STUDIES OF INSECTICIDE RESISTANCE IN FLORIDA MOSQUITOES

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The first instance of mosquito resistance to insecticides in Florida was DDT resistance in the salt-marsh mosquitoes *Aedes taeniorhynchus* and *A. sollicitans* reported by Deonier and Gilbert in 1950 (Deonier and Gilbert, 1950). Resistance to DDT was soon followed by resistance to BHC and then to dieldrin (Keller and McDuffie, 1952, and Keller and Chapman, 1953). As a result, satisfactory control of these species could not be obtained in some areas of the state from about 1950 to 1956. In 1955 malathion was first introduced as a mosquito adulticide (Gahan *et al.*, 1956) and by 1957 it was widely used throughout Florida.

It was at this time that the Florida State Board of Health, fearing resistance to malathion, developed a policy of limiting the use of malathion to adulticiding in non-breeding areas. It was felt that frequent treatment of large populations of larvae and adults in breeding areas would result in the rapid selection of resistant individuals with the subsequent development of a high degree of malathion resistance. As a result of the policy, malathion has found only very limited use as a larvicide in the state, but has been used extensively and with good results as an adulticide for 10 years. Since mosquitoes have been shown to develop resistance to malathion after a period of only 2 years of extensive use as a larvicide (Gjullin and Isaac, 1957), it appears that this policy has greatly prolonged the effectiveness of malathion in Florida.

In 1964 the Florida State Board of Health, realizing the necessity of obtaining reliable information on the current status of insecticides used in the State, began to obtain baseline data for various insecticides on susceptible mosquito species (Rogers and Rathburn, 1964). In the summer of 1965 came reports of poor mosquito control with malathion adulticides in Lee County. These reports were investigated concurrently and resistance confirmed by this laboratory and the U. S. Department of Agriculture (Gahan *et al.*, 1966).

Although the more important pest species in the state over the years have been *Aedes taeniorhynchus* and *A. sollicitans,* recent research on arboviruses has pointed to the importance of many other species, namely, *Culex nigripalpus, C. quinquefasciatus* and *Aedes infirmatus.* This study has attempted to obtain data on several species of mosquitoes from several areas of the state and with all of the insecticides presently used. It is hoped that this research will be expanded in the future to include more species from

more areas so that a reduction in susceptibility to any insecticide can be determined before widespread resistance is prevalent, thereby providing time to develop new tools to combat resistant species.

MOSQUITO COLLECTION AND TRANSPORTATION. Mosquitoes were collected in Bay and Walton Counties in western Florida; Marion and Polk Counties in central Florida; Pinellas, Hillsborough, Sarasota and Lee Counties on the west coast; and Volusia, Brevard and Indian River Counties on the east coast of the State.

Adult mosquitoes were collected by means of light and bait traps, and when numerous, by sweeping with insect nets. They were transported to the Laboratory in cages made from pint ice cream cartons. The cages, each containing up to 200 adults, were shipped in styrofoam ice chests. The mosquitoes were fed a sugar solution by means of cotton pads placed on the screened top portion of the cage and kept cool in transit by means of "canned" ice. They were shipped by means of airplane, bus or automobile.

MOSQUITO REARING. Upon arrival, the mosquitoes were placed in 12 x 9 x 8 inch screened cages, supplied with a sugar solution and blood-fed on anesthetized chickens. *Aedes* species were identified and separated prior to egg collection. Eggs of these species were collected on damp cotton balls wrapped with cheesecloth. The eggs were removed by washing in ice water to prevent hatching, placed in a thin layer on 1 x 1 inch organdy squares and held in petri dishes at room temperature for five to seven days prior to hatching. Eggs to be held longer than one week were stored in a refrigerator and removed 5 to 7 days prior to hatching. Nitrogen was bubbled through the water as a stimulant for hatching.

Eggs of the *Culex* species were collected from small bowls containing hay-infusion water placed in the cages the preceding day. *Culex* species were identified as larvae, one egg raft being reared per pan. Both *Aedes* and *Culex* species were reared in 15 x 10 inch enamel pans containing about 1000 ml. of water and fed a water solution of powdered liver and brewer's yeast.

TESTING METHOD. Larval tests were conducted with minor modifications according to procedures outlined by the World Health Organization (World Health Organization, 1960). One millimeter of the appropriate insecticide soltuion was pipetted under the surface of 200 ml. of water contained in each 600 ml. test beaker. Alcohol (95 percent) was used as the diluent for malathion, Baytex and DDT solutions. The solutions of Dibrom were prepared in acetone. Twenty-five third instar larvae, previously placed in 50 ml. of water in 50 ml. beakers, were then added to each test beaker. Each replication consisted of a series of five insecticide dosages plus a check of alcohol or acetone. Because of variations in the number of available larvae for testing, from one to four replications were conducted at a time. In order to reduce variations in susceptibility caused by rearing and testing, it was desired to have at least 16 replications obtained in at least four different tests for each species, area and insecticide used. However, due to variations in the number of larvae obtained for testing, this was not always possible.

Tap water was used in testing all *Culex* species since it was also used in rearing these species. Salt water of the same salinity as the rearing water was used in testing the *Aedes* species. The temperature of the water during testing was between 22° and 28° C. Larval mortality was determined at 24 hours. Larvae incapable of rising to or submerging from the surface upon probing were considered dead. Tests with control mortality above 10 percent or in which more than 5 percent of the larvae pupated during the experiment were discarded. The percent mortalities obtained for the treatments in each test were corrected for the amount of control mortality by means of Abbott's formula. The control mortality for all

TABLE 1.—Susceptibility of five species of mosquitoes from different areas of Florida to malathion, 1965–66.

County	Area	Lethal concentrations in p.p.m. 1965 LC50	1965 LC90	1965 Reps.	1966 LC50	1966 LC90	1966 Reps.
Aedes taeniorhynchus (Wied.)							
Lab. Colony		.029	.062	15	.025	.050	14
Hillsborough	MacDill AFB	.470	1.750	8	...	...	..
	Ruskin	.056	.290	3	...	...	..
	Big Bend	...	...	..	.044	.118	12
Sarasota	Siesta Key	.590	3.900	16	...	...	..
	Longboat Key	.420	2.800	10	...	...	..
	Casey Key	.160	.760	3	...	...	..
Lee	Sanibel Island	.343	2.200	19	.220	2.600	8
	Captiva Island	.570	4.600	19	...	...	..
	Ft. Myers	.140	.670	8	...	...	..
	Iona [1]	.114	.350	20	...	...	..
	Bonita Beach	.275	1.500	7	.105	1.050	5
Volusia	Turtle Mound	.044	.170	3	...	...	..
	Oak Hill	.078	.370	3	...	...	..
Brevard	Mims	.100	.780	3	...	...	..
	Merritt Island	.180	.460	4	...	...	..
	South Brevard	...	...	..	.080	.150	16
Indian River	Vero Beach	.082	.490	15	...	...	..
Aedes sollicitans (Walk.)							
Brevard	Allenhurst	...	...	..	.042	.086	16
	Titusville Beach	...	...	..	.075	.124	16
Culex nigripalpus Theob.							
Lab Colony		...	...	..	.045	.074	18
Bay	State Park	...	...	..	.038	.070	13
Marion	Orange Springs	...	...	..	.034	.044	21
Polk	Bartow	...	...	..	.082	.110	3
Pinnellas	Lake Maggiore	...	...	..	.038	.058	22
	Largo	...	...	..	.036	.059	24
Hillsborough	Temple Terrace	.044	.062	19	...	...	..
Lee	Sanibel	...	...	..	.050	.082	6
	Ft. Myers	...	...	..	.054	.084	5
	Orange River	...	...	..	.053	.084	6
Volusia	New Smyrna Beach	...	...	..	.044	.066	16
Culex salinarius Coq.							
Walton	Santa Rosa	...	...	..	.048	.060	8
Pinellas	Lake Maggiore	...	...	..	.067	.121	12
Culex quinquefasciatus Say							
Lab. Colony		...	...	..	.130	.215	28

[1] Average of tests of larvae collected in field and larvae reared from eggs of field collected adults.

tests averaged only 2 percent. Testing vessels were washed with detergent and rinsed in clear water and then acetone after each test.

RESULTS. The results of tests with malathion are shown in Table 1. These tests indicate an advanced degree of resistance of *Aedes taeniorhynchus* in the offshore islands of Sarasota and Lee Counties and in the peninsula of Hillsborough County occupied by MacDill Air Force Base. Tests conducted with malathion on *A. taeniorhynchus* larvae in 1966 indicate an increase in susceptibility in the two areas of Lee County that were sampled. This increase in susceptibility may possibly represent a reversion since malathion was not used in this area in 1966.

TABLE 2.—Susceptibility of five species of mosquitoes from different areas of Florida to Dibrom, Baytex and DDT, 1966.

County	Area	Lethal concentrations in p.p.m.								
		Dibrom			Baytex			DDT		
		LC50	LC90	Reps.	LC50	LC90	Reps.	LC50	LC90	Reps.
Aedes taeniorhynchus (Wied.)										
Lab. Colony	.....	.103	.185	20	.0005	.0017	4	.0094	.056	14
Walton	Santa Rosa [1]	...	...	..	...	...	..	.0047	.019	14
Brevard	South Brevard	.103	.141	12	...	...	..	...	...	..
Aedes sollicitans (Walk.)										
Walton	Santa Rosa	...	...	..	...	...	..	.0043	.019	4
Brevard	Allenhurst	.117	.180	4	...	...	..	...	...	..
	Titusville Beach	.090	.130	16	.0015	(2)	8	...	...	..
Culex nigripalpus Theob.										
Lab Colony	.....	.051	.067	12	.0032	.0051	4	.040	.097	6
Bay	State Park	...	...	..	...	...	..	.028	.070	6
Marion	Orange Springs	.067	.082	24	...	...	..	.014	.043	12
Pinellas	Lake Maggiore	.068	.090	16	.0028	.0044	12	...	...	..
	Largo	.054	.078	4	...	...	..	...	...	..
Volusia	New Smyrna Beach	.078	.098	12	...	...	..	...	...	..
Culex salinarius Coq.										
Bay	State Park	.060	.082	4	...	...	..	...	...	..
Walton	Santa Rosa	...	...	..	...	...	..	.014	.033	8
Pinellas	Lake Maggiore	.103	.169	4	...	...	..	...	...	..
Culex quinquefasciatus Say										
Lab. Colony	.....	.102	.121	12	.0032	.0050	8	.049	.103	4

[1] Larvae hatched from sod collected in field, 50% *Ae. sollicitans*.
[2] Insufficient data to accurately determine.

The only other areas in which there were indications of a reduction in susceptibility of *A. taeniorhynchus* to malathion in 1965 were in Brevard County and the mainland portions of Lee County. *A. sollicitans* obtained from Brevard County appear to exhibit the same degree of susceptibility to malathion as *A. taeniorhynchus* from this area. Previous research with adulticides has also demonstrated no significant difference in insecticide susceptibility between these two species (Rogers and Rathburn, 1958). From the data presented it appears that there is no resistance of *C. nigripalpus* or *C. salinarius* to malathion in any of the sampled areas. The reason for the high LC_{50} obtained with the colony *C. quinquefasciatus* when tested with malathion is not known. These mosquitoes were colonized in 1966 from specimens obtained from various parts of Bay County. Since none of the other species of mosquitoes collected from this area exhibit any reduction in susceptibility, it can only be assumed that this difference may be due to species variation.

Shown in Table 2 are the results of tests with Dibrom, Baytex and DDT. Although only limited data have been obtained with Dibrom and Baytex, there appears to be no resistance by any of the species tested from any of the areas to these chemicals. The colony of *A. taeniorhynchus* was established from a colony maintained since 1957 at the Entomological Research Center at Vero Beach. Although the colonized mosquitoes were collected in an area in which DDT resistance has been previously demonstrated, it appears that much of the original resistance to DDT may have been lost after the 10-year period of colonization.

The degree of DDT resistance is difficult to ascertain due to the lack of a susceptible strain. However, based upon the commonly accepted LC_{50} for DDT susceptible larvae of .002 to .004 p.p.m., the mosquitoes collected from certain areas of northwestern Florida may still be susceptible to DDT. Although larval data are lacking for many areas of the state, research with adulticides has shown that a high degree of resistance to DDT is prevalent throughout most of the state (Rogers and Rathburn, 1958; and Rogers and Rathburn, 1964). The *C. nigripalpus* collected in Marion County are from an area in which no mosquito control has been practiced and therefore may represent a DDT susceptible population. However, in the absence of baseline data with this species, no definite conclusions can be made. The colony *Culex nigripalpus* used in these tests were obtained from a colony originally established at the Entomological Research Center in 1964. Therefore this colony was established after the extensive use both of DDT and malathion in that area and represents a population with possibly some reduction in susceptibility to these insecticides.

DISCUSSION. This research did not encompass the entire state; the susceptibility of mosquitoes in many areas still remains to be determined. From present data, however, malathion resistance is limited to the *Aedes taeniorhynchus* populations of the offshore islands of Lee and Sarasota Counties and in peninsular Hillsborough County. These areas represent somewhat isolated and concentrated populations of mosquitoes which in most instances are the subject of intensive control operations. As was mentioned earlier, it is believed that these are the conditions which favor development of resistance, and therefore may account for the resistance in these areas.

It is well known that differences in temperature and availability and type of food cause differences in the susceptibility of mosquito larvae to insecticides. Because of this and other factors, larvae are naturally more difficult to kill in some areas than others. This natural variation or what is commonly referred to as "vigor tolerance" may result in a lethal concentration of an insecticide for a particular mosquito species that may be considerably higher in one area than an-

other or at one time of year than another. One of the benefits of a continuing study of this type is to establish these differences. However, by testing larvae reared under controlled conditions of food, temperature, larval density, type of water, etc., it was hoped to reduce these field variations to a minimum, thereby making the results obtained more comparable. The many unknown causes of variations in larval susceptibility were further reduced by the use of a considerable number of replications obtained over a relatively long period of time.

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MALE PHEROMONES OF *CULEX QUINQUEFASCIATUS*, *C. TARSALIS* AND *C. PIPIENS* THAT ATTRACT FEMALES OF THESE SPECIES

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Chemicals released by the females of many species of insects have been shown to attract males of the same species. Also males have been shown to produce chemicals that sexually excite females of the same species. Jacobson (1965), in a recent review, lists over 200 species which produce odors that promote mating.

Observations on the male behavior of *Opifex fuscus* by Kirk (1923), *Deinocerites cancer* Theobald by Haeger and Phinezee (1959) and *Culiseta inornata* Williston by Rees and Onishe (1951) suggest that sex attractants may be produced by the female of these species. Kliewer *et al.*, (1966) demonstrated that a sex attractant is produced in the female *C. inornata*.

The purpose of the present paper is to report the presence of male pheromones in *Culex pipiens quinquefasciatus* Say, *C. tarsalis* Coquillett, and *C. pipiens pipiens*.

MATERIALS AND METHODS. Tests for