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EVALUATION OF 1-OCTEN-3-OL AND CARBON DIOXIDE AS ATTRACTANTS FOR *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) IN SOUTHERN EGYPT[1,4,5]

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ABSTRACT. The effectiveness of 1-octen-3-ol (octenol) as an attractant for collecting medically important psychodids has never been reported. This study evaluated the effects of carbon dioxide (CO_2) and octenol released at 2 rates, individually and in combination, as attractants for adult sand flies in a small village in southern Egypt. Four sand fly species were collected: *Phlebotomus papatasi, P. sergenti, Sergentomyia palestinensis,* and *S. schwetzi.* Only *P. papatasi* was collected in numbers sufficient to allow statistical analysis. This study reaffirms that CO_2 is an effective attractant for female *P. papatasi* and also demonstrates that neither male nor female *P. papatasi* respond to octenol alone. Additionally, no synergistic attractancy for either females or males was observed when CO_2 and octenol were combined.

KEY WORDS 1-Octen-3-ol, carbon dioxide, attractants, *Phlebotomus papatasi,* Egypt

INTRODUCTION

The chemical 1-octen-3-ol (octenol) has been evaluated throughout the world as a potential attractant for a wide variety of hematophagous Diptera, including tsetse flies (Hall et al. 1984, Vale and Hall 1985), mosquitoes (Takken and Kline 1989, Kline et al. 1990, Kemme et al. 1993, Rueda et al. 2001), tabanids (French and Kline 1989, Nilssen 1998), bot flies (Anderson 1989), and biting midges (Kline et al. 1994, Ritchie et al. 1994). The combination of CO_2 and octenol also produces a synergistic response in some mosquito species (Kline et al. 1990, 1991) and in some *Culicoides* spp. (Kline et al. 1994, Ritchie et al. 1994). However, not all biting fly species are attracted to octenol. Kline and Mann (1998) reported that *Culex* spp. mosquitoes are not attracted to octenol alone or in combination with CO_2. Becker et al. (1995) also reported negative results in the Upper Rhine Valley, Germany, and Beavers et al. (1998) found that octenol was an unsuitable attractant for *Aedes caspius* (Pallus) and *Anopheles sergenti* (Theobald) in northern Egypt. Octenol has also been reported as an ineffective attractant for black flies (Simuliidae) (Atwood and Meisch 1993).

No studies have been published determining the response of sand flies to octenol. In Egypt, the most prevalent medically important sand fly species is *Phlebotomus papatasi* (Scopoli). This species is a major vector of *Leishmania major* Friedlin, the causative agent of Old World cutaneous leishmaniasis, as well as several arboviruses. There are a wide variety of methods currently used to collect adult sand flies in the field. In our efforts to collect live sand flies for arbovirus isolations and related studies, we have had the most consistent success with unlit Centers for Disease Control (CDC) light traps baited with dry ice as an attractant (Hanafi et al., unpublished data). Although relatively inexpensive and effective in trapping sand flies, dry ice as a CO_2 source can often be logistically impractical. It can be difficult to obtain and cumbersome to use and transport, and despite storage inside high-efficiency coolers, it survives poorly in the field, particularly in the desert environment of the Middle East. Small CO_2 tanks or canisters are often difficult to use in the field for many of the reasons described above, and they are also highly susceptible to theft. An alternative attractant that is effective without the negative aspects of dry ice would greatly enhance sand fly–borne disease research, surveillance, and control operations. In this paper, we present the results of a study that evaluated the response of *P. papatasi* to octenol under field conditions in southern Egypt.

MATERIALS AND METHODS

Field studies were conducted in Bahrif (24°10′0″N; 32°52′54″E), a small village located along the Nile River Valley approximately 15 km north of the city of Aswan and 1,000 km south of Cairo. The village and surrounding vicinity is predominantly agricultural in nature, utilizing an extensive irrigation network for vegetable crop, sugar

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cane, and palm tree production. The area's climate is hot and arid, with average annual rainfall measuring less than 1 cm and average daytime temperatures ranging from 38 to 45°C in June and from 27 to 35°C in October. This area was selected for the study because previous sand fly collections (Hanafi, unpublished data) had shown that relatively high numbers of *P. papatasi* adults were present year round.

Unlit, battery-operated CDC light traps (Sudia and Chamberlain 1962) mounted on 2-m metal poles at a height of approximately 1 m were used at each trap location. Treatments consisted of baiting the traps with either octenol or CO_2 (i.e., dry ice) alone, in 2 combinations (dry ice with a "high" release rate of octenol and dry ice with a "low" release rate of octenol), or with no attractant. Technical-grade octenol (American Biophysics Corp., East Greenwich, RI) was dispensed into and released from 5-ml microreaction vials via a pipe cleaner wick either in contact with, or protruding through, a neoprene septum as described by Kline et al. (1991). During this study, vials with the wick in contact with the septum released octenol at an average rate of 15 ± 3 mg/h and were defined as the low release (LR) rate treatment. Vials with the wick protruding from the septum released octenol at an average rate of 99 ± 9 mg/h and were defined as the high-release (HR) rate treatment. Dry ice was placed inside insulated 3.8-liter paint cans that were positioned immediately above the light trap opening. The average CO_2 release rate throughout the study was 71 ± 1 g/h.

The treatments for the field trials included: 1) no attractant, 2) dry ice (CO_2) alone, 3) LR octenol alone, 4) HR octenol alone, 5) LR octenol + CO_2, and 6) HR octenol + CO_2. A 6 × 6 Latin square design (Cochran and Cox 1957) was used, and the traps were rotated over 6 consecutive nights so that each treatment would occupy each of the 6 positions for a single night (total trap nights = 36). Traps were placed throughout the village no closer than 75 m apart to minimize the overlapping of plumes from the attractants. Traps were operated from approximately 1800 to 0630 h. Sand flies were removed from traps with battery-operated handheld aspirators and placed in vials that were then dropped into portable liquid nitrogen storage tanks. The specimens were brought to the laboratory, where they were mounted on microscope slides, sexed, and identified to species, and the results were tabulated. The initial trial was conducted in October 1998 and replicated in June 1999. Data were combined for analyses. The raw collection data were transformed to $\log(n + 1)$, and the effects of attractant, trap location, and date were evaluated by a 3-way ANOVA.

RESULTS

During the 2, 6-night trials, resulting in a total of 72 trap nights (36 trap nights/trial), 909 sand flies representing 4 species were collected: *P. papatasi, P. sergenti* Parrot, *Sergentomyia palestinensis* Adler and Theodor, and *S. schwetzi* Adler, Theodor and Parrot. *Phlebotomus papatasi* constituted approximately 95% (859/909) of all specimens collected, and of the 859 specimens, 394 were females and 465 males. As a result, only the *P. papatasi* collection data were analyzed (Table 1).

Phlebotomus papatasi females were significantly ($P < 0.05$, Tukey's studentized range test) more attracted to traps baited with dry ice than those baited with octenol alone. No significant differences were observed between the control traps and those baited with octenol alone. The statistical difference between control traps and those with the CO_2 + LR octenol combination was marginal ($P \leq 0.052$), whereas the difference between the control traps and those with the CO_2 + HR octenol combination was highly significant ($P \leq 0.01$). Females were also much more attracted to traps with CO_2 and octenol than octenol alone ($P \leq 0.01$). However, no synergistic effects between CO_2 and octenol for attracting female *P. papatasi* were observed.

For male *P. papatasi,* no significant differences were observed between controls and baited traps. However, dry ice was a significantly better ($P \leq 0.05$) attractant than octenol alone. Synergistic effects between CO_2 and octenol for attracting males also were not observed. In addition, no statistically significant differences were observed between the numbers of female and male sand flies collected with regard to either location or trap night.

DISCUSSION

This study reaffirms that CO_2 is an effective attractant for female *P. papatasi* and clearly demonstrates that male and female *P. papatasi* do not respond, either positively or negatively, to octenol. For male *P. papatasi,* the large difference in the average number of males collected between control traps and those baited with dry ice, although not statistically significant, indicate that males might also be attracted to CO_2. This possibility merits further testing.

Three other sand fly species were also collected during this study: *P. sergenti, S. palestinensis,* and *S. schwetzi.* The collection of several *P. sergenti* is significant because it was the 1st evidence that this medically important species had expanded its range into the southern Nile River Valley of Egypt (Hanafi et al. 2001). This sand fly is a known vector of *Leishmania tropica* (Ashford and Bettini 1987, Al Zahrani et al. 1988). *Leishmania tropica* is the causative agent of anthroponotic cutaneous leishmaniasis (ACL) or urban cutaneous leishmaniasis (CL), as well as the visceralizing form of leishmaniasis (Magill et al. 1993). Very little is known of the biology and ecology of either *S. palestinensis* or *S. schwetzi.* Sand fly collections in Senegal indicate that *S. schwetzi* inhabit termite mounds, an-

Table 1. Mean (SE) response of sand flies collected by unlit CDC light traps with no bait, CO_2 alone, octenol alone released at a low rate (LR), octenol alone released at a high rate (HR), CO_2 plus octenol (LR), and CO_2 plus octenol (HR) in Bahrif, Aswan Governorate, Egypt.[1]

			Attractant			
Species (sex)	Control	CO_2	Octenol (LR)	Octenol (HR)	CO_2 + octenol (LR)	CO_2 + octenol (HR)
Phlebotomus papatasi (F)	3.67 (1.12) A	10.75 (4.00) B	1.67 (0.61) A	1.50 (0.42) A	6.17 (1.67) AB	9.17 (1.86) B
Phlebotomus papatasi (M)	4.00 (1.32) A	14.83 (6.94) AC	3.08 (1.08) AB	2.58 (0.84) AB	7.75 (2.34) ABC	6.50 (1.46) AC
Phlebotomus sergenti (F)[2]	0	0.08 (0.08)	0	0.08 (0.08)	0.16 (0.11)	0
Phlebotomus sergenti (M)[2]	0	0	0	0	0.08 (0.08)	0
Sergentomyia palestinensis (F)[2]	0.33 (0.19)	0	0.17 (0.17)	0.17 (0.11)	0.25 (0.25)	0.17 (0.17)
Sergentomyia palestinensis (M)[2]	0	0	0	0.08 (0.08)	0.08 (0.08)	0
Sergentomyia schwetzi (F)[2]	0.17 (0.11)	0.50 (0.42)	0.08 (0.08)	0.17 (0.11)	0	0.33 (0.19)
Sergentomyia schwetzi (M)[2]	0.33 (0.11)	1.25 (0.90)	0	0	0.08 (0.08)	0

[1] n = 12 trap nights per treatment. Means in the same row followed by the same letter are not significantly different ($P > 0.05$).
[2] No statistical analyses conducted because of low numbers.

imal burrows, and rock crevices (Ba et al. 1998, 1999). Investigations on the ability of *S. schwetzi* to vector *L. major* found that the parasites did not develop beyond the procyclic promastigote stage, nor did they survive beyond 90 h in the sand fly, leading to the conclusion that *S. schwetzi* is not a vector of *L. major* (Lawyer et al. 1990).

Other published studies clearly indicate that not all host-seeking hematophagous diptera are attracted to octenol. Species even within the same taxonomic family, such as the Culicidae, respond differently to octenol or to octenol combined with CO_2 (Takken and Kline 1989, Kemme et al. 1993, Van Essen et al. 1994, Becker et al. 1995, Beavers et al. 1998, Kline and Mann 1998, Rueda et al. 2001). For these reasons, although we found octenol to be an ineffective attractant for *P. papatasi,* it should still be evaluated against other medically important sand fly species in both the New and Old World.

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