GEOGRAPHIC VARIATION OF RESISTANCE TO ORGANOPHOSPHATES, PROPOXUR AND DDT IN THE SOUTHERN HOUSE MOSQUITO, *CULEX QUINQUEFASCIATIS*, IN CALIFORNIA

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ABSTRACT. A survey of resistance in larvae of *Culex quinquefasciatus* conducted at three widely separated geographical areas of California, namely Coachella Valley, southeast Los Angeles and northern San Joaquin Valley, revealed high resistance to temephos and lower resistance to chlorpyrifos, methyl parathion and malathion at all locations. Likewise resistance to DDT was high in all areas despite its withdrawal from use since 1970. There was no obvious tolerance to propoxur. Tests for the presence of esterase-2^A have shown a close correlation between the frequency of this esterase and the level of organophosphate resistance.

INTRODUCTION

The southern house mosquito, *Culex quinquefasciatus* Say, is an important biting pest of man and animals and a vector of several diseases in various parts of the world (Harwood and James 1979). It breeds successfully in agricultural as well as urban environments and is widely distributed in central and southern California (Bohart and Washino 1978).

Populations of *Cx. quinquefasciatus* in California were shown to have developed resistance to DDT first in Orange County (Lewallen 1960) and later throughout the State. Organophosphate (OP) insecticide resistance was reported soon after introduction of these insecticides (Womeldorf et al. 1968). The status of resistance in this species was last examined in the Coachella Valley by Apperson and Georghiou (1974) and in the San Joaquin Valley by Georgiou et al. (1975). The linkage relationships between an organophosphate detoxifying esterase (gene *Est.* 2^A) and resistance in this species were established earlier by Georghiou and Pasteur (1978), Georghiou et al. (1980) and Pasteur and Georghiou (1981). A filter paper test devised by these investigators (Pasteur and Georghiou 1981) allowed for fast detection of *Est.* 2^A and hence of OP resistance. Because of increasing concern over the impact of resistance in control programs a more up-to-date survey of the spectrum and regional variation of resistance has become necessary.

MATERIALS AND METHODS

MOSQUITO STRAINS. The following strains (1–3) were collected from the field as larvae and pupae in large numbers (1000–3000) and were reared in the laboratory.

1. Coachella Valley strain (CV) was collected from an abandoned swimming pool at Coachella in August 1979.

2. Southeast Los Angeles strain (LA) was collected from a drain ditch within the Southeast Mosquito Abatement District in June 1980.

3. Northern San Joaquin Valley strain (NC) was collected from standing water near Stockton, California in August 1980.

Susceptibility tests on the field strains were performed in the F_1 and F_2 generations and the data were pooled.

4. S-Lab, a susceptible reference strain that originated from the San Joaquin Valley in 1950 and is being reared in this laboratory without exposure to insecticides.

LARVAL BIOASSAYS. Technical grades of temephos, chlorpyrifos, methyl parathion, malathion, DDT and propoxur were dissolved in acetone and used in larval bioassays. For these tests, 99 ml of water were placed in waxed paper cups and 20 late-third or early-fourth instar larvae were added. Each cup was dosed with 1 ml of the required insecticide concentration. For each insecticide 5 or more dosages were tested on at least 5 different days. Larval mortality was recorded after 24 hr. Because of genetic heterogeneity in the data, ld-p lines were drawn graphically, and the LC_{50} and LC_{95} values were read directly from the graphs. Calculation of ld–p lines by probit analysis (Finney 1971) was limited to the data of the susceptible reference strain, S-Lab.

ESTERASE DETECTION BY THE FILTER PAPER METHOD. The presence of *Est*-2^A in individual mosquitoes was detected by the following technique after Pasteur and Georghiou (1981). Individual adults were crushed on a glass plate in 50 μl drops of distilled water and the homogenates were transferred to filter paper strips. These were then immersed for 90 sec in a solution of 10 ml 1% α-naphthyl acetate and 90 ml of phosphate buffer (9.2 gm KH_2PO_4 + 4.8 gm Na_2PHO_4 per 1000 ml water). The strips were then dipped in a solution of 0.5% GBC salt for another 90 sec and fixed in 10% acetic acid.

The presence of the active esterase was indicated by a blue color.

RESULTS

The strains studied were not reproductively isolated as they were found to hybridize successfully. Filter paper tests on adults of each strain revealed that significant fractions of these populations (44–74%) were expressed as temephos-resistant genotypes (RR and RS) (Table 1). Based on these data, the frequencies of the R gene (*Est-2^A*), according to the Hardy-Weinberg equation, were 0.25, 0.33 and 0.49 in the LA, CV and NC strains, respectively. The results of bioassay tests with 6 insecticides on the S-Lab and the 3 field strains are given in Table 2.

RESISTANCE TO TEMEPHOS. Resistance levels to temephos were significantly higher in all areas. The NC population showed the highest resistance in comparison to the other populations, i.e., 34.6-fold at the LC_{50} and 86.8-fold at the LC_{95} (Table 2). But the ld-p line for temephos in this population lacked a distinct plateau and thus showed no partitioning of the population into distinct classes with respect to resistance, namely RR, RS and SS, possibly due to presence of minor, ancillary genes (Fig 1). In contrast, plateaus demarcating the approximate proportion of susceptible individuals were evident in the ld-p lines of the CV and LA populations (Fig. 1A). Resistance to temephos in the Los Angeles area seemed to have arisen after 1970 since data by Pelsue et al. (1972) for this area indicated an LC_{50} value of 0.0016 ppm. As the baseline LC_{50} used in the present study is higher, i.e., 0.0026 ppm, the actual level of resistance may in fact be higher.

RESISTANCE TO CHLORPYRIFOS. The LA population was more resistant to chlorpyrifos than the CV (Fig. 1B), i.e., 59.6X vs 21.3X the normal LC_{95}, respectively (Table 2). As with temephos, resistance to chlorpyrifos also appears to have occurred after 1970 in the Los Angeles area with reference to an LC_{50} of 0.006 ppm that was last reported by Pelsue et al. (1972).

RESISTANCE TO METHYL PARATHION. The CV population demonstrated a heterogeneous response to methyl parathion as evident in the plateau of the ld-p line separating the population into approximately 60% susceptible and 40% resistant genotypes (Fig. 1C). This can also be seen in the lower resistance level at the LC_{50} (2.4X) although at the LC_{95} this population registers considerably higher resistance (35.7X).

RESISTANCE TO MALATHION. The LA and NC populations demonstrated moderate levels of

Table 1. Frequencies of temephos-resistant individuals and of the *Est-2^A* gene in three populations of *Culex quinquefasciatus* in California.

Population	Females examined/ positive	Males examined/ positive	Total examined/ positive	Frequency of resistant individuals (RR + RS)	Frequency of *Est-2^A*
LA	50/23	115/50	165/73	0.44	0.25
CV	76/45	76/38	152/83	0.55	0.33
NC	70/49	78/60	148/109	0.74	0.49

Table 2. Resistance ratios toward various insecticides in three populations of *Culex quinquefasciatus* in California.

Insecticide	S-Lab			Southeast Los Angeles		Coachella Valley		Northern San Joaquin Valley	
	LC_{50}[a]	LC_{95}	Slope	RR_{50}[b]	RR_{95}	RR_{50}	RR_{95}	RR_{50}	RR_{95}
temephos	0.0026	0.0038	10.0	15.4	23.7	2.7	52.6	34.6	86.8
chlorpyrifos	0.0028	0.0047	7.1	11.4	59.6	2.7	21.3	—	—
methyl parathion	0.0036	0.0056	8.4	9.7	17.8	2.4	35.7	6.9	10.7
malathion	0.076	0.135	6.7	7.2	17.8	.53	1.5	5.3	10.4
propoxur	0.2	0.3	9.4	0.75	1.9	1.3	1.9	1.7	1.9
DDT	.022	.047	5.0	14.1	25.5	9.1	10.6	13.6	42.5

[a] LC_{50} and LC_{95} in ppm.

[b] RR (Resistance ratio at the indicated LC level) = $\frac{\text{LC of field population}}{\text{LC of S-Lab}}$

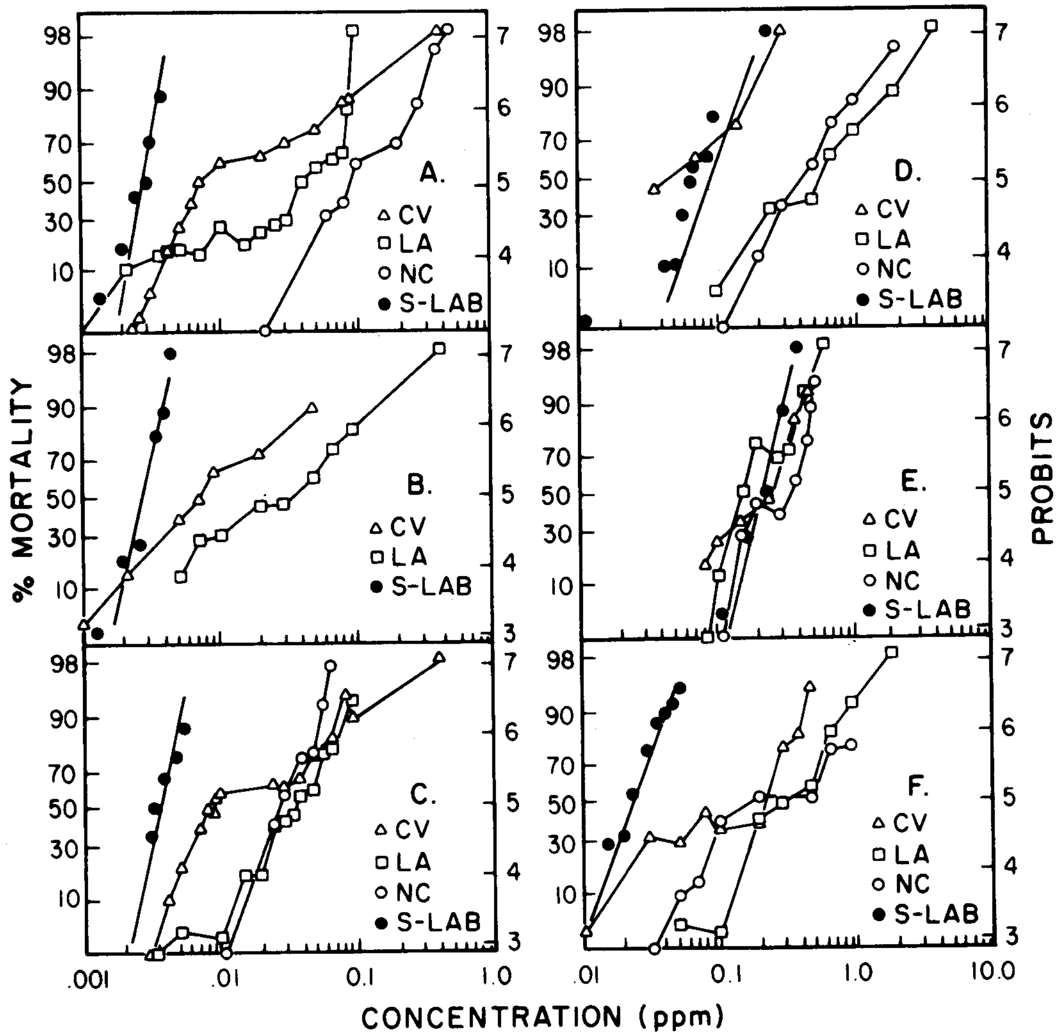

Fig. 1. Dose-response relationships of *Culex quinquefasciatus* toward various insecticides. Three locations in California: CV = Coachella Valley, LA = southeast Los Angeles, NC = northern San Joaquin Valley and in a susceptible laboratory strain (S-Lab). A. temephos, B. chlorpyrifos, C. methyl parathion, D. malathion, E. propoxur, F. DDT.

resistance to this insecticide, i.e., 17.8X and 10.4X the normal LC_{95}, respectively (Fig. 1D, Table 2). Resistance in the LA area seemed to have increased only slightly after 1967 in comparison with an LC_{50} of 0.083 ppm that was published by Pelsue et al. (1972). In contrast, no measurable resistance could be detected in the Coachella Valley collection, probably due to the absence of use of this insecticide for mosquito control in this are since 1974 (Fig. 2).

RESISTANCE TO PROPOXUR. The LA and NC populations showed no significant levels of resistance to propoxur at the LC_{50} (Fig. 1E). At the LC_{95}, a low level of resistance (1.9X) was noted in all three populations.

RESISTANCE TO DDT. Resistance to this long-abandoned insecticide was found to be still high in the three sampled areas, particularly in the NC population (42.5X at the LC_{95}) (Fig. 1F, Table 2). The LA and CV populations displayed resistance ratios of 25.5X and 10.6X, respectively, at the LC_{95} and lower ratios (14.1X and 9.1X) at the LC50.

DISCUSSION

The relative amounts of larvicidal oils and insecticides which were applied during 1970–81 for mosquito control in the three mosquito abatement districts that were sampled reflect to a certain extent the degree of selection pressure that is exerted on the mosquito population in each area (Table 3, Fig. 2). However, these statistics are deficient in that they do not show the size of the area treated or the frequency of applications. Additionally, they do not include the impact of selection by insecticides used prior to 1970 and of selection by agricultural chemicals. Thus, they are of value as qualitative rather than quantitative indicators of selection pressure.

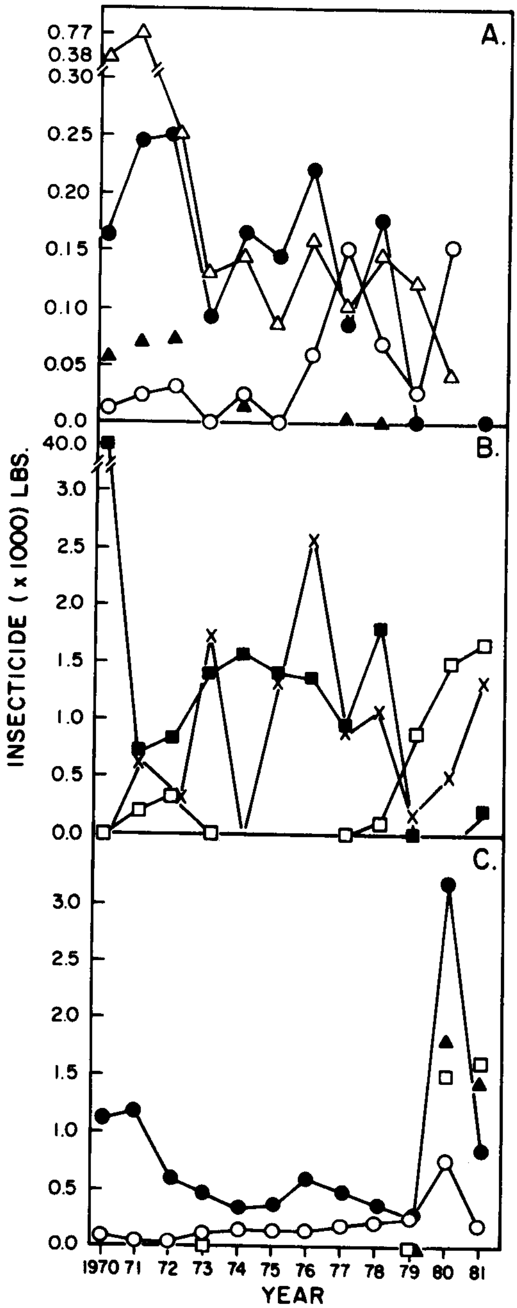

Fig. 2. The quantities of insecticides and oils applied for mosquito control in three mosquito abatement districts in California during 1970–81. A. Southeast Los Angeles, B. Coachella Valley, C. Northern San Joaquin Valley. temephos △, chlorpyrifos ○, methyl parathion □, malathion ▲, fenthion ●, parathion ■, naled ×.

In southeast Los Angeles oils were the most widely used larvicides during 1970–81, exceeding insecticides by a factor of 61.9. Among the insecticides, temephos was the one most commonly used (48.7%) followed by fenthion (32.6%) and chlorpyrifos (12.1%) (Fig. 2). Resistance in this District was particularly high at the LC_{95} for chloryprifos (59.6X) and temephos (23.7X) (Table 2). Resistance to DDT was still significantly high in the LA population (25.5X at the LC_{95}) (Table 2), despite the absence of use of this insecticide in mosquito and agricultural pest control for the past several years. It is evident that although strong reliance had been placed on oils in this area, selection pressure by insecticides was sufficiently high as to produce resistance at levels comparable to those observed in the Coachella Valley.

In the Coachella Valley the most frequently used insecticides for mosquito control during 1970–81 were parathion (76.4%), naled (16.0%) and methyl parathion (7.0%) (Fig. 2). Yet resistance to OPs was found to involve a variety of compounds at levels which were especially high for temephos (52.6X at the LC_{95}), chlorpyrifos (21.3X) and methyl parathion (35.7X).

The relatively high resistance toward methyl parathion might be expected in view of the use of this insecticide in moderate quantities by the Coachella Abatement District, and may have been enhanced by cross-resistance resulting from the extensive use of parathion. In addition, methyl parathion is being widely used on cotton in this area (Van Steenwyk et al. 1976) and may have exerted indirect selection against the mosquito population.

Since temephos and chlorpyrifos were used only occasionally and in very small quantities (0.048%) and 0.037%, respectively), resistance

Table 3. The relative use of insecticides and oils for mosquito control in three mosquito abatement districts during 1970–81.[a]

District	Insecticides lbs.	Oils gals.	Ratio insecticides: oils
Southeast	4,807	297,997	1:61.9
Coachella Valley	66,291	73,032	1:1.1
N. San Joaquin	19,453	163,960	1:8.4

[a] Data compiled from the Yearbooks of the California Mosquito Control Association.

to these insecticides is attributed mainly to cross-resistance from other OP insecticides.

In the northern San Joaquin Valley fenthion was the principal insecticide used for mosquito control (50.3%) and only after 1980 methyl parathion and malathion were used in substantial quantities, constituting 16.2% and 16.7% of the total, respectively (Fig. 2). Temephos was not reported as having been used in this area, yet the population was found to be highly resistant to this insecticide (86.8X at LC_{95}) and less resistant to methyl parathion (10.7X) and malathion (10.4X) (Table 2). It was also highly resistant to DDT (42.5X at LC_{95}). The observed temephos resistance may be due to cross-resistance from chlorpyrifos pressure as was seen earlier by Georghiou et al. (1975), and possibly from restricted use of temephos and other organophosphate insecticides at the collection site.

The continued presence of DDT resistance at relatively high levels in all three areas suggests that any consideration of use of pyrethroids in mosquito control must be approached with caution in view of the well known cross-resistance interrelationships of DDT and pyrethroids in this species (Priester and Georghiou 1980). With the exception of carbamates, multiresistance in *Cx. quinquefasciatus* is general in California and continues to be a significant phenomenon that requires continuous evaluation and management.

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