EFFECTS OF LARVAL ENVIRONMENTS ON ADULT RESISTANCE IN *ANOPHELES ALBIMANUS* WIEDEMANN[1]

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The resistance to dieldrin in the mosquito *Anopheles albimanus* Wiedemann is under genetic control (Rozeboom and Johnson, 1961, Davidson, 1963, and Gilotra, 1965); however, it is possible that the expression of this resistance may be modified by the environment. The purpose of this study was to determine whether certain factors in the larval environment, especially overcrowding, low and high temperatures, and a deficient diet, could influence the response of the adult mosquito to dieldrin.

MATERIALS AND METHODS. The mosquito used was the dieldrin resistant strain of *A. albimanus* from El Salvador. The eggs were removed from the colony cage within 12 hours of oviposition. They were allowed to hatch and first stage larvae were distributed in enamel pans 11½ x 7½ x 2 inches in size, containing about 1½ liters of dechlorinated tap water. These larvae were fed a powdered Purina laboratory chow supplemented with Fleischmann's yeast. The adults were exposed for 2 hours to dieldrin-impregnated papers in the WHO test cylinders. The test papers were impregnated with 0.2, 0.4, 0.8, 1.6, and 4.0 percent dieldrin. Following exposure, the mosquitoes were transferred to recovery cages made from ½ pint cardboard cylinders. They were maintained at about 80 percent humidity, and were fed 10 or 15 percent sugar solution. Mortality was determined after 24 hours.

Fat, which here refers to ether-soluble substances, was measured as follows. Three random samples of 1, 1, and 5 specimens were taken from a group of mosquitoes just before they were exposed to dieldrin test papers. Each sample was immediately frozen at —18° C., completely dried, weighed by means of a Roller Smith balance, subjected to two 6- to 8-hour periods of ether extractions in a Soxhlet apparatus, redried and reweighed. The difference in the two weights gave the amount of fat or ether-soluble substances. The average of the 3 samples was taken to calculate the percent of fat in the dry weight of a mosquito.

EXPERIMENTAL RESULTS. *Effect of larval crowding on dieldrin resistance in adult* A. albimanus. First stage larvae were distributed among the pans in groups of 100, 200, 400, 800, and 1,000 and reared to adults. The adults were separated according to sex prior to testing for susceptibility to dieldrin. In the first series of tests, the adults were 2 to 5 days old and were maintained on 10 to 15 percent sugar solution. Larval density had no effect on mortalities of adults exposed to the lower dieldrin concentrations. Mortalities ranged from 8.2 to 33.1 percent after exposure to 0.4 and 0.8 percent dieldrin concentrations, and there was no difference in the response of males and females. However, as shown in Table 1, the higher dosage of 4.0 percent dieldrin killed more males than females, and there was also an apparent effect of crowding. Pans with 100 or 200 larvae produced heavier adults (Table 1). These adults also contained slightly more fat and were

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less susceptible to dieldrin than were those from the 800 larval-density pans.

In the second series of tests with dieldrin, adults were ½ to 1½ days old and were unfed; sugar solution was withheld from them in order to avoid the building up of new fat reserves in the adult stage. Comparison of Tables 1 and 2 will show that the unfed adults were more susceptible than were those given sugar solution, with the exception of males reared from the 3 high density pans. Again there was a trend towards greater kill of the females from the high density pans, with the highest mortality in females reared from the 800 larval-density pans.

The decrease in kills of the adults reared from pans which had been seeded with 1,000 larvae may have resulted from early death of many of the crowded larvae, so that these adults may have been reared under less crowded larval conditions. The sugar-fed adults reared from these pans actually were heavier and contained more fat than did the adults from the pans with 800 larvae (Table 1). However, the data in Table 1 do not show a correlation between fat content and mortality.

TABLE 1.—Effect of larval densities on mortality of adult *A. albimanus* exposed for 2 hours to 4.0% dieldrin concentration.

Sex of adults	Larval Conc. No. larvae per pan	No. replicates	Mortality at 4.0% dieldrin [1]			Dry weight of a mosq. in mg [2]		% ether sol. lipids per dry weight of a mosquito	
			No. spec.	$\bar{x}$ % kill	S.E. $\bar{x}$ %	$\bar{x}$	S.E.	$\bar{x}$	S.E.
♀ ♀	100	3	74	31.4	8.0	0.465	0.036	20.8	1.6
	200	7	142	45.2	9.0	0.489	0.036	24.4	3.2
	400	3	75	54.6	3.7	0.342	0.017	18.8	1.6
	800	5	116	62.6	6.8	0.288	0.015	13.7	1.2
	1000	4	101	60.5	10.2	0.296	0.040	22.0	4.7
♂ ♂	100	3	75	42.6	7.4	0.305	0.034	22.1	3.1
	200	6	128	67.2	8.1	0.299	0.047	22.6	2.9
	400	3	72	77.8	4.0	0.227	0.004	20.9	1.1
	800	5	107	75.9	5.2	0.236	0.014	16.4	1.2
	1000	3	74	64.7	12.5	0.206	0.014	21.4	2.2

[1] Exposed to dieldrin paper for 2 hours and mortality counted after 24-hour recovery period.
[2] Each average is based on 3 to 7 replicates and each replicate consists of 3 samples.

TABLE 2.—Effect of larval densities on 24-hour mortalities of unfed adult *A. albimanus* after 2-hour contact with 4.0 percent dieldrin concentration.

Sex of adults	Larval concentration No. larvae per pan	No. repl.	No. specimens	% kill	
				$\bar{x}$	S.E.
♀ ♀	100	7	162	68.0	5.2
	200	7	169	73.9	5.6
	400	5	99	79.6	3.4
	800	2	27	84.6	7.7
	1000	8	163	70.0	3.4
♂ ♂	100	6	153	66.0	3.8
	200	4	84	85.4	3.0
	400	4	67	74.6	4.6
	800	2	27	75.3	1.6
	1000	8	160	61.9	2.9

TABLE 3.—Effect of breeding water temperature on 24-hour mortality of adult *A. albimanus* following 2-hour exposure to 4.0 percent dieldrin paper.

Sex of adults	Breeding water temperature in centigrades	Adults unfed, 1/ to 1½ days				Adults sugar fed, 2 to 5 days old				% kill—Difference between unfed and sugar-fed
		No. spec.	Repls.	% kill $\bar{x}$	% kill S.E.	No. spec.	Repls.	% kill $\bar{x}$	% kill S.E.	
♀♀	21	124	6	65.6	4.1	76	3	44.4	2.0	21.2[1]
	25	157	6	72.4	4.8	160	7	57.7	9.5	14.7
	34	131	6	69.2	6.7	88	4	46.8	7.6	22.4[1]
♂♂	21	95	6	72.1	4.8	72	3	53.6	5.9	18.5[1]
	25	155	6	70.5	4.8	149	7	59.5	9.3	11.0
	34	101	5	67.3	3.6	90	4	50.5	9.6	10.8

[1] Significant at 0.05 level.

Effect of breeding water temperature on dieldrin resistance in adult A. albimanus. In this experiment, the numbers of larvae were kept constant, from 200 to 250 per pan, but the rearing temperatures were 21, 25, and 34° C. The adults were allowed to emerge at room temparature. Sugar-fed and unfed males and females were exposed for 2 hours to 4.0 percent dieldrin. The results, given in Table 3, show that larval rearing temperatures were not correlated with differences in kills following exposure to the 4.0 percent dieldrin concentration. However, as in the previous experiment, the unfed adults showed consistently higher kill than the sugar fed adults.

Effect of deficient diet on dieldrin resistance in adult A. albimanus. Poorly nourished larvae should give rise to smaller adults with presumably smaller fat reserves. Larvae were reared at room temperature, at a density of 200 per pan. When they reached fourth stage, all additional food was withheld. Although few adults emerged from these pans, it was possible to conduct one test. These adults were fed sugar solution and were 2 to 7 days old at the time they were exposed for 2 hours to 4.0 percent dieldrin. Mortality rates for 23 females and 25 males were 62.2 and 82.6 percent respectively. The adults reared from well fed larvae in the 200 larval density pans showed lower kills, *i.e.,* 45.2 and 67.2 percent for females and males respectively (Table 1). However, these differences do not appear to be significant.

DISCUSSION. Susceptibility of insects to insecticides may be affected by body weight and certain environmental factors. Way (1954) reported that in the tomato moth, *Diataraxia oleracea* (L.), there was an 11- to 12-fold increase in DDT resistance with a 2-fold increase in body weight. Direct correlation of lipid contents and insecticide resistance has been reported for cockroaches (Munson and Gottlieb, 1953), house flies (Weismann, 1955), and *Anopheles* mosquitoes (Neri, Ascher, and Mosna, 1958, 1962). Both positive and negative correlation between pre- or post-treatment temperatures and resistance of various insects to insecticides has been observed by several authors. Seasonal fluctuations in susceptibility have been reported; for example, *A. maculipennis* is said to be more tolerant to DDT at the beginning of the hibernation period (Zulueta, 1957).

Problems in malaria eradication arise when adult *Anopheles* become more tolerant to residual insecticides. Because the larvae breed in various kinds of water, where they may be subjected to considerable differences in environmental conditions, it was thought to be of interest to determine whether these could affect the response of the adults to insecticides. In the present experiments, there was some correlation between larval crowding and adult mortality. The larger and heavier adults reared from the less crowded larvae were more tolerant to dieldrin; however, this did not appear to be correlated with greater fat content. Furthermore, feeding upon sugar increased the tolerance of the adults to dieldrin. Thus it would appear that in nature, the larval environment would have little, if any, effect on the efficacy of residual household insecticides in killing adult *Anopheles.*

SUMMARY. Larvae of *Anopheles albimanus* were reared under varying conditions of crowding, temperature, and nutrition. Adults reared from these larvae were tested for resistance to dieldrin. Optimum breeding conditions produced larger and heavier adults which were somewhat more tolerant to dieldrin; however, there was no correlation between this tolerance and fat content. Sugar-fed adults were more resistant to dieldrin than were unfed adults.

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