

SUSCEPTIBILITY OF *LUZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) TO SELECTED INSECTICIDES IN AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN VENEZUELA

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ABSTRACT. A field population of *Lutzomyia longipalpis* from La Rinconada, Lara State, an endemic focus of visceral leishmaniasis in Venezuela, was tested for susceptibility to organochlorine (DDT 2%), carbamate (propoxur 0.01%), organophosphate (malathion 2%, fenitrothion 1%, and pirimiphos methyl 1%), and pyrethroid (deltamethrin 0.06%, lambdacyhalothrin 0.06%, and permethrin 0.2%) insecticides. Susceptibility to the insecticides tested was evaluated in the field population of *L. longipalpis* and compared with a laboratory reference strain. The (LT₅₀) to propoxur and malathion insecticides for the field population was lower than the LT₅₀ for the laboratory reference strain, demonstrating high susceptibility to these compounds. A low level of resistance at LT₅₀ (<3-fold) was found for fenitrothion, pirimiphos methyl, and permethrin insecticides, but no resistance was detected at LT₅₀. No significant resistance at the LT₅₀ and LT₉₅ was detected for the pyrethroids deltamethrin and lambdacyhalothrin. The susceptibility levels of *L. longipalpis* to the insecticides tested are discussed in view of a future control program against endophilic vectors of leishmaniasis based on the use of pesticides.

INTRODUCTION

The leishmaniasis are endemic in Venezuela. From 1955 to 1994, more than 800 cases of visceral leishmaniasis (VL) and 37,000 cases of cutaneous leishmaniasis (CL) were registered. However, because many cases are unreported or undiagnosed, health authorities recognize that the true incidence may actually be higher than the figures stated in official statistics (Department of Dermatology, Ministry of Health 1986).

To date, the control of visceral leishmaniasis in Venezuela relies mainly on passive case detection and treatment in comprehensive health centers. Because leishmaniasis were traditionally considered rural and sylvatic diseases, no attempt was made to establish an organized vector control campaign. However, nowadays, these diseases have been recorded in periurban and urban areas where an increasing proportion of the population is at risk and an increasing number of cases are encountered (Scorza 1985).

At present, about 100 sand fly species are known in Venezuela. In the central area of the country, where the highest human population density is registered, *Lutzomyia longipalpis* and *Lutzomyia ovallesi* are recognized to be the main vectors of VL and CL, respectively. Such species show a remarkable anthropophilic behavior in peridomestic and domestic habitats (Amaral et al. 1961, Feliciangeli 1991), and transmission of leishmaniasis is thought to be intradomestic in some foci (Feliciangeli et al. 1994, Feliciangeli and Rabinovich 1997). These ob-

servations justify investigation on the feasibility of applying an organized program for domestic vector control based on the use of chemical insecticides.

For the control of phlebotomine sand flies in domestic and peridomestic environments, insecticides such as DDT, malathion, fenitrothion, propoxur and synthetic pyrethroids have been used in many countries (World Health Organization 1990). However, as a result of the pressure of insecticides employed for agricultural and health care, sand fly vector populations may have already developed resistance. In fact, many insects have demonstrated the ability to develop resistance toward a variety of insecticides used for their control (Georghiou and Lagunes-Tejeda, unpublished data). The available data show that 504 species are resistant, of which 58.8% are agricultural vectors and 41.2% are important vectors of animal and human diseases.

Although considerable information exists on the status of resistance in medical pests such as mosquitoes, few studies have been reported in the literature on the baseline susceptibility of sand flies to insecticides (Table 1).

Resistance to some insecticides has been detected in Old World populations of *Phlebotomus*. For example, DDT resistance has been reported in *Phlebotomus papatasi*, the vector of CL, from northeastern India (Kaul et al. 1978, Joshi et al. 1979, Rahman et al. 1982, Dhanda et al. 1983) and from Iran (Seyedi Rashti et al. 1992). Tolerance toward methoxychlor was found in this vector from Israel (Pener and Wilamovsky 1987). For the first time, resistance to DDT was detected in *Phlebotomus argentipes*, the vector of VL, from endemic areas in India (Mukhopadhyay et al. 1992).

As a prerequisite to an effective chemical control campaign against leishmaniasis vectors in Venezuela, a study was undertaken to establish baseline data on the susceptibility of *L. longipalpis* and *L. ovallesi* to insecticides currently used for vector control

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Table 1. Previous studies on the susceptibility baselines of phlebotomine sand flies to insecticides.

Source	Species	Country	Insecticide	Susceptibility
Schmidt and Schmidt 1969	<i>Phlebotomus papatasi</i>	Egypt, Sudan	DDT, dieldrin	Egyptian lab strain showed slight tolerance
Hassan et al. 1970	<i>P. papatasi</i>	Egypt	DDT, dieldrin	Highly susceptible
Kaul et al. 1978	<i>P. papatasi</i> <i>Phlebotomus argentipes</i>	India	DDT, dieldrin	<i>P. papatasi</i> was resistant to DDT and susceptible to dieldrin
Joshi et al. 1979	<i>P. papatasi</i> <i>P. argentipes</i>	India	DDT, dieldrin	<i>P. papatasi</i> was resistant to DDT and tolerant to dieldrin <i>P. argentipes</i> was susceptible to both
Rahman et al. 1982	<i>P. papatasi</i>	India	DDT, dieldrin	Resistant to DDT and dieldrin
Dhanda et al. 1983	<i>P. papatasi</i>	India	DDT, malathion, BHC	Resistant to DDT, susceptible to malathion and BHC
Pener and Wilamovsky 1987	<i>P. papatasi</i>	Israel	DDT, methoxychlor, permethrin	Susceptible to DDT and permethrin, tolerant to methoxychlor
Scorza and Marquez 1989	<i>Lutzomyia youngi</i>	Venezuela	DDT, lindane, dieldrin, malathion, propoxur	Highly susceptible to DDT and lindane, tolerant to dieldrin and propoxur
El-Sayed et al. 1989	<i>P. papatasi</i>	Cyprus	DDT	Studies on the baseline activity of possible DDT-resistance mechanisms
Munir et al. 1991	<i>P. papatasi</i> <i>Lutzomyia longipalpis</i>	Various ¹ Brazil	DDT	Studies on the baseline activity of possible DDT-resistance mechanisms
Mukhopadhyay et al. 1992	<i>P. argentipes</i>	India	DDT	Resistant
Seyedi Rashti et al. 1992	<i>P. papatasi</i>	Iran	DDT	Resistant
Refaat et al. 1993	<i>P. papatasi</i>	Egypt	DDT, BHC, malathion, propoxur, permethrin	Lab strain more susceptible to the 5 insecticides than were the wild-caught ones
Aboul Ela et al. 1993	<i>P. papatasi</i>	Egypt	DDT, BHC, permethrin, malathion, propoxur	Lab strain more susceptible than field strain to the 5 insecticides tested
Oliveira Filho and Melo 1994	<i>L. longipalpis</i>	Brazil	DDT, chlorpyrifos, malathion, propoxur, deltamethrin	Susceptible to all insecticides studied
Scorza et al. 1995	<i>L. youngi</i>	Venezuela	DDT, fenthion, malathion, propoxur, cypermethrin, deltamethrin, λ -cyhalothrin	Susceptible to DDT, λ -cyhalothrin, cypermethrin; tolerant to malathion, fenthion; "relatively resistant" to propoxur, deltamethrin

¹ The strains of *P. papatasi* originated in Afghanistan, Cyprus, India, Iraq, Saudi Arabia, Spain, Tunisia.

in the central areas of the country where these diseases are endemic.

The present paper reports the results of bioassay tests on one field population of *L. longipalpis* with 8 representative insecticides consisting of an organochlorine, 3 organophosphates (OPs), 1 carbamate, and 3 pyrethroids. Preliminary data of the study were presented to the XX International Congress of Entomology (Mazzarri et al. 1996).

MATERIALS AND METHODS

Study area: The investigation was carried out in the village of La Rinconada in the municipality of Curarigua, Lara State, western Venezuela. La Rinconada village, located 3 km away from Curarigua (69°55' W, 9°59' N; 600 m a.s.l.) and 96

km from Barquisimeto (Lara capital), is a rural settlement with 80 scattered houses and about 600 inhabitants. Houses are commonly constructed of adobe brick walls with a corrugated iron roof. The main economic activity of the inhabitants is agriculture, the main products of which are onions, corn, beans, and fruits. This life zone (annual mean temperature 24°C, annual mean precipitation 500 mm) is classified as tropical dry forest with a 5-month dry season (January–May) (Ewel and Madriz 1968).

Three VL cases occurred in La Rinconada in 1993, 2 of which were fatal. Although no insecticides in public health control programs have been reported to be used, insecticides of all groups have been employed for agricultural purposes in this area.

Table 2. Total numbers of female sand flies tested (5 replicates) for each insecticide for the field and laboratory strains of *Lutzomyia longipalpis*.

Insecticide	Field population				Laboratory strain			
	ET ¹	No. tested	C ²	Total	ET ¹	No. tested	C ²	Total
DDT 2%	8	1,000	125	1,125	7	850	125	975
Propoxur 0.01%	10	1,250	125	1,375	10	1,250	125	1,375
Malathion 2%	10	1,250	125	1,375	8	1,000	125	1,125
Fenitrothion 1%	9	1,125	125	1,250	9	1,125	125	1,250
Pirimiphos methyl 1%	8	1,000	125	1,125	8	1,000	125	1,125
Deltamethrin 0.06%	9	1,125	125	1,250	9	1,125	125	1,250
λ-cyhalothrin 0.06%	9	1,125	125	1,250	9	1,125	125	1,250
Permethrin 0.2%	8	1,000	125	1,125	8	1,000	125	1,125
Grand total		8,875	1,000	9,875		8,475	1,000	9,475

¹ Number of exposure times tested.
² Number of controls.

Sand fly field population: All phlebotomine sand flies were collected from April to June 1996 (a total of 36 collection nights) using 8 CDC light traps operating between 1800 and 0600 h. The traps were placed among volcanic stones that border the village at the west about 100 m away. Collected sand flies were kept in 11–12-cm³ cages held in plastic bags humidified by paper moistened with distilled water in order to avoid desiccation before the bio-

assays. After the tests, the identity of all field-exposed *L. longipalpis*, dead or alive, was confirmed by the morphological characters of the spermathecae and the cybarial armature.

Laboratory (S) strain: Because no international standard susceptible reference strains of phlebotomine sand flies are available, a laboratory colony of *L. longipalpis*, originally from La Rinconada and not exposed to insecticides for a period of 3 years, was used as a reference strain. This colony was maintained by one of us (M.D.F.) for 25 consecutive generations (23 ± 2°C, 80–85% RH) at the University of Carabobo (Nucleo Aragua, Maracay) unexposed to insecticides, following the rearing procedure of Killick-Kendrick et al. (1973).

Bioassay tests for susceptibility: A total of 19,350 female sand flies were used to evaluate the susceptibility of *L. longipalpis* to the 8 insecticides selected. Table 2 shows the number of females tested for each insecticide from both field and laboratory strains. The tests were based on the mortality rates observed at different exposure times. The bioassays were made on adult females that were exposed to insecticide-impregnated papers according to the World Health Organization protocols (World Health Organization 1981). Twenty five adult sugar-fed females at 1–2 days postcapture (field population) or 1–2 days old (laboratory strain) were transferred to the exposure tubes containing impregnated papers at the following standard concentrations of insecticides: DDT (2%), malathion (2%), fenitrothion (1%), pirimiphos methyl (1%), propoxur (0.01%), deltamethrin (0.06%), lambdacyhalothrin (0.06%), and permethrin (0.2%). Eight to ten exposure times (Table 2 and Figs. 1 and 2) for each insecticide, giving between 2 and 98% mortality, were tested on 5 different days. After exposure, the sand flies were transferred to holding tubes, and mortality was recorded after 24 h. The bioassay tests on the field population of *L. longipalpis* were carried out at the health service center of the village at ambient conditions (24.5°C and 70–80% RH),

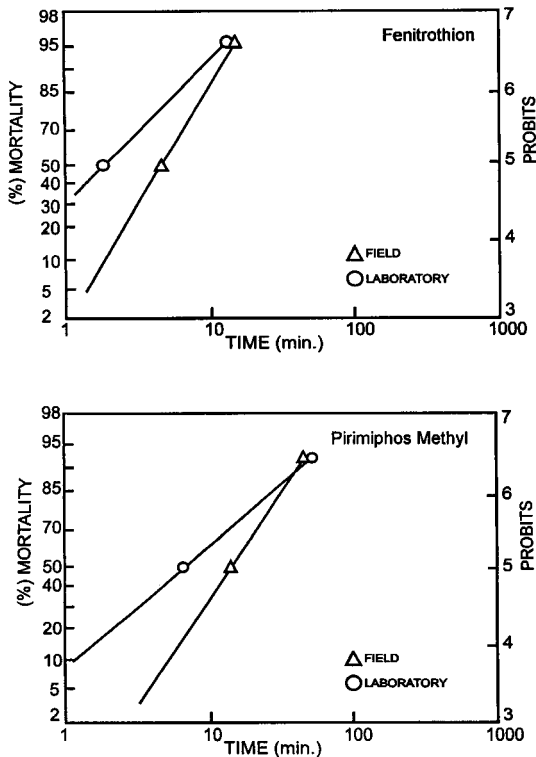


Fig. 1. Time-mortality regression lines for adults of *Lutzomyia longipalpis* laboratory and field strains for fenitrothion and pirimiphos methyl insecticides.

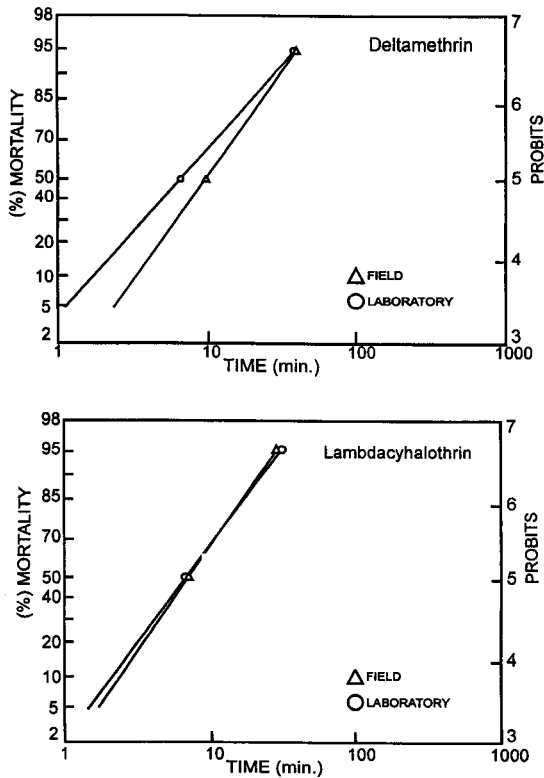


Fig. 2. Time-mortality regression lines for adults of *Lutzomyia longipalpis* laboratory and field strains for deltamethrin and lambda-cyhalothrin insecticides.

and the laboratory strain was exposed to insecticide-impregnated papers in standard conditions (23°C and 60–70% RH). The insecticide impregnated papers were prepared at the Vector Control Division (Ministry of Health, Venezuela) according to the protocols of World Health Organization (World Health Organization 1975). Data were subjected to probit analysis (Finney 1971) using a basic program (Raymond 1985). Resistance ratios were calculated at LT_{50} and LT_{95} by comparison with the laboratory strain. Resistance ratios were considered significant when the fiducial limits for both the laboratory and field populations did not overlap. On the basis of previous experience with mosquitoes, we considered resistance ratios greater than 5-fold as significant resistance in the population, whereas resistance ratios below 3-fold were considered a decline in susceptibility (Mazzarri and Georghiou 1995).

RESULTS

The values of LT_{50} , LT_{95} , and their fiducial limits as well as the calculated resistance ratios (RR) for the field population and laboratory strain of *L. longipalpis* to the 8 insecticides tested are reported in Table 3.

The field population showed susceptibility to the DDT (2%), having an LT_{50} lower than the LT_{50} of the laboratory strain and the LT_{95} for both strains close to 1 h. High susceptibility was found to propoxur (0.01%) and malathion (2%) as well, where the LT_{50} and LT_{95} values in the wild population were lower than those obtained for the laboratory strain.

For the other OPs tested (fenitrothion and pirimiphos methyl), the values at LT_{95} (14 and 43 min) were comparable with those obtained for the laboratory strain (28 and 47 min; Fig. 1). Similar results were found with the pyrethroids deltamethrin and lambda-cyhalothrin (Fig. 2). From the resistance ratios obtained at LT_{50} , it was evident that the field population possessed a decline in susceptibility (<3-fold) to the OPs fenitrothion and pirimiphos methyl, as well as to the pyrethroid permethrin.

DISCUSSION

The field population of *L. longipalpis* showed susceptibility to the insecticides tested and demonstrated LT values similar to those of the reference strain. Our results are in agreement with those obtained for the same species in Brazil (Oliveira Filho and Melo 1994). The carbamate propoxur, a toxic compound no longer used for vector control programs in public health in Venezuela, showed a high level of susceptibility in the field population, with more than 60% mortality at 10 min of exposure. Similar results were reported for the same insecticide for *P. papatasi* from Egypt (Aboul Ela et al. 1993).

Despite the long-term malaria campaign based on the use of DDT in Venezuela, no resistance was observed in the field population of *L. longipalpis* to this insecticide relative to the laboratory strain. To date, resistance to DDT has been reported in 2 vector species of phlebotomine sand flies. For the first time, resistance was described for *P. papatasi* (the Old World vector of CL) from northeastern India (Kaul et al. 1978, Joshi et al. 1979, Rahman et al. 1982, Dhanda et al. 1983, World Health Organization 1990) and more recently from Iran (Seyedi Rashti et al. 1992). Similarly, resistance to DDT was detected in *P. argentipes* (vector of visceral leishmaniasis) from endemic areas in India (Mukhopadhyay et al. 1992).

Limited data are available on the susceptibility of sand flies to OP insecticides (Table 1). Levels of tolerance to malathion were reported in *P. papatasi* from Israel and Egypt (Aboul Ela et al. 1993). However, in India, Dhanda et al. (1983) found susceptibility to malathion in the same species. According to our data, our field population of *L. longipalpis* is highly susceptible to malathion based on the lower LT values achieved compared with those of the laboratory strain. A similar phenomenon was observed by Schmidt and Schmidt (1969) in a wild population of *P. papatasi* from Egypt exposed to DDT, in which their laboratory colony conferred a

Table 3. Insecticide susceptibility of adult females of *Lutzomyia longipalpis* laboratory and field strains.¹

Insecticide and <i>L. longipalpis</i> strain	LT ₅₀	FL	LT ₉₅	FL	Slope ± SE
DDT 2%					
Laboratory	13.2	9.2–18.1	61.8	28.1–138.0	2.4 ± 0.93
Field	8.2	5.5–11.1	54.1	24.5–142.8	1.9 ± 0.28
Propoxur 0.01%					
Laboratory	11.4	10.3–13.1	79.2	62.4–106.8	1.95 ± 0.11
Field	6.3	6.2–7.3	26.3	22.0–31.4	2.6 ± 0.14
Malathion 2%					
Laboratory	10.1	8.2–13.1	47.3	24.2–1.6	2.4 ± 0.31
Field	7.2	6.2–7.4	32.1	27.2–40.4	2.4 ± 0.14
Fenitrothion 1%					
Laboratory	2.0	1.4–2.3	12.1	9.1–18.3	1.87 ± 0.19
Field	3.5	3.2–4.2	14.4	12.1–17.4	2.71 ± 0.18
	(RR = 1.78)*		(RR = 1.18)		
Pirimiphos methyl 1%					
Laboratory	4.4	3.6–5.2	48.2	36.1–71.4	1.58 ± 0.13
Field	11.1	9.2–13.0	43.1	30.5–61.8	2.7 ± 0.23
	(RR = 2.52)*				
Deltamethrin 0.06%					
Laboratory	6.2	4.1–8.3	37.4	21.0–69.6	2.03 ± 0.27
Field	9.1	6.5–11.6	38.5	20.5–72.6	2.54 ± 0.36
	(RR = 1.47)		(RR = 1.03)		
λ-cyhalothrin 0.06%					
Laboratory	7.2	6.3–7.4	29.0	24.1–35.4	2.6 ± 0.15
Field	7.5	7.3–8.1	27.5	24.2–33.2	2.9 ± 0.15
	(RR = 0.97)				
Permethrin 0.2%					
Laboratory	5.4	4.2–6.2	45.1	35–64.2	1.72 ± 0.14
Field	6.4	6.23–7.1	24.0	20.1–30.4	2.8 ± 0.21
	(RR = 1.19)*				

¹ LT = lethal time in minutes; FL = fiducial limits at the LT₅₀ and LT₉₅; RR = resistance ratio = LT₅₀, LT₉₅ field population/LT₅₀, LT₉₅ of reference strain; * significantly different from laboratory strain based on failure of 95% confident limits to overlap.

degree of "vigor tolerance" resulting in higher lethal concentration (LC) values than the field population. Resistance to fenitrothion and pirimiphos methyl was <3-fold at LT₅₀; however, no resistance was detected at LT₉₅. The decreased susceptibility observed for these insecticides might have resulted from the adult field population having been under agricultural insecticide pressure. In fact, in this area, the main economic activity is the cultivation of vegetables and fruits, and the associated crop pest control program depends on the use of OP insecticides.

No significant resistance (no overlapping fiducial limits) at LT₅₀ and LT₉₅ was detected for the pyrethroids deltamethrin and lambda-cyhalothrin. The results indicate that the field population is susceptible to these compounds. Similar results were obtained in Israel for *P. papatasi* with permethrin (Pener and Wilamovsky 1987) and in Venezuela for *Lutzomyia youngi* (Scorza et al. 1995), where the species showed high susceptibility to the pyrethroid lambda-cyhalothrin. The response of our field population to permethrin showed a decreased suscepti-

bility (RR = 1.19-fold), and because there is no history of permethrin for public health usage in this area, the change in susceptibility in the field population may be a result of agricultural pressure as well. It is worth mentioning that the lack of a true susceptible reference colony constitutes a limitation in detecting resistance in the field population. In fact, if the reference strain contains resistance to any of the insecticides, which may be stable in culture, misleading information may be obtained. However, the use of a field collected strain in culture for 3 years is better than no basis for resistance comparison.

Apart from the extensive studies on DDT resistance (Kaul et al. 1978, Joshi et al. 1979, Rahman et al. 1982, Pener and Wilamovsky 1987, Mukhopadhyay et al. 1992, Seyedi Rashti et al. 1992, Aboul Ela et al. 1993) in leishmaniasis vectors, there is no information on the status of susceptibility or mechanisms of insecticide resistance in sand fly vectors in Venezuela. In contrast, the insecticide resistance and its mechanism in mosquito vectors have been widely studied (Georghiou and Pasteur

1978, Georghiou et al. 1987, Mazzarri and Georghiou 1995). If we assume that similar mechanisms may occur in sand flies, then our results might indicate the absence of any resistance to organochlorine, OP, carbamate and pyrethroid insecticides in the field population of *L. longipalpis* tested.

In the absence of a specific control program for vectors of leishmaniasis in Venezuela, the susceptibility levels to insecticides obtained in the present investigation should constitute a baseline for further studies and may also be used as a guideline for the control campaign in domestic and peridomestic habitats.

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