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LABORATORY TESTS OF THE SUSCEPTIBILITY OF MOSQUITO LARVAE TO INSECTICIDES IN FLORIDA, 1968

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The surveillance program of the Florida State Board of Health for the detection of possible insecticide resistance in Florida mosquitoes was begun in 1963 (Rogers and Rathburn, 1964). Subsequently, two studies were made as a continuation of the program (Rathburn and Boike, 1967; Boike and Rathburn, 1968). This report deals with the further surveillance of Florida mosquitoes for their susceptibility to malathion, naled and fenthion. Initial data for Abate[1] and Dursban[2] are also included.

METHODS. The methods of collecting and handling the mosquitoes and testing procedures were generally the same as described by Rathburn and Boike (1967). With minor modifications the larval tests were performed according to procedures outlined by the World Health Organization (WHO, 1960). Rathburn and Boike (1969) showed that there was no significant difference between 600 ml. glass beakers and 400 ml. polypropylene beakers when using malathion, naled or fenthion; therefore, polypropylene beakers were used in most of the tests with these insecticides. However, they showed that there was a significant difference between the two types of test vessels with Abate; therefore, only glass beakers were used in tests with this insecticide. Tests of Dursban were conducted in glass beakers only.

One replication consisted of five insecticide dosages plus a check, 25 third instar larvae being used for each dosage. Tap water was used in testing all *Culex* species and *Aedes aegypti,* while 25 percent sea water was used with *Aedes taeniorhynchus.* Test beakers were washed with detergent, thoroughly rinsed and passed through two acetone baths after testing. The overall average water temperature was 74.0° F. with an average maximum temperature of 75.3° F. and an average minimum temperature of 72.8° F.

RESULTS. The results of tests with five insecticides against five species of mosquito larvae are shown in Table 1. Although the LC_{50} value of naled for *A. taeniorhynchus* from Mayport Naval Air Station (0.108 p.p.m.) is lower than reported in 1967 (0.196 p.p.m.), this may be due to species differentiation since the 1967 figure refers to tests with *A. taeniorhynchus* while the 1968 figure refers to a mixed population of 90 percent *A. sollicitans* and 10 percent *A.*

[1] American Cyanamid Co.
[2] The Dow Chemical Co.

taeniorhynchus. The LC_{50} and LC_{90} values of the population of *A. taeniorhynchus* and *A. sollicitans* from Merritt Island tested against malathion are somewhat high when compared to other areas. However, when compared to the LC_{50} and LC_{90} values of 0.180 and 0.460 p.p.m. respectively obtained from the same area in 1965 (Rathburn and Boike, 1967), there appears to be an increase in susceptibility to malathion of the mosquitoes from this area. Also, the LC_{50} and LC_{90} values of *A. taeniorhynchus* from Bonita Beach tested against malathion are lower than those previously reported. Except for these three instances, no significant variation in susceptibility was obtained when compared to results of the previous year. Baseline data for laboratory colonies of *A. taeniorhynchus* and *A.*

TABLE 1.—Susceptibility of mosquito larvae from various areas of Florida to five insecticides, 1968.

Insecticide	Species	County	Area	Lethal concentrations in p.p.m. LC_{50}	Lethal concentrations in p.p.m. LC_{90}	No. of reps.
Malathion	*Aedes taeniorhynchus*	Lab. Colony	Panama City	.021	.037	18
		Lab. Colony	Vero Beach	.022	.033	4
		Bay	State Park	.017	.038	7
		Brevard	Merritt Is.	.076	.250	12[a]
		Duval	Mayport N.A.S.	.046	.070	11[b]
		Duval	Marsh Area	.033	.047	13[a]
		Palm Bch.	W. Palm Bch.	.067	*	2
		Volusia	New Smyrna Bch.	.039	.062	2
		Lee	Bonita Bch.	.072	.280	7
	Aedes aegypti	Lab. Colony	Panama City	.090	.146	8
	Culex nigripalpus	Lab. Colony	Panama City	.027	.035	26
		Indian R.	Vero Beach	.038	.054	8
		Lee	Sanibel Is.	.036	.052	14
		Orange	Lake Apopka	.049	.092	14
		Palm Bch.	W. Palm Bch.	.042	.071	4
Naled	*Aedes taeniorhynchus*	Lab. Colony	Panama City	.075	.125	4
		Brevard	Merritt Is.	.122	*	12[a]
		Duval	Mayport N.A.S.	.108	.131	12[b]
		Duval	Marsh Area	.087	.113	23[a]
	Aedes aegypti	Lab. Colony	Panama City	.173	.269	16
	Culex nigripalpus	Lab. Colony	Panama City	.068	.087	40
		Indian R.	Vero Beach	.076	.092	8
		Lee	Sanibel Is.	.075	.094	18
		Orange	Lake Apopka	.068	.087	15
	Culex salinarius	Bay	State Park	.081	.098	8
Abate	*Aedes taeniorhynchus*	Lab. Colony	Panama City	.00123	.00166	8
		Lab. Colony	Vero Beach	.00073	.00116	4
	Aedes aegypti	Lab. Colony	Panama City	.00157	.00255	8
	Culex nigripalpus	Lab. Colony	Panama City	.00073	.00100	8
		Indian R.	Vero Beach	.00118	.00179	4
		Lee	Sanibel Is.	.00058	.00100	10
	Culex salinarius	Bay	State Park	.00056	.00098	4
Fenthion	*Aedes taeniorhynchus*	Lab. Colony	Panama City	.00094	.00178	8
	Culex nigripalpus	Lab. Colony	Panama City	.00300	.00420	12
	Aedes aegypti	Lab. Colony	Panama City	.00410	.00585	4
	Culex salinarius	Bay	State Park	.00290	.00380	4
Dursban	*Culex nigripalpus*	Lab. Colony	Panama City	.00064	.00079	16

* Insufficient data to accurately determine LC_{90}.
[a] Mixed population—Approx. 50% *A. taeniorhynchus*, 50% *A. sollicitans.*
[b] Mixed population—Approx. 90% *A. sollicitans*, 10% *A. taeniorhynchus.*

aegypti with Abate and fenthion and *C. nigripalpus* with Abate, fenthion and Dursban are also given in Table 1. The control mortality for all 396 replications averaged less than one percent.

DISCUSSION. The recent increase in susceptibility of *A. taeniorhynchus* from Lee County to malathion is of interest since tests performed in 1965 and 1966 indicated an advanced degree of resistance in certain areas of that county (Rathburn and Boike, 1967). In an attempt to correlate this increase in susceptibility with a decrease in usage of malathion, a comparison was made between the amount of malathion dispersed since 1964 and the yearly susceptibility levels of *A. taeniorhynchus* larvae. These figures are presented in Table 2. In general, malathion was used extensively in 1964 and early 1965. When resistance appeared in 1965 its use declined late in that year and also in 1966. Although malathion continued to be used in 1967 and 1968 it was primarily aimed at *Psorophora confinnis* which was the prevalent pest mosquito at that time. Although not conclusive, it appears that the trend in susceptibility of *A. taeniorhynchus* from resistant areas in Lee County was accompanied by a reduction in the amount of malathion dispersed. With the exception of Bonita Beach in Lee County, Mayport N.A.S., and Merritt Island, there appears to be little variation in the susceptibility of *A. taeniorhynchus* and *C. nigripalpus* to either malathion or naled from comparable areas tested the previous year. When tested against Abate, the susceptibility of *C. nigripalpus* from Indian River and Lee Counties was about the same as that of the laboratory colony.

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TABLE 2.—The number of pounds of malathion dispersed as aerial and ground fogs for the control of adult mosquitoes in two areas of Lee County, Florida, and the susceptibility to malathion of *Aedes taeniorhynchus* larvae from these areas, 1964–1968.

Area		1964	1965	1966	1967	1968
		Pounds of malathion dispersed[1]				
Sanibel-Captiva Is.	Aerial fog	18,582	9,576	3,623	10,331	0
	Ground fog	9,000[2]	10,629	1,981	1,208	0
	Total	27,582	20,205	5,604	11,539	0
Bonita Beach	Aerial fog	2,328	3,148	2,668	3,458	880
	Ground fog	7,500[2]	3,110	913	157	0
	Total	9,828	6,258	3,581	3,615	880
		Lethal concentration of malathion in p.p.m.				
Sanibel-Captiva Is.	LC_{50}	....	0.457	0.220	0.086	....
	LC_{90}	....	3.400	2.600	0.280	....
Bonita Beach	LC_{50}	....	0.275	0.105	....	0.072
	LC_{90}	....	1.500	1.050	....	0.280
Laboratory Colony	LC_{50}	....	0.029	0.025	0.030	0.021
	LC_{90}	....	0.062	0.050	0.047	0.037

[1] Ground fog dispersed as 4.1 gal. of malathion 95/100 gals. No. 2 diesel oil; aerial fog dispersed as 8 to 12 percent malathion by weight in No. 2 diesel oil.
[2] Estimated.

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A STUDY OF FACTORS AFFECTING THE SUSCEPTIBILITY OF MOSQUITO LARVAE TO INSECTICIDES IN LABORATORY RESISTANCE TESTS

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The delineation of variables is of great importance to any research and often is itself the object of extensive investigation. The variables encountered in testing the susceptibility of insecticides to mosquito larvae are many but in general are concerned with either the test organism or its environment. Those concerned with the test insects themselves are weight or size, sex, instar, age within instar and the time of year the larvae are tested. These, except for instar, are usually considered random variables and for the most part are uncontrolled. Variables of the other group are environmental in nature and are concerned with the type and quantity of water in which the larvae are tested, the presence or absence of food, the number of larvae per test vessel, the temperature of the test solution, the type of material and dimensions of the testing vessel, the number of replications and the amount and type of insecticide dilution. The importance of many of these variables is generally ignored even though there is considerable evidence to indicate that they may be responsible for large differences in published results.

Standardized tests (WHO, 1960) eliminate many of the above variables and others, such as sex differences and differences in age within instar, are beyond practical consideration. Although WHO procedures stipulate glass testing vessels, many research workers have substituted disposable paper or plastic testing vessels for them without conducting suitable research to first ascertain any difference. Kruse *et al.* (1952) demonstrated a considerably greater loss of DDT in paper than in glass or enameled containers. Curtis (1961), however, obtained little variation in the mortality of mosquito larvae with dilute solutions of DDT between test vessels of aluminum, glass, new polyethylene, paper or enamel vessels although, as he states, there may have been some reduction in toxicity of DDT in used polyethylene test vessels. Bransby-Williams (1965) demonstrated a seven-fold decrease in the effectiveness of fenthion in polyethylene lined containers when compared to unlined enamel pans. Thus it appears that the type of testing vessel may cause significant differences in larval mortality and it is likely that these differences will vary with the type of insecticide used.

World Health Organization procedures also specify that the average temperature of the water should be approximately 25° C. and that it must not be below 20° C. nor above 30° C. This is a wide range of