INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: RESPONSE OF *ANOPHELES FREEBORNI* AITKEN LARVAE TO ORGANOPHOSPHORUS COMPOUNDS

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INTRODUCTION

Organophosphorus resistance in culicine mosquitoes has been the subject of intensive study in California during the past 15 years, but very little has been done to determine the status of susceptibility of the anopheline species. Barr (1962) reported on the absence of work done on *Anopheles freeborni* Aitken, an important pest and potential malaria vector. Lewallen and Peters (1966) remarked that limited tests did not indicate high levels of insecticide resistance in the species but they did not offer substantiating data. Hazeltine and Kingsford (1970) presented graphic data for parathion, fenthion, and Dursban ®, principally from Butte County, California, and noted that parathion has been the primary larvicide for *A. freeborni* in ricefields in recent years.

The California mosquito larvicide susceptibility surveillance program, conducted cooperatively by local mosquito control agencies and the California Department of Public Health, monitors developing resistance in important species. The methods used have been described by Gillies and Womeldorf (1968). Field-collected late instar larvae are tested in the laboratory, or field-collected early instar larvae are reared to the fourth stage for testing. These procedures have been unsatisfactory with *A. freeborni*. Inasmuch as late instar larvae are difficult to collect in large numbers from ricefields, the major habitat, it has not been possible to collect sufficient larvae for comprehensive testing. Early instar larvae are much easier to capture, but rearing methods used successfully with *Culex* spp. and *Aedes* spp. were not satisfactory for *A. freeborni*. Larvae either die or are so weakened that test results are invalid. As a consequence, insufficient data have been obtained over the past several seasons to clarify the susceptibility situation of the species.

One of the research programs conducted by the University of California at Davis is concerned primarily with studies on ricefield mosquitoes. Since a major effort in this program has been focused on rearing *A. freeborni* in large numbers in the laboratory, the aid of these workers was enlisted to overcome the difficulty of rearing larvae in adequate numbers for testing at the fourth stage.

This paper describes collection, rearing, and testing procedures as applied during 1969, compares the toxicity of several organophosphorus insecticides and offers evidence of the susceptibility of *A. freeborni* to the materials tested.

MATERIALS AND METHODS

FIELD-COLLECTED LARVAE. Bailey and Gieke (1968) showed that population peaks of *A. freeborni* in ricefields occurred in late August and September, and in late winter and early spring in other habitats. Larvae were collected from ricefields and various other habitats during the appropriate seasons in Butte, Sutter, Yuba, and Placer counties within the central and lower Sacramento Valley. The sources were all in the main rice culture area of California and were within or adjacent to mosquito control districts.

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A. freeborni larval densities in ricefields were very low during the season of this study, typically not exceeding one per dip (Bailey and Gieke, *ibid.*). A larval concentrator as described by Womeldorf *et al.* (1963) facilitated collecting useful numbers. Water and larvae were dipped from the source into the concentrator. Debris was removed frequently since the larvae were observed to be susceptible to damage when crowded by accumulated floating material.

Transporting larval *A. freeborni* in ½ to 1-gal containers as described by Gillies and Womeldorf (*ibid.*) sometimes resulted in excessive mortality. The larvae were most safely carried from field to laboratory in shallow water (less than one inch) placed directly in a plastic ice chest (without ice). They were not damaged if the collection was taken into the laboratory within a short time (no more than one hour in hot weather) and the ice chest was protected from exposure to direct heat during the trip.

In the laboratory, fourth instars from mixed field collections were removed with a wide mouth dropping pipette and set aside for testing. Small larvae were reared as described below.

PROGENY OF FIELD-COLLECTED ADULTS. Approximately 500 *A. freeborni* females were collected at bi-weekly intervals with a mechanical aspirator (Bailey, 1966) in a rural area near Meridian, Sutter County, adjacent to two mosquito abatement districts. After capture, the females were transferred to a 3-gal cardboard container cage and transported to the laboratory. The top of each cage was covered with nylon mesh and the side fitted with a cotton sleeve to facilitate handling the adult mosquitoes.

Field-collected females which contained neither blood nor eggs were left in their cage or transferred to a second one. A small dish of tap water was left in the cage for oviposition. White mice were used for blood feeding, and pads soaked in 20 percent sucrose solution were provided as a supplementary food source. Blood-engorged and gravid females from the field collection were isolated in large glass tubes containing 10 ml of water and sealed with cotton plugs. Females were held until eggs were deposited in the water.

Eggs oviposited in the glass tubes or dishes were transferred to enamel pans (7 x 11.5 x 2 inches) containing tap water. Until the eggs hatched a glass cover was placed over the pan to minimize evaporation.

REARING. After hatching, 50 to 75 larvae were transferred to a smaller uncovered enamel pan (4.5 x 8.5 x 2 inches). The larvae were reared in these pans without aeration and fed pulverized Gaines nonfat dog food. The dog food was obtained directly from the manufacturer prior to the fat being added as in the commercial preparation. The rearing room was maintained at 78–80° F and lighted for 16 hours per day.

To have sufficient numbers of fourth instar larvae available for testing at one time, it was sometimes necessary to restrain pupation by holding the early fourth instar larvae in a 55° F cabinet.

TESTING PROCEDURES. Larvae reared at Davis were transported in clean water in a 1-gal container lined with a plastic bag to a laboratory in Sacramento where the tests were conducted. Tests were performed as described by Gillies and Womeldorf (*ibid.*): the procedures differ from those of the World Health Organization (1963) in that acetone is used in place of ethanol as a solvent for the technical insecticide, and 100 ml water in waxed paper cups are used instead of 250 ml water in glass jars. Average temperatures during the 24 hours of the test usually ranged between 21° and 24° C. A conservative criterion of mortality was used since the larvae normally responded rather erratically when touched with a probe. Only larvae that had very definite inability to move normally when probed were counted as moribund. Test results included in this paper include only those in which control mortality was less than 5 percent.

TREATMENT OF DATA. Probit analysis

Table 1. Log Concentration Probit Line Values for Four Insecticides From Batches of Fourth Instar Anopheles freeborni Larvae Obtained by Four Methods.

Source of Test Larvae	Test Date	Parathion			Methyl parathion			Fenthion			Dursban®		
		Slope	LC_{50}	95% C.L.	Slope	LC_{50}	95% C.L.	Slope	LC_{50}	95% C.L.	Slope	LC_{50}	95% C.L.
Progeny of Field Collected Adults	6 Feb. 69	2.9	.0097	.0080-.012									
	12 Mar. 69	3.2	.0045	.0035-.0058	2.6	.012	.0089-.016	4.0	.0065	.0051-.0082	3.1	.0013	.0010 -.0017
	21 Mar. 69	2.7	.0037	.0028-.0049	2.3	.014	.012 -.018	3.2	.0066	.0051-.0085	3.1	.00089	.00068-.0011
	1 Apr. 69	3.9	.0063	.0050-.0080				2.7	.0096	.0072-.013	4.6	.0025	.0020 -.0031
	11 Aug. 69	2.7	.0062	.0047-.0081									
	10 Nov. 69	3.5	.0072	.0061-.0086	2.6	.017	.014 -.021	2.8	.0093	.0077-.011	2.3	.0012	.00099-.0015
	23 Nov. 69	4.1	.0076	.0060-.0095	1.6	.020	.014 -.031	2.2	.0084	.0060-.011	2.1	.0012	.00088-.0017
	24 Nov. 69				2.8	.015	.013 -.018	2.6	.0074	.0060-.0089	2.3	.0019	.0016 -.0024
	4 Dec. 69	3.8	.0057	.0045-.0072	1.9	.0085	.0061-.012	2.8	.0064	.0049-.0084	1.8	.0013	.00095-.0019
	9 Dec. 69	3.8	.0061	.0048-.0077				3.3	.0061	.0048-.0079	3.0	.0012	.00089-.0015
Field Collected 4th Instars	21 Sept. 66	2.3	.0029	.0022-.0037									
	27 Sept. 66	2.2	.0026	.0018-.0035									
	5 Oct. 66	3.1	.0024	.0018-.0032									
	5 Apr. 69	2.4	.0097	.0066-.016									
	1 Apr. 69	2.3	.0086	.0063-.012	2.4	.016	.012 -.022						
	1 Apr. 69	4.3	.0049	.0039-.0062									
	16 Apr. 69	1.9	.015	.011 -.023									
	16 Apr. 69	2.4	.0080	.0059-.011	2.4	.035	.025 -.067				4.6	.0028	.0023 -.0053
Field Collected Small Instars	16 Apr. 69	4.5	.011	.0084-.013	2.8	.027	.020 -.041						
	9 Apr. 69	3.7	.0060	.0047-.0076	2.3	.015	.011 -.021						
	9 Apr. 69	4.0	.0040	.0032-.0051	2.0	.012	.0087-.017						
	22 Apr. 69	5.1	.010	.0082-.012	2.7	.021	.016 -.028				4.1	.0033	.0026 -.0043
	22 Apr. 69	6.7	.011	.0092-.013									
Primate Colony	12 Mar. 69	4.0	.0022	.0017-.0046									
	21 Mar. 69	3.2	.0026	.0020-.0034	3.6	.0062	.0049-.014				3.8	.00070	.00059-.00082
	1 Apr. 69				2.6	.0067	.0051-.0091	3.4	.0048	.0037-.0062			

Table 2. Log Concentration Probit Line Values for Three Insecticides From Batches of Fourth Instar Anopheles freeborni Larvae Obtained by Four Methods.

Source of Test Larvae	Test Date	Malathion			Abate			Phoxim		
		Slope	LC_{50}	95% C.L.	Slope	LC_{50}	95% C.L.	Slope	LC_{50}	95% C.L.
Progeny of Field Collected Adults	21 Mar. 69							3.8	.0019	.0015 -.0024
	1 Apr. 69							3.1	.0040	.0031 -.0053
	22 Sept. 69				3.0	.011	.0086-.016			
	10 Nov. 69				1.8	.0082	.0061-.013			
	23 Nov. 69	2.6	.13	.10 -.18						
	24 Nov. 69	2.7	.079	.065-.096						
	4 Dec. 69				2.1	.0052	.0038-.0073			
Field Collected 4th Instars	27 Sept. 66				4.1	.0053	.0036-.0067			
Field Collected Small Instars	16 Apr. 69				3.7	.0048	.0036-.010			
Primate Colony	21 Mar. 69							2.4	.00067	.00041-.00093
	1 Apr. 69							3.3	.0012	.00092-.0015

of the test data was performed in the Data Processing Center, State Department of Public Health. The computer program fits log concentration probit lines to the data by the maximum likelihood method, providing estimates of the slope and LC_{50}, and 95 percent confidence limits for the LC_{50} (Finney, 1952). An exposition of log concentration probit lines and interpretation of them in insecticide testing has been given by Hoskins (1960).

RESULTS AND DISCUSSION

Table 1 lists the results from 1969 tests on fourth instar *A. freeborni* larvae obtained by three methods and from a colony (Primate Center) at the National Center for Primate Biology, University of California, Davis, which has been maintained in the absence of insecticide pressure for many years. The results are of tests against parathion, methyl parathion, fenthion, and Dursban ®. Table 2 lists some additional data obtained against malathion, Abate ®, and phoxim (Baythion ®, Bay 77488).

COMPARISON OF TEST METHODS. Figure 1 presents, graphically, the range of log concentration-probit lines determined against parathion (the chemical for which the greatest quantity of data was obtained), comparing larvae obtained by three methods. Within each group, the slopes of the lines did not differ significantly, but the differences between slopes of the groups were statistically significant. The greatest variance of response, i.e., flattest slope, was seen among the field-collected fourth instar larvae.

The greatest range of mean lethal concentration (LC_{50}) levels was exhibited by the field-collected fourth instar larvae. In addition, this range bracketed the LC_{50} ranges for the other two groups.

The results of these determinations show that laboratory reared larvae, obtained as early instar larvae or as progeny of field-collected adults, tend to exhibit parathion LC_{50} levels similar to field-

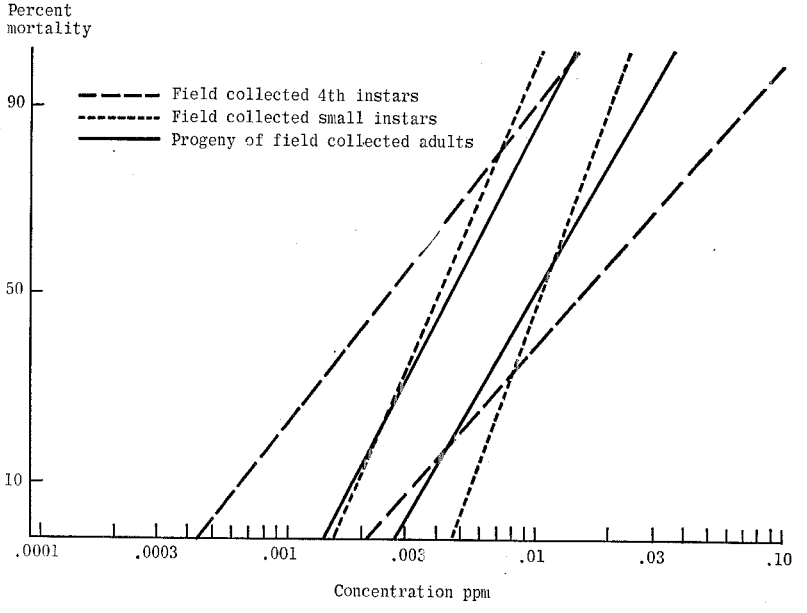

FIG. 1.—Parathion responses of 4th instar *Anopheles freeborni*.

collected fourth instar larvae. However, the slopes of their response lines can be expected to be steeper. Additionally similarity in LC_{50} values for the several methods of obtaining larvae can be seen in Tables 1 and 2 for methyl parathion, fenthion, Dursban ®, and Abate ®. Based on the above findings, it was concluded that data from tests with larvae obtained by any of the three methods can be utilized to determine susceptibility of *A. freeborni*.

COMPARATIVE TOXICITY OF LARVICIDES. Figure 2 illustrates the comparative toxicities of parathion, methyl parathion, fenthion, and Dursban ® to batches of progeny of field-collected adults large enough to permit testing two or more pesticides. The LC_{50} levels for Dursban ® are distinctly lower than for the other materials, but the parathion and fenthion values are quite similar in most cases, as shown by the overlap of the 95 percent confidence limits around the LC_{50}. Methyl parathion was the least toxic of the four materials. The data in Table 2 indicate that phoxim, which is not yet commercially available, may approach Dursban ® in its toxicity to *A. freeborni;* Abate ® probably resembles parathion and fenthion; and malathion is considerably less toxic than the other materials.

Our data on the response of *A. freeborni* larvae to parathion, fenthion, and Dursban ® appear to agree well with the graphic data presented by Hazeltine and Kingsford (*ibid.*) for tests in Butte County during 1966–68. Klassen *et al.* (1964) tested several larvicides against *A. quadrimaculatus*. Our results with *A. freeborni* were in the same range as theirs with Abate ® (listed as American Cyanamid 52160), parathion, and methyl parathion; but we did not find *A. freeborni* to show the elevated fenthion LC_{50} levels they reported for *A. quadrimaculatus*

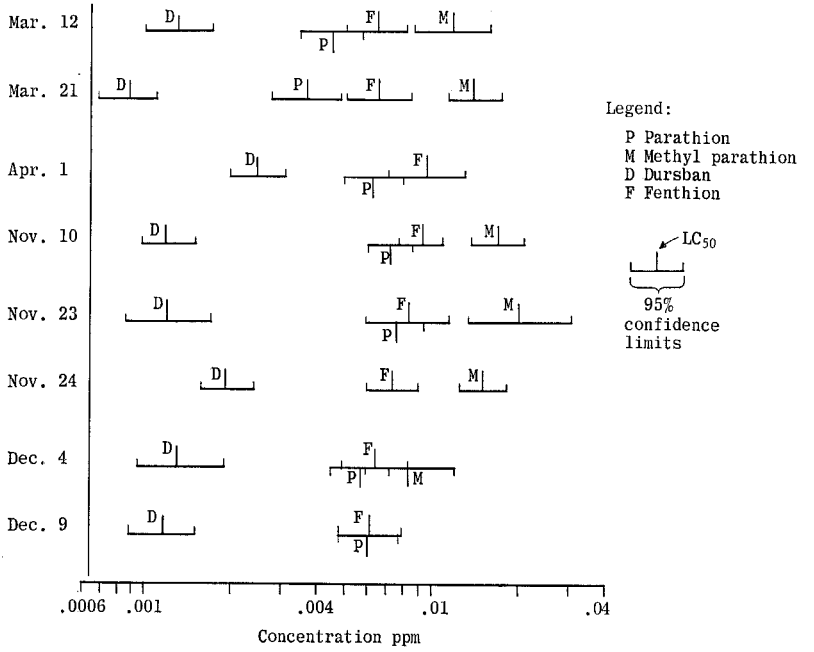

FIG. 2.—LC_{50} values with 95% confidence limits of four insecticides in batches of 4th instar progeny of field collected adult *Anopheles freeborni*.

(0.016 ppm for a laboratory strain; 0.072 for a field strain). Jakob and Schoof (1963) reported a fenthion LC_{50} for *A. quadrimaculatus* of 0.01 ppm, which was approached by many of our *A. freeborni* results. Our *A. freeborni* test results agree closely with those for malathion and parathion obtained by Georghiou and Metcalf (1961) on a dieldrin-resistant colony strain of *A. albimanus,* but our LC_{50} levels did not reach theirs for methyl parathion (0.096 ppm) nor for fenthion (0.016 ppm).

STATUS OF NONRESISTANCE. Figure 3 shows log concentration-probit lines representing the pooled data of tests of parathion against larvae from the Primate Center colony and progeny of field-collected adults. The data could be pooled because neither the LC_{50} levels nor the slopes were significantly different within each group. The difference between the LC_{50} values of the susceptible colony strain and the field material is about two-fold, within expectations for field and laboratory strains. The slopes of the lines are also very similar. A depressed line slope in a pressured population, as compared with an untreated one, signals incipient resistance in some species (Gillies *et al.,* 1968; Hoskins, 1960) so the similarity in slope between the field and laboratory material contraindicates genetically based physiological resistance against parathion, the most extensively used larvicide. Furthermore, inspection of the data in Tables 1 and 2 shows that the LC_{50} levels for Primate Center larvae tested against methyl parathion, fenthion, Dursban ®, and phoxim are only slightly lower than found for field-collected larvae or progeny of field-collected adults, and that the slopes are similar to the field material.

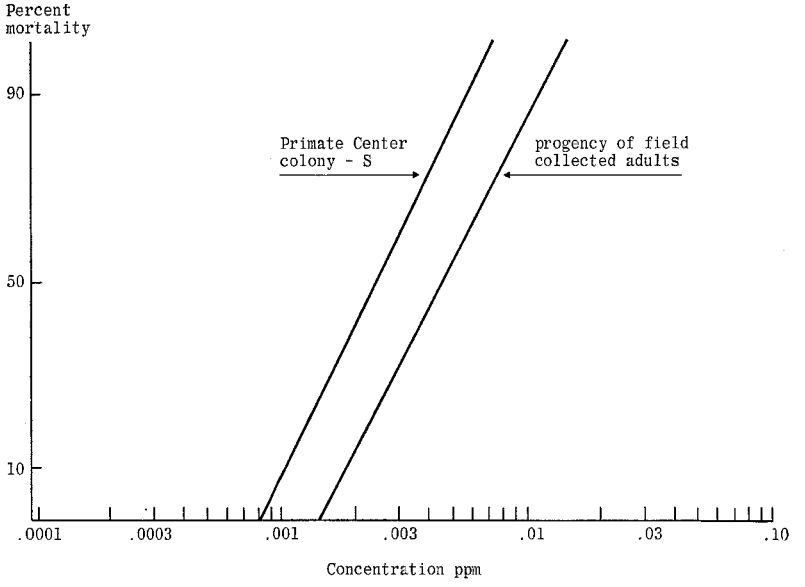

FIG. 3.—Comparison of parathion response of *Anopheles freeborni* 4th instars from a susceptible colony and progeny of field collected adults.

It is therefore concluded that no resistance has yet been discovered against the chemicals tested. From a worldwide

standpoint, Georghiou (1969) noted that no organophosphorus resistance has been demonstrated against any *Anopheles* spp.

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