INHERITANCE OF MALATHION RESISTANCE IN *AEDES TAENIORHYNCHUS* (WIEDEMANN) [1]

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ABSTRACT. Malathion resistance in *Aedes taeniorhynchus* (Wiedemann) was studied to determine its mode of inheritance. Dosage-mortality curves were calculated for a resistant (R) strain, a susceptible (S) strain, and reciprocal hybrids [F_1 (R x S) and F_1 (S x R)] from data obtained by topical application of malathion. These data were used to analyze the progeny of genetic crosses. The R males and R females were found to be 31- and 46-fold resistant at the LD-90, respectively. The results of genetic crosses suggested that malathion resistance was inherited, primarily as a single dominant, autosomal gene.

Malathion resistance in populations of *Aedes taeniorhynchus* (Wiedemann) in Florida have become a problem of increasing concern in recent years. The first reports of resistance were made by Glancey et al. (1966), Gahan et al. (1966), Rathburn and Boike (1967), and Lofgren et al. (1967). These reports indicated that F_1 larvae and/or adults of field-collected *A. taeniorhynchus* were resistant (>4×) to malathion in 4 counties on the west coast and in 5 counties on the east coast of Florida. Furthermore, the results of field applications by aircraft in 1966 showed a 10-fold increase in the amount of malathion needed for 90 percent reduction of adults of *A. taeniorhynchus* when compared to results obtained in 1959 (Glancey et al. 1966). Additional reports of malathion resistance in this species have since been reported by Boike and Rathburn (1968, 1969, 1972, and 1974) and Mount et al. (1971 and 1974). A review of these reports indicated that resistance is present along most of the east coast, the lower half of the west coast, and in the Florida Keys (Monroe County). No cross resistance to other organophosphate insecticides was observed in these studies.

Mount et al. (1974) reported the selection of a strain of *A. taeniorhynchus* that was highly resistant to malathion, but showed no cross resistance to other adulticides. The origin of this strain was a native population that was heterogeneous in susceptibility to malathion.

The objective of this present study was to determine the mode of inheritance of malathion resistance in *A. taeniorhynchus*. Although some generalizations can be made based on surveys for the detection of resistance, we believe that the mode of inheritance merits priority in assessing the

[1] This paper reflects the results of research only. Mention of a pesticide or a commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the USDA.

impact of resistance on trends in control of this important species of mosquito.

METHODS AND MATERIALS. The F_1 generation of the malathion-resistant (R) strain described by Mount et al. (1974) and a malathion-susceptible (S) strain colonized by Davis (1958) were used. The R strain originated from a cross of males of a field population (from Duval County, Florida) heterogeneous for malathion resistance to females of the S strain. This cross was necessary because the field-collected females would not mate readily in laboratory cages, a phenomenon characteristic of many species of mosquitoes. The F_1 and F_2 generations were not selected with malathion, but mosquitoes of these 2 generations were assayed for susceptibility to malathion by exposure to sprays containing various concentrations of the chemical in a wind tunnel, described by Mount and Pierce (1974). Mosquitoes of subsequent generations were treated as virgin adults with doses of malathion that caused 50 to 75% mortality. The S strain was established at our laboratory in 1957 and was subsequently maintained without any intentional exposure to pesticides. Rearing was conducted according to the procedures similar to those of Davis (1958) and Haeger (1958), but hog supplement was substituted for rabbit pellets.

The genetic analysis of resistance followed the basic guidelines presented by Tsukamoto (1963). Dosage-mortality regression lines were calculated by probit analysis (Finney, 1947) for the R and S strains and their reciprocal hybrids. The data for these calculations were obtained by topical application of malathion at doses ranging from 2.5 to 500 ng per adult with a calibrated repeatable dispenser (Microliter Syringe, Hamilton Co., Whittier, Calif.) that delivered an 0.5 μl volume of an acetone solution of malathion. Forty to 60 adults were treated with each dose, and a sufficient number of doses were used to fully define the nature of the dosage-mortality relationship. These data were then used as a basis for making appropriate crosses to determine the type of genic control of the resistance. Unfortunately, there are no useful genetic markers in *A. taeniorhynchus* that allowed a factorial analysis of variance of individual chromosome effect. Thus, we studied the presence of major gene action and ignored any ancillary genes that cause minor effects. Progeny from the backcrosses and F_2 crosses were assayed by using a series of doses of malathion. The dosage-mortality data for the defined genotypes, i.e., R, S, or hybrid, were used to calculate theoretical curves for the backcrosses and F_2 progeny, and these expected mortalities were compared with observed values by χ^2 analysis by two methods. The first χ^2 analysis was calculated for the data over the whole range of the assay doses, and the second was done with only those doses on the plateaus of the theoretical curves. In

Table 1. Probit analyses of dosage-mortality data obtained by topical application of malathion to resistant (R), susceptible (S), and reciprocal hybrids of *Aedes taeniorhynchus*.

Genotype	Slope	LD-50 (ng)	Fiducial limits (95%) Upper	Fiducial limits (95%) Lower	LD-90 (ng)	Fiducial limits (95%) Upper	Fiducial limits (95%) Lower
S ♀	1.96	5.7	7.6	3.9	10.9	29	8
R ♀	2.45	295	373	253	498	1025	388
F_1 ♀ (R ♀ x S ♂)	2.61	170	183	158	277	325	248
F_1 ♀ (S ♀ x R ♂)	2.41	161	176	148	276	334	243
S ♂	2.54	4.3	5.4	3.0	7.1	12.3	5.6
R ♂	3.08	145	167	117	220	290	190
F_1 ♂ (R ♀ x S ♂)	2.05	108	119	96	201	252	174
F_1 ♂ (S ♀ x R ♂)	1.66	89	101	76	192	250	163

each case, the χ^2 value was partitioned for deviation from expected and for heterogeneity between replicates, i.e., doses, as described by Mather (1957).

RESULTS. Probit analyses of the dosage-mortality assays are presented as regression lines in Figures 1 and 2 and summarized in Table 1. A comparison of the estimate for the LD-90 for the R and S trains showed the R males and R females were 31- and 46-fold resistant to malathion, respectively. The hybrids, F_1(R x S) and F_1(S x R), were resistant, thus indicating that the resistance was due to dominant gene action. A slight divergence was noted in the slopes of the regression lines for the hybrid males, but whether this was caused by a maternal or paternal effect cannot be determined accurately, although the smaller slope most probably resulted from a maternal effect since the slope of the line for the S female was less than the other genotypes. The response of the reciprocal F_1 hybrid females to malathion was similar as was expected.

Theoretical mortality curves for backcrosses to the S strain and F_2 crosses of the reciprocal hybrids are also shown in Figures 1 and 2. These curves represent the expected mortality values at various doses for a single, dominant, autosomal gene and contain a plateau that ranges from 25 to 75 ng per mosquito, allowing discrimination of the heterozygotes from the susceptible homozygote. When the

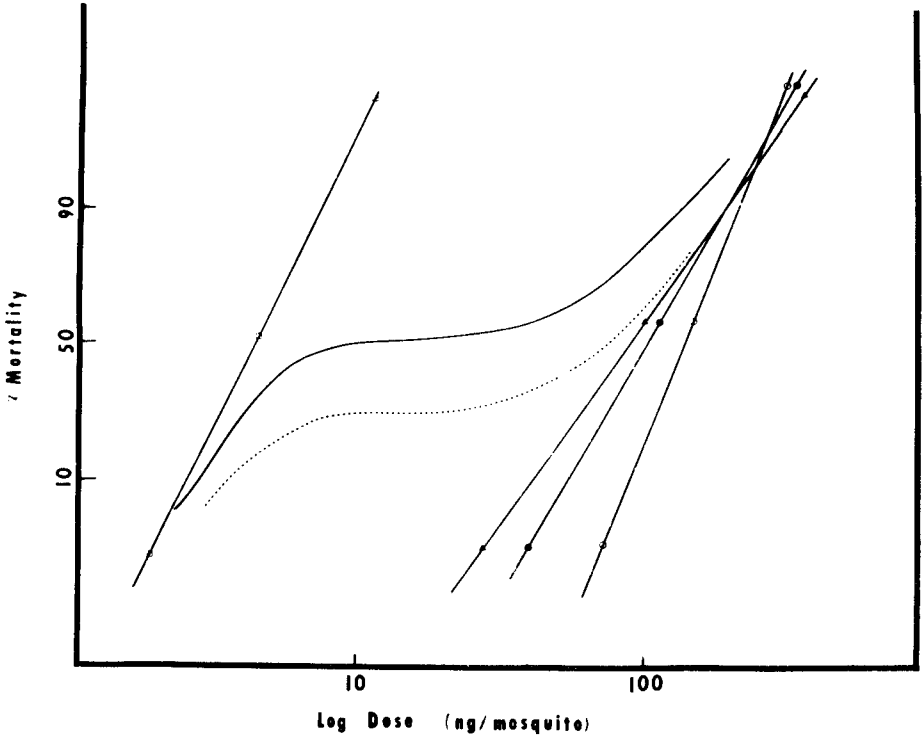

Fig. 1. Dosage-mortality regression lines for the R (○), S (□), and reciprocal hybrid (● and ▲) males treated topically with malathion. The expected dosage-mortality curves for F_2 (S ♀ x R ♂) (dotted line) and backcross (solid line) progeny are shown.

backcross data were anaylzed by χ^2, the observed kill agreed with the expected at the doses on the plateau, but the data did not fit the expected for all doses with some crosses (Table 2). In particular the progeny of the S ♀ x F_1 ♂ (S ♀ x R ♂) were more susceptible than expected from the theoretical model. The F_2 progeny were more susceptible than expected, especially at the higher doses, and a lack of fit to the theoretical was observed for both crosses. However, as in the case of the backcross data, there were no differences between observed and expected values at the discriminating doses.

DISCUSSION. Genetic studies can provide insight into the severity of a resistance problem because the mode of inheritance can be used to predict, to some extent, the effect of further selection of a field population that is heterogeneous for resistant genes. Even though continued selection will increase the frequency of the resistant type, the selection process requires time, the amount of which will depend on the number of genes affecting the resistance. If the resistance is polygenic, then the removal of selection pressure by switching to an alternate pesticide could in some cases be an effective means of avoiding a more severe resistance problem. Unfortunately, malathion resistance in *A. taeniorhynchus* is due to an autosomal, dominant gene, and sustained usage of malathion will select for a higher frequency of the resistance allele. There

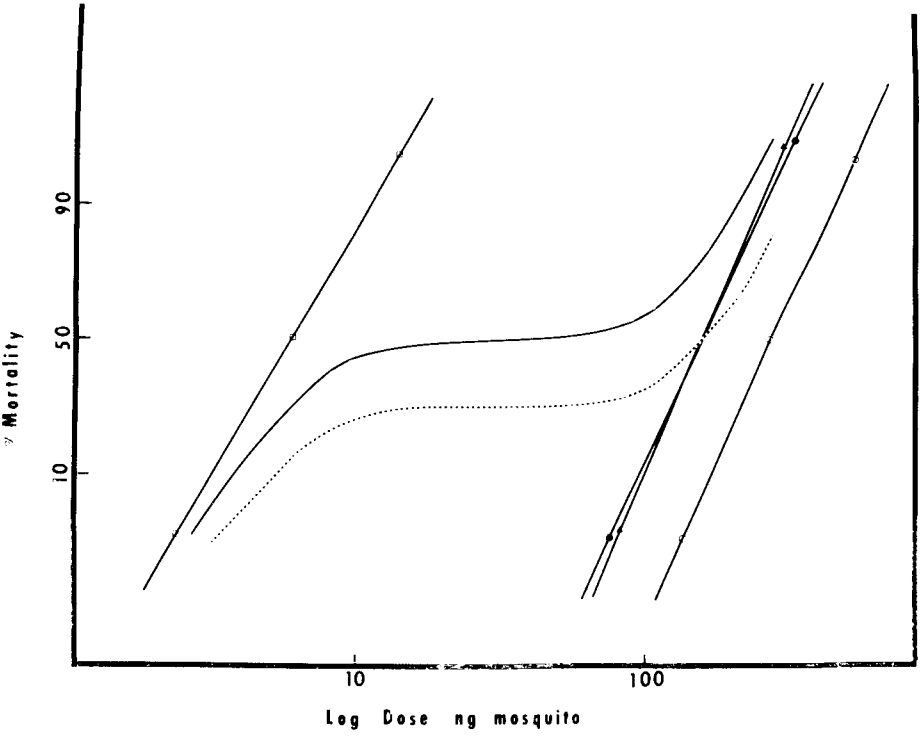

Fig. 2. Dosage-mortality regression lines for the R (○), S (□), and reciprocal hybrid (● and ▲) females treated topically with malathion. The expected dosage-mortality curves for F_2 (dotted line) and backcross (solid line) progeny are shown.

Table 2. χ^2 analyses of data on mortality of *Aedes taeniorhynchus* exposed to malathion (R=resistant, S=susceptible).

Cross	Observed and (expected) mortality (%) at doses (ng)								χ^2 Test for deviation	
	25	50	75	100	125	150	175	200	All doses	Discriminating doses
F_2(S♀ x R♂)										
Male	30(25)	50(34)	....	70(57)	77(69)	84(80)	92(87)	97(92)	12.04(P<.01)	1.24(P=.2-.3)
Female	33(25)	31(25)	22(26)	35(30)	27(36)	63(46)	62(55)	73(63)	9.63(P<.01)	0.54(P=.3-.5)
F_2(R♀ x S♂)										
Male	28(25)	30(25)	47(28)	66(52)	77(66)	92(77)	85(85)	95(91)	14.49(P<.01))	1.90(P=.1-.2)
Female	25(34)	25(30)	26(30)	30(23)	36(57)	46(40)	....	63(77)	5.81(P=.01-.02)	0.05(P=.8-.9)
S♀ x F_1♂ (R♀ x S♂)										
Male	45(50)	50(53)	60(60)	83(75)	87(83)	92(88)	93(93)	100(95)	0.64(P=.3-.5)	0.92(P=.3-.5)
Female	50(50)	51(50)	50(52)	52(55)	58(62)	63(70)	68(77)	95(84)	0.01(P=.9-.95)	0.41(P=.5-.7)
S♀ x F_1♂ (S♀ x R♂)										
Male	56(51)	57(57)	....	97(78)	....	96(91)	....	....	7.83(P<.01)	0.40(P=.5-.7)
Female	50(50)	49(50)	....	75(55)	....	90(70)	....	....	12.20(P<.01)	0.03(P=.8-.9)
F_1 ♀ (R♀ x S♂) x S ♂										
Male	53(50)	53(53)	72(60)	75(75)	90(83)	92(88)	98(93)	....	2.62(P=.1-.2)	0.13(P=.7-.8)
Female	55(50)	53(50)	73(50)	68(53)	55(60)	68(70)	98(80)	....	8.67(P<.01)	0.83(P=.3-.5)
F_1♀ (S♀ x R♂) x S♂										
Male	53(51)	59(57)	....	80(79)	....	92(91)	....	....	0.24(P=.5-.7)	0.10(P=.7-.8)
Female	44(50)	46(50)	....	55(55)	....	71(70)	....	92(84)	0.04(P=.8-.9)	1.61(P=.2-.3)

is little hope of disrupting such a simple inherited trait; however, a decrease in the resistance alleles could occur if the use of malathion was terminated. This would happen if the fitness of the resistant type was less than that of the susceptible type, which has often been the case in other resistance studies. A report of increased susceptibility by Boike and Rathburn (1969) suggested better survival for the susceptible type, although this observation could have been the result of interbreeding with neighboring unselected populations. Since the expression of resistance is also present in the hybrid, then the fixation of the resistance allele is improbable, because ineffective control would force abandoning malathion in spray operations. As an example, a frequency of 0.2 for the resistance allele corresponds to 64% kill, which is much less than is desirable for field control. The *A. taeniorhynchus* population from which Mount et al. (1974) selected a malathion-resistant strain had a frequency of 0.4 for the resistance allele which corresponds to only 40% kill at recommended doses of malathion.

As noted previously, malathion resistance was detected in many areas of coastal Florida as early as 1965, yet the use of this chemical continued in some areas. This resistance to malathion appears to have had a slower rate of development than has been observed in other species. The slow increase in frequency of the resistance allele probably stems from a low overall selection rate imposed by limited adulticiding in Florida. Ordinarily, adulticides for control of *A. taeniorhynchus* are applied in and around residential areas, and no larviciding is done with organophosphate insecticides. This practice of leaving breeding areas untreated could be an effective method of combating insecticide resistance; however, it must be strictly adhered to in the future to prolong or avoid the development of resistance to other adulticides in *A. taeniorhynchus*.

Our findings indicate that malathion resistance in *A. taeniorhynchus* is similar to malathion resistance in *Culex tarsalis* Coquillett as reported by several investigators. Malathion resistance in *C. tarsalis* was inherited as an autosomal dominant allele (Plapp et al. 1961), conferred no cross resistance to other organophosphate compounds (Darrow and Plapp 1960), was effectively negated by the addition of phosphate synergists to malathion (Plapp et al. 1963), and was due to a mutant carboxyesterase enzyme (Matsumura and Brown 1961; Bigley and Plapp 1962). This carboxyesterase enzyme was later characterized by Matsumura and Brown (1963).

Although we have not conducted a physiological study on the malathion resistance mechanism in *A. taeniorhynchus*, our data demonstrated the same type of specific inheritance which, apparently, does not confer cross-resistance to other types of organophosphorus insecticides. Moreover, malathion resistance in *A. taeniorhynchus* was effectively negated by some of the phosphate synergists used in Plapp et al. (1963) (unpublished data). This parallelism suggests that the resistance mechanism in *A. taeniorhynchus* involves an increased carboxyesterase activity which would be specific for malathion and similar insecticides.

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