MALATHION-SPECIFIC RESISTANCE IN *ANOPHELES STEPHENSI* FROM PAKISTAN

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ABSTRACT. A strain of *Anopheles stephensi* from Pakistan (MalR) was 8.7-fold resistant to malathion and 6.7-fold cross-resistant to phenthoate, but not to other carboxylesters; no cross-resistance to bendiocarb, fenitrothion, permethrin and carbaryl was detected. Resistance was not associated with elevated levels of general esterase activity, as determined by hydrolysis of naphth-l-yl acetate (α-NA), but was correlated with higher levels of malathion carboxylesterase (MCE) activity *in vitro*. The results suggest that a highly specific type of esterase mediated resistance, such as MCE, can best be detected by an enzyme specific assay rather than one for general esterase activity. A new, rapid and sensitive assay for mosquito MCE is given.

INTRODUCTION

Anopheles stephensi Liston is an important vector of malaria in parts of Asia and the Middle East. Malathion has been used as a control agent although resistance has been detected in *An. stephensi* near Lahore, Pakistan (Rathor and Toqir 1980). In this report, we determine the mechanism of resistance to malathion and develop a rapid and sensitive assay for this mechanism.

MATERIALS AND METHODS

Two mosquito strains were used in this study: Walter Reed (WR), a susceptible strain[3], was obtained from Walter Reed Army Institute of Research (WRAIR), Washington, D.C. in 1978. and has been reared here without selection pressure since then; MalR, a malathion resistant strain, was originally collected at Khano Harni, Pakistan in 1978 and was received here in 1983. The MalR strain has been maintained under routine laboratory selection with malathion applied to larvae.

The following chemicals were used: Malathion, 91% (American Cyanamid); permethrin, 95.5% (*cis:trans* ratio 41.9:53.6 ICI); bendiocarb, technical grade (FBC Limited); fenitrothion, 96.8% (Sumitomo); *S,S,S*-tribuytl phosphorothioate, 94% (DEF, Chemagro); triphenyl phosphate, 99+% (Aldrich); phenthoate, technical grade (Montedison); carbaryl 99.9+% (Union Carbide); naphth-l-yl acetate (α-NA, Sigma); fast garnett GBC salt (Sigma); alcoholdehydrogenase (Sigma); NAD-diaphorase (Sigma); and iodonitrotetrazolium violet (INT, Sigma).

Standard bioassay techniques for mid-4th instar larvae (Georghiou et al. 1966) were used. Twenty larvae were placed into 99 ml of water in waxed paper cups. One ml of acetone (control) or insecticide (in acetone solution) was added to the water to give the desired concentration. Mortality was assessed after 24 hours. Results were subjected to probit analysis (Finney 1952). Statistical significance was examined based on degree of overlap of the 95% confidence intervals (Scott and Georghiou 1984).

General esterase activity, as measured by hydrolysis of α-NA, was performed by the filter paper method of Pasteur and Georghiou (1981). Results were analyzed by a computerized method developed in this laboratory (A. Iseki, personal communication). The resulting histogram indicates the percent of individuals responding at a given level on a scale of 0% to 100%, where the 0% level is the staining intensity of a susceptible strain (C-lab, *Culex quinquefasciatus* Say) and 100% is the staining intensity of a highly resistant strain (Tem-R, *Cx. quinquefasciatus*).

The method of determining malathion carboxylesterase (MCE) activity is adapted from one originally used on rats (Talcott 1979). Levels of MCE were analyzed by rinsing 20 fourth instar larvae in distilled water and homogenizing in 0.5 ml sodium phosphate buffer (0.1 M, pH 7.5) containing 0.75% BSA in a glass-glass tissue homogenizer on ice. The homogenate was transferred to a test tube, the tissue homogenizer was rinsed with 0.5 ml buffer and the rinse was added to the test tube. Then 1.5 ml of incubation mixture (containing 437 μg/ml INT, 25 U/ml alcohol dehydrogenase and 0.1 U/ml NAD diaphorase) was added and the tube was centrifuged at 1500 *g* for 3 min at 10°C. Two ml of the supernatant was transferred to a glass cuvette and 5 μl

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[3] The WR strain has been maintained at WRAIR since January 1963. It was started from eggs received from P. G. Shute at the former Malaria Reference Laboratory in England. Mr. Shute's colony came from the Malaria Institute, Delhi, India in 1947. In the older literature the WR strain is known as the Delhi or India strain.

acetone (reference) or 5 µl of 150 mM malathion in acetone solution was added. The cuvettes were held at 31°C for 4 min to allow for temperature equilibration and then the change in absorbance at 500 nm was monitored. Five experiments, using the WR and MalR strains simultaneously, were carried out and the results averaged.

RESULTS

The bioassay data indicate that larvae of the MalR strain are resistant to malathion (8.7-fold) and phenthoate (6.7-fold), but not to any of the other pesticides tested (Table 1). Malathion resistance could be suppressed by the use of DEF (a general esterase inhibitor) or TPP (a carboxylesterase inhibitor), implying that the resistance mechanism was a carboxylesterase-mediated detoxification. Resistance to phenthoate could also be overcome by TPP, indicating that a carboxylesterase was also responsible for this resistance. As three of the other pesticides tested (i.e., bendiocarb, carbaryl and permethrin) also possess a carboxylester moiety, but were apparently unaffected by the resistance mechanism, it appears that the resistance mechanism is of a highly specific type (i.e., malathion carboxylesterase, MCE). This type of highly specific, carboxylesterase-mediated malathion resistance has been reported previously in several species, including *Tribolium castaneum* (Dyte and Rowlands 1968, Beeman 1983), *Musca domestica* (Matsumura and Hogendijk 1964), *Culex tarsalis* (Bigley and Plapp 1962, Matsumura and Brown 1963), *Nephotettix cincticeps* (Kojima and Kitakata 1963), *Lucilia cuprina* (Hughes et al. 1984) and implied to exist in *Blattella germanica* (van den Heuvel and Cochran 1965), *Chrysomya putoria* (Busvine et al. 1963) and *Anopheles stephensi* (Hemingway 1982).

Table 1. Relative toxicity of six insecticides to 4th instar larvae of the Walter Reed and MalR strains of *Anopheles stephensi*.

	Walter Reed	MalR	
Compound	LC_{50}[a]	LC_{50}[a]	RR[b]
Malathion	0.18	1.600	8.7*
Malathion + DEF	0.069	0.081	1.2
Malathion + TPP	0.20	0.201	1.0
Bendiocarb	1.7	1.5	0.91
Fenitrothion	0.027	0.035	1.30
Permethrin	0.022	0.011	0.50*
Phenthoate	0.045	0.303	6.7*
Phenthoate + TPP	0.017	0.014	0.8
Carbaryl	0.72	0.515	0.7*

[a] In units of PPM.
[b] Resistance Ratio: LC_{50} MalR/LC_{50} Walter Reed.
* Significantly different from 1.0 (P ≤ 0.05).

To determine if malathion resistance could be correlated with general esterase activity we measured the hydrolysis of α-NA in both the WR and MalR strains. The results shown in Fig. 1 indicate that resistance was not associated with higher levels of α-NA hydrolysis. This is in agreement with a previous study on malathion-specific resistance in *Tribolium castaneum* (Beeman 1983).

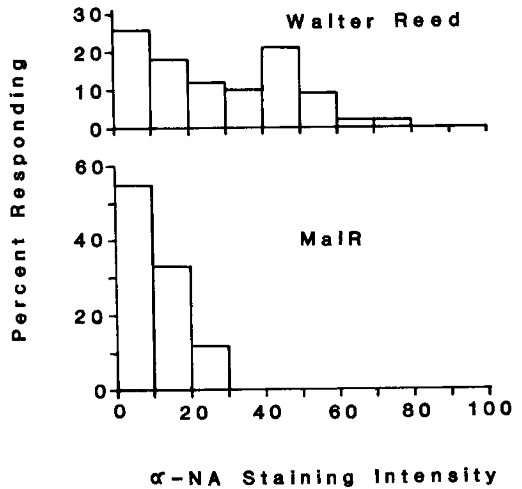

Fig. 1. Relative rate of α-NA hydrolysis in susceptible (WR) and resistant (MalR) strains of *Anopheles stephensi*.

Results of the MCE assay, as determined by the formation of malathion monoacid, are shown in Fig. 2. The MalR strain has greater MCE activity than the WR strain with 12.0 ± 1.4 and 6.7 ± 1.9 nmoles malathion hydrolyzed min^{-1} mosquito $equivalent^{-1}$, respectively, after 15 minutes (Table 2). This assay was found to be reproducible, rapid and easy to perform, with the greatest difference between the strains noted after 15 minutes (Table 2).

DISCUSSION

Malathion resistance in the MalR strain of *Anopheles stephensi* from Pakistan appears to be due to MCE-mediated detoxification. Cross-resistance was confined only to phenthoate, a malathion analog, and not to any other pesticide tested. The specific nature of this resistance mechanism is further exemplified by the fact that measuring general esterase activity could not detect the presence of MCE. Only after

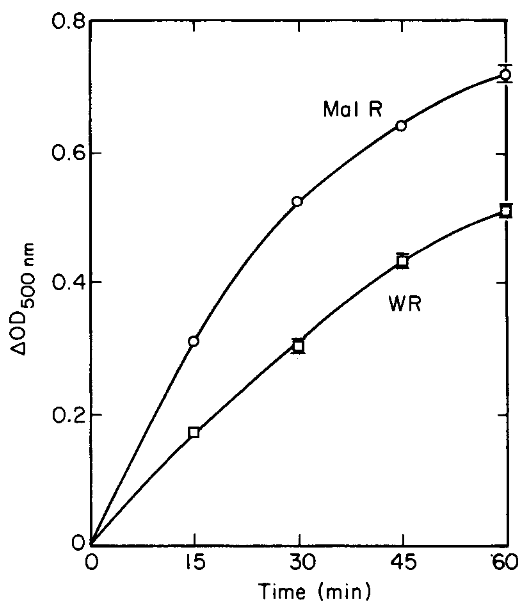

Fig. 2. MCE hydrolysis of malathion in susceptible (WR) and resistant (MalR) strains of *Anopheles stephensi*. Bars represent the S.E. of the mean except where the symbol exceeds the limits of the error.

using a more enzyme specific assay was it possible to confirm the nature of this resistance mechanism. These data help to further clarify the results of an earlier study on malathion resistance in *An. stephensi* in which the malathion resistance was suppressible by TPP and DEF while no increase in carboxylesterase activity could be detected using α-NA hydrolysis as the criterion (Hemingway 1982).

Based on our results with *An. stephensi* and those of Beeman (1983) on *Tribolium castaneum*, it appears that a highly specific type of esterase mediated resistance, such as MCE, can best be detected by enzyme specific assays rather than those for general esterase activity. Furthermore, it is possible that the MCE assay reported herein, run with phenthoate, might allow for determination of the kinetic parameters (i.e. K_m and V_{max}) of the reaction and thus a more complete understanding of the resistance mechanism.

Table 2. Comparison of malathion carboxylesterase activity in two strains of *Anopheles stephensi*.

Strain	Time (min)	Activity[a]	R/S[b]
WR	15	6.7±1.9	—
	30	5.8±1.4	—
	45	5.6±0.9	—
	60	5.0±0.5	—
MalR	15	12.0±1.4	1.8
	30	10.0±1.3	1.7
	45	8.3±1.1	1.5
	60	7.0±0.9	1.4

[a] In units of nmoles malathion hydrolyzed min^{-1} mosquito $equivalent^{-1}$. Values represent the $\overline{X}$±S.E.
[b] Value of MalR/WR.

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