RESPONSES OF DIELDRIN-RESISTANT LARVAE OF *ANOPHELES ALBIMANUS* WIEDEMANN TO DISCRIMINATING DOSAGES OF DIELDRIN [1]

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In their studies of dieldrin resistance in *A. albimanus,* Davidson (1963) and Gilotra (1965b) have established socalled discriminating dosages, but these are not absolute, and therefore do not assure complete separation of different genotypes as expected on the basis of simple Mendelian inheritance. The author has observed that frequently a dosage of 0.1 p.p.m. of dieldrin is not completely lethal to *A. albimanus* larvae which are heterozygous for dieldrin resistance, and that the proportion surviving this dosage varied from progeny to progeny. Somewhat higher dosages of 0.15 and 0.2 p.p.m. of dieldrin killed a part of the homozygous resistant larvae. The purpose of this investigation was to attempt to establish a test for dieldrin resistance that would separate with more certainty the heterozygous from the homozygous dieldrin-resistant *A. albimanus* larvae for possible use under the field conditions. To do so it was necessary to determine what proportions of *A. albimanus* heterozygotes are able to survive the intermediate dosage of dieldrin.

MATERIALS AND METHODS. The dieldrin resistant strain had been subjected to a number of generations of selection in the adult stage for dieldrin resistance (Rozeboom, 1963). The dieldrin susceptible strain was a subcolony of the Gorgas Memorial Laboratory strain (Hobbs, 1962). Crosses were made between predetermined genotypes by sib-mating. Inseminated females were then randomly isolated, and from them individual larval progenies were obtained, which were tested for dieldrin resistance. The method of sib-mating and testing individual progenies has been described elsewhere (Gilotra, 1965b). By this procedure it was possible to determine the pedigree of the test larvae and so avoid errors caused by heterozygosity in a presumed SS [3] or RR parent. Late third or early fourth stage larvae were exposed to dieldrin by the method recommended by the World Health Organization. Larvae were exposed to acetone suspensions of dieldrin. Tests were performed in 250 ml of water held in 400 ml beakers; no more than 30 larvae were placed in one breaker.

EXPERIMENTAL RESULTS. The experiments were started with RS sisters which were crossed with RR brothers or *vice versa.* These were taken to be RS because their larval siblings showed no kill at 0.01 p.p.m. of dieldrin and about 80 percent kill at 0.1 p.p.m. of dieldrin. Larval siblings of RR adults (used for cross breeding) were not killed at these dosages. Earlier tests had shown that exposure for 24 hours to 0.01 p.p.m. of dieldrin killed SS larvae only; 0.1 p.p.m. killed all SS and most of RS larvae, but neither of these dosages killed RR larvae.

Three backcrosses between the siblings of the RS and the RR were made. Since inheritance of dieldrin resistance in *A. albimanus* larvae is monofactorial, the individual progeny of any isolated female of

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[3] The homozygous resistant and susceptible genotypes will be referred to by the symbols RR and SS respectively, and the heterozygous genotype by RS.

this cross should be ½ RS and ½ RR. As 0.1 p.p.m. was not completely lethal to RS larvae, when the above progenies are exposed to this dosage, all RR and part of RS should survive, giving a less than 50 percent mortality. The results of the larvicidal tests of each individual progeny are presented in Table 1. Of 22 individual progenies, 20 showed less than 50 percent mortality, and in the other 2 progenies the larval kills were 51 percent and 56 percent. Thus it would appear that in the 20 progenies in which the kill was less than 50 percent, part of the RS larvae also survived.

The progenies of females E-2, E-4, and E-6 from Cross no. 2, and E-7 and F-7 from Cross no. 3 were tested with the 0.1 p.p.m. dosage, and the proportion of RS genotype in the survivors was calculated as follows. From the progeny of the E-7, 303 larvae were exposed to the dosage of 0.1 p.p.m. dieldrin; 114 or 37.6 percent were killed (Table 1), so that 189 or 62.4 percent survived. Now approximately half of the original 303 larvae, or 152, should have been RR. Therefore, of the 189 survivors, 37 (189–152) are assumed to be RS. The proportion of RS in the surviving population from E-7, therefore, theoretically is 37:189 or 20 percent. Similarly the proportions of RS larvae in the surviving population from E-2, E-4, E-6 and F-7 theoretically are 18, 26, 31, and 6 percent, respectively.

The larval progenies that survived 24-hour exposure to 0.1 p.p.m. of dieldrin were reared to adults, and these were placed in breeding cages. The progenies from the females E-4 and E-6 were small, and so the adults from them were combined, thus making 4 populations instead of 5 from the progenies of 5 females. These 4 independent populations were designated 'A'—from female E-2, 'B'—from females E-4 and E-6, 'C'—from female E-7, and 'D'—from female F-7. From these populations, 4, 4, 21, and 11 females respectively were isolated at random, and these in turn produced individual larval progenies. These progenies were tested at the 0.01 and 0.1 p.p.m. dosages. A 25 percent mortality at 0.01 p.p.m. and about 65 percent mortality at 0.1 p.p.m. of dieldrin would indicate a mating between RS x RS; no mortality at 0.01 p.p.m., and about 40 percent mortality at 0.1 p.p.m. of dieldrin should reveal that the larvae had been produced by a RS x RR cross; and the mating was assumed to have been RR x RR when neither of the dosages caused larval mortality. Results are presented in Tables 2 and 3.

Disregarding the possibility of multiple inseminations in the breeding cages, each individual progeny tested represented the genotype of two individuals. As an example, from the population C (progeny of female E-7) 21 individual progenies, produced by 42 mating partners, were tested. Of these individuals, 11 appear to be RS (Table 3). The proportion of heterozygous individuals among the larval siblings (also of female E-7) of these 42 adults was shown above to be 37/189 or 20 percent. Of the 42 adults, therefore, 20 percent or 8 individuals should theoretically also be heterozygous. The difference of 3 is not unreasonable (Table 4). Differences between the proportions of RS individuals as calculated from mortalities of larvae which were siblings of the parent females, and of larvae that are progenies of these females, were not significant (Table 4).

The individual progenies which showed no kill at 0.1 p.p.m. dieldrin were considered to be RR. These, when bred to adults, and then crossed with other adults, larval siblings of which also had shown no kill at the dosage 0.1 p.p.m., should produce larvae which are completely RR and, therefore, should not give any kill at the dosage of 0.1 p.p.m. in subsequent generations.

Thirty larvae were taken at random from each of the progenies of females no. 1, 2, and 3 from population A and of female No. 3 from population B. These progenies showed no kill at 24 hours of exposure to 0.1 p.p.m. dieldrin (Table 2). These larvae were pooled to produce one colony, RR-I, and similarly a second colony, RR-II, was also produced from the progenies of females 1, 2, 5 and 6 of

TABLE 1.—Larval mortalities after 24 hours of exposure to 0.1 ppm dieldrin in backcrosses to the RR P_1 population.

		RS x RR				RR x RS					
Cross No.	♀ No.	F-1	F-2	H-1	H-8	D-4	D-6	D-7	K-4	K-5	K-7
1	No. larvae tested	161	179	140	82	57	34	25	20	115	102
	Percent mort. [1]	34.2	46.4	47.9	51.2	35.1	26.5	28.0	20.0	55.6	37.2
	p [2]	<.001	.759	.612	.841	.025	.006	.027	.007	.227	.012
	♀ No.					E-2	E-3	E-4	E-6		
2	No. larvae tested					203	38	35	23		
	Percent mort. [1]					39.4	29.0	34.3	30.4		
	p [2]					.003	.009	.063	.061		
	♀ No.	E-1	E-2	E-7	E-8	F-1	F-6	F-7	F-8		
3	No. larvae tested	206	67	303	266	362	22	589	265		
	Percent mort. [1]	38.8	26.9	37.6	45.1	36.5	40.9	46.5	42.3		
	p [2]	<.001	<.001	<.001	.112	<.001	.397	.088	.011		

[1] Expected mortality for complete kill of RS larvae at this dosage is 50 percent.
[2] p is calculated on the basis of chi square test (Dixon and Massey, 1957), with 50 percent as the theoretical mortality.

TABLE 2.—Mortalities of larval progenies from the females of populations 'A' and 'B'; estimation of genotypes in parental crosses.

Population 'A'						Population 'B'					
	0.01 ppm		0.1 ppm				0.01 ppm		0.1 ppm		
♀ No.	No. larvae	% dead	No. larvae	% dead	Parental genotypes [1]	♀ No.	No. larvae	% dead	No. larvae	% dead	Parental genotypes [1]
1	17	0	84	0	RR x RR	1	22	27.3	26	73.1	RS x RS
2	14	0	120	0	RR x RR	2	..	..	24	0	RR x RR
3	17	0	187	0	RR x RR	3	18	0	129	0	RR x RR
4	286	23.8	265	56.0	RS x RS	4	..	..	28	25.0	RR x RR
Progenies tested=4 or 8 individuals						Progenies tested=4 or 8 individuals					
Heterozygous individuals as estimated from the dosages 0.01 and 0.1 ppm = 2						Heterozygous individuals as estimated from the dosages 0.01 and 0.1 ppm = 3					

[1] Genotypes shown in the column represent either the male or the female.

TABLE 3.—Mortalities of larval progenies from females of the populations 'C' and 'D'; estimation of genotypes in parental crosses.

Population 'C'						Population 'D'					
	0.01 ppm		0.1 ppm				0.01 ppm		0.1 ppm		
♀ No.	No. larvae	% dead	No. larvae	% dead	Parental genotypes [1]	♀ No.	No. larvae	% dead	No. larvae	% dead	Parental genotypes [1]
1	..	..	154	0	RR x RR	1	..	..	71	0	RR x RR
2	20	0	136	1.5	RR x RR	2	..	..	57	0	RR x RR
3	28	0	129	38.0	RR x RS	3	16	0	95	0	RR x RR
4	..	..	3	0	RR x RR	4	..	..	80	0	RR x RR
5	..	..	81	1.8	RR x RR	5	..	..	11	0	RR x RR
6	..	..	79	0	RR x RR	6	20	0	167	0	RR x RR
7	33	3.0	95	38.7	RR x RS	7	..	..	42	59.5	RR x RS
8	19	0	196	1.0	RR x RR	8	..	..	95	0	RR x RR
9	22	0	147	49.0	RR x RS	9	20	0	158	0	RR x RR
10	18	0	122	26.2	RR x RS	10	13	0	95	30.9	RR x RS
11	..	..	76	42.1	RR x RS	11	14	0	166	0	RR x RR
12	..	..	116	0	RR x RR						
13	25	0	175	37.1	RR x RS						
14	38	29.0	64	71.9	RS x RS						
15	19	0	136	33.8	RR x RS						
16	..	..	27	0	RR x RR						
17	..	..	74	0	RR x RR						
18	..	..	24	4.2	RR x RR						
19	..	..	157	0	RR x RR						
20	18	0	158	30.4	RR x RS						
21	20	0	28	42.7	RR x RS						
Progenies tested=21 or 42 individuals						Progenies tested=11 or 22 individuals					
Heterozygous individuals as estimated from the dosages 0.01 and 0.1 ppm = 11						Heterozygous individuals as estimated from the dosages 0.01 and 0.1 ppm = 2					

[1] Genotypes shown in the column represent either the male or the female.

TABLE 4.—Number of heterozygous individuals that survived the dosage 0.1 ppm; comparison of the estimates of heterozygous individuals by testing the progenies of isolated females and by testing the siblings of isolated females.

Population	No. individuals 2 indivs.=1 progeny	No. heterozygous individuals: By testing progenies of isolated females	No. heterozygous individuals: By testing siblings of isolated females	p
A	8	2	1	.294
B	8	3	2	.403
C	42	11	8	.292
D	22	2	1	.317

population C. To test for the presence of heterozygotes, 500 larvae in 26 replicates from the colony RR-I, and 1,043 larvae in 51 replicates from the colony RR-II, were exposed to 0.1 p.p.m. dieldrin. The former showed no mortality, and the latter had 0.02 percent kill. Therefore, these larvae evidently were all homozygous resistant (RR).

The results of this experiment confirmed the segregation of genotypes according to monofactorial inheritance, that part of RS larvae survive 0.1 p.p.m. of dieldrin, the number of which can be satisfactorily determined by testing individual progenies of isolated females.

DISCUSSION. For determination of relative frequencies of genotypes for insecticide resistance in a mixed population, establishment of discriminating dosages is highly desirable. Unfortunately, these dosages may not be completely effective. In the present study, an effort was made to be very certain of the genotypes of the test larvae by cross-checking mortalities of larvae produced by individual females with the mortalities of the larval siblings of the parent females. It was not possible to avoid an error of about 20 percent with RS larvae subjected for 24 hours to 0.1 p.p.m. dieldrin. If larvae from natural breeding places are equally susceptible to dieldrin (i.e., if the superior environmental factors in natural waters do not enhance resistance), one would experience a similar large error with this dosage. However, the SS component of a natural population should be completely susceptible to a 24 hour exposure with 0.01 p.p.m. dieldrin. It should be possible, therefore, to determine the relative frequencies of the heterozygous and homozygous resistant larvae by use of the Hardy-Weinberg formula.

The results of these tests are interpreted as support for the monofactorial hypothesis of inheritance of dieldrin resistance in *A. albimanus,* with intermediate resistance being afforded by a single gene. However, the survival of some of the heterozygous larvae at the dosage 0.1 p.p.m. of dieldrin indicates that factors in addition to the gene for resistance may be involved. In the present work, these did not appear to be in the larval environment, or to be associated with inherent biological characteristics (Gilotra, 1965a). There is, therefore, some support for the suggestion by Rozeboom (1963) that in addition to the major gene for dieldrin resistance, other ancillary genes, each with small effect, may also be making a contribution to resistance.

SUMMARY. 1. Tests for dieldrin resistance in *Anopheles albimanus* Wiedemann were made of individual progenies obtained by a method described as sib-mating and testing of individual progenies. Exposure for 24 hours to 0.01 p.p.m. dieldrin killed all SS larvae and the dosage of 0.1 p.p.m. killed all the SS and most of RS larvae, but neither of these dosages killed RR larvae.

2. Adults of known genotypes were obtained by testing a part of the larvae from an individual progeny with the discriminating dosages and rearing the other part to the adult stage. These adults were cross-bred to produce individual progenies for the insecticide tests.

3. The proportion of heterozygous larvae (RS) that survived the 0.1 p.p.m. dosage was determined by observing mortalities in (a) individual progenies produced by parents of known genotypes, and in (b) the larvae produced in turn by the survivors of the above tests.

4. It is suggested that an estimation of gene frequencies of natural populations of *A. albimanus* larvae can be determined by the mortality caused by 24 hours of exposure to 0.01 p.p.m. dieldrin.

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