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ANOPHELES ALBIMANUS: DEVELOPMENT OF CARBAMATE AND ORGANOPHOSPHORUS RESISTANCE IN NATURE[a]

by

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Abstract. Natural populations of Anopheles albimanus in the area of La Libertad in coastal El Salvador demonstrate a broad spectrum of resistance to carbamate and organophosphorus insecticides, including propoxur, carbaryl, parathion, malathion, and fenitrothion. This development appears to be the result of extensive and long-term use of organophosphorus, and to a lesser extent carbamate insecticides on agricultural crops, especially on cotton.

Carbamate and organophosphorus insecticides are currently being studied in the global malaria eradication effort as alternative chemicals for use against DDT and dieldrin-resistant *Anopheles* mosquitos where the situation so demands. Although more expensive and less persistent than DDT as residual sprays for the walls of buildings, many members of these types of chemicals possess the advantage of outstanding toxicity against adult and larval mosquitos. Such toxicity remains largely unaffected by existing DDT or dieldrin resistance, and until recently it appeared that selection pressure on *Anopheles* species did not result in resistance to these chemicals. This paper reports on the appearance of high levels of resistance to several carbamate and organophosphorus insecticides in a natural population of *Anopheles albimanus* in coastal El Salvador. The observed resistance was found in an area that has been under intensive treatment with organophosphorus insecticides for several years for cotton insect control and may be attributed to indirect selection pressure by these treatments.

The first signs of impending resistance to organophosphates in field populations of *Anopheles* were reported by the World Health Organization (WHO) in 1965 and consisted of suspected slight increases in tolerance to malathion in *A. albimanus* in Guatemala and Nicaragua.[3] Subsequently, Breeland et al. (1970) demonstrated that aerial applications of this chemical in the area of La Libertad, El Salvador, in March, 1969, were significantly less toxic to caged wild-caught adult *A. albimanus* than to similarly exposed adults of a laboratory colony. Assays with the standard WHO testing kit verified these conclusions. Breeland and his co-workers pointed out that the decrease in susceptibility to malathion in El Salvador occurred only in areas of intensive cotton cultivation. These areas are being subjected during six months of the year at almost weekly intervals, to heavy applications of several organophosphorus insecticides, including trichlorphon, parathion, methyl parathion, and malathion (Breeland et al., 1970).

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[3] WHO Inf. Circ. Insecticide Resist., Insect Behaviour and Vector Genetics, 56, 24 (1965).

Organophosphorus insecticides have been employed extensively on cotton in El Salvador for more than 10 years. Since A. albimanus breeds also in the vicinity of cotton fields (Breeland et al., 1970), it may be assumed that it is subjected to indirect selection pressure by these chemical materials. The reported field resistance to malathion in Anopheles is of significance in the global malaria eradication programme since this compound, together with the organophosphate fenitrothion and the carbamate propoxur are the only compounds among more than 1400 candidate materials which have been tested successfully through most stages of WHO's International Collaborative Programme for Evaluating and Testing New Insecticides (Wright et al., 1969).

For almost a decade, studies have been carried out in our laboratory at the University of California, Riverside, on the potentialities and mechanisms of resistance to promising new insecticides in mosquitoes. Intensive selection pressure in the laboratory on A. albimanus from Panama and Haiti using m-isopropylphenyl methylcarbamate, propoxur, and fenitrothion has resulted in only minimal resistance to these insecticides, not exceeding 3X the larval LC_{50}, despite the presence of DDT- and dieldrin-resistant genes in the parental populations (Georghiou, 1963a, 1963b, 1969; Georghiou & Calman, 1969). Such results have been considered encouraging indications that high resistance to carbamates or organophosphates in Anopheles would, if it ever developed, require extremely prolonged selection pressure.

Following publication of the WHO reports of resistance of Anopheles to organophosphates, we obtained a collection of this species from the area of Hacienda Melara, La Libertad, El Salvador, in June 1970, and colonized the insects in our laboratory at the University of California, Riverside. Our studies provided definitive evidence of the appearance of tolerance to organophosphates in larvae, amounting to a 3.2X increase in the LC_{50} value for parathion, 3.4X to methyl parathion, and 2.9X to malathion[1] (Ariaratnam & Georghiou, 1971). Moreover, when this larval population was subjected to rigorous selection pressure by propoxur, resistance rose within three generations to more than 100X for propoxur, 74.8X for carbaryl, 35.5X for parathion, 36.9X for methyl parathion, 20X for malathion, and 9.6X for fenitrothion[1] (Ariaratnam & Georghiou, 1971). Thus it was apparent that A. albimanus was indeed capable of developing resistance to carbamates and organophosphates and that the population of this species in the La Libertad area had attained a tolerance level which would readily lead to high resistance upon rigorous selection pressure by propoxur.

We have continued to follow up on the El Salvador case and can now report that additional samples collected from the same locality eight months later in February 1971 show considerably increased resistance to carbamates and organophosphates. The samples consisted of approximately 500 gravid females obtained from the field and permitted to oviposit at the CAMRS Laboratory[2] in San Salvador. Some 3000 eggs from these females were then shipped to our Riverside laboratory.

Larvae of the F_1 and F_2 generations resulting from the shipment were tested for susceptibility to a variety of insecticides. Parallel tests were performed on larvae of a strain of Haiti/Panama origin, which were susceptible to carbamates and organophosphates but resistant to dieldrin and moderately resistant to DDT. The adult colonies were maintained in 30 x 30 x 30 cm screened cages at 29°C and 60 to 70 per cent. R.H. and provided with white mice for blood meals. The larvae were kept in enamelled pans at 29°C and fed on a 50:50 mixture of brewers' yeast and Purina Laboratory Chow. Larvae were tested in the early fourth instar in groups of 20 in 250 ml capacity waxed paper cups containing 100 ml of water. The insecticides were prepared from technical or analytical grade samples in standard w/v acetone solutions of the desired concentrations and were applied at the rate of 1 ml per 100 ml of water. The results were then assayed 24 hours later. Tests were repeated on at least five

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[1] Ariaratnam, V. & Georghiou, G. P. (1971): Resistance to Carbamate and Organophosphorus Insecticides in *Anopheles albimanus* WHO/VBC/71.277 (unpublished document).

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different days and the data subjected to probit analysis (Finney, 1952) on an IBM 360/50 computer.

The results as summarized in Table 1 clearly indicate that the population of A. albimanus at Melara acquired considerable resistance to a variety of insecticides between June 1970 and February 1971. Resistance was highest for the carbamates propoxur and carbaryl and the organophosphates parathion, methyl parathion, malathion, and fenitrothion, and only slight for dichlorvos and Dursban(R) (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), whereas no resistance occurred with fenthion and Abate(R) (O,O, O',O'-tetramethyl O,O'-thiodi-p-phenylene phosphorothioate). This is in agreement with the characteristic subgroup specificity of organophosphorus resistance observed in other insects (March, 1960; Georghiou & Hawley, in press). A high degree of resistance is evident not only in increased LC_{50} values and resultant resistance ratios, but also in characteristically low slope values (b) of the log-dose probit-mortality regression lines. This is especially apparent in the case of the carbamates where b for carbaryl is 0.8 and for propoxur 0.43. Similar results were also obtained when resistance to propoxur and parathion was assayed in glass beakers instead of waxed paper cups. In this case there was a small decrease in the LC_{50} values of both the susceptible and resistant strains but there was no significant change in the level of resistance obtained.

High resistance, especially in the aquatic stage which is bioassayed by continuous exposure of the organisms for 24 hours, would suggest that a combination of increased metabolism and decreased penetration of the insecticide may be responsible for resistance. The role of reduced penetration in producing considerably magnified levels of resistance has been amply demonstrated in the house fly (Plapp & Hoyer, 1968; Georghiou, 1971). Until appropriate tests on metabolism are carried out in the present strain, it is not possible to offer a biochemical interpretation of the broad spectrum of resistance we have observed, especially in regard to the involvement of carbamates in such resistance. Studies with various synergists on the propoxur-selected strain mentioned earlier, which possesses a resistance spectrum closely resembling the one observed in the present study, suggest that mixed function oxidases and carboxyesterase enzymes are involved in metabolism[1] (Ariaratnam & Georghiou, 1971). Mixed function oxidases and a reduced rate of penetration have been shown to be responsible for propoxur resistance in larvae of Culex pipiens fatigans (Shrivastava et al. 1969).

Although the precise mechanisms of resistance in the present case are unknown, it is evident that the observed pattern of resistance to organophosphates corresponds closely to the extensive use of parathion, methyl parathion, and malathion on cotton in El Salvador during the past several years. Fenitrothion has not been employed in this area, however, and the 9.2X resistance observed for this insecticide may be a case of true cross resistance[2] in view of the close similarity of its molecule to that of methyl parathion. A similar situation exists in organophosphorus-resistant Aedes nigromaculis in California, in which a 75X cross resistance to fenitrothion was reported although this compound has not been used commercially in the State (Schaefer & Wilder, 1970). In the house fly, the concurrence of resistance to fenitrothion and methyl parathion has been shown to be due to enhanced degradation of the activation products fenitro-oxon and methyl paraoxon by phosphatase action (Hollingworth, Metcalf & Fukuto, 1967). The involvement of carbamates in such resistance is less well understood, however, when one considers the relatively limited use that has been made of these insecticides in the study area. It is possible that resistance to these materials has resulted from selection by carbaryl, which is applied to rice and occasionally to cotton and corn, and may have been enhanced by the broad spectrum organophosphorus resistance present in the population.

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[2] "Cross resistance" refers to those cases in which one mechanism confers protection against various toxicants.

The coexistence of propoxur, malathion, and fenitrothion resistance in *A. albimanus* in El Salvador, and its possible impact on malaria eradication in Central America, require careful assessment, since these three chemicals have been considered to be outstanding alternative insecticides for use against DDT and dieldrin-resistant anophelines.

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TABLE 1. INSECTICIDE RESISTANCE LEVELS IN *ANOPHELES ALBIMANUS*, EL SALVADOR, IN JUNE 1970 AND FEBRUARY 1971

Insecticide	Haita/Panama strain (Susceptible)		El Salvador strains					
			June 1970			February 1971		
	LC_{50}*	slope	LC_{50}*	slope	RR**	LC_{50}*	slope	RR**
Propoxur	.39	7.7	.53	7.1	1.4	64.1	.43	164.4
Carbaryl	.89	6.6	1.02	5.9	1.1	15.7	.8	16.9
Parathion, methyl	.0065	3.0	.022	3.5	3.4	.17	2.1	26.2
Parathion, ethyl	.0031	6.8	.0098	3.1	3.2	.057	1.9	18.4
Malathion	.085	2.9	.25	2.7	2.9	1.1	1.8	12.9
Fenitrothion	.025	3.8	.045	5.3	1.8	.23	2.9	9.2
Dichlorvos	.11	7.1	.11	7.0	1.0	.29	5.9	2.6
Dursban	.0063	4.7	-	-	-	.015	3.6	2.4
Fenthion	.023	6.0	.026	5.8	1.1	.028	4.1	1.2
Abate	.005	2.6	.0049	2.6	1.0	.006	3.0	1.2

* in parts per million

** Resistance ratio = $\frac{LC_{50} \text{ El Salvador strain}}{LC_{50} \text{ Susceptible strain}}$