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MALATHION RESISTANCE IN *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* AFTER ITS USE IN *AEDES AEGYPTI* CONTROL PROGRAMS

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ABSTRACT. The continued widespread use of malathion in *Aedes aegypti* control programs in Latin America has generated insecticide resistance to this chemical in *Culex quinquefasciatus* but not in *Ae. aegypti.* To determine the extent of this resistance, the susceptibility of *Cx. quinquefasciatus* and *Ae. aegypti* from several countries to malathion was evaluated. Bioassay results indicated that all *Ae. aegypti* strains evaluated from Cuba, Venezuela, Costa Rica, and Jamaica were susceptible to malathion in spite of the historical use of this insecticide in *Ae. aegypti* control programs in these countries. In contrast, a high level of resistance to this insecticide was found in *Cx. quinquefasciatus* from Venezuela, Colombia, Brazil, and Cuba. Synergist assays indicated that neither esterases nor mixed-function oxidases (MFOs) were involved as the resistance mechanism to malathion in any of the *Ae. aegypti* strains tested. In *Cx. quinquefasciatus,* synergist assays confirmed that esterases played an important role in malathion resistance but MFOs were not involved in causing malathion resistance in this species. Biochemical assays showed that both resistance mechanisms were present in the *Ae. aegypti* and *Cx. quinquefasciatus* populations. Acrylamide electrophoresis gels revealed that all *Ae. aegypti* strains had a strongly staining, clear band, named A4, and had a relative mobility (Rm) value of 0.7. Analysis if the results of this study suggested that malathion could continue to be used for the emergency control of *Ae. aegypti,* the mosquito vector for dengue and dengue hemorrhagic fever in the Americas, but that malathion is probably not effective for the control of adult *Cx. quinquefasciatus* in urban areas. Therefore, control operations should integrate nonorganophosphate insecticides such as pyrethroids for control of these 2 species found in the urban environment.

KEY WORDS Malathion resistance, resistance mechanisms, *Aedes aegypti, Culex quinquefasciatus*

INTRODUCTION

Insecticides have had an important role in control programs for *Aedes aegypti* (L.) in most countries in the Americas. A high level of resistance to dichlorodiphenyltrichloroethane (DDT) and other organochlorine insecticides has developed and has led to the use of organophosphates (OPs). The most widely used OPs are temephos as a larvicide and fenitrothion and malathion as adulticides. Although spray programs using malathion have been active in some areas of the Caribbean for 15 years or more, only a low to moderate level of resistance (5- to 10-fold) has been reported in field populations (Georghiou et al. 1987, Rawlins and Ragoonansingh 1990, Mekuria et al. 1991, Rawlins and Ou Hing Wan 1995, Rawlins 1998).

An intensive *Ae. aegypti* control campaign was started in Cuba in 1981 and malathion was the principal insecticide used between 1981 and 1986. *Aedes aegypti* was successfully controlled by this campaign, but this resulted in the widespread colonization of typical *Ae. aegypti* breeding sites by *Culex quinquefasciatus* Say (Bisset et al. 1985, 1987). Extensive use of malathion for *Ae. aegypti* control has selected for malathion-resistant populations of *Cx. quinquefasciatus* Say in Cuba. These resistant populations have elevated nonspecific esterase, which confers OP resistance, and altered acetylcholinesterase (AChE), which confers OP resistance and cross-resistance to propoxur (Bisset et al. 1991).

In this publication, results of studies of the level of malathion resistance in *Ae. aegypti* and *Cx. quinquefasciatus* from several countries in Latin America and the mechanisms involved in resistance are reported.

MATERIALS AND METHODS

The following strains of *Ae. aegypti* were tested: Santiago de Cuba and Ciudad de la Habana (collected in 1997 from these 2 localities in Cuba where they were quite abundant); Tachira, Apure, Miranda, and Aragua (collected in 1997 in Venezuela and maintained in the laboratory without exposure to insecticides); Costa Rica and Jamaica (collected in 1997 from these 2 countries where they were quite abundant); and Rockefeller (a laboratory strain of Caribbean origin, colonized in the early 1930s, and provided by the Centers for Disease Control and Prevention laboratory in San Juan, Puerto Rico). The following strains of *Cx. quinquefasciatus* were tested: Santiago de Cuba and Ciudad de la Habana (collected in 1996 from these cities in Cuba where they were abundant); Medellín (collected in 1996 in Colombia where it was abundant); Miranda (col-

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lected in 1996 in Venezuela where it was abundant); Brazil (collected in 1995 in Río de Janeiro, where it was abundant; and Slab (a laboratory strain of *Cx. quinquefasciatus* provided by the University of California at Riverside).

To evaluate resistance in these strains, malathion (97%; American Cyanamid Co., Princeton, NJ) was used. Synergists can be indicators of resistance mechanisms and act by inhibiting certain enzymes that are responsible for insecticide detoxification. Two synergists were used in this study: piperonyl butoxide (PB; produced by Mc Laughlin Gormley King Co., Minneapolis, MN) and *S,S,S*-tributyl phosphorotrithioate (DEF; produced by Mobay, Kansas City, KS).

Bioassay procedure

The standard larval bioassay procedure described by Georghiou et al. (1987) was used to establish complete dosage–mortality regression lines. Briefly, 20 early 4th-stage larvae of uniform size were placed in plastic cups containing 99 ml of tap water and 1 ml of insecticide solution of the desired concentration in acetone. Five or more concentrations of malathion, prepared in standard (weight/volume) acetone solution were used in at least 5 replicates on different days. The control cup was treated with 1 ml of acetone. Mortality was determined 24 h after insecticide treatment. Results were subjected to probit analysis by the method of Finney (1971) using a basic program (Raymond, 1985). Resistance ratios (RRs) were calculated at the median lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) by comparison to the susceptible reference strain.

In the bioassays with the synergists, the procedure described above was followed except that 0.5 ml of the required concentration of each synergist solution in acetone was applied to each cup 4 h before application of the desired concentration of insecticide. The total volume of acetone solvent applied per cup did not exceed 1 ml. Synergists were used at sublethal concentrations corresponding to 0.008 mg/liter for PB or 5.0 mg/liter for DEF. At these concentrations, no mortality was observed with the synergist alone.

Biochemical tests

In *Cx. quinquefasciatus,* the total esterase activity was studied using the method of Peiris and Hemingway (1990). Esterase activity in *Ae. aegypti* was determined in early 4th instars with a modification of the above procedure reported by Rodríguez et al. (2001). Twenty microliters of each homogenized larva were added in 96-well microtiter plates and mixed with 200 μl of 0.7 mM β-naphthylacetate substrate. After 10 min, 40 μl of Fast-blue was added and the optical density (OD) was read at 570 nm in a Labsystems iMS Reader MF (Labsystems, Helsinki, Finland). Values of OD above 1.227 (>mean + 3 SD Rockefeller strain) were considered as high esterase activity.

The activity of normal and propoxur-inhibited AChE was measured in *Cx. quinquefasciatus,* following the method of Rodríguez et al. (1993). Standard solutions of 1×10^{-2} M solutions of 100% pure propoxur were prepared in acetone. These were diluted 1:3 with phosphate buffer (0.02 M, pH 7.5) immediately before use. Solutions of 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) (1.36 mg/ml) and acetylthiocholine iodide (1.08 mg/ml) in phosphate buffer were prepared fresh on the day of testing. One fourth of the mosquito homogenate was used to assay normal uninhibited AChE activity, and a further 0.25 ml was used for the propoxur inhibition studies. Two replicate aliquots of homogenate from a single mosquito were placed in a microtiter plate and 10 μl of propoxur solution were added to 1 replicate. After 2 min, 25 μl of the acetylthiocholine iodide solution plus 20 μl of the DTNB solution were added to all replicates. The enzyme reaction was then allowed to run for 30 min, and the absorbance was read at 420 nm in a Labsystems iMS Reader MF. The activity of normal and propoxur-inhibited AChE was measured in *Ae. aegypti* with the same methodology. Control AChE activity was compared with the activity in wells pre-exposed to propoxur, then results were assessed. In this way, each individual mosquito acted as its own control and variations in AChE activity due to age, sex, or general condition were automatically corrected for. In both laboratory tests, inhibition studies were only undertaken with propoxur, because laboratory tests suggested this gave accurate discrimination, and the remainder of the homogenate could then used for other purposes. An estimate of resistance gene frequencies for esterase and AChE mechanisms were calculated from the number of homozygous susceptible individuals for each assay, assuming the population was in Hardy–Weinberg equilibrium.

Electrophoresis

To detect esterases possibly involved on insecticide resistance, homogenates of *Ae. aegypti* larvae were subjected to 10% polyacrylamide gel electrophoresis (PAGE) and larval homogenates of *Cx. quinquefasciatus* were subjected to 7.5% PAGE. Electrophoresis was performed using Tris-borate/ethylenediaminetetraacetic acid (pH 8.6) as the buffer and the gel were run at 150 V. Esterases were detected using a stain solution consisting of 4 ml of α-naphthylacetate, 4 ml of β-naphthylacetate, and 10 ml of Fast-blue solution (0.03 g of Fast-blue salt + 3 ml of distilled water + 7 ml of 5% sodium dodecyl sulfate) in 50 ml of phosphate buffer at pH 7.5. Enzyme activity was stopped by replacing the staining medium with 7% acetic acid.

Table 1. Lethal concentration that gave 50% (LC_{50}) and 90% (LC_{90}) mortality and resistance ratios (RRs) calculated for malathion in field-collected *Aedes aegypti* from Latin America compared with the susceptible Rockefeller strain of *Ae. aegypti*. Slopes of the probit lines are given as an indication of the relative homogeneity of the data.

	Malathion				
Strains	LC_{50} (ppm) (range)	LC_{90} (ppm) (range)	RR_{50}	RR_{90}	Slope (± SD)
Santiago de Cuba	0.789 (0.729–0.855)	1.407 (1.224–1.759)	1.77	0.82	5.10 (±0.66)
Ciudad de la Habana	0.512 (0.472–0.545)	0.748 (0.699–0.821)	1.15	0.44	7.76 (±0.91)
Jamaica	0.578 (0.534–0.627)	0.918 (0.821–1.07)	1.29	0.54	6.37 (±0.63)
Costa Rica	0.614 (0.567–0.655)	0.949 (0.873–1.06)	1.37	0.55	6.77 (±0.78)
Apure	0.370 (0.33–0.402)	0.710 (0.637–0.816)	0.83	0.41	4.47 (±0.41)
Aragua	0.530 (0.471–0.597)	1.060 (0.885–1.395)	1.19	0.62	4.27 (±0.55)
Miranda	0.104 (0.077–0.13)	0.290 (0.224–0.433)	0.23	0.17	2.88 (±0.45)
Táchira	0.342 (0.305–0.382)	0.696 (0.585–0.916)	0.77	0.41	4.14 (±0.54)
Rockefeller	0.445 (0.38–0.531)	1.712 (1.243–2.825)	—	—	2.19 (±0.27)

RESULTS

Insecticide resistance data for the organophosphorus insecticide malathion in field-collected *Ae. aegypti* and *Cx. quinquefasciatus* from Latin American countries are presented in Tables 1 and 2, respectively. The LC_{50} and the RRs are also shown.

The low values of RRs (<2-fold) to malathion indicated that all the *Ae. aegypti* strains were susceptible to this chemical (Table 1). However, the values of the RR for all *Cx. quinquefasciatus* strains tested indicated a high level of resistance to this insecticide (RR > 11-fold; Table 2). The highest RR values were observed in the *Cx. quinquefasciatus* strains from Cuba (Santiago de Cuba, 186×, and from Ciudad de la Habana, 184×). The RR values in the strains from Brazil (19.3×), Medellín (11.0×), and Miranda (16.1×) were lower, but were still significant.

Analysis of results of assays with synergists (Table 3) indicated that the synergism ratio (SR) in all *Ae. aegypti* strains tested using DEF was low (<5-fold), except the Aragua strain where the SR value was 6.02, higher than the Rockefeller SR value (1.809). A similar result was observed using the PB synergist, where the only strain with an SR value higher than that of the Rockefeller strain (1.48) was Aragua (2.73), indicating that neither esterases nor mixed-function oxidases (MFOs) resulted in resistance to malathion in any of the *Ae. aegypti* strains tested.

In *Cx. quinquefasciatus* (Table 4), the synergist DEF reduced resistance to malathion in all strains,

Table 2. Lethal concentration that gave 50% (LC_{50}) and 90% (LC_{90}) mortality and resistance ratios (RRs) calculated for malathion in field-collected *Culex quinquefasciatus* from Latin America compared with the susceptible Slab strain of *Cx. quinquefasciatus*. Slopes of the probit lines are given as an indication of the relative homogeneity of the data.

Strains	LC_{50} (ppm) (range)	RR_{50}	LC_{90} (ppm) (range)	RR_{90}	Slope (± SD)
Miranda	0.224 (0.191–0.261)	16.11	0.438 (0.371–0.535)	7.30	4.42 (±0.4)
Medellín	0.472 (0.44–0.506)	33.96	0.663 (0.607–0.753)	11.05	8.69 (±1.0)
Brazil	0.609 (0.553–0.668)	43.81	1.159 (1.006–1.424)	19.32	4.59 (±0.523)
Santiago de Cuba	2.890 (2.30–3.72)	207.91	11.04 (7.88–17.35)	184.06	2.20 (±0.20)
Ciudad de la Habana	1.890 (1.478–2.438)	135.97	11.17 (7.709–18.581)	186.16	1.66 (±0.159)
Slab	0.0139 (0.00358–0.0218)	—	0.06 (0.047–0.097)	—	2.01 (±0.547)

Table 3. Synergism of malathion by *s,s,s*-tributyl phosphorotrithioate (DEF) or piperonyl butoxide (PB) on early 4th-instar *Aedes aegypti* field and laboratory strains from Latin America.

Strains	Malathion + DEF			Malathion + PB		
	LC_{50} (range)	SR[1]	Slope (± SD)	LC_{50} (range)	SR[1]	Slope (± SD)
Santiago de Cuba	0.778 (0.71–0.86)	1.01	3.80 (±0.36)	1.11 (1.0–1.25)	0.71	3.77 (±0.38)
Ciudad de la Habana	0.757 (0.709–0.81)	0.67	6.19 (±0.65)	0.76 (0.655–0.962)	0.67	2.92 (±0.44)
Jamaica	0.250 (0.219–0.287)	2.32	4.69 (±0.38)	0.54 (0.49–0.578)	1.07	6.19 (±0.72)
Costa Rica	0.503 (0.47–0.534)	1.22	7.42 (±0.65)	0.77 (0.718–0.818)	0.80	6.47 (±0.78)
Apure	0.621 (0.539–0.746)	0.59	2.73 (±0.36)	0.48 (0.45–0.51)	0.77	6.52 (±0.57)
Aragua	0.088 (0.08–0.097)	6.02	6.08 (±1.82)	0.19 (0.16–0.23)	2.73	3.92 (±0.42)
Miranda	0.349 (0.32–0.375)	0.29	6.64 (±0.82)	0.29 (0.263–0.332)	0.35	3.98 (±0.46)
Táchira	0.183 (0.147–0.238)	1.87	1.89 (±0.22)	0.52 (0.474–0.577)	0.65	4.27 (±0.54)
Rockefeller	0.246 (0.215–0.27)	1.81	6.66 (±0.92)	0.30 (0.268–0.331)	1.48	4.75 (±0.49)

[1] SR, synergism ratio = LC_{50} insecticide alone/LC_{50} insecticide + synergist.

especially in the Cuban strains from Ciudad de la Habana and Santiago de Cuba, where the resistance was reduced from 1.89 to 0.0636 and 2.89 to 0.09 mg/ml, at the LC_{50}, respectively. These reductions in resistance level confirmed that esterases play an important role on the enzymatic detoxification of these 2 strains. The results with PB indicated that MFOs were not involved in malathion resistance in *Cx. quinquefasciatus,* as indicated by the lower SR values in all strains except the susceptible Slab strain (4.63).

Biochemical assays

Analysis of results of biochemical assays showed that both resistance mechanisms were present in the *Ae. aegypti* and *Cx. quinquefasciatus* populations. In both, OD values above 1.227 (> mean + 3 SD susceptible reference strain) were considered as high esterase activity. The same criterion was used to detect susceptible and resistant individuals in *Cx. quinquefasciatus.* For the AChE mechanism, resistant individuals were those displaying residual enzymatic activities higher than 60% in both species. The gene frequency for elevated esterase was higher than that for the AchE mechanisms in all *Ae. aegypti* strains (Table 5) and the same results were obtained for *Cx. quinquefasciatus* (Table 6), although the AChE frequency was higher for *Cx. quinquefasciatus* than for *Ae. aegypti.* The high esterase gene frequency and the higher AChE frequency correlate well with the high level of malathion resistance observed in all Cx. *quinquefasciatus* strains. Nevertheless, the esterase gene was present at a high frequency in *Ae. ae-*

Table 4. Synergism of malathion insecticide by *s,s,s*-tributyl phosphorotrithioate (DEF) or piperonyl butoxide (PB) on early 4th-instar *Culex quinquefasciatus* strains from Latin America.

Strains	Malathion + DEF			Malathion + PB		
	LC_{50} (range)	SR	Slope (± SD)	LC_{50} (range)	SR	Slope (± SD)
Miranda	0.168 (0.11–0.21)	0.75	3.13 (±0.71)	0.287 (0.21–0.35)	0.78	3.79 (±0.85)
Medellín	0.076 (0.069–0.083)	6.21	5.39 (±0.85)	0.790 (0.743–0.836)	0.59	9.14 (±1.2)
Brazil	0.075 (0.069–0.081)	8.7	4.85 (±0.91)	0.620 (0.57–0.66)	0.98	7.20 (±1.3)
Santiago de Cuba	0.09 (0.03–0.4)	32.11	6.01 (±0.61)	1.221 (0.7–1.26)	2.37	4.11 (±0.86)
Ciudad de la Habana	0.0636 (0.059–0.0679)	29.71	6.23 (±0.573)	4.72 (3.61–6.46)	0.4	1.81 (±0.19)
Slab	0.049 (0.044–0.055)	0.28	3.54 (±0.438)	0.003 (0.00007–0.01)	4.63	0.79 (±0.23)

Table 5. Resistance gene frequencies for altered acetylcholinesterase (AChE) and elevated esterase-based resistance mechanisms in *Aedes aegypti* larvae collected from Latin America. Values in parentheses are numbers of larvae analyzed.

Collection site	Elevated esterase	Altered AChE
Santiago de Cuba	1.0 (288)	0.043 (288)
Ciudad de la Habana	0.9 (288)	0.016 (288)
Jamaica	0.0 (288)	0.089 (288)
Costa Rica	1.0 (288)	0.054 (288)
Apure	1.0 (288)	0.11 (288)
Aragua	0.42 (288)	0.0053 (288)
Miranda	0.76 (288)	0.016 (288)
Táchira	0.77 (288)	0.032 (288)
Rockefeller	0.0 (288)	0.0 (288)

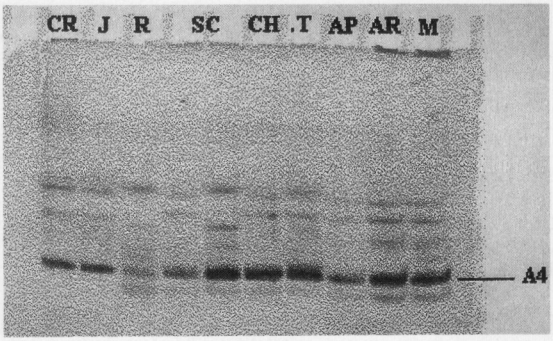

Fig. 1. Esterase patterns observed in *Aedes aegypti* from Costa Rica (CR), Jamaica (J), Rockefeller (R), Santiago de Cuba (SC), Ciudad de la Habana (CH), Tachira (T), Apure (AP), Aragua (AR), and Miranda (M).

gypti in all strains tested without resistance to this chemical, indicating that the esterases present in the *Ae. aegypti* strains tested were not involved in malathion resistance.

Electrophoresis

The esterase bands were numbered according to their specific reaction with α- or β-naphtylacetate in esterase A or B, respectively, and were numbered according to their relative mobility (Rm), which was calculated as the distance of the band from the origin, divided by the total distance of the band traveled in 45 min. Visual examination of the gels revealed that all *Ae. aegypti* strains exhibited a band, here named A4, with an Rm value of 0.7. The esterase A4 band was strongly stained in all the strains, except for the reference susceptible strain (Fig. 1). Different *Cx. quinquefasciatus* strains showed different electrophoretic patterns. The Brazil strain was used as a standard for *Culex* because this strain had all the esterase bands, including A2, B2, B1, A6, B5, and B6, whereas Miranda, Medellín, Ciudad de la Habana, and Santiago de Cuba had quite similar esterase patterns, with bands B1, A6, and B6. All of these bands were strongly stained in all the strains, except for the reference susceptible strain (Slab) (Fig. 2).

Table 6. Resistance gene frequencies for altered acetylcholinesterase (AChE) and elevated esterase-based resistance mechanisms in *Culex quinquefasciatus* larvae from Latin America. Values in parentheses are numbers of larvae analyzed.

Collection site	Elevated esterase	Altered AChE
Miranda	1.0 (288)	0.1 (288)
Medellín	1.0 (288)	0.3 (288)
Brazil	1.0 (288)	0.5 (288)
Santiago de Cuba	0.54 (288)	0.7 (288)
Ciudad de la Habana (Río Quibú)	1.0 (200)	0.4 (200)
Slab	0.0 (288)	0.0 (288)

DISCUSSION

All of the *Ae. aegypti* strains that were assayed had less than 2-fold resistance to malathion. Similarly, Rawlins and Ou Hing Wan (1995) found that 11 (32.3%) of 34 strains of larval *Ae. aegypti* from 17 Caribbean countries assayed for susceptibility to malathion had 2-fold or less resistance. The other strains (67.7%) had developed only moderate levels of resistance (between 5- and 10-fold). In none of the strains did the LC_{50} approach the World Health Organization (WHO 1981) recommended diagnostic dosage of 1.0 mg/liter. Georghiou et al. (1987) reported only low to moderate levels of resistance to malathion in *Ae. aegypti* strains that they tested. These results are probably related to the small amount of malathion that has been used for larval control and its infrequent use during emergencies, if at all, against adult *Ae. aegypti.*

Thus far, no laboratory confirmation has been made of reduced susceptibility to malathion in bloodfed *Ae. aegypti* in the Caribbean. However, malathion is one of the principal insecticides recommended for use in emergency situations for dengue control (PAHO 1982); therefore, demonstrating

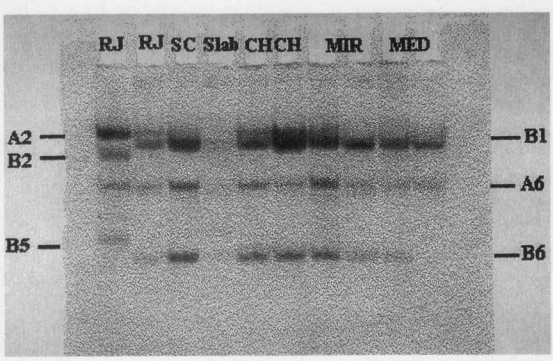

Fig. 2. Esterase pattern observed in *Culex quinquefasciatus* from Brazil (RJ), Santiago de Cuba (SC), Susceptible reference strain (Slab), Ciudad de la Habana (CH), Miranda (MIR), and Medellín (MED).

that this species is still susceptible to this chemical is important.

Selection with malathion in the Mettupalayam strain of *Ae. aegypti* induced only a 3-fold increase in tolerance to malathion even though the parent strain was quite tolerant to this insecticide (Madhukar and Pillai 1970). A 5-fold increase in resistance was obtained when the Panang strain of *Ae. aegypti* was selected in the laboratory with malathion for 8 successive generations (Brown and Abedi 1960). It is noteworthy that *Ae. aegypti* strains from Miranda, Santiago de Cuba, or Ciudad de la Habana had no malathion resistance, whereas high levels of resistance were observed in *Cx. quinquefasciatus* collected from the same localities.

All *Cx. quinquefasciatus* strains that were assayed for malathion susceptibility had very high resistance levels, ranging from 16.1 in Miranda to 208 in Santiago de Cuba. Similarly, Bisset et al. (1991) reported high levels of resistance to malathion in *Cx. quinquefasciatus* populations from within a 100-km radius of Ciudad de la Habana. Selection with malathion for 22 consecutive generations in the laboratory increased the level of malathion resistance to 1,208-fold (Díaz et al. 1993).

Early work on the development of resistance to OPs in larval *Ae. aegypti* suggested that in the case of malathion, detoxification by esterases was of little importance and that physical mechanisms such as decreased absorption were important (Ziv and Brown 1969). The possible role of detoxifying enzymes was 1st noted by Chen and Sudderuddin (1978) when they found that *Ae. aegypti* larvae, which were generally more tolerant of the organophosphorus compounds, showed higher carboxylesterase activity, as evidenced by the Michaelis constant (Km) value.

The results of the present study with larval mosquitoes showed a stronger band than did the susceptible Rockefeller strain. Quantification of the Rm indicated that the stronger band (Rm = 0.7) was present in all of the strains. This esterase was named esterase A4 because of its Rm. No evidence exists to correlate the presence of this esterase band with malathion resistance in field strains because no resistance was found in any of the populations tested. The role of esterase A4 must be defined in future studies. Using acrylamide gel electrophoresis, Field et al. (1984) found that the malathion-resistant Villa Palmeras (VP) strain (10-fold) had markedly stronger band intensity than the unselected TM strain of *Ae. aegypti,* caused by the locus previously described as esterase 6 (Field and Hitchen 1981). Mazarri (1994) observed that esterase A5, with an Rm of 0.61, was observed in higher frequency (81–100%) in resistant individuals, but not in the susceptible Rockefeller strain. She confirmed involvement of elevated esterase with a synergist test, but this is not the case with the *Aedes* in this study.

In sharp contrast, the involvement of 2 types of esterases (A and B) with increased activity toward naphthylacetate in OP insecticide resistance is now well documented in the *Culex pipiens* complex. Esterases B1, A2–B2, and the new C esterase have been previously described in the American region. Esterase B1 was 1st reported in a laboratory colony derived from mosquitoes collected in 1974 in California (Georghiou and Pasteur 1978). Esterase B1 was latter reported from other states (Pasteur and Georghiou 1989) and Bisset et al. (1990) observed it in Cuba. Esterases A2–B2 were 1st described in Tanzania (Curtis and Pasteur 1981). In 1984, these esterases were 1st observed in California in the American region (Raymond et al. 1987), and were later found in Cuba (Bisset et al. 1990).

Before 1986, *Cx. quinquefasciatus* from Cuba were resistant to malathion because of the presence of elevated esterase B1 (Bisset 1990, 1991), resulting from extensive malathion applications as both an adulticide and a larvicide by the *Ae. aegypti* eradication campaign. After 1986, malathion was replaced by a number of pyrethroids. Subsequently, 2 new esterases (A6 and B6; Rodríguez 1995) appeared, not only in Cuba, but also in *Cx. quinquefasciatus* from Venezuela, Colombia, and Brazil.

The *Ae. aegypti* esterase patterns from different Latin American countries are similar, as they are in *Cx. quinquefasciatus.* Whether these identical esterase patterns are the result of migration or of similar insecticide use practices is under investigation.

Elevated esterase mechanisms have generally been selected in *Cx. quinquefasciatus,* and the esterases are well classified in the Caribbean region. Elevated esterase activity associated with chlorpyrifos and temephos resistance, respectively, has been reported in *Ae. aegypti* from Venezuela (Mazarri and Georghiou 1995) and Trinidad (Vaugan and Ffrench-Constant 1998) and more recently was reported by biochemical and sinergist studies of the role of the esterases in temephos resistance in *Ae. aegypti* (Wirth and Georghiou 1999). The malathion resistance in *Cx. quinquefasciatus* is higher when altered AChE mechanisms are present together with elevated esterase levels than where they are separate (Bisset et al. 1991).

The development of pyrethroid resistance, together with OP and carbamate resistance, is well established in field populations (Bisset et al. 1990) and has obvious implications for *Culex* control in Cuba. If pyrethroids are applied continuously over the next few years, resistance in *Cx. quinquefasciatus* could rise to a level where operational efficacy of pyrethroids will be lost. Therefore, by carefully regulating their use, the active life and efficacy of pyrethroids in Cuba may be prolonged indefinitely. The presence of relatively high frequencies of 2 mechanisms of OP resistance, 3 years after the cessation of malathion treatment (Bisset et al. 1991) suggests that it is not feasible to revert to malathion applications to eliminate foci of pyrethroid resistance. However, pirimiphos methyl is relatively unaffected by the malathion resistance mechanisms,

even when these resistance factors have been selected. Because pirimiphos methyl is also fully effective against pyrethroid-resistant specimens of *Cx. quinquefasciatus* in Cuba, this insecticide would be appropriate for use in a rotational scheme for resistance management (Bisset et al. 1991).

This suggests that use of malathion for emergency dengue control could be continued, but that malathion cannot be used for *Cx. quinquefasciatus* control in the Caribbean region. Monitoring of insecticide resistance to detect any further significant increases in resistance is recommended. Control operations should also integrate the use of other non-OP insecticides, such as pyrethroids, for successful control of the 2 species in urban environments.

REFERENCES CITED

Bisset JA, Navarro A, Marquetti MC. 1985. La abundancia larval de mosquitos urbanos durante la campaña de erradicación de *Aedes aegypti* y del Dengue en Cuba. *Rev Cubana Med Trop* 37:161–168.

Bisset JA, Navarro A, Marquetti MC. 1987. Algunos aspectos del nicho ecológico de *Aedes aegypti* y *Culex quinquefasciatus*. *Rev Cubana Med Trop* 39:191–194.

Bisset JA, Rodríguez M, Díaz C, Ortíz E, Marquetti MC. 1990. The mechanisms of organophosphate and carbamate resistance in *Culex quinquefasciatus* (Diptera: Culicidae) from Cuba. *Bull Entomol Res* 80:245–250.

Bisset JA, Rodríguez MM, Hemingway J, Díaz C, Small GJ, Ortíz E. 1991. Malathion and pyrethroid resistance in *Culex quinquefasciatus* from Cuba: efficacy of pirimiphos-methyl in the presence of at least three resistance mechanisms. *Med Vet Entomol* 5:223–228.

Brown AWA, Abedi ZH. 1960. Cross-resistance characteristics of a malathion-tolerant strain developed in *Aedes aegypti*. *Mosq News* 20:118–124.

Chen YP, Sudderuddin KI. 1978. Toxicological studies of insecticides on *Culex quinquefasciatus* Say and *Aedes aegypti* (L.). *Southeast Asian J Trop Med Public Health* 9:378–383.

Curtis CF, Pasteur N. 1981. Organophosphate resistance in vector populations of the complex *Culex pipiens* L. (Diptera, Culicidae). *Bull Entomol Res* 71:153–161.

Díaz C, Bisset J, Rodríguez MM. 1993. Selección de una cepa de *Culex quinquefasciatus* homocigótica para la resistencia al insecticida organofosforado malation. *Rev Cubana Med Trop* 45:193–196.

Field WN, Hitchen JM. 1981. Linkage relationship between an esterase locus and group II markers in the yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 18:61–64.

Field WN, Hitchen JM, Rees AT. 1984. Esterase activity in strains of *Aedes aegypti* (Diptera: Culicidae) tolerant and susceptible to the organophosphate insecticide malathion. *J Med Entomol* 21:412–418.

Finney DJ. 1971. *Probit analysis* Cambridge, United Kingdom: Cambridge University Press.

Georghiou GP, Pasteur N. 1978. Electrophoretic esterase patterns in insecticide resistant and susceptible mosquitoes. *J Econ Entomol* 71:201–205.

Georghiou GP, Wirth M, Tran H, Saume F, Knudsen AB. 1987. Potential for organophosphate resistance in *Aedes aegypti* in the Caribbean area and neighboring countries. *J Med Entomol* 24:290–294.

Madhukar BVR, Pillai MKK. 1970. Development of organophosphorus resistance in Indian strains of *Aedes aegypti* (L.). *Bull WHO* 43:735–742.

Mazarri, MB. 1994. *Insecticide resistance in two field populations of* Aedes aegypti *(L.) from Venezuela*. Master's thesis. University of California, Riverside. 119 pp.

Mazarri MB, Georghiou GP. 1995. Characterization of resistance to organophosphate, carbamate, and pyrethroid insecticides in field populations of *Aedes aegypti* from Venezuela. *J Am Mosq Control Assoc* 11:315–322.

Mekuria Y, Gwinn TA, Williams DC, Tidwell MA. 1991. Insecticide susceptibility of *Aedes aegypti* from Santo Domingo, Dominican Republic. *J Am Mosq Control Assoc* 7:69–72.

PAHO [Pan American Health Organization]. 1982. *Emergency vector control after natural disaster* PAHO Sci. Publ. 419. Available from: Pan American Health Organization, Washington, D.C.

Peiris HTR, Hemingway J. 1990. Mechanism of insecticide resistance in a temephos selected *Culex quinquefasciatus* (Diptera: Culicidae) strain Sri Lanka. *Bull Entomol Res* 80:49–55.

Rawlins SC. 1998. Spatial distribution of insecticide resistance in Caribbean populations of *Aedes aegypti* and its significance. *Pan Am J Public Health* 4:243–251.

Rawlins SC, Ou Hing Wan J. 1995. Resistance in some Caribbean populations of *Aedes aegypti* to several insecticides. *J Am Mosq Control Assoc* 11:59–65.

Rawlins SC, Ragoonansingh R. 1990. Comparative organophosphorus insecticide susceptibility in Caribbean populations of *Aedes aegypti* and *Toxorhynchites moctezuma*. *J Am Mosq Control Assoc* 6:315–319.

Raymond M. 1985. Presentation d'un programme d'analyse log-probit pour micro-ordinateur. *Cah ORSTOM Ser Entomol Med Parasitol* 22:117–121.

Raymond M, Pasteur N, Georghiou GP, Mellon RB, Wirth MC, Hawley M. 1987. Detoxification esterases new to California in organophosphate-resistant *Culex quinquefasciatus* (Diptera:Culicidae). *J Med Entomol* 24:24–29.

Rodríguez MM, Bisset J, Díaz C, Ortíz E. 1993. Determinación de fenotipos de acetilcolinesterasa modificada en *Culex quinquefasciatus* por el Sistema Ultramicroanalítico (S.U.M.A). *Rev Cubana Med Trop* 45:122–127.

Rodríguez MM, Bisset J, Lauzan L, Milá L, Molina D, Soca A. 2001. Adaptación de los métodos en placas de microtitulación para la cuantificación de la actividad de esterasas y glutation-*s*-transferasa en *Aedes aegypti* (L.) *Rev Cubana Med Trop* (in press).

Rodríguez MM, Bisset J, Mastrapa L, Díaz C. 1995. Asociacion de la resistencia a insecticidas organofosforados, carbamatos y piretroides con los mecanismos de resistencia observados en cepas de *Culex quinquefasciatus* de Ciudad de la Habana. *Rev Cubana Med Trop* 47:154–160.

Vaugan AD, Ffrench-Constant R. 1998. Biochemical monitoring of organophosphorus and carbamate insecticide resistance in *Aedes aegypti* mosquitoes from Trinidad. *Med Vet Entomol* 12:318–321.

Wirth MC, Georghiou GP. 1999. Selection and characterization of temephos resistance in a population of *Aedes aegypti* from Tortola, British Virgin Island. *J Am Mosq Control Assoc* 15:315–320.

WHO [World Health Organization]. 1981. *Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides* WHO/VBC/81.807. Geneva: World Health Organization. 6 pp.

Ziv M, Brown AWA. 1969. Resistance potentialities of *Aedes aegypti* and *Culex quinquefasciatus* to organophosphorus and other insecticides. *Bull WHO* 41:941–946.