ECOLOGY, BEHAVIOR AND BIONOMICS

Electroantennographic Responses of the *Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) to 1-octen-3-ol

ADSON L. SANT'ANA[1], ALVARO E. EIRAS[1] E REGINALDO R. CAVALCANTE[1,2]

[1]*Depto. Parasitologia, Universidade Federal de Minas Gerais, ICB/UFMG, Av. Presidente Antônio Carlos, 6627, Pampulha, 31270-901, Belo Horizonte, MG*
[2]*Depto. Parasitologia e Microbiologia, Universidade Federal do Piauí, CCS/UFPI Campus Ininga, Teresina, PI*

Neotropical Entomology 31(1): 013-017 (2002)

Respostas Eletroantenográficas de *Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) a 1-octen-3-ol

RESUMO – Octenol (1-octen-3-ol) é um cairomônio utilizado por vários grupos de insetos hematófagos para a localização de hospedeiros vertebrados. No entanto, o seu efeito sobre *Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva) nunca foi estudado. O presente trabalho avaliou as respostas eletroantenográficas (EAG) de fêmeas de *L. longipalpis.* Como estímulo controle utilizou-se corrente de ar, pulsos de ar e pulsos de solvente (hexano). As concentrações logarítmicas de 1-octen-3-ol testadas foram de 10 a 10^6 ηg/50μl de solvente. Observaram-se respostas olfativas significativas de 1-octen-3-ol a partir de 10^3ηg/50μl, tendo a maior amplitude na concentração 10^6ηg/50μl (-3,33mV). Foi observada dose-dependência, ou seja, quanto maior a concentração, maior foi a resposta eletrofisiológica. Estes resultados demonstram, pela primeira vez que *L. longipalpis* pode detectar a presença do 1-octen-3-ol em corrente de ar. O possível uso de 1-octen-3-ol como cairomônio é discutido para a espécie.

PALAVRAS-CHAVE: Insecta, quimiorecepção, olfação, semioquímicos, leishmaniose visceral.

ABSTRACT – Octenol (1-octen-3-ol) is a kairomone used by haematophagous insects to locate their vertebrate hosts. However, effect of 1-octen-3-ol on *Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva) has never been studied. The present work evaluated the electrophysiological (EAG) responses of female *L. (Lutzomyia) longipalpis.* Air current, air pulse and solvent (hexane) pulse were used as control stimuli. The logarithmic concentrations of 1-octen-3-ol 10 a 10^6 ηg/50μl of solvent were tested. Significant olfactory responses were observed in the concentration of 1-octen-3-ol from 10^3 ηg/50ul with the greatest response at concentration of 10^6 ηg/50ul (-3,33mV). Dose-dependency was observed, as the concentration increased, so did the electrophysiological response. These results demonstrated, for the first time, that *L. longipalpis*, can detect the presence of 1-octen-3-ol in air current. The possible use of 1-octen-3-ol as kairomone for this species is discussed.

KEY WORDS: Insecta, chemoreception, olfaction, semiochemicals, visceral leishmaniasis.

Kairomones are odors that benefit the receptor organism (ex: haematophagous insect) and are disadvantageous to the emitter (ex: host), in interspecific interactions (Nordland 1984). Kairomones (host-odor) are used by haematophagous insects in the process of searching and locating the host for blood feeding, following a sequence of behavior (Eiras 2001). The 1-octen-3-ol kairomone (octenol) is atractive for the tse-tse fly *Glossina* (*Glossina*) *morsitans* (Westwood) (Diptera: Glossinidae) and was identified from volatile compounds emanated from bovines (Hall *et al*. 1984). The attraction of the tse-tse fly and other haematophagous insects by 1-octen-3-ol baited traps was also reported for mosquitoes (Takken & Kline 1989, Kline *et al*. 1990, Becker *et al*. 1995), black fly (Atwood & Meisch 1993), screwworm (Cork 1994), tabanides (Hayes *et al*. 1993), and culicoides (Kline *et al*. 1994).

The sand fly *Lutzomyia* (*Lutzomyia*) *longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) is a phlebotomine of great importance because it is the main vector of *Leishmania chagasi* (Cunha & Chagas), the etiological agent of the visceral leishmaniases in the New World. Only females possess the haematophagic behavior. It is a species of wide geographic distribution, comprising the North and South Americas, from Mexico to North-Eastern Argentina and Paraguay. The primary hosts and the main reservoirs of the parasite are foxes in the wild environment, and dogs in the peri-domestic habitat. *L. longipalpis* also feeds on pigs, cats, horses, birds, humans and opossums, and the feeding preference varies according to its environment (Dougherty *et al*. 1999).

The biology of *L. longipalpis* in its natural habitat is practically unknown due to its behavior and to the sites of development of the larval phase (Forattini 1973). Adults monitoring is performed by means of light traps, generally the CDC type (Sudia & Chamberlain 1962) or traps baited with birds or small mammals (Disney 1966). Several studies on development of attractants, such as sexual pheromones (Ward *et al*. 1990) or oviposition attractants (El-Naien & Ward 1991), have been carried out in the search for improving the efficiency and specificity of the traps used for monitoring. Up to the present, the use of 1-octen-3-ol as a possible attractant for *L. longipalpis* has not been published.

The electroantennographic (EAG) technique that consists in measuring electrical activities of the insect antennae in response to olfactory stimulus has already been used for decades to evaluate the sensitivity of the chemical receptors to semiochemicals. Up to the present, the effect of 1-octen-3-ol on the chemoreceptors of *L. longipalpis* is unknown. The objective of this research work was to evaluate the electrophysiological responses of *L. longipalpis* females' antennae to 1-octen-3-ol, aiming its use as possible kairomone in traps.

Materials and Methods

***L. longipalpis* Rearing.** The insects were reared in laboratory and maintained in insectary at 25±1ºC, RH 80% and 12h photoperiod. The colony used was in the 26th generation and was initiated in July 1996 with specimens obtained from an open rearing from Teresina County, State of Piaui, North of Brazil. The rearing technique used was based on the methodologies described by Young *et al*. (1981) and Modi & Tesh (1983).

Preparation of the Antennae for the Assays. The antennae used in the tests were obtained from two- to seven-day old females, fed on a honey solution. The insects were individually anesthetized at –20ºC for 2 min. The antennae were amputated at the base and at the tip using a micro-scalpel under stereomicroscope. The antennae were individually tested and each antennae tip was placed in the extremity of a microcapillary tube (10 µl) with the aid of a micro-handler. Each microcapillary tube contained a microelectrode and a KCL solution (0.1 N) in its interior (Van der Pers & Minks 1993). Three antennae were analyzed with five replications totaling 15 replications for each treatment, totalizing 135 observations.

Olfactory Stimuli. 1-octen-3-ol (Lancaster Synthesis, 98% purity) was diluted in hexane (Carlo Erba, HPLC degree of purity) to obtain logarithmic concentrations from 10 ηg to 10^6 ηg. Fifty micro-liters of the solution to be tested were than transferred to a 2 cm^2 filter paper, and after solvent evaporation, the filter paper was placed into a 15 cm Pasteur pipette.

Electroantennogram Response (EAG). Microelectrodes were connected to an amplifier and to a potentiometer (IDAC Box Model ID-01B, Synthec, the Netherlands), which records the potential of action in the antennae nerves (Van der Peers & Minks 1993). An air current was produced, purified and calibrated to a continuous flow of 5 µl/min., with 0.3 seconds of stimulus duration (Stimulus Controller C5-05, Synthec, the Netherlands). Data were stored in the EAG for Windows V2.6 program and than transferred to statistical programs (Systat 8.0) for linear regression analysis. Data received logarithmic transformation followed by ANOVA and Tukey test for means.

Results and Discussion

The EAG technique has been used for moths (Lepidoptera) (Van der Pers & Minks 1993), mosquitoes (Diptera: Culicidae) (Knols *et al*. 1997, Du & Millar 1999), *Culicoides impuctatus* (Goethwebuer) (Diptera: Ceratopogonidae) (Blackweel *et al*. 1996), aphids (Homoptera) (Park & Hardie 1998), and *L. longipalpis* (Dougherty *et al*. 1999). Electrophysiological techniques have been extensively used to identify host-odors (kairomones) for several haematophagous insects (Hall *et al*. 1984) and the screwworm (Cork 1994). However, little is known about the sensorial physiology of some groups of dipterans such as anopheline and mainly phlebotomine.

Some examples of electrophysiological graphs obtained during this study are shown in Fig. 1. The highest potential of action recorded was -3.33 mV in the concentration of 10^6 ηg, and the lowest was 0.097 mV in the concentration of 10 ηg.

The antennae response to the controls remained stable (mean variation of 0.16 – 0.19 mV) with no significant differences among them (P=1.000). All concentrations were significantly different from the controls. No significant differences were observed among the concentrations of 10 ηg to 10^5 ηg. However, significantly higher responses were observed only at 10^6 ηg when compared with the lower concentrations of 10 ηg and 10^2 ηg (Fig. 2). The responses in the EAG for 1-octen-3-ol, which were able to significantly stimulate the chemoreceptors (or chemical receptors) present in the *L. longipalpis* antennae, occurred in a dose-dependent manner.

The regression analysis of the effect of 1-octen-3-ol concentrations (10^2 ηg to 10^6 ηg) on the electrophysiological response (mV) indicated that the concentrations follow a linear pattern, in which the electrophysiological response is directly correlated to concentration ($R^2 = 0.98$; $P<0.001$) (Fig. 3).

The carbon dioxide is incontestably considered the most important kairomone among the haematophagous insects. Nevertheless its use is limited due to its high cost and the need of constant maintenance (Eiras 2001). The kairomone 1-octen-3-ol has shown evidences of attraction in traps for species of Glossinidae and zoophilic species of Muscidae, Tabanidae, Ceratopogonidae and Culicidae as well as some indications of effect in some species of Simulidae (Cheke & Garms 1987). Takken & Kline (1989) and Kline *et al*. (1994)

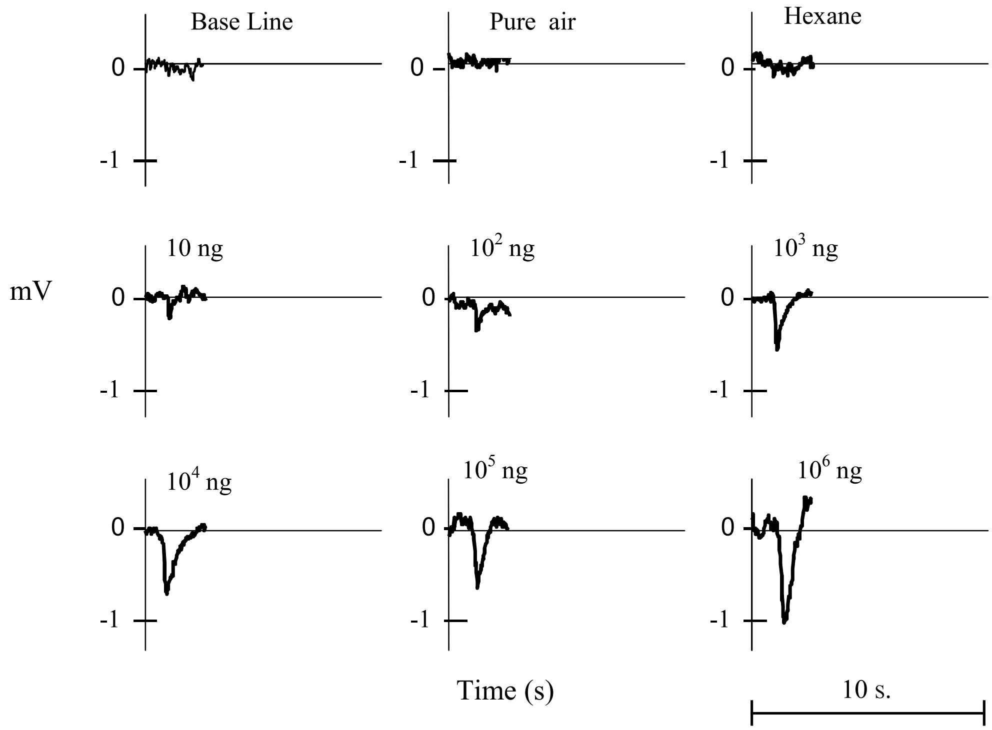

Figure 1. Electroantennograms of *L. longipalpis* females as a response to 1-octen-3-ol.

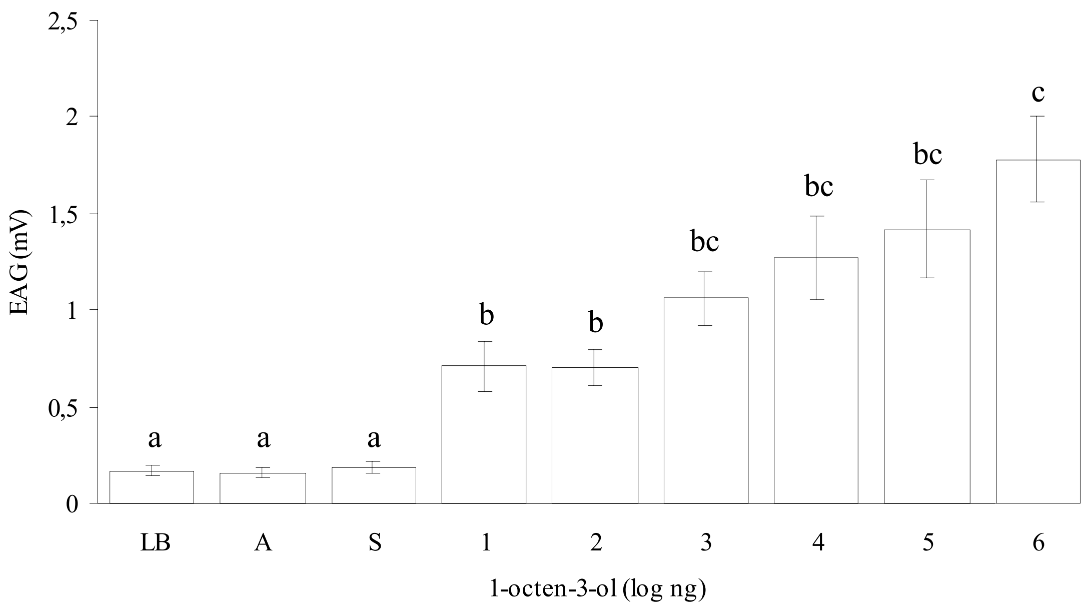

Figure 2. Electroantennographic responses of *L. longipalpis* females to 1-octen-3-ol. Vertical bars represent the standard error. Columns followed by the same letter are not different by the Tukey test (P<0.05). LB = base line; A = purified air; S = solvent.

reported that *Culicoides furens* (Poey) is attracted by carbon dioxide and by 1-octen-3-ol, and that the combination of both promoted a synergistic effect in traps. These studies used a relatively high rate of 1-octen-3-ol release (2-3mg/h). Gibson & Torr (1999) pointed out that there is no evidence of response in phlebotomine for 1-octen-3-ol. However, in the present work it was demonstrated that the *L. longipalpis* antennae possess chemoreceptors for this compound.

Dougherty *et al*. (1995) were the pioneers in carrying out research on electrophysiology and in detecting the ascoide

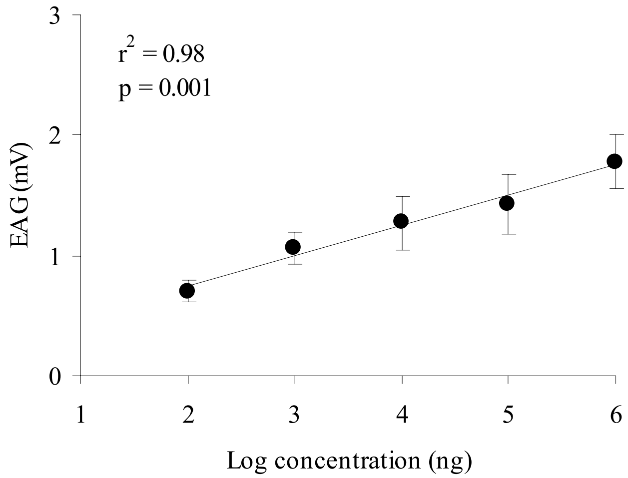

Figure 3. Linear regression analysis of the electroantennographic responses of *L. longipalpis* females to the kairomone 1-octen-3-ol. Vertical bars represent the standard error.

sensilla in the *L. longipalpis* antennae, which are sensitive to sexual and oviposition pheromones. Dougherty *et al*. (1999) evaluated the behavioral and electrophysiological responses of *L. longipalpis* to different kairomones extracted from a primary host, the fox *Vulpes vulpes.* They found neural receptors in the ascoide sensilla of this insect antennae, which responded in a dose-dependent manner to synthetic extracts of odor glands of this canine. Sixty constituents such as ketones, alcohols, aldehydes, and carboxylic acids were also identified as olfactive stimulants.

Concerning visual response, Mellor *et al*. (1996) have demonstrated that the sensitive spectrum of *L. longipalpis* is similar to that of the tse-tse fly *G. morsitans* and the mosquito *Aedes aegypti* (Linnaeus). Temperature and relative humidity play an important role in locating the host (Nigam & Ward 1991) as well as do volatiles from the host (Hamilton & Ramsoondar 1994), carbon dioxide, hamster urine, and chicken uropygial gland (Nigam & Ward 1991). Killick-Kendrick *et al*. (1986) demonstrated that *Phlebotomus ariasi* (Tonnoir) (Diptera: Psychodidae) was attracted to human odors. Blackwell *et al*. (1996) observed electrophysiological and behavioral responses to 1-octen-3-ol in ceratopogonideans, and were able to increase the capture of these insects in the field by using this compound in relatively low dose (0.11 mg/day) as baits.

Olfactive and electrophysiological responses of a species of great parasitological importance such as *L. longipalpis*, as other phlebotomines, are scarcely investigated by researchers all over the world. In the present work it was demonstrated that chemical receptors for 1-octen-3-ol do exist and confer a special character of this semiochemical to this insect. These results demonstrate that *L. longipalpis* apparently detects the presence of 1-octen-3-ol in air currents. Further behavioral and field studies are needed in order to determine the orientation of *L. longipalpis* in response to 1-octen-3-ol aiming its possible use for detection and/or monitoring these vector insects.

Acknowledgements

The authors wish to thank CNPq, CAPES and IFS (Sweden) for the financial support.

Literature Cited

Atwood, D.W.R. & M.V. Meisch. 1993. Evaluation of 1-octen-3-ol and carbon dioxide as black fly (Diptera: Simulidae) attractants in Arkansas. J. Am. Mosq. Control. Assoc. 9: 143-146.

Becker, N., M. Zgonda, D. Petric & M. Ludwig. 1995. Comparison of carbon dioxide, octenol and a host-odour as mosquito attractants in the Upper Rhine Valley, Germany. Med. Vet. Entomol. 9: 377-380.

Blackwell, A., C. Dyer, A.J. Mordue, L.J. Wadhams & W. Mordue. 1996. The role of 1-octen-3-ol as a host-odour attractant for the biting midge, *Culicoides impuctatus* Goetghebuer, and interactions of 1-octen-3-ol with a volatile pheromone produced by parous female midges. Physiol. Entomol. 21: 15-19.

Cheke, R.A. & R. Garms. 1987. Trials of attractants to enhance biconical trap catches of *Simulium yahense* and *Simulium sanctipauli*. S. l. Tropic. Medic. Parasitol. 38: 62-63.

Cork, A. 1994. Identification of eletrophysiologycally-active compounds for new world screwworm, *Cochiomyia hominivorax*, in larval wound fluid. Med. Vet. Entomol. 8: 151-159.

Disney, R.H.L. 1966. A trap for phlebotomine sandflies attracted to rats. Bull. Ent. Res. 56: 445-451.

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

Dougherty, M.J., P.M. Guerin & R.D. Ward. 1995. Identification of oviposition attractants for the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae) in volatiles of feces from vertebrates. Physiol. Entomol. 20: 23-32.

Du, Y.J. & J.G. Millar. 1999. Electroantennogram and oviposition biossay response of *Culex quinquefasciatus* and *Culex tarsalis* (Diptera: Culicidae) to chemicals in odors from bermuda grass infusions. J. Med. Entomol. 36: 158-166.

Eiras, A.E. 2001. Mediadores químicos entre hospedeiros e insetos vetores de doenças médico-veterinárias, cap. 12. p. 99-122. In E.F. Vilela & M.T.D. Lúcia (eds.) Feromônios de insetos: biologia, química e emprego no manejo de pragas. Editora Holos, 206p.

El Naien, D.A. & R.D. Ward. 1991. Response of the sand fly *Lutzomyia longipalpis* to an oviposition pheromone associated with conspecific eggs. Med. Vet. Entomol. 5: 87-91.

Forattini, O.P. 1973. Entomologia Médica, 4°. volume: Psychodidae. Phlebotominae. Leishmanioses. Bartonelose, Editora Edgar Blucher Ltda. e Editora da Universidade de São Paulo, 658p.

Gibson, G. & S.J. Torr. 1999. Visual and olfactory responses of haematophagous diptera to host stimuli. Med. Vet. Entomol. 13: 2-23.

Hall, D.R., A. Beevor, B.F Cork & G.A. Vale. 1984. 1-octen-3-ol: a potent olfactory stimulant and attractant for tse-tse isolated from cattle odours. Insect. Sci. Appl. 5: 335-339.

Hamilton, J.G.C. & T.M.C. Hamsoondar. 1994. Attraction of *Lutzomyia longipalpis* Lutz & Neiva (Diptera: Psychodidae) to human skin odours. Med. Vet. Entomol. 8: 375-380.

Hayes, R.O., O.W. Doame, G. Sakolsky & S. Benick. 1993. Evolution of atractants in traps for greenhead fly (Diptera: Tabanidae) collections on cape-cole, Massachussets, Salt Marsh. J. Am. Mosq. Control. Assoc. 9: 436-440.

Killick-Kendrick, R., T.J.M. Wilkes, I. Bailly, T. Bailly & L.A. Righton. 1986. Preliminary field observations on the flight speed of a phlebotomine sandfly. Transac. Roy. Soc. Trop. Med. Hyg. 80: 138-142.

Kline, D.L., D.V. Hagan & 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., W. Takken, J.R. Wood & D.A. Carlson. 1990. Field studies on the potencial of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. Med. Vet. Entomol. 4: 383-391.

Knols, B.G.J., J.J.V.A. Van Loon, A. Cork, R.D. Robinson, W. Adam, J. Meijerink, De Jong & W. Takken. 1997. Behavioral and electrophysiological responses of the female malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) to Limburger cheese volatiles. Bull. Entomol. Res. 87: 151-159.

Mellor, H. E., J.G.C. Hamilton & M. Anderson. 1996. Spectral sensitivity in the eyes of male and female *Lutzomyia longipalpis* sandflies. Med. Vet. Entomol. 10: 371-374.

Modi, G.B. & R.B. Tesh. 1983. A single technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. J. Med. Entomol. 30: 568-569.

Nigam, Y. & R.D. Ward. 1991. Male sandfly pheromone and artificial host as atractants for female *Lutzomyia longipalpis* (Diptera: Culicidae). Physiol. Entomol. 16: 305-312.

Nordland, R.T. 1984. Terminology of semiochemicals: a review, p.7-25. In The role of semiochemicals in insect pest control. Academic Press, New York, 306p.

Park, K.C. & J. Hardie. 1998. An improved aphid electroantennogram. J. Insec. Physiol. 44: 919-928.

Pers, J.N.C. Van der & A.K. Minks. 1993. Pheromone monitoring in the field using single sensillum recording. Entomol. Exp. Appl. 68: 237-245.

Sudia, W.D. & R.W. Chamberlain. 1962. Battery operated light trap, an inproved model. Phlebotomine. Mosq. News 22: 126-129.

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

Ward R.D., I.E. Morton, R.P. Brazil, S. Trumper & A.L. Falcão. 1990. Preliminary laboratory and field trials of a heated pheromone trap for sandfly *Lutzomyia longipalpis*. Mem. Inst. Oswaldo Cruz 85: 445-452.

Young, D.G., P.V. Perkins & R.G. Endris. 1981. A larval diet for rearing phlebotomine sand flies (Diptera: Psychodidae). J. Med. Entomol. 18: 446.

Received 10/04/2001. Accepted 10/12/2001.