MULTIPLE RESISTANCE IN *ANOPHELES ALBIMANUS*

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ABSTRACT. A comparison of the susceptibility status of adults of *Anopheles albimanus* from Panama and El Salvador showed the former to be highly susceptible to all the insecticides tested. In contrast the El Salvador population showed a broad spectrum of resistance involving DDT, propoxur, and a number of organophosphates but remained susceptible to fenthion and the 2 pyrethroid insecticides, permethrin and decamethrin. Exposures of the resistant population to combinations of malathion and the synergists TPP, PB, SV_1 and DEF produced results suggestive of the involvement of at least 2 detoxication mechanisms, carboxylesterase and mixed function oxidases.

INTRODUCTION

The first appearance of malathion resistance, accompanied by cross resistance, in a field population of *Anopheles albimanus* Wiedemann from El Salvador, was reported by Breeland et al. (1970). Ariaratnam and Georghiou (1971) demonstrated a broad spectrum of resistance, involving organochlorines, organophosphates and carbamates in a population of the species from El Salvador which initially showed organochlorine resistance, some tolerance to a number of organophosphates and carbamates and which was subsequently selected in the laboratory with propoxur. By the use of synergists these authors were able to show that a variety of detoxication mechanisms were involved. While suggesting the carboxylesterase (CE) involvement in malathion resistance, carbamate resistance was attributed to the mixed function oxidase system. The strong antagonism produced by piperonyl butoxide (PB) on phosphorothioate compounds was pointed out to be a result of the inhibition of the activation process. Lack of synergism of the phosphates was attributed to an attack on the thionate prior to its conversion to the phosphate and/or a non-oxidative degradation process. A low level of synergism of paraoxon with *S, S, S*-tributyl phophorotrithioate (DEF) was considered to indicate a demethylation mechanism. The role played by factors other than metabolism, such as reduced penetration of insecticides (Ariaratnam and Georghiou 1975) and insensitive acetylcholinesterase (Ayad and Georghiou 1975), in the multiple resistance have been subsequently demonstrated in this species. These observations were mainly based on studies carried out on the larval stages of the populations concerned and Georghiou (1972) associated the resistance with the intensive use of insecticides for agricultural purposes.

Davidson and Sawyer (1975) working with both adults and larvae confirmed the broad spectrum of resistance in El Salvador *An. albimanus* and while finding no evidence of fenthion resistance in adults an increased tolerance was evident in the larvae.

This paper compares the response shown by adult mosquitoes of 2 populations of *An. albimanus* from Panama and El Salvador towards DDT, 8 organophosphates, propoxur and 2 pyrethroid insecticides, permethrin and decamethrin. Most of these compounds are candidate insecticides for public health vector control programmes. It also reports on attempts to determine the nature of the detoxication mechanisms involved in the malathion resistance in adults of the El Salvador population.

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MATERIALS AND METHODS

The 2 populations of *Anopheles albimanus* used were:

PALB—a population from Panama maintained in the laboratory at the London School of Hygiene and Tropical Medicine since 1959 and previously in the United States.

FERNS/RR—a population provided by Mr. Su Yung Liu, WHO Entomologist, from El Salvador in 1974. Adults of this population had been selected in the laboratory with propoxur for some time before transfer to London.

The standard impregnated papers as supplied by the World Health Organization for the adult insecticide susceptibility test were used in the case of DDT, malathion, fenitrothion, fenthion and propoxur. Permethrin (cis/trans isomers 25:75) and decamethrin papers were supplied by Mr. P. R. Chadwick of Wellcome Research Laboratories, Berkhamsted, England. They were prepared using a silicone as non-volatile solvent. The impregnated papers of chlorphoxim, phoxim, pirimiphos-methyl, iodophenphos and phenthoate were prepared locally from pure or near pure samples using dioctyl phthalate as the solvent. Acetone served as the volatile solvent.

The synergists triphenyl phosphate (TPP) and O,O-dimethyl O-phenyl phosphorothioate (SV_1) were supplied by Dr. F. J. Oppenoorth of the Laboratory for Research on Insecticides, Wageningen, The Netherlands. Piperonyl butoxide (PB) and *S,S,S*-tributyl phosphorotrithioate (DEF) were supplied by Dr. R. M. Sawicki of Rothamsted Experimental Station, Harpenden, England. Impregnated papers of these synergists were prepared locally using the maximum non-toxic dosages of the appropriate compound.

Rearing, maintenance of the mosquito colonies and testing were carried out in the insectaries of the Ross Institute, London School of Hygiene and Tropical Medicine where controlled temperatures (25–28°C), RH (70–80%) and a 12 hr photoperiod existed. The standard procedures for rearing anopheline mosquitoes were followed. The larvae were maintained at temperatures of 28–31°C and fed on ground Farex, a proprietary baby food. Larval feeding was adjusted depending on the larval density and stage of development to ensure the production of uniform sized larvae, pupae and emerging adults. Both males and females had access to approximately 20% glucose solution. The females were also fed on guinea pig blood.

The susceptibility status of the 2 populations to the different insecticides were determined by the standard WHO adult susceptibility test using less than one day old males and unfed females. The concentration of the different insecticides used were those considered to discriminate susceptibility from resistance. Mortalities were determined usually after 1 hour exposure, followed by a 24 hr recovery period. Exposures to the synergists were done in the same way and followed by exposure to malathion. A comparable sample of the population was tested simultaneously with malathion alone.

RESULTS AND DISCUSSION

Table 1 compares the susceptibility status of the 2 populations PALB and FERNS/RR to DDT, permethrin, decamethrin, malathion, fenitrothion, phenthoate, fenthion, chlorphoxim, phoxim, pirimiphos-methyl, iodophenphos and propoxur. Both populations were highly susceptible to fenthion and there was no apparent difference in the response to permethrin and decamethrin in the 2 populations. With decamethrin, a 2 hour exposure was required for a complete kill in both populations. In contrast to the highly susceptible PALB, the FERNS/RR population showed resistance to DDT, malathion, phenthoate, fenitrothion, chlorphoxim, phoxim, pirimiphos-methyl, iodophenphos and propoxur.

Table 2 and Figure 1 shows the mortalities in the FERNS/RR population after

Table 1. Percentage mortalities at "discriminating dosages" of various insecticides, (usually after 1 hour's exposure) in the 2 populations of *Anopheles albimanus* from Panama (PALB) and El Salvador (FERNS/RR).
(Numbers of mosquitoes tested are given in parentheses).

Insecticide	Concentration	*An. albimanus* population			
		PALB		FERNS/RR	
malathion	5%	100	(233)	39	(1,164)
phenthoate	10%	95***	(87)	24***	(74)
fenitrothion	1%	99	(454)	14	(155)
				27**	(75)
chlorphoxim	4%	100	(138)	60	(211)
phoxim	2.5%	100	(59)	89	(83)
pirimiphos-methyl	1%	100	(143)	58	(319)
iodophenphos	10%	100	(102)	27	(158)
fenthion	2.5%	100	(60)	100	(118)
		100*	(29)	100*	(38)
DDT	4%	100	(126)	92	(333)
		100*	(79)	—	
permethrin	0.2%	97	(238)	93	(266)
decamethrin	0.001%	81	(206)	85	(336)
		100**	(17)	100**	(89)
propoxur	0.1%	100	(341)	46	(61)

* 30 minute exposure.
** 2 hour exposure.
*** 45 minute exposure.

different periods of exposure to malathion and to malathion following pre-treatment with TPP, PB, SV_1 and DEF. Only tentative conclusions can be drawn from them as straight-line relationships between dosage and mortality are not always apparent. TPP appeared to produce slight synergism at all dosages tested suggesting a possible involvement of carboxylesterase (CE) in malathion resistance. Response to PB/malathion treatment varied producing slight evidence of both synergism and antagonism at different dosages. If the involvement of mixed function oxidases (mfo's) is confined to the well established process of conversion of the P=S bond to P=O in the activation of malathion to the toxic analogue malaoxon (Metcalf 1969), then pre-treatment with PB which will inhibit this activation step should produce only continued antagonism with malathion at all the dosages tested. On the other hand if this enzyme system is additionally involved in the detoxication of malathion, the nature of the response to the PB/malathion sequence will depend on the balance of the opposing processes of activation and detoxication of malathion. The net result could be one of synergism, antagonism or even ineffectiveness. The observations made therefore suggest the possible involvement of both mfo's and CE in the detoxication of malathion, a conclusion reached from similar studies made with a comparably organophosphate multi-resistant population of *An. culicifacies* Giles from India (Herath and Davidson 1981a) and in contrast to that reached from observations with a population of *An. stephensi* Liston from southern Iran (Herath and Davidson 1981b).

Synergist SV_1 (known to inhibit carboxylesterases and oxidases) enhanced the toxicity of malathion to a level greater than that produced by TPP. This may substantiate the involvement of both CE and mfo's. The broad spectrum of resistance exhibited by this population may be to some extent a result of cross-resistance conferred by the mfo involvement. There was no obvious synergism with DEF. It

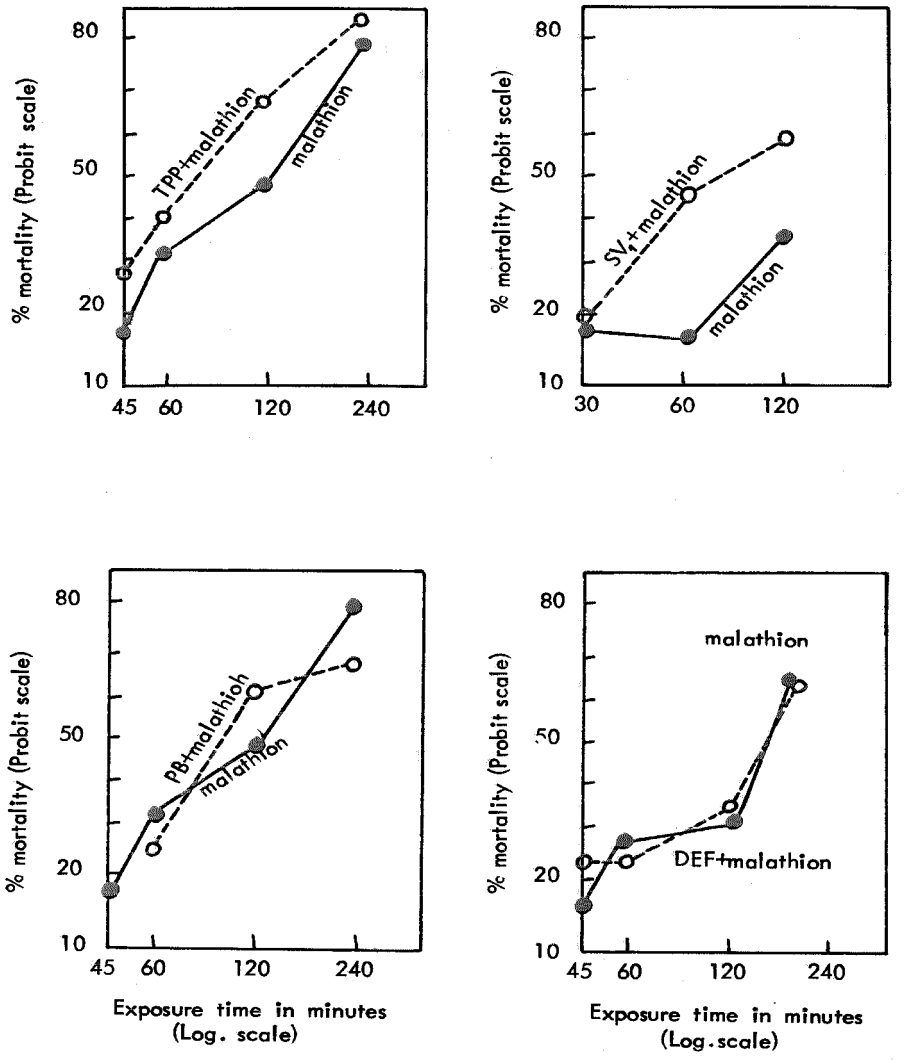

Fig. 1. The mortality relationship in *Anopheles albimanus (FERNS/RR)* exposed to malathion alone and to malathion after previous exposure to the synergists TPP, SV, PB and DEF.

Table 2. Results of exposures of the FERNS/RR strain of *An. albimanus* from El Salvador to malathion alone and to malathion following pretreatments with TPP, PB, DEF and SV_1.

Type of exposure	Exposure time in minutes															
	30			45			60			120			180			240		
	D*	T**	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%
malathion 5.0%	—			11	66	17	73	232	32	186	392	47				116	147	79
TPP + malathion 5.0%	—			18	64	28	84	211	40	238	351	68	—			128	154	83
PB + malathion 5.0%	—				—		33	130	25	145	233	62	—			94	139	68
malathion 5.0%	—			5	34	15	42	157	27	81	264	31	74	116	64		—	
DEF + malathion 5.0%	—			9	39	23	33	145	23	77	231	33	98	154	64		—	
malathion 5.0%	3	17	18		—		5	31	16	24	68	35		—			—	
SV_1+ malathion 5.0%	3	16	19		—		19	43	44	35	59	59		—			—	

D* number of dead mosquitoes.
T** number of mosquitoes tested.

must be stressed however, that these findings need further substantiation before definite identification of all the mechanisms involved can be made.

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