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LABORATORY EVALUATION OF SUSCEPTIBILITY OF NATURAL SUBPOPULATIONS OF *AEDES AEGYPTI* LARVAE TO TEMEPHOS

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ABSTRACT. *Aedes aegypti* showed the ability to develop resistance to different insecticides, including temephos, the most widely used larvicide. The objectives of this work were to 1) determine the resistance value of 4 natural subpopulations of *Ae. aegypti*, identified by their different haplotypes, to the insecticide temephos "Abate 1G (1%)"; 2) determine the lethal concentration $(LC)_{50}$ and LC_{90} values by using the Rockefeller strain as control; and 3) estimate the resistance ratios. Mosquito samples were collected in Catamarca, Córdoba, and Posadas (Argentina) and in Yacuiba (Bolivia). Six insecticide concentrations were tested. The Rockefeller strain and the Posadas sample showed susceptibility to the diagnostic concentration (0.012 mg/liter), whereas the mortality in Catamarca was 87%. In the Yacuiba and Córdoba collections, mortality was 74% and 75%, respectively, indicating resistance. These results were coincident with those of the Probit analysis from which the highest resistance ratios were estimated for the last 2 subpopulations (5.2 and 4.9, respectively). Before this study, no information was available about the existence of resistance in natural populations of *Ae. aegypti* in the studied area.

KEY WORDS *Aedes aegypti*, dengue, larvae susceptibility, temephos

INTRODUCTION

Aedes aegypti (L.), the main urban vector of dengue and dengue hemorrhagic fever, was considered eliminated from Argentina in 1964, after the participation of the country in a continental eradication program implemented by the Pan-American Heath Organization (Ousset et al. 1967). The reinfestation was reported by 1986, in the northeast (NE); at present, the vector is found in most of the Argentinean territory (Carbajo et al. 2001). In the northwest (NW) of the country, several outbreaks of dengue have been reported, with high morbidity rates (Blanco et al. 2001).

Present control programs of this mosquito include the use of larvicides with low toxicity in mammals and residual effect, mainly the organophosphate temephos. In 1960, the first cases of *Ae. aegypti* resistance to organophosphate and carbamate insecticides were recorded in Puerto Rico (Fox and García Mola 1961) by genetic analyses (Hemingway and Ranson 2000).

One of the factors that must be taken into account when analyzing any arthropod-transmitted disease is the geographic distribution of the genetic heterogeneity present in the vector populations, which can be correlated with epidemiologically important genetic variation, such as differences in insecticide resistance, vector competence, and feeding habits (Tabachnick 1991).

Rondan Dueñas et al. (2002) and Rondan Dueñas (2005) found differences in the composition of *Ae. aegypti* haplotypes in different regions of Argentina and adjacent countries by using restriction fragment length polymorphism of mitochondrial DNA. Haplotypes 2 and 3 were predominant in Posadas (Misiones Province, NE Argentina). Haplotypes 14, 15, 16, and 17 were the most frequent in San Fernando del Valle de Catamarca (Catamarca Province, NW Argentina). Haplotypes 2 and 3, and haplotypes 1, 5, and 19 in lower proportion, characterized the mosquito populations of Córdoba city (central Argentina), and haplotype 6 was uniquely found in Yacuiba (south Bolivia). If these subpopulations present different haplotype composition, that is, they are genetically distinct, at least some of them also may differ in other genetically determined properties, such as susceptibility to insecticides.

The objectives of this work were to determine the resistance level of 4 natural subpopulations of *Ae. aegypti* (Posadas, Catamarca, Córdoba, and Yacuiba) to the insecticide temephos "Abate 1G (1%)"; determine, under laboratory conditions, the LC_{50} and LC_{90} values by using the Rockefeller strain as control; and estimate the resistance ratios.

MATERIALS AND METHODS

Mosquito samples were obtained in 3 Argentine cities (San Fernando del Valle de Catamarca, 28°28′S, 65°47′W, Catamarca Province; Córdoba City, 31°24′S, 64°11′W, Córdoba Province; and Posadas, 27°23′S, 55°55′W, Misiones Province),

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Table 1. Mean percentage and SD of *Aedes aegypti* larval mortality treated with temephos ("Abate 1G"). The number of larvae per cohort was 20; 10 replications of each treatment were performed.

Treatment (mg/liter)	Population				
	Posadas	Yacuiba	Catamarca	Córdoba	Rockefeller
0.001	0 ± 0	0 ± 0	6 ± 0.11	5 ± 0.08	32 ± 0.27
0.0015	16 ± 0.17	10 ± 0.12	25 ± 0.28	1 ± 0.02	55 ± 0.26
0.003	45 ± 0.24	16 ± 0.21	48 ± 0.41	3 ± 0.03	84 ± 0.15
0.006	85 ± 0.05	64 ± 0.10	99 ± 0.025	56 ± 0.08	99 ± 0.02
0.012	99 ± 0.02	74 ± 0.25	87 ± 0.16	75 ± 0.16	99 ± 0.01
0.024	99 ± 0.03	89 ± 0.09	99 ± 0.025	91 ± 0.12	100 ± 0
Control	0	0	0	0	0

and a sample from Bolivia (Yacuiba, 22°2′S, 63°42′W). The Rockefeller strain was used as control. Laboratory colonies were established from eggs and larvae and maintained at 25 ± 5°C and a photoperiod of 12:12 (L:D) h. Mass rearing methodology was based on Domínguez et al. (2000). Larvae for the assays were obtained from the laboratory colonies.

The organophosphate temephos "Abate 1G 1%" suspended in distilled water was tested in 6 concentrations estimated on the bases of the diagnostic concentration (DC) of 0.012 mg/liter (WHO 1992). The DC kills all individuals or produces 99.9% mortality in 24 h. The DC, 1 higher concentration (0.024 mg/liter), and 4 lower concentrations (0.006, 0.003, 0.0015, and 0.001 mg/liter) were assayed to determine the LC_{50} and LC_{90} values for each *Ae. aegypti* subpopulation. We used cohorts of 20 3rd instars in 250 ml of water placed in plastic trays. Ten replicates of each treatment and controls with water were performed for each subpopulation. Larval mortality in each cohort was recorded after 24 h of treatment.

Probit regression analysis was carried out to estimate the LC_{50} and LC_{90} values for each subpopulation (Finney 1971, EPA 1992). The resistance ratios (RRs) were estimated, based on LC_{50} and LC_{90} values, as the quotient between the LC of each subpopulation and the LC of the Rockefeller strain. Also, the confidence intervals (CIs) for LC_{50} and LC_{90} values were obtained.

RESULTS AND DISCUSSION

The Rockefeller strain (control) showed 99% mortality to the temephos diagnostic concentration (0.012 mg/liter), confirming its susceptibility to this insecticide (Table 1), following the criteria of WHO. The sample from Posadas, with 99% mortality, would be considered susceptible to temephos. Mortality values between 80% and 98% suggest an incipient alteration in susceptibility to the insecticide, as for the Catamarca sample (87% mortality to the DC of 0.012 mg/liter). In the Yacuiba and Córdoba subpopulations, the mortality values were lower than 80% (74% and 75%, respectively), under the limit from which a population can be considered resistant to the insecticide.

The LC_{50} and LC_{90} values with their respective RRs and the slope values (*b*) of the regression lines between the mortality Probit as a function of the log concentration for each subpopulation studied and the susceptible reference strain are presented in Table 2. The results obtained with the Probit analysis were consistent with results of the DC: the Yacuiba subpopulation, which showed the lowest percentage of mortality, had the higher RR (RR_{90} = 5.2). The sample from Córdoba presented a similar value (RR_{90} = 4.9), whereas the other two subpopulations showed much lower levels of resistance. However, the RR of the Posadas subpopulation may be underestimated because it was determined with the F_2 generation, which was maintained in the laboratory without selection pressure by insecticides.

Table 2. Ranks for LC_{50} and LC_{90} values, slope of line (*b*), resistant ratios (RRs), and 90% confidence intervals (CIs) for LC_{50} and LC_{90} values to temephos ("Abate 1G") estimated for different subpopulations of *Aedes aegypti*.

	Rockefeller	Posadas	Catamarca	Yacuiba	Córdoba
LC_{50}	0.0017 ± 0.0010	0.0036 ± 0.0022	0.0039 ± 0.0030	0.0065 ± 0.0049	0.0078 ± 0.0036
RR_{50}		2.1	2.3	3.8	4.6
LC_{50} CI	0.0013–0.0015	0.0030–0.0035	0.0030–0.0036	0.0063–0.0080	0.0062–0.0078
LC90	0.0047 ± 0.0021	0.0092 ± 0.0053	0.0119 ± 0.0056	0.0243 ± 0.0190	0.0228 ± 0.0087
RR_{90}		1.9	2.5	5.2	4.9
LC_{90} CI	0.0036–0.0045	0.0073–0.0094	0.0099–0.0135	0.0268–0.040	0.0198–0.0265
b	2.8 ± 0.4	3.1 ± 0.5	2.5 ± 0.8	2.4 ± 0.4	2.7 ± 0.2

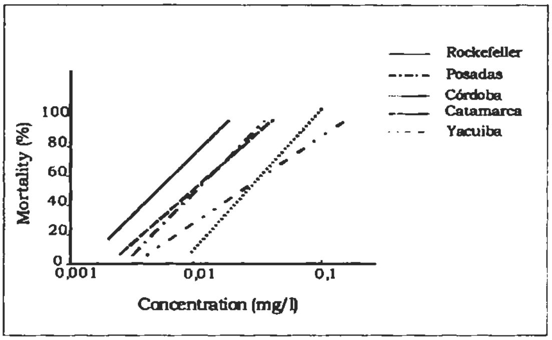

Fig. 1. Probit analysis of mortality as a function of concentration-log of temephos to 4 subpopulations of *Aedes aegypti*. The Rockefeller strain is included as susceptible control.

The resistance levels observed were lower than those found in Brazil, Cuba, Venezuela, and other Caribbean countries (Bisset et al. 2001, Pereira Lima et al. 2003, Alvarez et al. 2004, Bobadilla-Utrera et al. 2004). However, they are coincident with the values reported for Brazil several years ago (Macoris et al. 2003), when the Brazilian Ministry of Health decided to review the strategies of *Ae. aegypti* control, on the basis that the programs focused on the use of chemicals did not manage to control the vector (Barbosa da Silva et al. 2002).

When comparing the Probit-log regression lines, those corresponding to the 4 subpopulations studied deviate to the right of the reference susceptible strain line, which indicates resistance (Fig. 1). Three groups are clearly differentiated: the control (Rockefeller strain); a group formed by Posadas-Catamarca; and a group including Yacuiba-Córdoba, which showed resistance. There was no overlap between the CIs of the 3 groups, indicating that they are significantly different.

According to the results obtained for the Bolivian subpopulation, in which haplotype 6 occurs up to the northwestern frontier of Argentina in the Salta Province (Rondan Dueñas et al. 2002), routine monitoring for resistance would be necessary in this area. Determination of the angular coefficient and RRs would allow monitoring of temporal changes in the susceptibility to chemical products.

Although the RRs of other *Ae. aegypti* subpopulations remain to be determined in Argentina, the existence of resistant subpopulations must be taken into account by the National Public Health authorities.

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