SUSCEPTIBILITY OF VARIOUS LARVAL INSTARS OF *CULEX P. QUINQUEFASCIATUS* SAY TO INSECTICIDES [1]

M. S. MULLA [2]

Department of Entomology, University of California, Riverside

Field and laboratory larviciding tests against mosquitoes are generally directed against the fourth instar larvae (Isaak 1956, Lewallen 1958, Mulla 1961, Mulla *et al.* 1960, 1961). The fourth instar larvae are considered by many workers to be more tolerant to larvicides than the younger instar larvae and, therefore, practically all larvicidal evaluation studies have been conducted on the response of the fourth instar larvae to candidate insecticides. Materials that would cause fourth instar larval mortality at a specific dosage are generally believed to produce a similar if not a greater response in the younger instar larvae.

The common belief that fourth instar larvae are more tolerant to organophosphorus and other insecticides than the younger larvae, has not been supported by experimental evidence. Information on the susceptibility level of various instar larvae to the newer insecticides is lacking. From the standpoint of screening programs and field evaluation studies, information on the susceptibility level of various instar larvae to insecticides could be of some value.

In laboratory screening tests it is often difficult to obtain a pure stock of fourth instar larvae for establishing dosage response lines of test materials. A number of third instar larvae are usually mixed with the fourth instar larvae; separation of these is time-consuming and often impractical. The accuracy and reproducibility of the dosage response lines may be influenced among other things by the number of third instar larvae present, as well as by the degree of their susceptibility to insecticides. If the susceptibility level of the third instar larvae to insecticides approximates that of the fourth instar larvae, the variation introduced due to the presence of third instar larvae may be very small; on the other hand, if the susceptibility level of the third instar larvae is appreciably greater than that of the fourth instar larvae, the variation introduced by heterogeneous populations may be of greater magnitude. Variation of this nature is also encountered in field evaluation work where, at times, the third instar larvae are not easily differentiated from the early fourth instar larval population.

In field mosquito larvicidal treatments it is desirable to know the susceptibility level of various larval instars to the larvicidal materials. After application, the larvicides disappear due to physical and chemical processes, and the administered concentration of the larvicide decreases. Some larvicides degrade faster than others. The initial dosage applied for the control of fourth instar larvae also affects younger instar larval populations present at the time of treatment. In most cases the treated breeding source is subject to repeated reinfestation; first instar larvae may appear within a few days after treatment. If these are highly susceptible to the larvicidal material employed, complete kill may be obtained, despite the fact that the concentration of the toxicant in the water was diminished markedly. Stability of larvicidal materials and the level of susceptibility of the first instar larvae to these will determine the extent of control following a specific application.

To obtain information on the susceptibility level of various larval instars, the current studies were initiated in the laboratory against first, second, third and

---

[1] Paper No. 1326, University of California Citrus Experiment Station, Riverside, California.

[2] The able assistance of H. Axelrod of the Department of Entomology, University of California, Riverside, is duly acknowledged.

fourth instar larvae of the mosquito *Culex pipiens quinquefasciatus* Say.

METHODS AND MATERIALS. Larvae from a susceptible colony of *Culex p. quinquefasciatus* Say were used. The colony had been reared for several years without being exposed to selection pressure from insecticides. First instar larvae were obtained by placing egg rafts in tap water. Soon after hatching, the larvae were transferred to tap water in cups by means of a fine plastic screen scoop. Second and third and fourth instar larvae were removed from colony trays and transferred to the cups in a similar manner.

The materials were evaluated in the same manner as described elsewhere (Mulla *et al.* 1960, 1961). Twenty-five larvae of each instar were added to 100 ml. of tap water in 6-ounce paper cups. Acetone solutions of the toxicants were added to the cups after addition of the larvae.

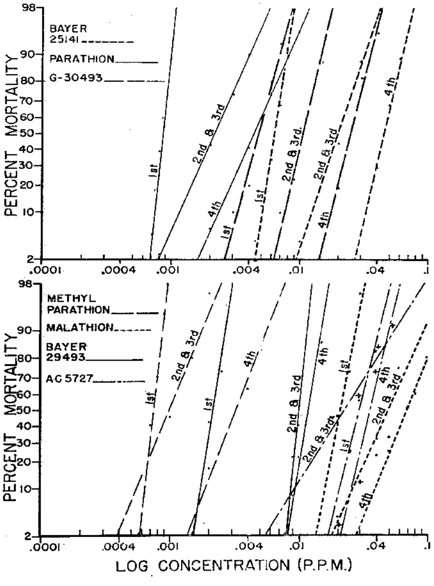

FIG. 1.—Dosage response lines of insecticides against various larval instars of *Culex p. quinquefasciatus* Say.

Larval mortality was recorded after an exposure period of 24 hours at a temperature of 78–80° F. Each cup was replicated twice and each material was run 2 or 3 times on different days.

The average 24-hour mortality of the larvae for each concentration of each material was plotted on a probit log paper. The points were visually joined by a straight-line. LC_{50} and LC_{90} levels were read off these dosage response lines.

RESULTS AND DISCUSSION. Eleven insecticides were evaluated against the various larval instars of *Culex p. quinquefasciatus* Say. Of these insecticides one was an organochlorine, two were carbamates and the others were organophosphate insecticides. The dosage response lines of these eleven materials against the first, second and third and fourth instar larvae are presented in Figures 1 and 2.

The first instar larvae were highly susceptible to parathion. The dosage response line of this material against the newly hatched larvae was steep (Fig. 1). The dosage response lines of parathion against the second and third, and fourth stage larvae were not as steep as the line against the first stage. The dosage response lines of G-30493 [*O,O*-dimethyl *S*(-3,4-dichlorophenylthio) methyl phosphorodithioate] against the various larval instars indicated similar slopes. Bayer 25141 [*O,O*-diethyl *O-p*-(methylsulfinylphenyl) phosphorothioate] showed high activity against the first instar larvae with a relatively steep dosage response line. Dosage response lines of Bayer 25141 against the second and third, and fourth instar larvae became flatter.

Methyl parathion, like parathion, manifested a high degree of activity against first instar larvae with a steep dosage response line (Fig. 1). The dosage response lines of methyl parathion against second and third, and fourth instar larvae were flatter and showed approximately the same slope. Bayer 29493 [*O,O*-dimethyl *O*-[4-(methylthio)-*m-tolyl*] phosphorothioate] proved highly effective against the first instar larvae but showed decreased activity against second and third, and fourth instar

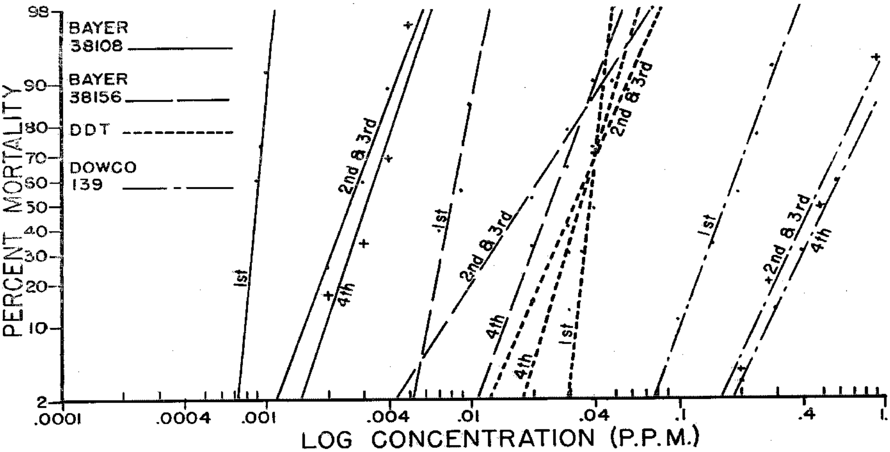

FIG. 2.—Dosage response lines of insecticides against various larval instars of *Culex p. quinquefasciatus* Say.

larvae. Essentially no difference in activity of Bayer 29493 was determined against second and third, and fourth instar larvae. The degree of activity of AC-5727 (*m*-isopropylphenyl *N*-methylcarbamate) was essentially the same against all larval instars. Malathion showed more activity against the first instar larvae but its spectrum of activity against the second and third, and fourth instar larvae was, for all practical purposes, the same.

Bayer 38108, like parathion and methyl parathion, showed higher activity against the newly hatched larvae (Fig. 2). How-

TABLE 1.—Susceptibility of various instar larvae of *Culex p. quinquefasciatus* Say

	LC_{50} (PPM)			LC_{90} (PPM)			Susceptibility Fold			
							LC_{50}[a]		LC_{90}[a]	
Material	First	Second and third	Fourth	First	Second and third	Fourth	First	Second and third	First	Second and third
Methyl parathion	.00074	.0010	.0032	.00086	.0017	.0055	4.3	3.2	6.4	3.2
Parathion	.00085	.0022	.0043	.00096	.0040	.0081	5.0	1.9	8.4	2.0
Bayer 38108	.00089	.0025	.0030	.0010	.0042	.0048	3.4	1.2	4.8	1.1
Bayer 29493	.0021	.010	.012	.0026	.0115	.0150	5.7	1.2	5.8	1.3
G-30493	.0047	.010	.030	.0068	.0145	.034	6.4	3.0	5.0	2.4
Bayer 25141	.006	.020	.045	.0075	.032	.062	7.5	2.2	8.3	1.9
Bayer 38156	.008	.019	.025	.010	.046	.042	3.1	1.3	4.2	0.9
Malathion	.020	.041	.069	.027	.11	.12	3.5	1.7	4.5	1.1
AC-5727	.030	.024	.036	.042	.058	.050	1.2	1.5	1.2	0.9
DDT	.038	.032	.035	.046	.060	.053	0.9	1.1	1.2	0.9
Dowco 139	.17	.48	.56	.29	.93	1.15	3.3	1.2	4.0	1.2

[a] Compared to fourth instar larvae.

ever, it manifested no marked differential activity against the second and third and fourth instar larvae. Similarly, Bayer 38156 (*O*-ethyl *S*-*p*-methylphenyl ethylphosphorodithioate) showed high activity against first instar larvae. Its activity against the second and third and fourth instar larvae, was lower but practically the same against these instars. DDT indicated a similar range of activity against all larval instars. Dowco 139 or Zectran (4-dimethylamino-3,5-xylol-*N*-methylcarbamate) showed higher activity against the newly hatched larvae, but the range of activity was lower and essentially the same against the other larval instars.

The degree of susceptibility to insecticides of first, second and third and fourth instar larvae may be easily visualized in terms of their LC_{50} and LC_{90} concentrations (Table 1). An analysis of the degree of susceptibility to toxicants of first, second and third instar larvae, as compared to the susceptibility of the fourth instar, indicates some interesting trends. It is obvious from Table 1 that the various larval instars do not show a uniform degree of susceptibility to all the toxicants used.

The first instar larvae were found more susceptible than the fourth instar larvae (both at LC_{50} and LC_{90} levels) to all the insecticides tested except AC-5727 and DDT. The first and fourth instar larvae were equally susceptible to AC-5727 and DDT.

The second and third instar larvae were found to be more susceptible (both at LC_{50} and LC_{90} levels) to methyl parathion, parathion, G-30493, and Bayer 25141 than the fourth instar larvae. The degree of susceptibility of second and third and fourth instar larvae to Bayer 38108, Bayer 29493, Bayer 38156, malathion, AC-5727, DDT and Dowco 139 (Zectran), within the range of experimental variation, was essentially the same.

Literature Cited

Isaak, L. W. 1956. The results of field testing some new mosquito larvicides. Proc. & Papers, Calif. Mosq. Contr. Assoc. Ann. Conf. 24:59–60.

Lewallen, L. L. 1958. Larviciding tests against mosquitoes in irrigated pastures of the San Joaquin Valley, California. Proc. & Papers, Calif. Mosq. Contr. Assoc. Ann. Conf. 26:88–90.

Mulla, M. S. 1961. Mosquito control in olive vats. Mosq. News 21(1):39–43.

Mulla, M. S., Axelrod, H., and Isaak, L. W. 1961. Effectiveness of new insecticides against mosquito larvae. Mosq. News 21(3):216–224.

Mulla, M. S., Isaak, L. W., and Axelrod, H. 1960. Laboratory and field evaluation of new insecticides against mosquito larvae. Mosq. News 20(3):256–61.

The Eighth Annual Conference of the **ILLINOIS MOSQUITO CONTROL ASSOCIATION** will be held on March 30–31, 1962, at the Dixie Governor Motel, East Hazelcrest, Illinois. The program will include scientific papers, descriptions of adequate mosquito abatement techniques, demonstration of equipment and other features. The location is readily accessible to all individuals who travel by automobile from Indiana, Michigan, Wisconsin, and Ohio. Details concerning the meeting can be obtained from Harvey J. Dominick, Secretary-Treasurer, Illinois Dept. of Public Health, Div. of Sanitary Engineering, Springfield, Illinois or from Dr. Robert Hedeen, Manager, South Cook County Mosquito Abatement District, P. O. Box 30, Harvey, Illinois.