CO_2, one with 1-octen-3-ol and CO_2, and one baited with human hair

in ethanol. The three sets of trials/sets were run on nights over the following days: August 7-10, August 24-27, and September 10-14, 2006 in Bahrif village, Aswan, Egypt. The distance of the closest site (2) to the Nile River bank was 324 m inland. Traps were placed at > 10 m apart. For our study, the distance between trap sites 1 and 2 was 25 m, and this was the smallest distance between any two traps. Trap site 3 (centrally located relative to the other sites) was 82 m from site 2, 84 m from site 4, and 104 m from site 1. Site 4 was the located farthest North and was 166 m from site 1 and 186 m from site 2. Trap nights began around 1700 local time each evening and concluded in the morning at approximately 0700.

Lures

Carbon dioxide was delivered via placing ~1 kg dry ice in Igloo thermos containers (~2 L) (J.W. Hock, Gainesville, FL). The 1-octen-3-ol solution (95% v/v in acetone) was prepared as a 100 ml stock solution comprised of 5 ml of reagent grade 1-octen-3-ol and the remainder acetone in a volumetric flask. In the field, approximately 5 ml of the stock solution was transferred to a 5 ml reaction vial and sealed with a cap containing a rubber septum. The septum was pierced by a 23 gauge pointed Luer needle to allow for evaporation rates of approximately 0.5 ml/trap night (range of 0.2-2.0 ml). The human hair sample (approximately 2.54 cm^2) was clipped or shaved off an individual daily and placed into a 5 ml reaction vial. To this, absolute ethanol was added to bring the total volume to 5 ml in the vial. The vial was sealed with a cap containing a rubber septum. The septum was pierced from the inside by a 23 gauge pointed Luer needle to allow for evaporation rates of approximately 0.5 ml/trap night (range of 0.2-2.0 ml).

The vials containing solution were hung at the base of the outlet of the MMX trap using cotton string of ~0.32 cm diameter such that the needles were inside the trap outlet and directly in the air flow. For the two traps that were unbaited, empty reaction vials were used in place of those with solution. The trap assembly was suspended using a fiber cord, such that the CFG air flow exit of the MMX was about 0.67 m above the ground. When possible, cords were tied to palm trees in the natural environment, or Shepherd's hooks (~2 m tall) were used when palm trees were not feasible for trap suspension.

Evaluations

All collections were performed in the morning so we could estimate the amount of solution that had evaporated overnight. This was expressed as a rough approximation by estimating the volume lost. The vials were then placed near the trap location selected for the upcoming evening test and these were replenished with fresh solution in the afternoon. To the octenol vial, we just added additional solution to bring the volume back up to 5 ml. The hair/ethanol in the second vial was completely replaced.

The trap assemblies with CO_2 dispensing containers were transported as a single assembly to a sheltered worksite to remove the trapped insects. The remaining dry ice from the dispenser was removed and placed in a large plastic trash bag (~144 L volume). The trap assembly containing insects was also placed in the same bag and the bag was sealed. Each filled bag was set aside for 15-20 min to knock down all insects in the trap. After this period, the trap assembly was removed and insects aspirated using a hand-held aspirator (Hausherr's, Toms River, NJ). The collected insects were placed into 20 mL vials for transportation and later identification under a microscope.

Data Analysis

All data were log_{10} (n+1) transformed prior to data analysis. The data were analyzed for treatment (no CO_2, CO_2, CO_2 + 1-octen-3-ol, CO_2 + human hair), site location (1-4), and time effects (repetitions 1-12) using 3-way ANOVA (SAS Institute, 2003). Means in treatment catches were separated with Duncan's Multiple Range Test (α = 0.05) (SAS Institute, 2003).

RESULTS AND DISCUSSION

Lures

Significant differences were present in the main effects of the model (F=7.35; df=17, 47; $P < 0.0001$). There were highly significant differences between the lures used (F=19.98; df=3, 47; $P < 0.0001$) and between the sites of the study (F=17.13; df=3, 47, $P < 0.0001$). As expected in a Latin square design, significant differences were not observed over time (between repetitions) of this study (F=1.23; df=11, 47; P=0.3097). A total of 2152 sand flies were collected during the 3 tests (Table 1). The only significant

difference observed was that for an unbaited trap compared to a trap containing CO_2, with or without additional baits. Alexander (2000) noted that the use of CO_2 in studies increased the distance, and therefore numbers of sand flies attracted to a trap. It was disappointing that although the trap baited with CO_2 and human hair caught the highest numbers of sand flies, the catch was not significantly different than CO_2 + octenol, nor simply CO_2 alone.

Table 1. Total and mean numbers of sand flies captured with each lure at each site.

Treatment	Total	Mean ± SE[1]
Hair + CO_2	760	63.33 ± 13.68a
Octenol + CO_2	607	50.58 ± 9.49a
CO_2	608	50.67 ± 8.89a
Unbaited	177	13.92 ± 2.97b
Total	2152	

[1]Values with the same letter are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range Test (SAS Institute, 2003).

Location

There were no significant differences in catches for site location 1 compared to location 2, nor for site 3 compared to site 4. However, significant differences existed between each of these pairs, (1, 2) compared to (3, 4). This was expected based on the site locations; sites 1 and 2 were located in one section of the village where significant goat and human activity occur, sites 3 and 4 were more remotely located. Site 3 was located farthest away from human dwellings, across a field used for trash disposal which quite often contained goats feeding in this area during daylight hours. The site was located near vegetation next to an irrigation stream used by inhabitants of the village. Site 4 was located between dwellings, also near this irrigation stream, but in an area where there was less trash and lower rates of traverse by livestock. One reason that goats were expected to play some role in location of increased sand fly populations for trapping is based on findings by Johnson et al. (1993) that goats (and sheep) were the most attractive animal baits for studies with *Phlebotomus guggisbergi* in Kenya. Additionally, sites 1 and 2 were the most "enclosed" in a courtyard type area with constructed walls around the perimeter. Possibly, this may be partly due to the same parameters that resulted in larger collections of *Phlebotomus papatasi* in enclosed spaces (bunkers) than out in the open desert (Hanafi et al., 2007). Therefore, one of the reasons for differences in trap catch in our study may be for similar reasons.

Table 2. Numbers of Diptera other than sand flies captured using various lures.

Species / Treatment	*Ochlerotatus caspius*	*Culex Perexiguus*	*Culex pipiens*	*Culex antennatus*	*Culex poicilipes*	*Anopheles pharoensis*	*Musca crassirostris*
Hair + CO_2	227	12	29	9	2	0	41
Octenol + CO_2	386	13	71	19	0	2	59
CO_2	388	73	69	18	3	3	57
Unbaited	4	5	5	1	0	2	1
Total	1015	103	174	47	5	7	158

Other Flies

In addition to sand flies, mosquitoes and Muscid flies were also trapped (Table 2). *Ochlerotatus caspius* Pallas were collected in greatest numbers (227-388 summed over all trials) in traps that employed lures. *Musca crassirostris* Stein was also collected in traps baited with lures. In order of decreasing numbers caught were *Culex pipiens* (L.), *Cx. perexiguus* Theobald, Cx. *antennatus* Becker, *Anopheles pharoensis* Theobald, and *Cx. poicilipes* Theobald.

CONCLUSIONS

It was clear that the use of any chemical lure, including carbon dioxide alone, resulted in trapping of sand flies at levels significantly greater than those collected in an unbaited MMX trap. The Latin square design compensated for time effects in the data, and there were significant differences in site location. The addition of odors and chemicals thought to be host-specific did not significantly increase the collection of sand flies in traps over numbers caught by release of CO_2 alone. Future experiments will involve additional lures, trap placement effects on catch, and examination of the use of diodes, in conjunction with optimal lures and trap placements, to enhance trap collection.

ACKNOWLEDGMENTS

REFERENCES CITED

Alexander, B. 2000. Sampling methods for phlebotomine sandflies. Med. Vet. Entomol. 14:109-122.

Alexander, B. and Maroli, M. 2003. Control of phlebotomine sandflies. Med. Vet. Entomol. 17:1-18.

Burkett, D.A., Knight, R., Dennett, J.A., Sherwood, V., Rowton, E., and Coleman, R.E. 2007. J. Med. Entomol. 44:381-384.

Guernaoui, S., Boussaa, S., Pesson, B., and Boumezzough, A. 2006. Nocturnal activity of phlebotomine sandflies (Diptera: Pyschodidae) in a cutaneous leishmaniasis focus in Chichaoua, Morocco. Parastiol Res. 98:184-188.

Hanafi, H.A., Fryauff, D.J., Modi, G.B., Ibrahim, M.O., and Main, A.J. 2007. Bionomics of phlebotomine sandflies at a peacekeeping duty site in the north of Sinai, Egypt. Acta Tropica 101:106-114.

Hoel, D.F., Butler, J.F., Fawaz, E.Y., Watany, N., El-Hossary, S.S., and Villinski, J. 2007. Response of phlebotomine sand flies to light-emitting diode-modified light traps in southern Egypt. J. Vector Ecol. 32:302-308.

Hoogstraal, H., Dietlein, D.R., and Heyneman, D. 1962. Leishmaniasis in the Sudan Republic: 4. Preliminary observations on man-biting sandlfies (*Psychodidae: Phlebotomus*) in certain Upper Nile endemic areas. Trans. Roy. Soc. Trop Med. Hyg. 56:411-422.

Johnson, R.N., Ngumgi, P.M., Mwanyumba, J.P., and Roberts, C.R. 1993. Host feeding preference of *Phlebotomus guggisbergi*, a vector of *Leishmania tropica* in Kenya. Med. Vet. Entomol. 7:216-218.

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

Maroli, M. and Khoury, C. 2006. Current approaches to the prevention and control of leishmaniasis vectors. Vet. Res. Commun. 30:49-52 (Suppelement 1)

Orshan, L., Szekely, D., Schnur, H., Wilamowski, A. Garler, Y., Bitton, S., and Schlein, Y. 2006. Attempts to control sand flies by insecticide-sprayed strips along the periphery of a village. J. Vector Ecol. 31:113-117.

Oshaghi, M.A., McCall, P.J., and Ward, R.D. 1994. Response of adult sandflies, *Lutzomyia longipalpis* (Diptera: Pyschodidae), to sticky traps baited with host odour and tested in the laboratory. Ann. Trop. Med. Parasitol. 88:439-444.

Pinto, M.C., Campbell-Lendrum, D.H., Lozovei, A.L., Teodoro, U., and Davies, C.R. 2001. Phlebotomine sandfly responses to carbon dioxide and human odour in the field. Med. Vet. Entomol. 15:132-139.

Rebollar-Tellez, E.A., Hamilton, J.G.C., and Ward, R.D. 1999. Response of female *Lutzomyia longipalpi*s to host odour kairomones from human skin. Physiol. Entomol. 24:220-226.

SAS Institute. 2003. SAS/STAT User's Guide, version 9.1, Cary, NC: SAS Institute.

Takken, W. and Kline, D.L. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. J. Am. Mosq. Control Assoc. 5:311-316.

Veronesi, E., Pilani, R., Carrieri, M., and Bellini, R. 2007. Trapping sand flies (Diptera: Psychodidae) in the Emilia-Romagna region of northern Italy. J. Vector Ecol. 32:313-318.