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ORGANOPHOSPHORUS RESISTANCE IN *AEDES NIGROMACULIS* IN CALIFORNIA

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Organophosphorus insecticides, including EPN, parathion, and malathion, were first introduced in California between 1952 and 1954 to control larvae of *Aedes nigromaculis* (Ludlow), a pest of irrigated pastures, which by 1951 had already shown resistance to chlorinated hydrocarbon insecticides (Gjullin and Peters, 1952). Control failures with parathion became evident by 1958 in Kings County (Lewallen and Brawley, 1958), and that this was due to acquired tolerance was proved for material from Kings and Tulare counties (Lewallen and Nicholson, 1959). By 1960 it was evident that a very high degree of resistance to parathion was present in Tulare County (Lewallen, 1961). It was in the Delta Mosquito Abatement District of northwestern Tulare County and in Kings County that methyl parathion was substituted to control these resistant larvae, but its initial success was followed by control failures in the Delta district in 1962. The purpose of this investigation was therefore to examine cross-resistance data in order to ascertain whether this was a case of general organophosphorus resistance to all OP compounds employed, i.e., parathion, methyl parathion, fenthion (Baytex) and malathion.

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METHODS. Larvae were collected in the field and transported in the field water to the laboratory for test in the late third and early fourth instars. The required amount of insecticide was dissolved in acetone, and 1 ml. of this solution was added to 100 ml. of distilled water in 4-oz. waxed Dixie cups. Lots of 20 larvae were examined for 24 hours at 70° F., and the percentage mortality was scored from the sum of the moribund and the dead. A succession of concentrations was employed (e.g., 0.01, 0.02, 0.05, 0.08, 0.1 p.p.m.) so that a dosage-mortality line could be obtained and the LC_{50} level established.

RESULTS. The LC_{50} level of parathion (Table 1) ranged from 0.0006 p.p.m. for an untreated locality at Porterville in Tulare County to 0.170 p.p.m. on the Webb property in Tulare County. These compare with 0.00004 p.p.m. as being the LC_{50} levels obtained in 1958 at Pinedale and Kerman in

Table 1.—LC_{50} values in p.p.m. of four organophosphorus insecticides for *Aedes nigromaculis* larvae collected in 1963.

	Parathion	Methyl parathion	Fenthion	Malathion
Porterville I, Tulare	.0006	..	.00011	..
Lost Hills, Kern *	.0010	.00070	..	.011
Porterville II, Tulare	.0015	.00130	.00026	.033
Porterville III, Tulare	.0017	.00093	..	.034
Crocker-Huffman 17, Merced	.0017	.00100	.00072	.013
Rahilly, Merced	.0018	.00089	..	..
Robin Bell, San Joaquin	.0018	.00078	.00110	.011
Fulvio, Stanislaus	.0027	.00130	.00082	.019
Parrish, Kern *	.0027	.00110	.0012	.022
Johnson, Merced	.0031	.00085	..	.013
Clover, Stanislaus	.0031	.0012	.0011	.030
Lemoore, Kings	.0050	.0033	.0018	.037
Thornburg, Kern	.0068	.0040	..	.052
Heitt, Kern	.008	.0024	.0013	.047
Filippini, Stanislaus	.012	.0015	.0025	.050
Justeson, Sutter	.016	.0032	.0012	..
Mill Creek, Tulare	.030	.0034	.0017	..
Burner I, Tulare	.034	.0031	.0020	.086
Parker, Kern *	.037	.0021	..	.066
Burner II, Tulare	.046	.0033	.0024	..
Overland I, Tulare	.058	.0047	.0022	.072
Overland III, Tulare	.060	.0050	.0024	..
Overland II, Tulare	.078	.0068	..	..
Goshen, Tulare	.082	.0150	.0026	.088
Berberia, Tulare	.089	.0045	.0024	.120
Webb I, Tulare	.096	.0150	..	..
Webb II, Tulare	.130	.0083	.0023	.076
Webb III, Tulare	.170	.0056	..	.080

* Determinations of L. W. Isaak, 1962.

Fresno County (Lewallen and Nicholson, 1959). Thus the larvae at Webb have increased in resistance to parathion by approximately 4,000 times.

The LC_{50} levels of methyl parathion ranged from 0.0007 p.p.m. from an untreated locality at Lost Hills in Kern County to 0.0150 p.p.m. on the Webb property, an increase of approximately 20 times. Those of fenthion ranged from 0.00011 p.p.m. at Porterville to 0.0026 p.p.m. at Goshen in Tulare County, again an increase of approximately 20 times. Those of malathion ranged from 0.011 p.p.m. at Lost Hills to 0.120 p.p.m. on the Berberia property in Tulare County, an increase of approximately 10 times.

The slopes of the dosage-mortality lines for parathion from which the LC_{50} values were obtained (Fig. 1) were quite steep, the slope (change in probits per 10-fold change in dosage) being 7.1 for the susceptible Porterville material. These lines, being steep and showing no signs of the inflexions that develop where resistance is due to single factors as with DDT or

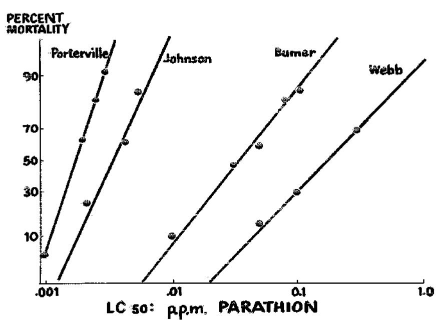

Fig. 1.—Dosage-mortality relationships to parathion of 4 samples of *A. nigromaculis* populations with different resistance levels.

dieldrin, indicate that this organophosphorus-resistance is due to multiple factors. The slopes become gradually shallower as resistance increases, suggesting the gradual emergence of a principal genetic factor.

Cross-resistance tests made on 8 samples of various levels of resistance to parathion (Fig. 2) indicate that they

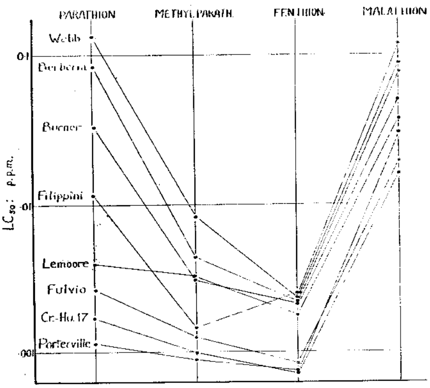

FIG. 2.—Cross-resistance levels to 4 organophosphorus compounds of 8 samples of *A. nigromaculis* in California in 1963.

showed a corresponding level of resistance to methyl parathion, fenthion and malathion. When the LC_{50} levels of malathion are plotted against those of parathion (Fig. 3), they are found to show a linear

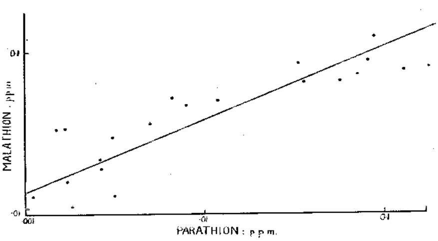

FIG. 3.—LC_{50} values for malathion compared to those for parathion; *A. nigromaculis*, California, 1963.

relationship where a 100-fold increase of parathion is accompanied by a 10-fold increase of malathion; the relationship between the two insecticides is sufficiently close to give a correlation coefficient of 0.89. Even the sample from northern San Joaquin County, where malathion has always been used, and where parathion has never been used, shows this same relationship between parathion and malathion LC_{50} levels.

When the LC_{50} levels of methyl parathion are plotted against those of parathion (Fig. 4), they also show a linear relation-

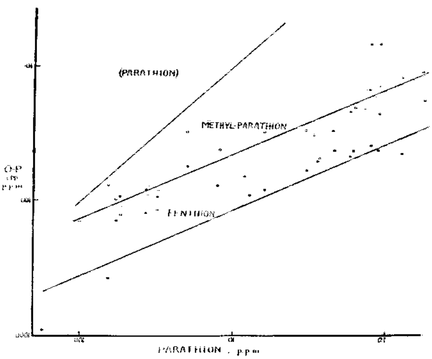

FIG. 4.—LC_{50} values for methyl parathion and fenthion (Baytex) compared to those for parathion; regression of parathion on parathion inserted for the purpose of comparing slopes.

ship, with a correlation coefficient of 0.92; again the increase of methyl parathion is 10-fold for a 100-fold increase of parathion. A similar increase in cross-resistance to fenthion is also shown, with a correlation coefficient of 0.84.

It has been the general experience in the field that when the LC_{50} of parathion rises above 0.005 p.p.m., partial control failures result when parathion is applied as 0.075–0.1 lb. per acre. When methyl parathion is substituted, control failures result when the LC_{50} of methyl parathion is in excess of 0.004 p.p.m., corresponding to an LC_{50} of 0.05 p.p.m. of parathion.

When the susceptibility test figures are divided into 3 categories—less than 0.005, more than 0.05, and 0.005 to 0.05 p.p.m. for the LC_{50}—and plotted on a map (Fig. 5), it is seen that the highest resistance is in Tulare County, with moderate resistance in Kings, Kern, southern Fresno, and northern Sutter counties.

Discussion. The cross-resistance figures made it clear that the resistance in *A. nigromaculis* is a nonspecific organophosphorus-resistance extending equally to all the 4 OP compounds employed. It is also equally inducible by any one of the 4 compounds; for example, in San Joaquin County it was malathion that increased the parathion-tolerance level, and in Tulare County the use of methyl parathion increased the parathion LC_{50} from 0.0011 p.p.m. in 1962 to 0.089 p.p.m. in 1963. This situation is different from that in the house fly, where there is a distinction between the resistance to ethyl-substituted compounds, such as parathion and diazinon and that to methyl-substituted compounds such as malathion (Oppenoorth and van Asperen, 1960), and in *Culex tarsalis* Coquillett in which there is a specific

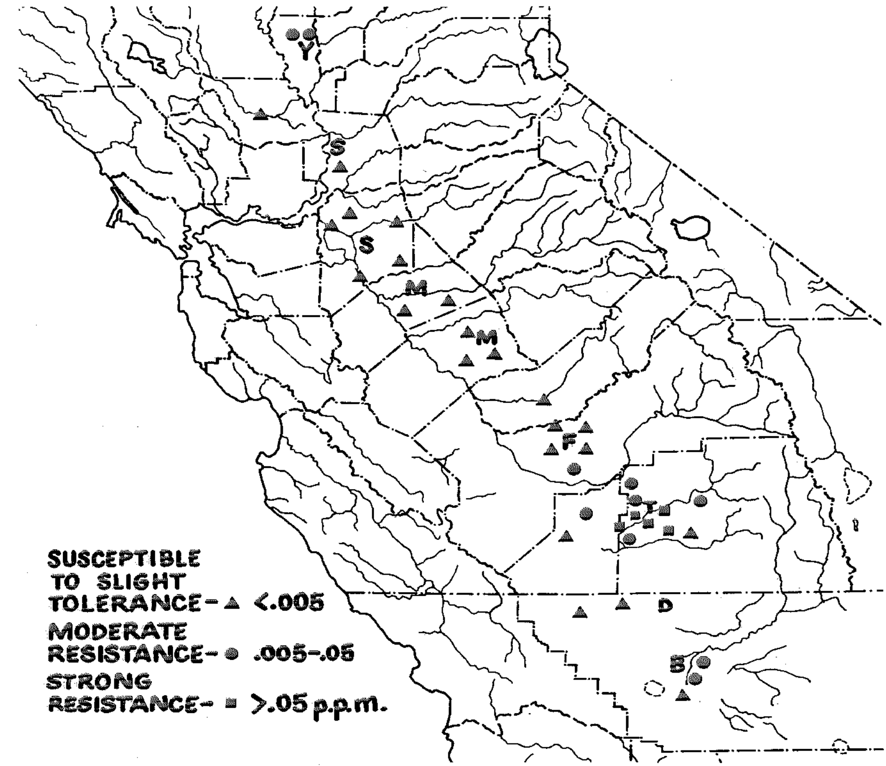

Fig. 5.—LC_{50} levels of parathion for *A. nigromaculis* in the Central Valley, California, 1963. See note p. 345 for additional explanation.

malathion-resistance not extending to any other organophosphorus compound (Darrow and Plapp, 1960); these resistances are all due to detoxication by specific enzymes. Evidently in *A. nigromaculis* the Californian operations have developed a nonspecific OP-resistance of polyfactorial genetic origin; this may be comparable to the resistance developed by parathion in *Aedes aegypti* (L.) (Matsumura and Brown, 1963), which involves neither increased detoxication nor decreased absorption.

SUMMARY. Organophosphorus-resistance in *Aedes nigromaculis* in California is a single entity, reaching a maximum (by 1963) of 4,000 times to parathion, 20 times to methyl parathion and fenthion, and 10 times to malathion. The control failures with methyl parathion observed in Tulare county 2 years after its initially successful substitution for parathion are due simply to further growth in this nonspecific OP-resistance.

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NOTE. In Fig. 5, the letters refer to key cities within the counties, north to south: Y=Yuba City, Sutter Co.; S=Sacramento, Sacramento Co.; S=Stockton, San Joaquin Co.; M=Modesto, Stanislaus Co.; M=Merced, Merced Co.; F=Fresno, Fresno Co.; T=Tulare, Tulare Co.; D=Delana, Kern Co.; B=Bakersfield, Kern Co.

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OBSERVATIONS ON *CULEX NIGRIPALPUS* THEOBALD IN A TYPICAL HAMMOCK AREA OF NORTH CENTRAL FLORIDA

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INTRODUCTION. During the late summer of 1962 an epidemic of St. Louis encephalitis struck the Tampa-St. Petersburg area of Florida resulting in 17 deaths. A previous outbreak occurred in 1959 in the same area, claiming five lives (Bond, 1961). At that time the mosquito *Culex nigripalpus* was implicated epidemiologically as the vector (Beadle, 1960). Only recently, however, has the virus been isolated from this species, which is thought to be an important vector in the transmission of the disease in Florida (Anonymous, 1962). This has resulted in considerable attention to studies of the biology and bionomics of the species in Florida.

The following observations were made during a study of the seasonal distribution and abundance of mosquitoes in different plant communities of a typical hammock