

RECALCITRANCE OF *CULEX TARSALIS* COQUILLET TO SELECTION PRESSURE BY PROPOXUR^{1, 2}

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ABSTRACT. A population of *Culex tarsalis* Coquillett from the Coachella Valley of California, exhibiting a wide spectrum of resistance to organophosphates, failed to develop resistance to the carbamate profoxur through nine generations of selection pressure. Outcrossing the F₄ selected generation to the natural population and

selecting for 7 additional generations was also unsuccessful in eliciting a positive response. Such recalcitrance of *C. tarsalis* to selection by profoxur in the laboratory is in accord with the absence of carbamate resistance in multiresistant populations of this species in California.

INTRODUCTION. A procedure frequently employed in attempting to forecast the "life expectancy" of a new chemical consists of subjecting a representative sample of the target population to selection pressure in the laboratory. Provided the study is performed on a newly-colonized strain of broad genetic background, which has not been narrowed by excessive inbreeding, the results can be highly significant. They should indicate, for instance, the responsiveness of the population to selection by the chemical and may also provide an indication of the level of attainable resistance. The resistant population may be further utilized in studies of the mechanisms of resistance, genetics, synergism, cross resistance, and other aspects of the phenomenon. Such studies are essential for understanding the dynamics of resistance and for selecting appropriate countermeasures. Here we report the results of selection of *Culex tarsalis* Coquillett by profoxur, in view of demonstrated effectiveness of this carbamate against organophosphorus-resistant populations of this species (Georghiou *et al.* 1969, Apperson and Georghiou 1974).

Culex tarsalis is frequently the target of chemical control operations in California

because of its tendency to attain population densities of nuisance proportions and its ability to transmit western and St. Louis encephalitis.

Since the early 1950's control of *C. tarsalis* has been effected primarily by organophosphate (OP) insecticides, the principal compounds being malathion, parathion, methyl parathion, fenthion, EPN, Abate® and chlorpyrifos, listed approximately in descending order of quantities used. This order also indicates the approximate order of introduction of each material into the control program. Malathion resistance was confirmed in 1956 in Fresno County (Gjullin and Isaak 1957) and within a decade it involved populations throughout the Central Valley (Womeldorf *et al.* 1972).

Intensive chemical control operations following the unusually wet season of 1969, utilizing mainly aerial applications of parathion, methyl parathion, fenthion and chlorpyrifos, resulted in the appearance of multiresistance toward these insecticides in the Central Valley (Georghiou *et al.* 1969). In 1971 such resistance was also found in the Coachella Valley (Apperson and Georghiou 1974).

Despite the involvement of several OP compounds in resistance, the affected populations were found to be susceptible to carbamates (Georghiou *et al.* 1969, Apperson and Georghiou 1974) and this susceptibility was maintained despite further intensive selection of the population in the laboratory by methyl parathion (Apperson and Georghiou, in preparation). It was

¹ Diptera: Culicidae.

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therefore considered essential to test the potentiality of development of resistance to carbamates by specific selection pressure in the laboratory.

MATERIALS AND METHODS. The population under study was colonized utilizing approximately 6000 adults collected as larvae at Thermal, Calif. on November 16, 1971, and Mecca, Calif., on November 16, 1971, and March 24, 1972. The resistance spectrum of the population determined shortly after colonization, was found to include chlorpyrifos (resistance ratio of larvae at $LC_{95}=26.3x$), chlorpyrifos-methyl (174.1x), fenitrothion (92.3x), fenitrothion (17.3x), methyl parathion (13.3x), malathion (11.6x), Abate (9.5x) and parathion (6.4x) (Apperson and Georghiou 1974). The amenability of this population to laboratory selection was demonstrated with methyl parathion which raised resistance to this compound to a 90 x level (Apperson and Georghiou, in preparation).

The rearing, testing and selection procedures, except for slight modifications, were as described in detail previously by Georghiou *et al.* (1966). Rearing was at 28°-30° C, 60% RH and a 16:8 hour light:dark photoperiod. Adults were fed on chicks and larvae on a mixture of finely ground Purina Laboratory Chow and hydrolyzed yeast in equal proportions. Air was bubbled into each rearing pan to prevent scum formation. Bioassay tests involved 24-hr treatment of groups of twenty 4th instar larvae in paper cups containing 100 ml of water.

Larval selection consisted of exposing groups of fifty 4th-instar larvae in 100 ml of water in glass beakers containing the required concentration of chemical. Adult selection involved 1-hr exposure of groups of 20 mosquitoes, less than 24 hours old, to treated glass fiber filter paper in shell vials (Georghiou and Gidden 1965, Apperson and Georghiou 1973).

Upon completion of 4 generations under pressure, part of the F_4 generation was outcrossed to a new collection obtained from the same localities as the original.

Outcrossing was done reciprocally and was intended to broaden the genetic background of the strain and thus to enhance the chances of positive response to selection. The two crosses involved 569 individuals of the F_4 and 874 of the field population. The F_1 generations of these crosses were combined and the F_2 and subsequent generations were selected as in Series I. A total of 9 generations was selected in Series I and 7 generations in Series II. In addition to larval selection, 5 generations of Series I and 2 generations of Series II were also selected in the adult stage.

RESULTS AND DISCUSSION. Data on the selection pressure applied and on the susceptibility of larvae and adults of successive generations, presented in detail in Table 1, indicate no evidence of development of resistance. The larval LC_{50} was 0.14 ppm in the parental generation, 0.19 in the F_9 of Series I and 0.21 in F_{10} of Series II. There has also been no significant change in the slope of the dose-mortality regression lines thus indicating the lack of incipient resistance (Fig. 1). Similar evidence is also present in the data on adult susceptibility. An effort was made during these selections to maintain populous colonies to avoid excessive inbreeding while at the same time applying relatively high selection pressure. The average number of larvae treated per generation was 4414 in Series I and 2773 in Series II, while the selection pressure was at an average level of 75% and 48% mortality in the two series, respectively. Experience had indicated that colonies severely stressed by carbamate insecticides suffer a substantial decline in fecundity and fertility (Georghiou 1965).

It is not possible to offer a positive explanation for the recalcitrance of *C. tarsalis* to develop resistance to propoxur, or to postulate that the species lacks entirely the potentiality for development of such resistance. However, the failure of the twice colonized population to manifest evidence of resistance must be considered as encouraging from the standpoint of useful-

TABLE I. Extent of selection pressure by propoxur on successive generations of *Culex tarsalis* and effect on susceptibility levels.

Generation	Larvae				Adults				Total selection pressure (%)
	Number treated	Conc. (ppm)	% kill	LC ₅₀ (ppm)	Number treated	Conc. (γ/cm ²)	% kill	LC ₅₀ (γ/cm ²)	
Series I									
P ₁	2900	.07	40	.14	40
F ₁	7000	.12	67	.17	67
2	5600	.15	80	.16	80
3	6960	.15	65	.20	1483	.4	29	..	75
4	8750	.15	72	.23	1573	.4	52	.32	87
5	3980	.15	64	.22	489	.4	94	.34	98
6	1500	.15	57	..	643	.32	67	..	86
7		—not selected—							
8	1250	.2	55	55
9	1790	.15	64	.19	272	.32	72	.19	90
Series II: following outcrossing of F ₄									
F ₄ x P ₂									
F ₁		—not selected—							
2	8860	.15	51	.19	1525	.32	79	.36	90
3		—not selected—							
4	2268	.15	42	42
5	4100	.2	59	.19	295	.32	70	.24	70
6	766	.15	28	28
7	1050	.2	33	33
8	997	.2	24	24
9	1371	.3	48	48
10		—not selected—							

ness of propoxur in the immediate future.

It is of interest to note that carbamate resistance has been demonstrated in two species of mosquitoes, namely *Culex quinquefasciatus* Say (= *fatigans* Wied.) and *Anopheles albimanus* Wied. In the former, a 25.4 fold resistance in larvae and 8.4 fold resistance in adults was induced by laboratory selection pressure (Georghiou *et al.* 1966). Such resistance was due to NADPH₂-dependent mixed function oxidase (MFO) enzymes which were shown to catalyze the metabolism of propoxur and other carbamates faster in the R than the S strains (Shrivastava *et al.* 1970, 1971). Evidence of carbamate metabolism by MFO action was also obtained by demonstration of *in vivo* synergism of propoxur by piperonyl butoxide (p.b.) (Georghiou *et al.* 1966). In contrast, only small amounts of hydrolysis products were observed *in vitro* (Shrivastava *et al.* 1971) and only slight synergism was noted *in vivo* by the esterase inhibitor tri-*o*-cresyl-

phosphate (Georghiou *et al.* 1966).

In *Anopheles albimanus* a considerably higher level of propoxur resistance (>1000 fold) which was observed in laboratory-stressed colonies (Ariaratnam and Georghiou 1971) and also occurred in the field (Georghiou *et al.* 1972), was found to be due primarily to reduction in sensitivity of acetylcholinesterase (AChE) to inhibition by propoxur (as well as by parathion) (H. Ayad and G. P. Georghiou, in preparation). In this case, metabolism of propoxur was negligible (Ariaratnam 1973) and its synergism by p.b. could be demonstrated only at extremely high concentrations of propoxur (>500 ppm) (Ariaratnam and Georghiou 1971).

Thus resistance to propoxur was based on oxidative rather than hydrolytic reactions in *C. quinquefasciatus* and to reduced AChE sensitivity rather than oxidative metabolism in *A. albimanus*. None of the above mechanisms could be demonstrated in *C. tarsalis*. AChE determinations did

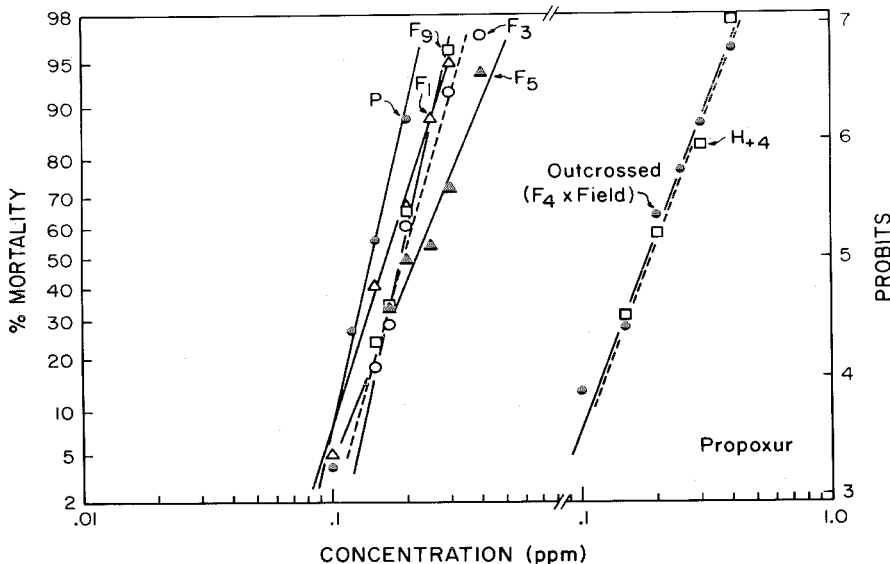


FIG. 1. Dosage-mortality regression lines for successive generations of *Culex tarsalis* selected by propoxur.

not show differences in sensitivity between a parathion-R (also derived from the Coachella strain) and a susceptible (Bakersfield) strain (Apperson 1974). Synergism was negligible with p.b., but very pronounced when either tributylphosphorotrithioate or triphenylphosphate was employed (Apperson 1974). It may, therefore, be tentatively stated that the recalcitrance of the present population of *C. tarsalis* to selection by propoxur is due to unavailability of those mechanisms (high MFO activity, AChE insensitivity) which have enabled *C. quinquefasciatus* and *A. albimanus* to develop resistance to the carbamates.

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