PARATHION-RESISTANT *AEDES NIGROMACULIS* IN CALIFORNIA

LAWRENCE L. LEWALLEN AND LOREN M. NICHOLSON [1]
California State Department of Public Health,
Bureau of Vector Control, Fresno

Laboratory investigations on fourth instar larvae of the pasture mosquito, *Aedes nigromaculis* (Ludlow), from an area that had experienced difficulty in achieving control with parathion applications during 1958 showed a relatively high degree of physiological resistance to this compound (Lewallen and Brawley, 1958). On the basis of these preliminary results further investigations appeared to be in order to substantiate the original findings and establish the magnitude of parathion resistance or susceptibility in strains collected from other areas of California.

Mosquito larvae reared in the laboratory under standard conditions are more uniform in response to chemical tests than larvae collected directly from the field. Tests on field collected larvae are sometimes questionable since factors such as variation in nutrition, exposure to agricultural chemicals and transportation fatigue, can markedly influence results. In order to produce *A. nigromaculis* larvae reared under uniform conditions, it was necessary to collect adult females in the field which were capable of supplying fertile eggs. Attemps to colonize this species have failed thus far due to its reluctance to mate in cages.

METHODS AND MATERIALS. *Collecting Procedure.* Strains of susceptible *A. nigromaculis* were obtained from irrigated pastures near Pinedale and Kerman (Fresno County) where insecticides have not been used in a mosquito control program. For comparison, *A. nigromaculis* was collected from the Kings Mosquito Abatement District near Hanford (Kings County) and the Tulare Mosquito Abatement District near Tulare (Tulare County). Organophosphorus compounds have been used in mosquito control programs for the past five to six years in both of these districts.

The same procedures in collecting, handling, rearing, testing, and analyzing the data were observed for all four strains.

Adult females were collected in irrigated pastures, usually during the early morning hours. A mouth aspirator was used to transfer mosquitoes attracted to the collector's bare arms into quart glass jars lined with damp paper toweling. Unfed females were allowed to engorge on the collector's arms before being taken.

Rearing Procedure. At the laboratory, one to three females were placed in shell vials about 3½ inches long by ⅞ inch in diameter lined with a piece of damp paper toweling 3¼ inches long by 1 inch wide. The vials were plugged with a piece of cellucotton. A test tube rack inclined at a 45 degree angle held the vials. Within three to five days, eggs were deposited on the damp toweling.

Dead mosquitoes were removed from the vials, and vials containing newly deposited eggs were placed upright in a gallon glass jar filled to about three inches with sawdust saturated with water. A few phenol crystals were added to the sawdust to prevent development of mold.

The jar was placed in an incubator held at 80° F. for six days to "condition" the eggs. After "conditioning," the paper toweling was removed from the vials, and the eggs washed off into enamel pans 10 inches by 16 inches by 2½ inches deep

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containing distilled water filled to a depth of 1½ to 2 inches. Eggs from approximately 10 vials were placed in each pan.

Hatching of the eggs usually occurred within from five minutes to twenty-four hours at about 70° F., the same temperature as maintained for larval rearing and egg deposition.

Larval food consisted of livestock pellets [2] that had been boiled in distilled water for five minutes to kill deleterious organisms; a thick paste resulted. Chunks of paste about the size of a pea were introduced into the larval pans as needed. Air was constantly bubbled through the water while the larvae were developing. Tests with insecticides were performed when the larvae had developed to early fourth instar.

Testing method. Technical or purified samples of insecticides were made up as weight/volume solutions in acetone. Either 1.0 percent or 0.1 percent stock solutions were made, depending on the amount of material available for testing. Aliquots of the stock solutions were diluted further with acetone (usually in tenfold increments) to produce a range of concentrations which killed 5 to 95 percent of the larvae treated within 24 hours.

Individual test units consisted of one hundred ml. of distilled water in a four-ounce paper cup [3] containing 20 fourth instar larvae which had been transferred from the rearing pans by means of a small piece of 16 mesh aluminum screen. Care was taken not to transfer excess water from the rearing pans to the cups. A control was run with each group of treated larvae. Mortality in the controls was consistently nil.

One ml. of the desired concentration of insecticidal solution was pipetted into each test unit, each concentration being replicated three times in each test. Average mortalities were based on three test series giving a total of nine replications for each concentration.

[2] Misco Mills, Bozeman, Montana.
[3] Dixie Cup Company, Anaheim, California.

Treated larvae were held at 70° F. ± 4° for 24 hours and received no food during this interval. Mortality was determined by the criterion that larvae which responded normally when probed were alive; all moribund larvae were included in the dead counts.

Average percent mortalities were plotted on log-probit graph paper as dosage in parts per million vs. percent mortality. A minimum of three points (usually four) was employed in obtaining a straight line which was fitted to the points by eye. LC_{50} and LC_{90} values were determined from the line.

An analysis of variance test was performed on the data obtained on parathion for the four strains studied. There was more variation between the three tests (different collection dates of *A. nigromaculis* females) than within each test (same collection date). This demonstrates the reproducibility of results on material which is reared at the same time. It does not demonstrate, however, whether the discrepancy between successive collections is due to variations which occur in the laboratory or in the field.

RESULTS. The results of tests conducted with parathion against the four strains of *A. nigromaculis* in this study are given in table 1.

TABLE 1—Response of fourth instar *Aedes nigromaculis* to parathion

Strain	LC_{50} in p.p.m.	LC_{90} in p.p.m.
Pinedale	0.000035	0.0001
Kerman	0.00004	0.00009
Tulare	0.00012	0.0025
Hanford	0.00008	0.0068

Since the data for the Pinedale and Kerman strains are in agreement, it is assumed that this represents the normal response of the species to parathion, and consequently the Pinedale strain has served as a reference point in making comparisons with strains suspected of being parathion resistant. In comparison with the susceptible Pinedale strain, the Hanford strain

requires twice as much parathion for a 24 hour mortality of 50 percent and sixty-eight times as much for a 90 percent mortality. The Tulare strain is three times as resistant at the LC_{50} level and twenty-five times as resistant at the LC_{90} level as the susceptible Pinedale strain. This indicates a significant degree of physiological resistance to parathion which undoubtedly is responsible for failures of field applications.

Cross-resistance tests. In view of the high degree of parathion resistance in the two strains investigated, the question of substitute organophosphorus insecticides poses the problem of cross-resistance. To determine the degree of cross-resistance to other organophosphates, limited laboratory tests were conducted observing the same procedures outlined for parathion. The results obtained are presented in table 2.

TABLE 2—Organophosphorus cross-resistance tests with parathion-resistant *Aedes nigromaculis*

Compound	Susceptible Strain (Pinedale) 24 hr.		Resistant Strain (Hanford) 24 hr.	
	LC_{50} in p.p.m.	LC_{90} in p.p.m.	LC_{50} in p.p.m.	LC_{90} in p.p.m.
Korlan	0.01	0.028	0.041	0.058
Malathion	0.01	0.03	0.0058	0.03
Guthion	0.0074	0.01	0.0064	0.01
Trithion	0.015	0.033	0.031	0.052

Graphical analysis indicates cross-resistance in the results obtained with Korlan and Trithion. Field tests with Korlan and Trithion against parathion-resistant *A. nigromaculis* larvae required higher dosages to give complete kill than in areas where parathion resistance did not exist in this species. This evidence lends support to the laboratory findings that cross-resistance to Korlan and Trithion may exist in the parathion-resistant strain.

No cross resistance to Guthion or malathion was indicated in the analysis of laboratory tests. It appears likely that these compounds would successfully control parathion-resistant *A. nigromaculis,* although resistance to these compounds might appear more quickly than in a normal population.

Methyl parathion (Metacide) has been used successfully in controlling parathion-resistant *A. nigromaculis* in field operations carried out by the Kings Mosquito Abatement District.

Further studies on the response of these strains to other insecticides are contemplated.

SUMMARY. A method is described for laboratory rearing of *Aedes nigromaculis* larvae from eggs produced by field collected females. This method was used to obtain uniform larvae for parathion resistance tests since the species has not yet been colonized.

Tests with parathion on fourth instar larvae from two areas that have not been treated with insecticides for mosquito control provided a reference point for comparison with populations presumed resistant to parathion. Tests on larvae from two mosquito abatement districts where parathion has been used in control programs for five to six years indicated that the LC_{50}'s to this compound had increased two to three fold. On the basis of LC_{90} comparisons the larvae from parathion treated areas were 25 and 68 times as resistant to parathion as the susceptible strains.

Cross-resistance tests indicated that parathion-resistant *A. nigromaculis* larvae may also be slightly resistant to Korlan and Trithion but not to malathion or Guthion.

Methyl parathion (Metacide) has been used successfully in field operations to control parathion-resistant *A. nigromaculis.*

Literature Cited

LEWALLEN, L. L., and BRAWLEY, JOHN H. 1958. Parathion resistant *Aedes nigromaculis.* California Vector Views 5(8):56.