PARATHION-RESISTANT *Aedes nigromaculis* IN CALIFORNIA

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Laboratory investigations on fourth in-
star 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 re-
sistance or susceptibility in strains collected
from other areas of California.

Mosquito larvae reared in the laboratory
under standard conditions are more uni-
form in response to chemical tests than
larvae collected directly from the field.
Tests on field collected larvae are some-
times questionable since factors such as
variation in nutrition, exposure to agri-
cultural 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. Attempts 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. nigro-
maculis* were obtained from irrigated
pastures near Pinedale and Kerman
(Fresno County) where insecticides have
not been used in a mosquito control pro-
gram. For comparison, *A. nigromaculis*
was collected from the Kings Mosquito
Abatement District near Hanford (Kings
County) and the Tulare Mosquito Abate-
ment District near Tulare (Tulare
County). Organophosphorus compounds
have been used in mosquito control pro-
grams 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 col-
lector'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 ¾ inch in
diameter lined with a piece of damp paper
toweling 3½ inches long by 1 inch wide.
The vials were plugged with a piece of
cellulocotton. 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 de-
posited 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.
containing distilled water filled to a depth of 1 1/2 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 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 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.

Treated larvae were held at 70°F ± 4°F 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<sub>50</sub> and LC<sub>90</sub> 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. nigromaculatus 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. nigromaculatus in this study are given in Table 1.

<table>
<thead>
<tr>
<th>Strain</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; in p.p.m.</th>
<th>LC&lt;sub&gt;90&lt;/sub&gt; in p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinedale</td>
<td>0.000035</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kerman</td>
<td>0.00004</td>
<td>0.00009</td>
</tr>
<tr>
<td>Tulare</td>
<td>0.00012</td>
<td>0.0003</td>
</tr>
<tr>
<td>Hanford</td>
<td>0.000068</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

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 LC90 level and twenty-five times as resistant at the LC99 level as the susceptible Pineland 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 organophosphorous 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](image)

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 LC90's to this compound had increased two to three fold. On the basis of LC99 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**