ARTICLES

RECENT STUDIES OF MOSQUITO RESISTANCE TO INSECTICIDES IN CALIFORNIA [1]

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The warm weather and the frequent irrigations in the San Joaquin Valley of California provide ideal conditions for the production of mosquitoes. In the Valley are large grass pasture areas, many of which contain ladino clover. *Aedes nigromaculis* (Ludl.), which is the most important species, propagates mainly in these situations. Large numbers of this species are also found in low grassy spots in alfalfa fields. Thurman *et al.* (1951) have shown that pasture lands may be irrigated as many as 18 times during a season and that a new generation of *A. nigromaculis* is produced after each irrigation. *A. dorsalis* Meig. is also abundant in grass pastures during the spring months. *Culex tarsalis* Coq. breeds in situations where water accumulates, especially in rice fields, around duck clubs, and in cotton fields after the fall irrigation. *C. quinquefasciatus* Say builds up large populations rapidly in dairy drains and sewer farms in the summer and fall months. Several other less important species are also to be found in the Valley.

DDT mosquito larvicides were first used in the San Joaquin Valley in 1945, and in 1946–47–48 this insecticide was used for nearly all control work. The resistance of mosquitoes to DDT was first reported by Smith (1949) in Kern County, but failures with this larvicide had also been reported in other districts. In a survey of resistance to DDT in *A. nigromaculis* larvae in 1949, Bohart and Murray (1950) found that the average larva from three treated fields in Tulare County was about 10 times as resistant as the average larva from three untreated fields in Kings County.

In 1949, some of the districts began to use toxaphene exclusively, and other districts used toxaphene for some conditions and DDT for others. Benzene hexachloride has been used for special situations since it became available, and lindane has had similar use since 1950. Gamtox, a product containing gamma benzene hexachloride, has also been used in some districts. Toxaphene, benzene hexachloride, and lindane, as well as DDT, failed to give control in certain areas in 1950–51.

In 1951 laboratory tests were made at Bakersfield, Visalia, Fresno, and Merced to determine the degree of resistance that had developed to DDT, toxaphene, lindane, and aldrin in the more important species in these districts. These tests were begun on April 4 and continued until October 20.

Methods and Materials.—Larvae were gathered from breeding places that had been controlled with chlorinated hydro-

[1] A cooperative project between the Bureau of Entomology and Plant Quarantine, the Bureau of Vector Control of the California Department of Public Health, and several mosquito-abatement districts in California. Several Bureau of Vector Control employees, including Grafton Campbell and Malcolm White, assisted with the project, as well as personnel of the San Joaquin Valley mosquito-abatement districts.

carbon insecticides and also from untreated adjacent areas. They were collected with dippers and transported to the laboratories in 1-gallon vacuum jugs. The resistance to the insecticides was based on the parts per million of insecticide necessary to give 50 per cent mortality of the larvae.

The tests were made with early fourth-instar larvae. To 200 cc. of tap water[1] in a glass container sufficient acetone solution of the insecticide was added to give the desired concentration. Twenty larvae were then placed in each container, and two replications were used for each collection. The solutions were maintained at 80° F., and larval mortality was determined after 24 hours.

Each insecticide was tested at four or five different strengths on each collection of larvae. The dosages were adjusted to obtain kills of more than 1 per cent and less than 100 per cent in as many tests as possible. The number of parts per million of each insecticide required to give 50 per cent kill is based on the results of two replications made with from two to eight collections of larvae.

In several counties tests were made in both the spring and fall months to obtain information on seasonal differences in resistance. Larvae were taken from the same general area in both seasons.

From each location where larvae were collected, 1-gallon samples of water were taken and sent to the California Department of Public Health for plankton determinations and analysis of dissolved chemicals.

Discussion of Results.—The results of the tests with *Aedes* larvae are given in table 1.

The resistance to DDT of *A. nigromaculis* larvae from the treated areas was from three to seven times as great as that of larvae from untreated check areas. In all tests except the one made in September in the Tulare District the resistance was from five to seven times greater than that of the larvae from the check area. The low resistance indicated at Tulare is due to the high LD-50 obtained for DDT in the Kings County check area. It is possible that the larvae in this check area were subjected to DDT in some way during the summer, since the LD-50 requirement was over twice as much as for other check areas and for the May test in this area.

In August the *A. nigromaculis* larvae in the Peacock-Canfield area in the Kern District were more than three times as resistant to DDT as the larvae taken in April. This was apparently due, at least in part, to the application of DDT by the syphon method as reported by Geib and Smith (1949). By this method the emulsion is delivered into the irrigation water before it flows onto the fields and kills the larvae as they hatch. The method was used in this district in 1949, but discontinued when larvae became resistant and could not be controlled with 0.25 p.p.m. The 1951 applications were made in June and July to determine whether the larvae were still resistant. In these tests 0.25 p.p.m. was effective for two irrigations but failed in the third. In the next two irrigations 0.35 and 0.5 p.p.m. also failed to give control. Larvae from this district were also about twice as resistant to toxaphene, lindane, and aldrin as were the larvae from the check. Except in two tests with toxaphene, all larvae from treated areas showed less than twice as much resistance to these materials as those from untreated areas.

Larvae taken from an alkali-flat area in the Delta District in September were found to be about three times as resistant to toxaphene as were larvae from the check area. The managers of this and other districts have found that the failure of toxaphene in such areas occurs in the fall months, and coincides with a reduced permeability of the soil and more standing water. Normal permeability is restored by the following spring, and toxaphene is again effective at normal dosages.

Larvae from this alkali-flat area were

[1] The tap water used in the different areas showed no significant variation in percentage of kill.

also tested in the water in which they were breeding and in mosquito-breeding water from a nonalkali area. Toxaphene was equally effective in both types of breeding water, but the mortality was higher than that obtained in tap water. It therefore appears that failure of toxaphene in control operations in this area is due to the resistance of the larvae and not to any effect of the alkaline water on the toxaphene emulsions.

A. dorsalis larvae required slightly larger doses of DDT, lindane, and aldrin for 50 per cent kill than did *A. nigromaculis*. Larvae of *A. dorsalis* from the Hilmar area in the Merced District showed a DDT resistance 12 times that of the larvae from the check area. This result was comparable to one with *A. nigromaculis* larvae obtained from this area in which resistance to DDT was fairly high and no resistance to toxaphene was indicated. Heavy applications of both these materials have failed to give control of larvae in this area.

As shown in table 2, *C. tarsalis* larvae in

TABLE 1.—Dosages required for 50 per cent kill of *Aedes* larvae in San Joaquin Valley as determined in duplicate tests of from two to eight larval collections from each area.

Area [1]	Time of Tests	LD-50 in p.p.m.			
		DDT	Toxaphene	Lindane	Aldrin
		Aedes nigromaculis			
Kern County:					
Peacock-Canfield [2]	April	0.025	0.020	0.005	0.0046
Lane-Trout	April	.077	.05	.0069	.007
Sargent Ranch (check) [2]	April	.0106	.0113	.0042	.004
Peacock-Canfield	August	.0909	.0454	.0154	.010
Canfield	August	.066	.033	.0105	.0079
Sargent Ranch (check)	August	.0120	.0209	.0063	.0045
Tulare and Kings Counties: [3]					
Delta and Tulare Districts	May	.059	.0204	.0063	.0037
Kings County (check)	May	.0114	.0200	.0147	.0031
Delta alkali flat	September	.0952	.0769	.0086	.0061
Tulare District	September	.0571	.0204	.0085	.0056
Kings County (check)	September	.0370	.0238	.0087	.0056
Fresno, Madera, and Kings Counties: [3]					
Consolidated and Fresno Districts	May–June	.0999	.0190	.0058	.0040
Madera District	May–June	.0833	.0175	.0192	.0054
Kings County (check)	May	.0114	.0200	.0147	.0031
Merced and Fresno Counties:					
Near Merced, Merced District	July–Aug.	.0909	.0213	.0067	.0050
Fresno County (check)	July–Aug.	.0149	.0147	.0060	.0038
Hilmar area, Merced District	July–Aug.	.1111	.0190	.0074	.0011
		Aedes dorsalis			
Kern and Kings Counties:					
Sargent Ranch (check)	April	.026	.04	.019	.0071
Greenfield Ranch	April	.090	.043	.028	.0079
Kings County (check)	May	.026	.021	.022	.0046
Merced County:					
Near Merced, Merced District	July–Aug.	.1111	.050	.0263	.020
Hilmar area, Merced District [4]	July–Aug.	.3333	.0454	.0277	.0076

[1] All areas were treated except those indicated as checks.
[2] 22 per cent of larvae were *A. dorsalis.*
[3] Untreated portions of Kings and Fresno Counties were used as checks for Tulare, Fresno, Madera and Merced Counties.
[4] 27 per cent of larvae were *A. vexans.*

TABLE 2.—Dosages required for 50 per cent kill of *Culex* larvae in San Joaquin Valley as determined in duplicate tests of from two to four larval collections from each area.

Area [1]	Time of Tests	LD–50 in p.p.m. DDT	Toxaphene	Lindane	Aldrin	Heptachlor
		Culex tarsalis				
Kern County:						
Poso Creek (check)	April	0.0093	0.0143	0.0118	0.0012	......
Peacock Ranch	April	.077	.0147	.0100	.0023	......
Poso Creek (check)	August	.0154	.0303	.040	.0031	0.0018
Paloma field and Stockdale Road [2]	August	.0555	.0370	.0312	.0023	......
Pin Tail Duck Club	August	.0303	.2857	.020	.1250	......
Ware Duck Club	August	.1539	1.00	.479	.6666	2.4
Fresno and Kings Counties:						
Fresno and Consolidated Dist.	June	.0641	.0175	.0164	.0016	......
Consolidated Districts	October	.0833	.0217	.0345	.0022	......
Kings County (check)	October	.0333	.0175	.0213	.0016	......
		Culex quinquefasciatus				
Merced County:						
Cannery Waste [3]	July	.0555	.0277	1.0 [3]	.0057	......
Tulare and Kings Counties:						
Dairy drain, McCuen Ranch	September	.0769	.2222	.1389	.0166	.1429 [4]
Dairy drain, #2 ranch	September	.0250	.0779	.0526	.0073	.0714
Visalia sewer farm	September	.0455	.0909	.0526	.0130	......
Fresno and Madera Counties:						
Firebaugh sewage plant	October	.0777	.0714	.05	.0108	.0117 [4]
Madera sewage plant	October	.0711	.1111	.0713	.0192	.0151 [4]
Consolidated District	October	.0588	.0713	.0370	.0041	......

[1] All areas were treated except those indicated as checks.
[2] 34 per cent of larvae were *C. quinquefasciatus*.
[3] 45 per cent of larvae were *C. tarsalis*.
[4] One larval collection.

the Kern District were found in April to have a resistance to DDT eight times that found in the check area and no resistance to the other three insecticides. Lower resistance to DDT and none to the other insecticides were found in a few tests in other districts during the summer. This pattern was completely changed when tests were made in August in the duck club area of the Kern District, where applications of aldrin and toxaphene were failing to give control. The larvae from the Ware Duck Club were found to be ten times as resistant to DDT, approximately 33 times as resistant to toxaphene, 11 times as resistant to lindane, and 215 times as resistant to aldrin as larvae in the check areas. These larvae were approximately 1,300 times as resistant to heptachlor as the larvae from Poso Creek. Heptachlor and lindane had not been used for control in this area. A few tests with parathion indicated that larvae from the duck-club area might be slightly more susceptible to this material than the untreated larvae from Poso Creek.

Larvae from the Pin Tail Duck Club, less than half a mile from the Ware Club, were 2, 9, 5, and 40 times as resistant, respectively, to DDT, toxaphene, lindane, and aldrin as larvae from the Poso Creek area. The lower resistance found in the Pin Tail Club area is apparently not due to variations in insecticide applications, for both club areas have been treated with the same insecticides for several years.

Analysis of water samples (table 3) from the Pin Tail Duck Club showed that this area had approximately the same amount of dissolved chemicals as the water from Paloma field about 11 miles away in the Kern District, where *C. tarsalis* showed moderate resistance to DDT only. Water from the Ware Duck Club, where the high resistance in *C. tarsalis* larvae was found, had 49 times as much chloride, 141 times as much sulphate, 6 times as much calcium, 16 times as much magnesium, and 17 times as much sodium as water from the Pin Tail Club. This analysis strongly suggests that these chemicals may be a cause of the high resistance found in the larvae at the Ware Club, and that the lower resistance in the larvae from the Pin Tail Club, which is less than ½ mile distant, may be due to migration of adults from the Ware Club. Further tests will be necessary to determine whether resistance in the area has developed in this way.

Water from the Poso Creek area was also high in sodium, chloride, and sulphate, but no insecticide had been applied here. It is possible that larvae develop high resistance in waters containing large amounts of these chemicals only when repeated applications of insecticides have been made.

Larvicide applications on the duck-club area have been almost entirely by plane. DDT dosages ranged from 0.2 to 1.3 pounds per acre during the period from 1946 to 1948. Toxaphene applications of 0.22 to 0.46 pound were made in 1949 and 1950. Some applications of DDT at 0.31 to 0.46 pound and of aldrin at 0.048 to 0.082 pound per acre were also made in 1950.

One of the duck clubs was sprayed with DDT at the rate of 1 pound per acre after the resistance tests had been made. This dosage was effective, but when the area was later resprayed with the same dosage it was ineffective. Application of 1.5 pounds of dieldrin per acre in this area was also ineffective.

In two lots of mixed *C. quinquefasciatus* and *C. tarsalis* larvae from Paloma field and from near Stockdale Road, 11 and 30 miles, respectively, from the duck-club area, resistance to DDT was only three times that of larvae from the untreated area, and no resistance to other insecticides was indicated.

C. quinquefasciatus larvae from various situations were tested during the summer

TABLE 3.—Analysis of water samples from areas in Kern County where *Culex tarsalis* larvae were developing.

Test	Pin Tail Duck Club	Ware Duck Club	Paloma Field	Poso Creek (Check)
pH	7.9	7.1	8.1	8.4
		Parts per million		
Bicarbonate	198	122.4	115	204.7
Carbonate	3	0	0	10.5
Hydroxide	0	0	0	0
Hardness (as calcium carbonate)	132	1138	55	127.5
Chloride	41.6	2060	9.6	614
Nitrate nitrogen	0			
Organic nitrogen	0.5	0.2	0.1	0.5
Sulphate	2.8	396	6.9	34.6
Phosphate	3.4	1.2	0.1	0.7
Calcium	38.2	223	20.8	37.6
Soluble iron	1.5	0.4	0.6	0.6
Magnesium	8.8	142	0.1	8.2
Manganese	0.1	1	0	0.2
Sodium (calculated)	59.9	1060	66.7	455
Total solids	395.4	4001.5	306.6	1399.2

and fall, but definite comparisons with untreated larvae could not be made because such larvae were not found in the Kings County untreated area or at the Firebaugh sewage plant, as anticipated. Larvae taken from a dairy drain at the McCuen Ranch in Kings County showed considerable resistance to toxaphene, DDT, lindane, and heptachlor. This dairy was found to have been treated every two weeks with benzene hexachloride. Lower resistance was indicated in larvae from a second drain in this area from a dairy that had been treated with DDT. Although Firebaugh is not in a control district, the larvae taken from the sewage plant appeared to have considerable resistance, and it was found that the water was occasionally treated with one of the chlorinated hydrocarbon insecticides.

C. quinquefasciatus larvae from the Visalia sewer farm showed considerable resistance to the insecticides tested. Benzene hexachloride, which had been used in control operations against these larvae, had failed just prior to these tests. A high resistance to lindane was also found in larvae from a cannery-waste pond in the Merced District, where this insecticide was no longer effective.

Mosquito-abatement managers in a number of districts in the San Joaquin Valley find that larvicide applications are less effective against *A. nigromaculis* in the late summer and fall than against larvae produced earlier in the year. In two of the untreated check areas only fractionally larger amounts of insecticides were required in the fall than in the spring except for a threefold increase in DDT in one area. The large increase in DDT may have been due to contamination of the area with this insecticide. The reduced effectiveness of larvicides in fall months which has been noted in control operations has been shown to be associated with alkali conditions in some areas. Other undetermined factors may also be involved.

Resistance to insecticides other than those used against them has been demonstrated in mites, mosquitoes, house flies, and *Drosophila*. In a review of the literature on insect resistance Babers and Pratt (1951) conclude in part,

"It is not possible apparently to cover all cases, but the following generalities begin to appear:

(1) A very high resistance to one insecticide confers a certain amount of cross tolerance for other insecticides of widely dissimilar nature.

(2) When the level of resistance is low, the resistance is almost specific and is limited to other insecticides of closely related structures.

. . .

(8) Once resistance to any insecticide is developed in house flies, the development of resistance to other insecticides proceeds at an accelerated rate."

The resistance of *C. tarsalis* in the Kern District has been of about the same general pattern. The high tolerance of these larvae for heptachlor is believed to represent the most extreme degree of resistance so far recorded in mosquitoes.

Failures that have already occurred with benzene hexachloride and lindane against *C. quinquefasciatus* larvae indicate that chlorinated hydrocarbon insecticides may soon become completely ineffective against this species in areas that are repeatedly treated.

Larvae of *A. nigromaculis* and *A. dorsalis* in the San Joaquin Valley, which are now partially resistant, will undoubtedly become very resistant to chlorinated hydrocarbon insecticides if they continue to be used against them. No estimate of how long this will take is possible on the basis of present information.

The high resistance developed in *Culex* larvae that were repeatedly treated and the rapid acceleration in the rate of resistance of *A. nigromaculis* in the Peacock-Canfield area, where newly hatched larvae were treated with DDT, indicates that resistance to chlorinated hydrocarbon insecticides may develop more rapidly in young larvae.

Summary—The resistance of several important species of mosquitoes in the San Joaquin Valley of California to DDT, toxaphene, lindane, and aldrin was deter-

mined in laboratory tests of larvae from treated and untreated areas. Tests were made in six counties in the Valley—Kern, Tulare, Kings, Fresno, Merced, and Madera.

Aedes nigromaculis larvae from the treated areas were found to be from three to seven times as resistant to DDT as larvae from the untreated areas. Resistance to toxaphene was less than twice that of larvae from the untreated area except in two areas, in which resistance was from two to three times that of the larvae from the untreated area. Little or no resistance to lindane and aldrin was shown except in one area in the Kern District, where it was twice that of the larvae from the untreated area.

In a few tests with *Aedes dorsalis,* resistance to DDT was 3 to 12 times that of larvae from the untreated area, but no resistance to the other insecticides was indicated.

Culex tarsalis larvae from a duck club in the Kern District, where applications of toxaphene and aldrin were failing, were found to be 10, 33, 11, 215, and 1300 times as resistant, respectively, to DDT, toxaphene, lindane, aldrin, and heptachlor as larvae from an untreated area. Larvae from a nearby duck club had a smaller range of resistance. Parathion was about equally effective on both treated and untreated larvae. DDT at 1 pound per acre was effective against these larvae in the first application and failed in the second. Dieldrin was ineffective at 1.5 pounds per acre.

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FURTHER OBSERVATIONS ON SEXUAL DIMORPHISM IN MOSQUITO PUPAE (DIPTERA, CULICIDAE)

By STANLEY J. CARPENTER[1]

Moorefield (1951) pointed out obvious structural differences in the sexes of mosquito pupae as exhibited by the structure of the tenth abdominal segment (genital pouch) which is attached to the sternite of the eighth segment and lies ventral of the paddle. Moorefield illustrates these sexual characters as observed in eight species of mosquitoes representing five genera studied as follows: *Aedes stimulans* (Walker), *A. trivittatus* (Coquillett), *A. vexans* (Meigen), *Anopheles punctipennis* (Say), *Culex apicalis* Adams, *C. restuans* Theobald, *Culiseta inornata* (Williston) and *Psorophora ferox* (Humboldt).

The writer has checked and verified this character for separating the sexes of mosquitoes in the pupal stage by examining both males and females with associated

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