RESEARCH NOTE

Insecticide Resistance in a *Culex quinquefasciatus* Strain from Rio de Janeiro, Brazil

Tania González/++, Juan A Bisset, Cristina Díaz, María M Rodríguez, Mauro Blanco Brandolini*/+

Departamento de Control de Vectores, Instituto de Medicina Tropical "Pedro Kouri", Autopista Novia del Mediodía, Km 6, e/Carretera Central y Autopista Nacional, Habana, Cuba *Instituto de Biologia do Exército, Rua Francisco Manuel 102, 20911-270 Rio de Janeiro, RJ, Brasil

Key words: insecticide - resistance - *Culex quinquefasciatus* - esterases - acetylcholinesterase - Rio de Janeiro - Brazil

Culex quinquefasciatus Say 1823 is a vector of equine encephalitis and filariasis, and is one of the mosquito species most studied for insecticide resistance (E Zerva 1988 *Parasitol Today 4*: 53-57). Studies to detect incipient resistance in the field, and its mechanisms, are very important to design effective strategies to avoid its development.

This study determined the levels and main mechanisms of insecticide resistance in a *Cx. quinquefasciatus* population from Rio de Janeiro. Eggs were collected in areas of Benfica, in May of 1994. They were carried to the laboratory where the larvae and adults were reared to creat the laboratory colony. We used the insecticide susceptible laboratory strain "Bleuet" from the University of Montpellier, France, as the reference strain. This susceptible strain was used to calculate the resistance ratios (RR) (M Raymond et al. 1985 *Génét Sél Evol 17*: 73-88, M Raymond & N Pasteur 1986 *J Econ Entomol 79*: 1452-1458).

Eight insecticides were tested: malathion, chlorpyriphos, pirimiphos-methil, propoxur, cypermethrin, deltamethrin, lambdacyhalothrin and DDT, using the bioassay methods of GP Georghiou et al. (1966 *Bull WHO 35*: 691-708). Tests with synergists were carried out with S.S.S tributyl phosphotritiated (DEF), an inhibitor of esterases and piperonyl butoxide (PBO), an inhibitor of acetylcholinesterase. The sinergists were applied 4 hr prior to the insecticides at doses of 0.05 and 0.5 ppm, respectively. If these enzymes are involved in the resistance to an insecticide, the effect will be higher when using the compound that inhibit them. This means that DEF and PBO can sinergize the action of the insecticide. Microplate test were conducted in order to determine gene frequencies for increased esterases (HTR Peiris & J Hemingway 1990 *Bull Entomol Res 80*: 453) and modified acetylcholinesterase (J Hemingway et al. 1982 *Pest Biochem Physiol 17*: 149-155). The frequency of resistant genes was calculated using the Hardy-Weinberg expression: $GF=1-(SS/T)^{1/2}$, where SS are the susceptible individuals and T is the total number tested (GH Hardy 1908 *Science 28*: 49-50). Esterase phenotypes were determined using polyacrylamide gel electrophoresis (PAGE) (JA Bisset et al. 1991 *Med Vet Entomol 5*: 223-228). Bands were classified as A or B according to substrate preference for the enzyme (1 or 2 naphthylacetate) and numbered under the basis of their migration rates compared with those reported internationally (C Mouchés et al. 1986 *Science 233*: 778-780, M Raymond et al. 1991 *Nature 350*: 151-153, F Poiré et al. 1992 *Biochem Genet 30*: 13-260).

The results of bioassays showed that the Benfica strain was susceptible to none of the tested insecticides (RR>2 in all cases). It was resistant to DDT (RR>10) and highly resistant to chlorpyriphos (RR>>10) (Table I). Sinergism tests were carried out for chlorpyriphos, propoxur and lambdacyhalotrhin (Table II). The bioassays results for three insecticides demonstrated synergism when using PBO and DEF, therefore suggesting that both mixed function oxidase and increased esterases could be acting as resistance mechanisms. However, PBO was more active for propoxur and DEF for chlorphyriphos. Enhanced oxidative metabolism appears to be a major resistance mechanism for all insecticide classes, except cyclodienes in mosquitoes, esterases are important in resistance to organophosphorus insecticides and occasionally pyrethroids (RT Roush 1993 *Parasitol Today 9*: 174-179). Our results corroborate this statement.

Ninety four single larvae were analyzed in the microplate test; one was found susceptible in the test for increased esterases and six had modified acetylcholinesterase. Gene frequency for increased esterases was 0.90, supporting the importance of

++Present address: ICIDCA, Vía Blanca No. 804, Carretera Central, San Miguel del Padrón, Habana, Cuba
+Corresponding author. Telefax: +55-21-264.5782. E-mail: mblanco@zaz.com.br
Received 12 February 1998
Accepted 4 November 1998

TABLE I

Toxicity of three organophosphates, one carbamate, three pyrethroids and DDT (in this order in the table) to *Culex quinquefasciatus* larvae from a Rio de Janeiro (Benfica) strain

Insecticide	Bleuet	Benfica strain			
	LD_{50}	LD_{50}	LD_{90}	Slope	RR
Malathion	0.15	0.33 (0.03)	0.68 (0.20)	4.9	2.2
Chlorpyriphos	0.0005	0.04 (0.06)	0.09 (0.02)	3.7	78.9
Pirimiphos-methil	0.026	0.11 (0.03)	0.25 (0.20)	4.4	4.4
Propoxur	0.51	2.64 (0.61)	7.64 (3.20)	2.7	5.1
Cypermethrin	0.0008	0.003 (0.0005)	0.01 (0.006)	2.1	3.4
Deltamethrin	0.0003	0.0009 (0.0001)	0.003 (0.001)	2.3	3.2
Lambdacyhalothrin	0.0003	0.006 (0.002)	0.006 (0.002)	2.6	6.0
DDT	0.025	0.29 (0.03)	0.76 (0.20)	3.1	11.8

Bleuet: susceptible reference strain; LD_{50} and LD_{90} are expressed in ppm ( ): +/- standard deviation; RR: resistance ratio refered to Bleuet LD_{50}; $2<RR<10$ ⇨ the strain has lost susceptibility to the insecticide, is evolving to resistance; $RR>10$ ⇨ resistant strain to the tested insecticide.

TABLE II

Sinergism rates (SR) to one organophosphate, one carbamate and a pyrethroid (in this order in the table) in larvae from a Rio de Janeiro (Benfica) *Culex quinquefasciatus* strain compared with those of a susceptible strain (Bleuet)

Insecticide	Benfica strain		Bleuet	
	DEF	PBO	DEF	PBO
Chlorpyriphos	51.4	3.4	0.7	0.9
Propoxur	11.0	457.5	0.4	0.8
Lambdacyhalothrin	33.3	40	0.8	0.6

SR: insecticide LD_{50} / insecticide + sinergist LD_{50}; $SR > 1$ ⇨ inhibition of specific detoxification enzymes because of sinergism; DEF: S.S.S. tributyl phosphotritiated; PBO: piperonyl butoxide.

these enzymes as resistance mechanisms in this strain. Esterases can degrade carbamate, organophosphates and pyrethroids (CL Terriere 1984 *Ann Ver Entomol 29*: 71-78). Moreover, gene frequency for modified acetylcholinesterase was also high (0.75). It has been found that resistance to organophosphorus and carbamate insecticides is commonly due to a less sensitive acetylcholinesterase (Roush *loc. cit.).*

Five different esterases were detected in the homogenates of 70 single mosquito larvae analyzed in PAGE: A_2, A_6, B_1, B_2 and B_6 (data not shown). It would be important to study the relationship between these enzymatic patterns and the resistance to differents types of insecticides. The majority of currently used insecticides are susceptible to the attack of esterases, but is necessary to investigate the specificity of the enzymes on its substrate.

The data of this work suggest that when designing a control strategy, the pyrethroids may provide better control than cyclodienes, most of organophosphates and carbamates. Malathion resistance is not yet extensive in the population, so insecticide rotation of pyrethroids and malation may be the productive strategy. However, the high level of cross reactivity will likely limit the duration and efficacy of these pesticides and monitoring will be required to manage this mosquito population.