

RESISTANCE OF *CULEX TRITAENIORHYNCHUS* GILES AND *CULEX QUINQUEFASCIATUS* SAY TO MALATHION ON OKINAWA WITH NOTES ON SUSCEPTIBILITY TO OTHER INSECTICIDES¹

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Insecticides have been used extensively on Okinawa since 1945 for the control of mosquitoes. DDT was utilized as the insecticide of choice from 1945 to 1959. Gentry (1957) reported resistance in *Culex quinquefasciatus* Say to DDT and other chlorinated hydrocarbon insecticides on Okinawa, and in 1959 its use as a mosquito larvicide and adulticiding fog was discontinued and replaced by malathion. Malathion was utilized for both larviciding and adult fogging until 1965 when, based on the results reported here, together with the results of field tests conducted in 1963 by Gahan, *et al* (1965) and in the summer of 1964 by Lofgren, *et al* (1966), malathion was replaced by fenthion as an adulticidal fog material (Pennington 1966). Malathion continues to be used for larviciding.

Sporadic insecticide resistance tests have been conducted on Okinawa, but it was not until the World Health Organization insecticide resistance testing method came into general use that routine procedures for testing were established on Okinawa. Sufficient numbers of reliable tests to establish base line data on malathion against larval mosquitoes were not conducted until 1962, four years following introduction of malathion as the standard mosquito control insecticide, and not until 1963 against adult mosquitoes.

¹Fenthion, naled and "Sumithion" (o, o-dimethyl o-4-nitro-m-tolyl phosphorothiolate). Mention of a proprietary product does not necessarily imply endorsement of this product by the U. S. Army.

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METHODS

The standard World Health Organization (1960 and 1963) methods for determining resistance of mosquito larvae and adults were used during all tests. Mosquito larvae were collected from the field in various locations throughout the southern half of Okinawa. One test consisted of four replicates of each concentration of insecticide. In some larval tests all four replicates were conducted simultaneously, and in others two replicates were conducted one day and two replicates the following day, but mosquitoes from the same collection or area of collection were always used to complete each test. A succession of concentrations was used which would allow log concentration probit mortality regression lines to be obtained and LC 50 and LC 90 levels to be established. Adult mosquitoes were reared from field collected larvae and fed on 10 percent sugar water solution. Adults at least 3 days old, and not more than 12 days old, were used to conduct all tests. Each test consisted of four replicates of each concentration. The LC 50 and LC 90 levels of both larval and adult mosquitoes were determined by the method described by Finney (1952) using the concentration-mortality probit analysis.

RESULTS AND DISCUSSION

Culex tritaeniorhynchus. The results of insecticide susceptibility tests with malathion against *Culex tritaeniorhynchus* larvae conducted from 1962 through the first one-half of 1967 are summarized in Table 1.

The results show that there was very little increase in the amount of insecticide required to kill 50 percent and 90 percent

TABLE 1.—Summary of insecticide susceptibility tests with malathion against *Culex tritaeniorhynchus* larvae, 1962-1967.

Year	ppm LC 50	Fold Increase	ppm LC 90	Fold Increase	Number of Tests Conducted
1962	.0089	Base line	.0202	Base line	6
1963	.0094	1.1	.0386	1.9	5
1964	.0575	6.5	.3680	18.2	11
1965	.2716	30.5	.7848	38.8	21
1966	.0737	8.3	.3622	17.9	5
1967 (Jan-Jun)	.4306	48.4	1.1539	57.1	11

of the mosquito test population in 1963 over the base line established in 1962. During 1964 there was a noticeable increase in resistance, and by 1965 the level of resistance had increased to over three times that which is considered indicative of resistance, i.e., an increase of 10 times over the original LC 50. During 1966 the results show an apparent decrease in resistance. However, evaluation of the individual tests showed that most of the tests were conducted using mosquitoes which were younger than those routinely used, which could account for the apparent drop in the level of resistance since the younger mosquitoes are more susceptible to insecticides. Tests conducted during the first half of 1967 show, rather than a decrease in resistance as in 1966, an increase of 18 and 19 fold in the LC 50 and LC 90 levels over that obtained during 1965. The 1967 results show a dramatic 48.4 fold increase at the LC 50 level over the base line established in 1962 and a 57.1 fold increase at the LC 90 level. The results of tests conducted with malathion against adult *Culex tritaeniorhynchus* are shown in Table 2.

The results show that by 1963, after five years of using malathion, resistance of a high order was already apparent in the adult mosquito population. In field tests conducted on Okinawa in 1963 with caged adult *Culex tritaeniorhynchus*, Gahan, *et al* (1965) showed that a malathion fog of 6 oz/gal (45 gr/l) produced only 75 percent mortality of the test specimens. Lofgren, *et al* (1966) in similar tests conducted in 1964 obtained an average of only 59 percent mortality of caged specimens with a 12 oz/gal (90 gr/l) concentration of malathion fog, and Pennington (1966) in tests conducted during 1965 obtained an average of only 42.9 percent with a 12 oz/gal (90 gr/l) concentration. An overall evaluation of the laboratory results of susceptibility tests against both adults and larvae coupled with the data obtained by field tests shows *Culex tritaeniorhynchus* on Okinawa to be highly resistant to malathion.

Culex quinquefasciatus. The results of tests with malathion against *Culex quinquefasciatus* larvae are summarized in Table 3.

The results show that from the few tests

TABLE 2.—Summary of insecticide susceptibility tests with malathion against *Culex tritaeniorhynchus* adults, 1963-1967.

Year	% LC 50	% LC 90	Number of Tests Conducted
1963	2.773	11.563	6
1964	2.622	11.084	4
1965	5.1671	12.264	8
1966	2.747	6.049	4
1967 (Jan-Jun)	3.138	88% mortality at 12.8% concentration	1

TABLE 3.—Summary of insecticide susceptibility tests with malathion against *Culex quinquefasciatus* larvae, 1962-1967.

Year	ppm LC 50	Fold Increase	ppm LC 90	Fold Increase	Number of Tests Conducted
1962	.0155	Base line	.0379	Base line	4
1963	.0120		.0393		2
1964	.0563	3.6	.1867	4.9	7
1965	.3273	21.1	.9937	26.2	18
1966	.3335	21.6	.9747	25.7	12
1967 (Jan-Jun)	.5190	33.5	1.1811	31.2	11

conducted during 1963 there was no significant change in the level of susceptibility over the base line established in 1962. During 1964 there was a noticeable decrease in susceptibility, and this trend toward resistance allows a determination that the population had become intermediately resistant to malathion. Tests conducted in 1965 showed a marked increase in resistance requiring 20.1 times as much malathion to obtain an LC 50 and 26.2 times more material to obtain the LC 90 than was required in 1962. There was no noticeable change in the level of resistance during 1966; however, tests during the first half of 1967 show a further increase in the level of resistance to a 33.5 fold increase at the LC 50 level and 31.2 fold increase at the LC 90 level which is more than three times that which is usually indicative of resistance. The results of tests conducted with malathion against *Culex quinquefasciatus* adults are summarized in Table 4.

The results of adult tests show that there was a noticeable increase in the level of resistance from 1963 to 1964, and the level

increased again in 1965 only to return to approximately the 1964 level during 1966. Only two tests were conducted during the first half of 1967. The first test showed a highly resistant population in which an average of only 70 percent mortality was obtained with a 12.8 percent concentration. The second test of another population conducted 12 days later showed that although this population was resistant, the level of resistance was much lower than that of the first population tested. In field tests with caged adult *Culex quinquefasciatus* conducted in 1963, Gahan, *et al* (1965) showed that a 6 oz/gal (45 gr/l) malathion fog produced an average mortality of only 84 percent of the test specimens, and Lofgren, *et al* (1966) conducting the same type of test in 1964 reported a mortality of 60 percent with a 4 oz/gal (30 gr/l) malathion fog. In tests conducted in 1965, Pennington (1966) reported 89 percent mortality at 6 oz/gal (45 gr/l), and in 1966 Pennington and Armstrong (1968) showed an average mortality of only 78 percent with the same concentration of malathion fog. In the overall

TABLE 4.—Summary of insecticide susceptibility tests with malathion against *Culex quinquefasciatus* adults, 1963-1967.

Year	% LC 50	% LC 90	Number of Tests Conducted
1963	.633	1.914	7
1964	1.698	3.514	3
1965	1.941	4.563	11
1966	1.156	3.474	2
1967 Test #1	5.509	70% mortality at 12.8% concentration	
1967 Test #2	.816	6.294	

TABLE 5.—Summary of insecticide susceptibility tests with fenthion against *C. tritaeniorhynchus* and *C. quinquefasciatus* larvae, 1962–1967.

Year	<i>Culex tritaeniorhynchus</i>			<i>Culex quinquefasciatus</i>		
	LC 50	LC 90	No. of Tests	LC 50	LC 90	No. of Tests
1962	.0024	.0058	6	.0067	.0328	4
1963	.0014	.0032	4	.0013	.0036	2
1964	.0086	.0264	5	.0050	.0137	6
1965	.0039	.0074	12	.0066	.0180	11
1966	.0029	.0060	5	.0057	.01029	7
1967 (Jan-Jun)	.0030	.0065	11	.0049	.0092	8

evaluation of susceptibility of *Culex quinquefasciatus* to malathion, the gross increase in the larval LC 50 and LC 90 levels from 1962 to the present, and the high LC 50 and LC 90 levels demonstrated in adult tests, together with the poor results obtained with this material in field tests with caged mosquitoes, clearly indicates that *Culex quinquefasciatus* are resistant to malathion on Okinawa.

SUSCEPTIBILITY TO OTHER INSECTICIDES

Fenthion. Laboratory susceptibility tests with this material against *Culex tritaeniorhynchus* and *Culex quinquefasciatus* have been conducted since 1962, and field tests against caged adult mosquitoes were conducted during 1963 through 1966 (see references). The results of laboratory tests are shown in Table 5.

Fenthion was not used on Okinawa until May 1965, when it was introduced for use as a fog material for mosquito adulticiding. All tests conducted previous to this time may be considered base line data. The results show that both mosquitoes are susceptible to fenthion and

that *C. tritaeniorhynchus* are somewhat more susceptible than *C. quinquefasciatus*. The results from 1962 through the first half of 1967 show some minor fluctuations in LC 50 and LC 90 values but no significant changes in either species. The LC 50 and LC 90 base line levels are established as follows: *C. tritaeniorhynchus* LC 50 .0041, LC 90 .0118; *C. quinquefasciatus* LC 50 .0043, LC 90 .0167. The results of field tests with caged adult mosquitoes conducted in 1963 through 1966 (see references) are shown in Table 6.

The results of field tests demonstrate the effectiveness of fenthion against *Culex tritaeniorhynchus* and *Culex quinquefasciatus* on Okinawa.

“Sumithion” (0,0-dimethyl 0-4-nitro-methyl phosphorothiolate). This material, although available on the local market and probably used to a limited extent in agriculture, has not been employed in mosquito control operations. Lofgren, *et al* (1966) in limited laboratory susceptibility tests conducted in 1964 reported the LC 50 and LC 90 for *C. tritaeniorhynchus* to be .0045 ppm and .0066 ppm respectively, and for *C. quinquefasciatus* .0049 ppm and

TABLE 6.—Results of insecticidal fogging field tests with fenthion against caged *C. tritaeniorhynchus* and *C. quinquefasciatus* on Okinawa, 1963–1966.

Year	Concentration gr/liter	<i>C. tritaeniorhynchus</i> Average % Mortality	<i>C. quinquefasciatus</i> Average % Mortality
1963	15	95	99
1964	15	99.7	100
1965	15	98.6	100
1966	15	96.9	100

TABLE 7.—Results of insecticidal fogging field tests with "Sumithion" against caged *C. tritaeniorhynchus* and *C. quinquefasciatus* on Okinawa, 1964-1966.

Year	Concentration gr/liter	<i>C. tritaeniorhynchus</i> Average % Mortality	<i>C. quinquefasciatus</i> Average % Mortality
1964	7.5	94	97
	15	97	100
1965	7.5	93.5	98.6
	15	99.6	100
1966	7.5	92.2	97.7
	15	99.5	100

.0078 ppm respectively. In more extensive laboratory tests conducted in 1965 the following results were obtained: *C. tritaeniorhynchus* LC 50 .0044, LC 90 .0095; *C. quinquefasciatus* LC 50 .0096, LC 90 .0229. These later values, although somewhat higher in some instances, parallel those obtained by Lofgren and have established a base line of susceptibility for these two species. Table 7 shows the results obtained by field tests with "Sumithion" on Okinawa reported elsewhere (see references).

The results in Table 7 show very similar response during each year's testing and together with the laboratory data show that the above two species of mosquitoes are susceptible to "Sumithion."

Naled. This material is not available on the local market nor has it been used in mosquito control operations. Naled has recently been added to the list of Federal supply items for military use and may become available for use in mosquito programs, therefore, to establish base line data prior to any use of this material, laboratory susceptibility tests were conducted during the first half of 1967. The LC 50 and LC 90 base line levels for larvae were established as follows: *C. tritaeniorhynchus* .0093 and .0228, and *C. quinquefasciatus* .0318 and .0524.

SUMMARY

Laboratory susceptibility tests with malathion against larvae and adult *Culex tritaeniorhynchus* and *Culex quinquefasciatus* conducted from 1962 to 1967 coupled with field testing from 1963 through 1966 have shown the development of resistance in both of these mosquito species to malathion on Okinawa.

Culex tritaeniorhynchus larvae showed a 48.4 fold increase in resistance at the LC 50 level and 57.1 fold increase at the LC 90 level from 1962 to 1967. Adult tests showed an LC 50 of 2.7 percent and an LC 90 of 11.6 percent in 1963, and in 1967 the LC 50 was 3.1 percent and only 88 percent mortality could be obtained with a 12.8 percent concentration. Field tests with caged adult mosquitoes showed very poor effectiveness producing an average of only 59 percent mortality with a 90 gr/l fog concentration during 1964 and only 43 percent mortality during tests in 1965.

Culex quinquefasciatus larvae showed a 33.5 fold increase in resistance at the LC 50 level and 31.2 fold increase at the LC 90 level from 1962 to 1967. Adult tests showed less resistance but usually requiring from 1 to 2 percent concentration to

TABLE 8.—Base line levels of susceptibility.

Insecticide	<i>C. tritaeniorhynchus</i> Base line		<i>C. quinquefasciatus</i> Base line	
	LC 50	LC 90	LC 50	LC 90
fenthion	.0041	.0118	.0043	.0167
"Sumithion"	.0044	.0095	.0096	.0229
naled	.0093	.0228	.0318	.0524

obtain an LC 50 and from 2 to 5 percent concentration to obtain an LC 90. Some populations show higher resistance than others. In field tests of caged adults conducted from 1963 through 1965, poor effectiveness was shown with average mortalities of only 83 percent with a 45 gr/l fog concentration.

Base line levels of susceptibility were established for fenthion, naled and "Sumithion" for *Culex tritaeniorhynchus* and *Culex quinquefasciatus* larvae by laboratory susceptibility testing. These levels are shown in Table 8.

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