

## Cross-Resistance to Pyrethroid and Organophosphorus Insecticides in the Southern House Mosquito (Diptera: Culicidae) from Cuba

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**ABSTRACT** A sample of the southern house mosquito, *Culex pipiens quinquefasciatus* Say, from Cuba was subjected to lambda-cyhalothrin selection to evaluate the usefulness of this pyrethroid insecticide for mosquito control. High resistance developed after 6 generations of selection. Little or no cross-resistance was observed to other pyrethroids (deltamethrin and cypermethrin), to a carbamate (propoxur) and to some organophosphates (chlorpyrifos and pirimiphos-methyl), but high cross-resistance was found to malathion (organophosphate). Possible resistance mechanisms responsible for this phenomenon are discussed.

**KEY WORDS** *Culex pipiens quinquefasciatus*, insecticide resistance, cross-resistance, pyrethroid, malathion

ORGANOPHOSPHORUS INSECTICIDES HAVE been used against members of the *Culex pipiens* complex (mainly *Cx. p. pipiens* L. and *Cx. p. quinquefasciatus* Say) in many countries, and the corresponding resistance genes have been identified (e.g., Pasteur et al. 1981, Raymond et al. 1986, Bisset et al. 1990, Poirié et al. 1992, Bourguet et al. 1996). In Cuba, malathion (an organophosphate) was used against *Cx. p. quinquefasciatus* for 7 yr until 1986 when, because of resistance, it was replaced by cypermethrin. Malathion still was used in some rural areas around La Havana (Bisset et al. 1990, 1991; Rodriguez et al. 1993). This malathion resistance was caused by at least 2 resistance mechanisms, an overproduced esterase B and an insensitive acetylcholinesterase (Bisset et al. 1990). These mechanisms apparently were not able to impart cross-resistance to pyrethroids (Bisset et al. 1991). Cypermethrin resistance was detected in the central area of La Havana in 1990 (Rodriguez et al. 1993); the physiological mechanism responsible for this resistance is not known. Before using an alternative insecticide, possible cross-resistance to already existing resistance mechanisms should be investigated, as was done for pirimiphos-methyl (Bisset et al. 1991).

Here, we report the selection of a strain with a distinct pyrethroid, lambda-cyhalothrin. High resistance was achieved in few generations, and cross

resistance was observed in organophosphate and carbamate insecticides as well as to other pyrethroids.

### Materials and Methods

**Insects.** Two strains of *Cx. p. quinquefasciatus* were used—S-LAB, a susceptible reference strain colonized by Georghiou et al. (1966), and SANTIAGO, a natural population collected from Santiago de Cuba (Cuba) in May 1995. This population was mass-selected during 6 generations by exposing 4th instars to lambda-cyhalothrin doses that induced 90% mortality. Each generation is designated as SAN-Fx (x being the generation number).

**Insecticide Bioassays.** Resistance characteristics of SANTIAGO and SAN-F1 to SAN-F6 larvae were analyzed by bioassays on 4th instars in cups holding 99 ml of tap water and 1 ml of an insecticide solution in acetone. Seven insecticides were tested—3 organophosphates (chlorpyrifos, malathion, and pirimiphos-methyl), 3 pyrethroids (deltamethrin, cypermethrin, and lambda-cyhalothrin), and 1 carbamate (propoxur). In each test, sets of 20 larvae were exposed to different insecticide doses for 24 h. Whenever possible, 5 replicates of 5 doses (20 larvae per dose), giving between 2 and 98% mortality, were performed with each insecticide. To standardize the bioassays, the final concentration of acetone was adjusted to 1%. Mortality data were analyzed by using the log-probit program of Raymond et al. (1993) based on Finney (1971).

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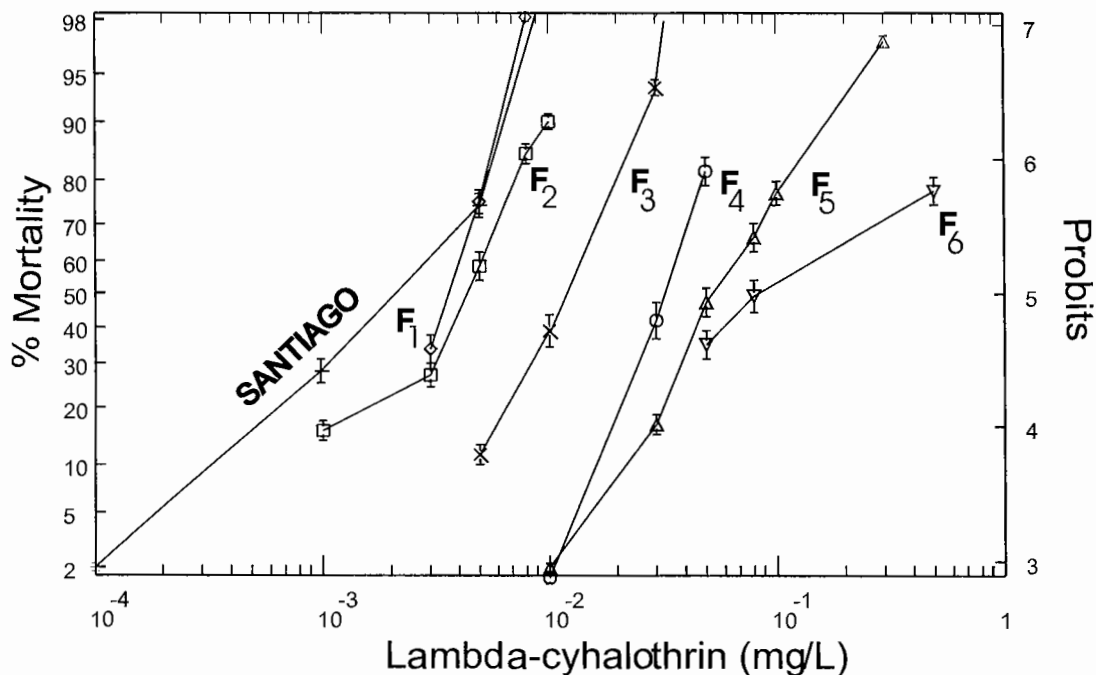


Fig. 1. Lambda-cyhalothrin mortality lines of the field sample of SANTIAGO and the 6 subsequent selected generations, SAN-F1 to SAN-F6 (indicated as F<sub>1</sub> to F<sub>6</sub>).

### Results and Discussion

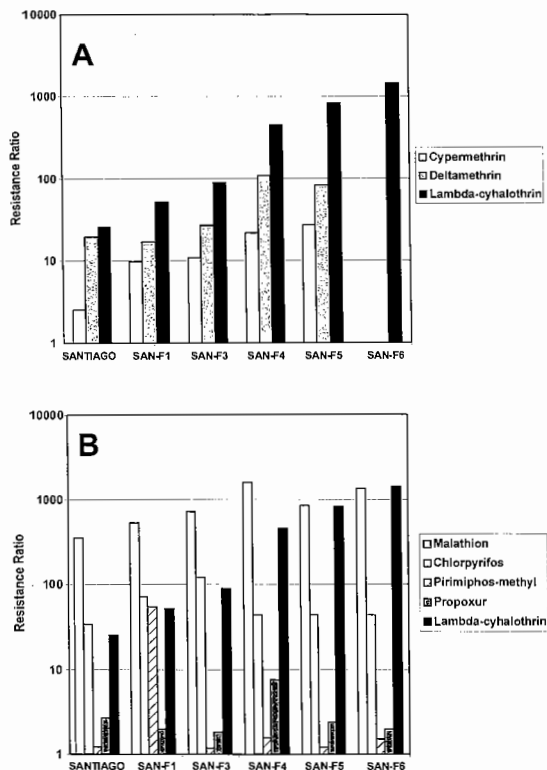
**Selection of Resistance in Santiago Mosquitoes.** Fig. 1 shows the lambda-cyhalothrin resistance observed in successive generations of selection. The LD<sub>50</sub> increased in the natural population SANTIAGO from 0.0018 to 0.011 ppm after 3 generations of selection, then to 0.058 ppm in SAN-F5. Further lambda-cyhalothrin selection (SAN-F6) increased the LD<sub>50</sub> to almost 0.1 ppm. This progression of LD<sub>50</sub>s from SANTIAGO to SAN-F6 was not caused by the progressive decrease of a plateau (Fig. 1). This indicated that >1 locus probably was involved, and that several resistant genotypes were selected progressively. The increase in the slope of the SAN-F6 mortality line indicated that further selection was probable.

**Cross-Resistance.** The increase in resistance to lambda-cyhalothrin during 6 generations was associated with an increase in resistance to other pyrethroids (Fig. 2A) but at a much lower level, indicating that within this insecticide family, a rather specific lambda-cyhalothrin resistance mechanism was present in SAN-F6. For organophosphate or carbamate insecticides, an increased resistance to malathion was associated significantly (Spearman rank correlation = 0.829,  $P < 0.05$ ) with lambda-cyhalothrin selection (Fig. 2B), but not to pirimiphos-methyl (Spearman rank correlation = 0.09,  $P > 0.5$ ), chlorpyrifos (Spearman rank correlation = 0.03,  $P > 0.5$ ), or propoxur (Spearman rank correlation = -0.77,  $P > 0.5$ ). It was unlikely that 2 distinct resistance mechanisms were selected, 1

providing resistance to lambda-cyhalothrin and the other to malathion, in the absence of cross-resistance.

The high resistance to lambda-cyhalothrin observed after only 6 generations of selection indicated that this insecticide was not a good candidate for long-term mosquito control in Cuba. This conclusion was based on the widespread malathion resistance existing in Cuba and the cross-resistance between lambda-cyhalothrin and malathion observed in the current study. Any pyrethroids used in this area should be evaluated carefully for possible cross-resistance to malathion and pirimiphos-methyl. Resistance to the former is widespread in Cuba, whereas the latter remains a good alternative to both malathion and pyrethroid resistance (Bisset et al. 1991).

Two organophosphate resistance mechanisms are known in Cuba, an overproduced esterase B and an insensitive AChE (Bisset et al. 1990). The latter is unlikely to be responsible for the lambda-cyhalothrin resistance observed, because the Cuban insensitive AChE also provides high resistance to propoxur (Bisset et al. 1990), which was not observed here. In addition, no increase of propoxur resistance was observed during the lambda-cyhalothrin selection. The former could not be excluded, because the frequency of overproduced esterases also increased during the selection process (unpublished data). Moreover, cross-resistance to pyrethroids and organophosphates caused by an overproduced esterase previously has been report-



**Fig. 2.** Evolution of resistance during the selection of SANTIAGO with lambda-cyhalothrin. The resistance ratio is the  $LC_{50}$  of a resistant strain divided by the  $LC_{50}$  of the susceptible strain. (A) Resistance to other pyrethroids; data for SAN-F6 are missing for cypermethrin and deltamethrin. (B) Resistance to some organophosphates and to propoxur (carbamate).

ed in aphids (Devonshire and Moores 1982). Further work is required to identify the resistance mechanism involved.

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