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RESISTANCE IN SOME CARIBBEAN POPULATIONS OF *AEDES AEGYPTI* TO SEVERAL INSECTICIDES

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ABSTRACT. Thirty-four strains of *Aedes aegypti* larvae from 17 Caribbean countries were bioassayed for sensitivity to temephos, malathion, fenitrothion, fenthion, and chlorpyrifos. There were fairly high levels of resistance in Tortola (10–12-fold resistance) and Antigua (6–9-fold resistance) strains to temephos and to fenthion (Tortola, 7–10-fold; Antigua, 6–10-fold resistance). Most other strains showed some resistance to malathion, fenitrothion, and chlorpyrifos, but only moderate levels. Adult populations of *Ae. aegypti*—Aruba, Jamaica, Trinidad, Puerto Rico, St. Lucia, and Antigua strains—also showed moderate resistance to malathion. Mosquito control field data supported the laboratory findings. Doubling the diagnostic dosage of temephos for larval *Ae. aegypti* was only partially effective against a more resistant strain, and even so, the chemical lost its limited efficacy over a short period of time. Integrated strategies for *Ae. aegypti* control to mitigate the negative effects of insecticide resistance in the Caribbean strains are suggested.

INTRODUCTION

Aedes aegypti (Linn)., the only known vector for dengue, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) in the Caribbean, as well as a potential vector of urban yellow fever, occurs in nearly every Caribbean territory (Nathan 1993). It thus poses a significant public health threat in an environment where dengue types 1, 2, and 4 are known to be endemic (Nathan and Knudsen 1991). In the Caribbean, the most valuable intervention for *Ae. aegypti* control has been community participation in eliminating container breeding of the mosquito in the peri-domestic situation through source reduction. This strategy has worked to some extent, but some *Ae. aegypti*-producing containers are not disposable (Rosenbaum et al. 1995), thus other intervention methods such as chemical control are needed.

The organophosphorus (OP) insecticides temephos and malathion have been used throughout the Caribbean during the last 15–20 years for routine control of *Ae. aegypti* (Georghiou et al. 1987). Temephos (Abate®) as 1% sand-core granules is applied for larval control and is widely used in storage containers or potable water, especially in areas of unreliable water supply. Malathion, on the other hand, is used as an adulticide in thermal fogging or in ultra-low volume (ULV), principally in times of high mosquito or dengue prevalence. Overall, insecticide use in most Caribbean countries has been sporadic, often depending on the availability of the chemical. Thus, the selection pressure for the development of OP resistance may not have been as intense as 15–20 years of insecticide use may suggest.

(Georghiou et al. 1987) reported larval resistance to temephos, malathion, fenthion, and propoxur in some of the *Ae. aegypti* strains collected from 28 sites in the Caribbean region. More recently, Mekuria et al. (1991) considered the problem of insecticide resistance in *Ae. aegypti* in the Dominican Republic to be serious enough to warrant consideration of control measures other than the use of chemicals. Because of the continuing dependence on chemical control of *Ae. aegypti* in the Caribbean, it is important to monitor trends of OP resistance in this mosquito. Also, it is essential to determine whether the emergence of resistance is at a level that would make the use of OP insecticides ineffective. In the present study 34 *Ae. aegypti* populations from 17 Caribbean countries were studied for their susceptibility to some organophosphates.

MATERIALS AND METHODS

Laboratory studies: Thirty-four strains of *Ae. aegypti* collected throughout the Caribbean region within the last 3 years and maintained at the Caribbean Epidemiology Centre (CAREC) in Port of Spain were tested. Also, a known insecticide-susceptible Trinidad strain, the CAREC strain (Georghiou et al. 1987, Rawlins and Ragoonansingh 1990), which had been kept at CAREC for the past 10 years without exposure to any chemicals, and the Rock strain, a susceptible population from a California laboratory, were used as our reference susceptible strains. Generally, susceptibility tests were run on F_{2-4} generations.

Standard larvicidal and adulticidal kits and procedures for testing insecticide resistance in mosquitoes (World Health Organization 1981a, 1981b) were used. In larvicidal studies, 3 replicates of 25 4th-instar larvae were exposed to ranges of concentrations of temephos, malathion, chlorpyrifos, fenitrothion, or fenthion.

Table 1. Susceptibility of Caribbean strains[1] of larval *Aedes aegypti* to organophosphorus insecticides.

Country	Location	Temephos		Malathion		Fenitrothion		Fenthion		Chlorpyrifos	
		LC_{50}[2]	R.R.[3]	LC_{50}	R.R.	LC_{50}	R.R.	LC_{50}	R.R.	LC_{50}	R.R.
Anguilla	Little Dix (ex rock hole)	0.0133	1.4	0.0657	1.0	0.0106	0.8	—	—	0.0055	1.2
	Little Dix	0.0248	2.7	0.1260	1.9	0.0161	1.2	—	—	0.0054	1.2
	The Valley (ex tree hole)	0.0263	2.8	0.0737	1.1	0.0124	0.9	—	—	0.0052	1.2
	Valley Health Center	0.0430	4.6	0.0782	1.2	0.0191	1.5	0.0094	1.8	0.0066	1.5
Antigua	All Saints Village	0.0474	5.1	0.2505	3.8	0.0429	3.3	0.0490	9.6	—	—
	Barnes Hill	0.0854	9.2	0.1913	2.9	0.0259	2.0	0.0355	7.0	0.0091	2.0
	Gray's Farm	0.0554	6.0	0.1295	1.9	0.0322	2.5	0.0282	5.5	—	—
Aruba	Oranjestaad	0.0367	4.0	0.4843	7.3	0.0263	2.0	0.0166	3.3	—	—
Bahamas	Nassau	0.0245	2.6	0.3267	4.9	0.0211	1.6	0.0745	14.6	—	—
Barbados	Fontabelle	0.0239	2.6	0.2405	3.6	0.0394	3.0	0.0434	8.5	—	—
Dominica	Wesley	0.0513	5.5	0.1447	2.2	0.0281	2.2	0.0174	3.4	—	—
	Deepwater Harbour	0.0327	3.5	0.1061	1.6	0.0291	2.2	—	—	—	—
	Roseau	0.0875	9.4	0.1517	2.3	0.0384	3.0	—	—	—	—
	Canefield Airport	0.0729	7.8	0.0948	1.4	0.0311	2.4	0.0213	4.2	0.0184	4.1
Grenada	Grande Anse	0.0817	8.8	0.3243	4.9	0.0287	2.2	0.0212	4.2	—	—
Guyana	Georgetown	0.0214	2.3	0.1031	1.5	0.0177	1.4	0.0200	3.9	—	—
	Mabaruma	0.0157	1.7	0.1255	1.9	0.0283	2.2	0.0076	1.5	0.0080	1.8
Jamaica	Richmond Park	0.0317	3.4	0.2459	3.7	0.0255	2.0	0.0077	1.5	0.0318	7.1
	Hughenden Park	0.0284	3.1	0.1967	3.0	0.0248	1.9	0.0129	2.5	—	—
Puerto Rico	San Juan, Puerto Nuevo	0.0246	2.7	0.2225	3.3	—	—	—	—	—	—
St. Kitts	Basseterre	0.0134	1.4	0.0966	1.5	0.0141	1.1	0.0118	2.3	—	—
St. Lucia	Castries	0.0628	6.7	0.1822	2.7	0.0385	2.7	0.02437	4.8	0.0391	8.7
St. Maarten	St. Maarten	0.0429	4.6	0.3013	4.5	—	—	—	—	—	—
St. Vincent	Kingstown	0.0338	3.6	0.2249	3.4	0.0340	2.6	0.0218	4.3	—	—
Tortola B.V.I.	Emmanuel Reef	0.0945	10.2	0.1432	2.1	—	—	0.0374	7.3	0.0233	5.2
	Sea Cow's Bay	0.1126	12.1	0.1676	2.5	—	—	0.0528	10.3	0.0152	3.4
Trinidad	St. James	0.0413	4.4	0.3216	4.8	0.0365	2.8	0.0174	3.4	—	—
	Caroni (Chin Chin)	0.0376	4.0	0.2354	3.5	0.0425	3.3	0.0244	4.8	—	—
	Point Fortin	0.0435	4.7	0.2555	3.8	0.0523	4.0	—	—	—	—
Suriname	Reneprojeht	0.0133	1.4	0.1294	1.9	0.0260	2.0	0.0109	2.1	0.0074	1.7
	Diakenessen	0.0147	1.6	0.2304	3.5	0.0172	1.3	0.0110	2.2	—	—
	Lands Hospital	0.0183	2.0	0.1670	2.5	0.0318	2.5	0.0133	2.6	0.0069	1.5

Table 1. Continued.

Country	Location	Temephos LC_{50}[2]	Temephos R.R.[3]	Malathion LC_{50}	Malathion R.R.	Fenitrothion LC_{50}	Fenitrothion R.R.	Fenthion LC_{50}	Fenthion R.R.	Chlorpyrifos LC_{50}	Chlorpyrifos R.R.
Venezeula	Maracay—23 de Enero	0.0132	1.4	0.2504	3.8	0.0113	0.9	0.0053	1.0	—	—
	El Pinonal	0.0171	1.8	0.2991	4.5	—	—	—	—	—	—
Reference	CAREC colony	0.0093	1.0	0.0668	1.0	0.0130	1.0	0.0051	1.0	0.0045	1.0
	Susceptible California Rock	0.0071	0.8	0.0545	0.8	0.0071	0.06	0.0035	0.7	0.0036	0.8

[1] Strains collected and tested 1990–94.
[2] LC_{50} = dosage (mg/liter) required to kill 50% of the larval sample.
[3] Resistance ratio (R.R.) based on the susceptible CAREC colony strain.

Mortalities were determined after 24 h exposure, and the results were probit analyzed (Finney 1964). The resistance ratios were determined by comparison to the reference CAREC strain. The tests for each strain were repeated on several occasions.

Two- to 4-day-old bloodfed females were exposed to 5% malathion-impregnated surfaces (World Health Organization 1981b) for varying periods of time. They were then kept for 24 h on insecticide-free surfaces, after which mortality was determined. The data from at least 3 replicates were probit analyzed to provide LT_{50} and LT_{90} values, which were compared to those of the reference CAREC strain in order to obtain resistance ratios for each strain. In the interest of saving space, only the LC_{50} and LT_{50} and their resistance ratios are presented in the tables.

Field studies: Tests were designed to show whether resistance to temephos in *Ae. aegypti* larvae was likely to impact negatively on the use of the chemical for *Ae. aegypti* control. The efficacy of temephos against a moderately and a more severely temephos-resistant strain was tested in field situations. Temephos at 0.02 mg/liter and at 0.04 mg/liter was added to 2 200-liter barrels of water. Three replicates of 20 4th-instar larvae were enclosed in floating fluid-penetrable containers at the water surface in each barrel, in devices similar to the one reported by Chadee (1989). After 24 h, the larvae were removed, and mortality was assessed. Three replicates in a control drum (no temephos) were also assessed. New larvae were introduced twice weekly and the toxicity of temephos to *Ae. aegypti* at the surface was determined.

After 3 wk, when surface mortality had dropped close to 0%, the drums were stirred so as to briefly resuspend the temephos granules. After these had resettled, new larvae were added to the surface larval containers, and the toxicity assessment was repeated at weekly intervals until mortality had dropped close to 0%.

RESULTS

Larvicidal studies: Insecticide resistance data for temephos, malathion, fenitrothion, fenthion, and chlorpyrifos in *Ae. aegypti* larvae are presented in Table 1. The LC_{50} and resistance ratios, which compare a strain's LC_{50} with that of the reference susceptible strain, are also shown.

The data for temephos indicate a fair level of prevalence of elevated insecticide resistance in most Caribbean strains. Only the Little Dix (rock hole) strain of Anguilla (1.4), Mabaruma strain of Guyana (1.7), Reneprojeht (1.4) and Diakenessen (1.6) strains of Suriname, Venezuela strains 23 de Enero (1.4) and El Pinonal (1.8), and the St. Kitts Basseterre strain (1.4) showed resistance of 2.0-fold or less. At the higher extreme, the Tortola strains, Sea Cow's Bay (12.1) and Emmanuel Reef (10.2), were most resistant to this insecticide, followed by Barnes Hill, Antigua (9.2) > Grand Anse, Grenada (8.8) > Castries, St. Lucia (6.7) > Gray's Farm, Antigua (6.0). All other populations showed a magnitude of resistance to temephos intermediate between these 2 groups.

Resistance to malathion was much less intense. The Oranjestaad population of Aruba showed the highest level of resistance, but this was a mere 7.3-fold resistance. Similarly, resistance to fenitrothion was not very marked either. The Point Fortin (4.0) and Caroni (3.3) strains of Trinidad > All Saints Village, Antigua strain (3.3) > Roseau, Dominica (3.0) and Fontabelle, Barbados (3.0) were the most resistant populations.

Conversely, resistance to fenthion was significant, and generally strains that had high resistance to temephos also had resistance to fenthion. Nassau, Bahamas (14.6) > Sea Cow's Bay, Tortola (10.3) > All Saints Village, Antigua (9.6) > Emmanuel Reef, Tortola (7.3) were the most resistant strains. Against chlorpyrifos, only Castries, St. Lucia (8.7) > Richmond Park, Jamaica (7.1) > Emmanuel Reef, Tortola (5.2) and Canefield Airport, Dominica (4.1) showed some resistance.

Adulticidal studies: In the adult stage, most populations showed a susceptibility of less than 2-fold resistance to malathion (Table 2). Only Hughenden Park, Jamaica (4.0) > St. James, Trinidad (3.8) > Castries, St. Lucia (3.3) > Puerto Nuevo, Puerto Rico (3.2) > Emmanuel Reef, Tortola (3.1) > Urlings, Antigua (3.0) showed significant resistance.

Field studies: When larvae of a highly temephos-resistant strain of *Ae. aegypti* (LC_{90} = 0.082 mg/liter, resistance ratio = 4.6) and a moderately resistant strain (LC_{90} = 0.063 mg/liter, resistance ratio = 2.7) were exposed to water treated with temephos granules at 0.02 mg/liter and 0.04 mg/liter, there was significant difference in toxicity at the water surface level. Immediately, all of the more sensitive larvae died (Table 3), whereas some of the resistant ones survived (78.3% mortality at 0.02 mg/liter; 95% mortality at 0.04 mg/liter) on day 1. By day 11 mortality in the less resistant strain in the 0.02- and 0.04-mg/liter drums was still high at 70 and 85.0%, respectively, whereas in the more resistant strain this had dropped to 8.3 and 11.6%, respectively.

Resuspending the temephos granules by stirring had a marked effect on the less resistant larvae at the surface, producing almost complete mortality on day 25, and on day 32, 78.3% and 93.3% in 0.02- and 0.04-mg/liter barrels, respectively. In the more resistant larvae, however, there was only 1.6 and 8.3% mortality at these 2 concentrations on day 32. Thereafter, in the more resistant population, mortality was either absent or close to 0% in disturbed or undisturbed temephos, but up to day 39 there was still 25.0% mortality at the surface in the other group.

DISCUSSION

It seems reasonable to expect that 15–20 years of selection pressure with temephos in drum environments in the Caribbean region (Georghiou et al. 1987) may cause the emergence of massive levels of resistance to this insecticide. But in many locations, temephos is only occasionally used and source reduction is the major intervention method for *Ae. aegypti* management. This must be considered when reviewing the susceptibility patterns of the various *Ae. aegypti* strains to the insecticides assayed.

Temephos: Seven of 34 strains (20.6%) showed resistance ratios of about 2-fold or less to this chemical. Using the World Health Organization (1981a) recommended diagnostic dose of 0.02 mg/liter as a susceptible baseline value, all other 27 strains exhibit some levels of resistance to the insecticide. However, the levels of resistance shown here are quite moderate, with a maximum level of 12.1-fold resistance detected in the Sea Cow's Bay (Tortola) population. Using the same reference susceptible strain as did Georghiou et al. (1987), the Sea Cow's Bay population showed 15.9-fold resistance to temephos. This is to be compared with 46.8-fold resistance reported for a similar population by Georghiou et al. They only found one other highly resistant strain—Antigua (47.4)—for which we detected 5.1–9.2-fold resistance. The significance of our findings is that there is an increasing proportion of populations with enhanced levels of resistance over what was reported 8 years ago by Georghiou et al. (1987). Apart from the susceptible 20.6% of our populations mentioned above, virtually all the other strains fall into the resistant group, though moderately so.

Malathion: Eleven of the 34 (32.3%) populations assayed for susceptibility to malathion had 2-fold or less resistance to this chemical. All the others (67.7%) had developed only moderate levels of resistance. In none of the strains did the LC_{50} approach the World Health Organization (1981a) recommended diagnostic dosage of 1.0 mg/liter. Similarly, Georghiou et al. (1987) reported only low to moderate levels of resistance to malathion. This is probably related to very little malathion use against the larval stage, and

Table 2. Susceptibility to malathion in Caribbean strains[1] of adult *Aedes aegypti*.

Country	Location	LT_{50}[2]	R.R.[3]
Anguilla	The Valley (ex tree hole)	3.9	0.7
	Little Dix (ex rock hole)	5.1	0.9
	Crocus Bay (ex tree hole)	6.2	1.1
	Valley Health Center	5.4	0.9
Antigua	All Saints Village	7.5	1.3
	Barnes Hill	12.2	2.1
	Gray's Farm	9.0	1.5
	Urlings	17.3	3.0
Aruba	Oranjestaad	19.0	3.3
Bahamas	Nassau	6.0	1.0
Barbados	Fontabelle	15.2	2.6
Dominica	Wesley	10.6	1.8
	Deepwater Harbour	14.2	2.5
	Canefield Airport	6.8	1.2
Grenada	Grande Anse	13.0	2.2
Guyana	Mabaruma	8.8	1.5
	Georgetown	9.6	1.7
Jamaica	Hughenden Park	23.4	4.0
	Richmond Park	16.2	2.8
Puerto Rico	San Juan, Puerto Nuevo	18.5	3.2
St. Kitts	Basseterre	9.6	1.7
St. Lucia	Castries	19.1	3.3
St. Maarten	St. Maarten	15.8	2.7
St. Vincent	Kingstown	12.1	2.1
Tortola	Emmanuel Reef	18.0	3.1
	Sea Cow's Bay	12.3	2.1
Trinidad	St. James	21.8	3.8
	Caroni (Chin Chin)	15.1	2.6
	Point Fortin	18.2	3.1
Suriname	Reneprojeht	9.6	1.7
	Diakenessen	6.0	1.0
	Lands Hospital	13.8	2.4
Venezuela	Maracay—23 de Enero	7.4	1.3
	El Pinonal	15.1	2.6
Reference	CAREC colony	5.8	1.0
	Susceptible California Rock	3.2	0.5

[1] Strains collected and tested 1990–94.
[2] LT_{50} = time (minutes) of exposure to 5% malathion surfaces required to kill 50% of the adult sample.
[3] Resistance ratio (R.R.) based on the susceptible CAREC colony.

only rare use in emergencies, if at all, against the adult stage.

Fenitrothion: About 34.5% of our populations (10 of 29) are still susceptible to fenitrothion. But the 65.5% that show some resistance are all in the moderate 2- to 4-fold resistance levels. In none of the strains did the LC_{50} value reach the World Health Organization (1981a) diagnostic dosage of 0.06 mg/liter. Fenitrothion has not been used very commonly for mosquito control in the Caribbean (Rawlins, 1993, unpublished data). Thus, this moderate level of resistance may have been selected as cross-resistance due to other OP insecticides. Fenitrothion could conceivably be a replacement larvicide for chemicals that cease to be effective due to resistance.

Fenthion: Only 4 of 25 of our populations (16%) showed 2-fold or less resistance to fenthion. But considering that the World Health Organization (1981a) recommended diagnostic dose for this chemical is 0.05 mg/liter, the LC_{50} of 24 of 25 populations (96%) may fall below this threshold. However, our Tortola, Bahamas, and Antigua populations that were not highly resistant 8 years ago (Georghiou et al. 1987) now show the highest resistance to this chemical. This may also be a result of cross-resistance to temephos selection pressure.

Chlorpyrifos: Fifty-four percent (7 of 13) of

Table 3. Toxicity of 1% temephos (Abate®) sand granules (undisturbed and resuspended) to larvae of moderate and high insecticide-resistant strains of *Aedes aegypti* evaluated in drum containers.[1]

Day of treatment	% mortality in containers with undisturbed Abate granules				% mortality in containers with resuspended Abate granules			
	Moderately resistant strain[2]		Resistant strain[3]		Moderately resistant strain[2]		Resistant strain[3]	
	0.02 mg/liter	0.04 mg/liter	0.02 mg/liter	0.04 mg/liter	0.02 mg/liter	0.04 mg/liter	0.02 mg/liter	0.04 mg/liter
1	100.0	100.0	78.3	95.0	—	—	—	—
4	91.6	100.0	55.0	81.6	—	—	—	—
8	71.6	78.3	38.3	21.6	—	—	—	—
11	70.0	85.0	8.3	11.6	—	—	—	—
22	0.0	25.0	0.0	3.3	—	—	—	—
25	—	—	—	—	100.0	100.0	—	—
29	0.0	11.6	0.0	0.0	—	—	—	—
32	—	—	—	—	78.3	93.3	1.6	8.3
36	0.0	0.0	0.0	0.0	—	—	—	—
39	—	—	—	—	6.6	25.0	0.0	5.0
43	0.0	0.0	0.0	0.0	—	—	—	—
46	—	—	—	—	0.0	3.3	0.0	1.6
50	0.0	0.0	0.0	0.0	—	—	—	—
53	—	—	—	—	0.0	0.0	0.0	0.0

[1] All larvae in floating cups.
[2] Moderately resistant strain LC_{90} (temephos) = 0.063 mg/liter.
[3] Resistant strain LC_{90} (temephos) = 0.082 mg/liter.

all strains assayed for susceptibility to this chemical showed 2-fold or less resistance. This may be due to absence of use of this insecticide for *Ae. aegypti* control in the Caribbean. Chlorpyrifos too may be a potential larvicide for *Ae. aegypti* control in the region.

Adulticidal studies: Up to the present, there have been no laboratory confirmed data on reduced susceptibility to malathion of bloodfed adult Caribbean *Ae. aegypti.* But because malathion is one of the main insecticides recommended for use in emergency situations for dengue control (Pan American Health Organization 1982), it is important to demonstrate that our mosquito populations are still susceptible to this chemical. Forty-four percent of the strains were susceptible (not more than 2-fold resistance) to malathion. The other 56% showed only moderate—up to 4-fold—resistance. This suggests that malathion could continue to be used for emergency adulticidal operations. Monitoring to detect any further significant increase in resistance would be necessary. Adulticidal operations should also integrate the use of other non-OP insecticides such as pyrethroids.

Field studies: The drum studies with 2 strains of *Ae. aegypti* with different sensitivities to temephos indicate that in the practical situation of mosquito larvae control there is a loss of insecticide efficacy, with resistance demonstrated in the laboratory. In fact, the findings by Mekuria et al. (1991) that alternative methods to the use of chemicals for mosquito control be considered is a very worthwhile suggestion. In our more resistant population, insecticide failure was noted from day 1 at the diagnostic and even double the diagnostic doses.

Other insecticides with low mammalian toxicity must be evaluated for use against *Ae. aegypti.* Alternative biological control tools that are dependable against *Ae. aegypti* are also needed. However, these should only be aids to our main armament of environmental sanitation—source reduction—in the fight against *Ae. aegypti.*

The floating cup device (Chadee 1989) for evaluating insecticide efficacy in the field did not prove very useful in our studies. Immediately after day 1, the effect of the chemical was lost to some of the larvae at the water surface (Table 3), and the effect approached 0% by 3 wk. Stirring the granules in drums improved the efficacy, but we believe that observing the natural movement of free larvae in drums is the most effective, though time-consuming, method of testing the chemical's efficacy.

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