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Octenol as Attractant to *Nyssomyia neivai* (Diptera: Psychodidae: Phlebotominae) in the Field

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ABSTRACT The kairomone octenol is known as attractive to hematophagous Diptera such as mosquitoes, tsetse flies, and midges. There is little evidence that traps baited with octenol are also effective in attracting phlebotomine sand flies. The present report evaluated octenol in modified Centers for Disease Control and Prevention (CDC) traps in two experiments: 1) modified CDC trap without light and 2) modified CDC trap with light. The traps were baited with octenol at concentrations of 0.5, 27, and 43 mg/h in Rincão locality, São Paulo, Brazil. Traps without octenol were used as controls. The sand fly *Nyssomyia neivai* (Pinto) (=*Lutzomyia neivai*) (Diptera: Psychodidae: Phlebotominae) was the prevalent species (99.9%) in both experiments. The results of the experiments showed that traps baited with octenol at 27 and 43 mg/h caught significantly more *N. neivai* than control and octenol at 0.5 mg/h with and without light. This is the first report that shows that octenol itself is attractive to *N. neivai* and associated with light traps significantly increases the catches.

KEY WORDS *Nyssomyia neivai*, octenol, kairomones, attractiveness, sand flies

Phlebotomine sand flies, vectors of leishmaniasis, are extensively collected in the field with light traps (Alexander 2000). However, the search for volatile attractants such as pheromones or kairomones (host odors), without the presence of light, is a very desirable target considering that phototropism can vary among different phlebotomine sand flies species (Davies et al. 1995). Moreover, the development of traps that attract females in different physiological states is an important tool for monitoring or evaluating control measures.

Sex pheromones have been identified in males of some sand flies species (Brazil and Hamilton 2002). The response of *Lutzomyia longipalpis* (Lutz & Neiva) males and females to natural and synthetic male sex pheromone has been evaluated in laboratory (Morton and Ward 1989, Spiegel et al. 2005) and in the field (Bray et al. 2009). Kairomones from live host (hamsters) and human hand odor extract also were found to be attractive to sand flies (Rebollar-Tellez et al. 1999, Bray and Hamilton 2007). However, only few studies have evaluated the role of the host compounds individually for sand flies attraction (Dougherty et al. 1999, Pinto et al. 2001, Andrade et al. 2008). Octenol (1-octen-3-ol) has been evaluated for attractancy to zoophilic hematophagous Diptera species, showing positive or negative responses depending on the group or the species evaluated (Gibson and Torr 1999). Studies carried out with octenol and Old World sand flies species have not shown signs of attractiveness (Cameron et al. 1991, Beavers et al. 2004), but for New World sand flies it has shown relative attractiveness (Andrade et al. 2008, Mann et al. 2009).

Light traps baited with octenol seem to be more attractive to *Nyssomyia intermedia* (Lutz & Neiva) (=*Lutzomyia intermedia*) than to *L. longipalpis* (Andrade et al. 2008). The species *Nyssomyia neivai* (Pinto) was previously designated as a junior synonym of *N. intermedia* (Young and Duncan 1994). However, some differences have been satisfactory to separate them as distinct species (Marcondes 1996). Recently *N. neivai* was found naturally infected by *Leishmania* species (Pita-Pereira et al. 2009), and it is incriminated as a vector of cutaneous leishmaniasis in some areas of South America (Cordoba-Lanus et al. 2006, Casanova et al. 2009, Marcondes et al. 2009). The current study had the objective of evaluating the possible attraction of octenol, with and without light, to *N. neivai*.

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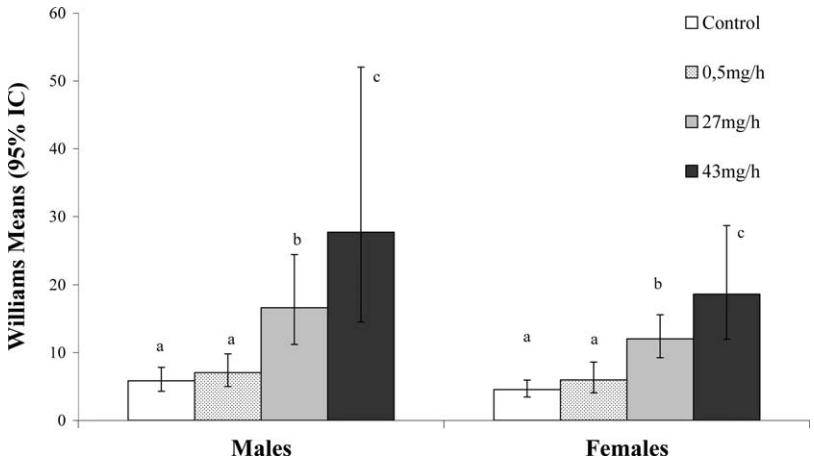

Fig. 1. Williams' means of males and females of *N. neivai* captured at modified CDC traps without light baited with octenol at release rates of 0.5, 27, and 43 mg/h. Controls are CDC traps without light and octenol lure. Means with the same letter (for the same sex) are not significantly different ($n = 12$ traps per treatment).

Materials and Methods

Trap Sites. The experiments were carried out in Rincão (locality of Araraquara), a small town located at northeast center of São Paulo, Brazil, at the edge of the western Paulista Plateau (21° 35′13″ S, 48° 04′15″ W). The area is crossed by the Mogi-Guaçu River, and its landscape is composed of residual forest and sugarcane (*Saccharum* spp.) plantations. The area is used for fishing and is considered endemic for human cutaneous leishmaniasis. *Leishmania braziliensis* (Vianna) was isolated from a local resident (Pinto et al. 2005).

Octenol. The kairomone octenol was released in three rates: 0.5, 27, and 43 mg/h. A commercial polymer (AgriSense BCS, Cardiff, United Kingdom) was used to dispense the lowest octenol release rate (0.5 mg/h). Octenol (≥98.0% pure by gas chromatography; Aldrich Chemical, Milwaukee, WI) was dispensed into and released from microreaction vials. The release rates were calculated by weighing octenol vials at the beginning and the end of the experiment. The release rates were obtained by one (27 ±1.3 mg/h) or two wicks (43 ± 2.1 mg/h) of 2 cm, each extended through a small hole at in the plastic lid according to Van Essen et al. (1994). Octenol dispensers were positioned on the outside of the modified Centers for Disease Control and Prevention (CDC) trap between the fan and the screen. The sand flies species names are in accordance with the classification system of Galati (1995, 2003), followed by the corresponding nomenclature of Young and Duncan (1994) in brackets when cited for the first time.

Experiment 1 (Traps Without Light). During four consecutive nights in January 2006, modified CDC light traps (Pugedo et al. 2005), without the light bulbs, were baited with octenol dispensed at the following release rates: 0.5, 27, and 43 mg/h. A trap without octenol was the control. The treatments were rotated in four positions during four nights following a Latin square design (4 × 4). This experiment was repeated three times during the period (12 traps per night). Traps were set up 20 m apart in a straight line for ≈10 m along the river from 1800 to 0600 hours.

Experiment 2 (Traps With Light). One week later, the same procedure was repeated during four consecutive nights. All the modified CDC traps had their light bulbs on during the experiment. The same positions and the same Latin square design were maintained. The control was the modified CDC trap with light and without octenol.

Statistical Analysis. Data were transformed to $\ln(x + 1)$ and analyzed using General Linear Models in computer package SAS (SAS Institute 1999). The best fit to the model was determined by taking out predictors variables that did not explain it (nights and positions; Crawley 1993). Traps that stopped working during the night were considered as missing data and received the appropriate statistical treatment. The sand fly catch data presented in figures are Williams'-transformed geometric means (Williams 1937).

Results

Trials Without Light. In total, 3,316 sand flies were captured during the first four nights without light using 48 traps (12 traps per treatment), and they were identified as *N. neivai.* The total number of males (2,403) was significantly higher that of females (913). There was an increase in *N. neivai* catches according to the highest octenol rates released for both sexes, and the differences among treatments were statistically significant ($F = 29.00$; $P < 0.0001$). Traps baited with octenol released at rates of 27 and 43 mg/h caught significantly more sand flies than the control and octenol groups at 0.5 mg/h (Fig. 1). Trap positions were significantly different for sand flies catches ($F = 27.27$, $P < 0.0001$).

Trials With Light. In total, 75,805 sand flies were caught during the four nights. Because the total num-

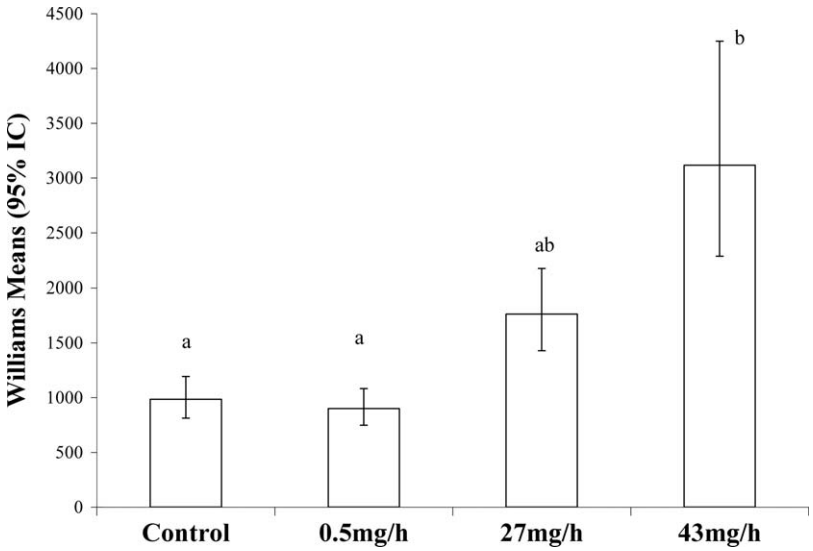

Fig. 2. Williams' means of *N. neivai* captured at modified CDC light trap baited with octenol (0.5, 27, and 43 mg/h). Controls are CDC traps with light. Means with the same letter are not significantly different ($n = 12$ traps per treatment).

ber of sand flies was extremely high, it was determined that only a 20% sample from each trap would be identified to the species level, by using the method of clarifying and analyzing the internal structures. The total of 15,161 (99.99%) were identified as *N. neivai*, and only two specimens (0.01%) of *Brumptomyia* sp. The remainders were observed according to external structures (palps and color of the thorax). If there was any doubt of the specimen not being *N. neivai*, it was prepared for clarification. The results showed the same response pattern of sand flies as in the previous experiment. An increase in the number of sand flies caught with the increase of octenol release rate was observed ($F = 8.40$, $P = 0.0005$) (Fig. 2). In absolute numbers, the highest octenol rate (43 mg/h) collected twice number of sand flies (28,156; Williams' mean = 3,117) than the control (14,029; Williams' mean = 984), and a synergistic effect was observed. Octenol at concentration of 27 mg/h caught almost twice *N. neivai* as many as control (23,296; Williams' means = 1,762). Trap positions also were significantly different ($F = 2.45$, $P = 0.03$).

Discussion

This study shows for the first time that octenol is itself attractive to the *N. neivai* species in field conditions, even in the absence of light. Octenol is a result of the biodegradation of lipids (Walinder et al. 2008), and it can be found in a wide range of biological sources, such as fungi (Matsui et al. 2003), bacteria (Viehweg et al. 1989), bovines and ruminants (Hall et al. 1984, Takken and Knols 1999), human sweat (Bernier et al. 2000), and human breath (Xue et al. 2008). In human breath, octenol can be used as a biomarker of liver cancer because the octenol levels increase in the presence cancer (Xue et al. 2008).

The attraction potential of octenol has been documented for different species of hematophagous insects such as mosquitoes (Kline et al. 1990), midges (Ritchie et al. 1994), tsetse flies, stable flies (Hall et al. 1984, Torr et al. 2006), and recently sand flies (Andrade et al. 2008). Differences in attraction to octenol between zoophilic and anthropophilic insect species has been documented. In the field, octenol is attractive to several zoophilic mosquito species (Kline et al. 1990, Van Essen et al. 1994) and to species of tsetse flies with a preference for bovines. The predominant distribution of the sand fly *N. neivai* extends from the edges of residual gallery forests to peridomiciliary areas (Casanova et al. 2005, Casanova et al. 2009). Such behavioral flexibility requires opportunistic feeding habits to find available hosts (Casanova 2002, Dias-Sversutti et al. 2007).

The predominance of *N. neivai* in the study area corroborated previous surveys carried out in the Mogi Guaçu River Valley region in areas with residual gallery forest (Casanova et al. 2005, 2009; Odorizzi and Galati 2007).

There are few evidences that Old World sand flies do not respond to traps baited with octenol, for example, the species *Phlebotomus argentipes* (Annandale & Brunneti) (Cameron et al. 1991) and *Phlebotomus papatasi* (Scopoli) (Beavers et al. 2004) did not respond to octenol at the release rates ranged from 0.5 to 100 mg/h. However, studies for New World sand fly species have reported the role of octenol as a possible attractant (Andrade et al. 2008). A study that combined visual and olfactory attractants has shown that *Psathyromyia shannoni* (Dyar) (=*Lutzomyia shannoni*) was attracted to octenol with 1-hexen-3-ol and red light-emitting

diode light (Mann et al. 2009). In the current study, we observed a dose-dependent response where there was an increase of *N. neivai* captures with increasing of octenol release rates.

Field experiments using light traps and kairomones (octenol, lactic acid, caproic acid, and ammonia) have indicated that *N. intermedia* is more strongly attracted to octenol than is *L. longipalpis* (Andrade et al. 2008). The attractiveness of *N. neivai*—the other species of the *N. intermedia* complex (Marcondes 1996)—to octenol corroborates Andrade et al. (2008).

All previous studies with traps baited with octenol in the field were associated with light. However, in the current study, octenol alone increased the catch rate in a dose-dependent response, and when light was added to the traps, a strong increase of sand flies captures was observed. The massive number of sand flies, specifically *N. neivai*, collected with octenol in light traps (75,805) compared with octenol without light (3,316) show the importance of visual cues. Such differences are probably due to physiological characteristics of this species because *N. intermedia* (the sister species of *N. neivai*) is highly phototropic (Campbell-Lendrum et al. 1999a). The attractiveness of *N. neivai* to octenol is probably physiologically explained by the presence of specific chemoreceptors on the antennae. Electrophysiological studies with antennae of *L. longipalpis* females showed responses of this species to octenol (Sant'ana et al. 2002). Volatile substances such as octenol may disperse and attract insects in the field to and this depends on wind direction and wind speed. However, the attractive range of kairomones or pheromones for sand flies is ≈2 m (Alexander 2000), whereas the attractive range of light is at least 2.5 m for *N. intermedia* and *Nyssomyia whitmani* (Antunes & Coutinho) (=*Lutzomyia whitmani*) (Campbell-Lendrum et al. 1999b) or even 6 m for *Pintomyia youngi* (Feliciangeli & Murillo) (=*Lutzomyia youngi*) (Valenta et al. 1995).

The modified CDC light traps baited with 43 mg/h octenol showed a synergistic effect (Fig. 2). Twice as many *N. neivai* captures compared with the control (light only) was observed. This increase of the efficiency in collecting sand flies is very desirable for monitoring sand fly populations, and it also can be used to evaluate vector control measures. According to our data, octenol and light are recommended tools to increase *N. neivai* catches.

The current study shows that the pattern of attractiveness was similar in both situations (traps baited with octenol in the presence or absence of light). Further studies should be conducted to evaluate higher release rates higher than 43 mg/h octenol. We concluded that traps baited with octenol alone on field conditions increased captures of *N. neivai* with or without light sources. Studies that evaluate other kairomones associated with octenol could improve trapping systems for *N. neivai* in the field and should be evaluated in the future.

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