INSECTICIDE SUSCEPTIBILITY TESTS OF *AEDES TAENIORHYNCHUS* AND *CULEX NIGRIPALPUS* IN FLORIDA, 1974–1976[1]

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ABSTRACT. F_1 larvae and adults from wild populations of *Aedes taeniorhynchus* (Wied.) and *Culex nigripalpus* Theob. in Florida were tested against malathion, naled, and fenthion, and compared to the susceptible laboratory strains. The areas tested were based on previous results and also included new areas for additional susceptibility information. Both larval and adult forms of *Ae. taeniorhynchus* were found to be resistant to malathion. F_1 adults from one of the Florida Keys were 40 times more tolerant to malathion at the LC_{50} level based on paired tests with the laboratory susceptible strain. No resistance was detected when larvae or adults from some of these resistant areas were tested against naled or fenthion. F_1 larvae of *Cx. nigripalpus* were as susceptible as the laboratory strain when tested against malathion and naled; however, adults from some areas were up to 5X less susceptible to malathion.

INTRODUCTION

In a continuing effort to control 2 principal mosquito species in Florida, knowledge of their susceptibility to various insecticides is essential. Susceptibility levels of *Aedes taeniorhynchus* (Wied.) and *Culex nigripalpus* Theob. to several insecticides have been reported by Rogers and Rathburn (1964), Rathburn and Boike (1967), Boike and Rathburn (1968, 1969, 1972, 1975), Gahan et al. (1966), and Mount et al. (1971). This paper includes additional studies on the tolerance of first generation larvae and adults of these 2 species to malathion, naled, and fenthion for the years 1974–76.

MATERIALS AND METHODS

Samples of wild adult mosquitoes from various areas in the state were sent to this laboratory where both F_1 larvae and adults were tested for insecticide resistance according to the methods previously described by Rathburn and Boike (1967), Boike and Rathburn (1969, 1972, 1975). Test results from each area were statistically analyzed by probit analysis in order to obtain the LC_{50} and LC_{90} values, their 95% confidence limits, and the slope and standard error of the regression line.

RESULTS

Results of larval tests are shown in Tables 1 and 2 and adult tests in Tables 3 and 4. In both larval and adult tests, each area was pair tested with the susceptible laboratory strain; however, since little variation occurred with the laboratory strain and in the interest of space, all replications were combined for a given year. Areas tested are arranged in the tables in increasing LC_{50} values. The resistant ratios cited in the text were based on paired tests and not on the average LC_{50} and LC_{90} values shown in the tables.

MALATHION VS *Ae. taeniorhynchus*. During the 3-year period, populations of *Ae. taeniorhynchus* showed a continuing lack of susceptibility to malathion, especially in the coastal areas of Florida and the Keys. This was observed previously by Boike and Rathburn (1975) and noted again during 1975 and 1976 (Tables 1 and 3). In 1975, F_1 larvae from Charlotte County were 11X less susceptible than the laboratory strain at the LC_{50} level and 16X at the LC_{90} level. During 1976, F_1 larvae of adult mosquito populations sampled from the southwestern coastal area (Longboat Key,

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Table 1. Susceptibility of *Aedes taeniorhynchus* (Wied.) larvae to malathion, naled, and fenthion 1974–76.

			Lethal concentration in μg/ml.					
County	Area	Reps.	LC_{50}	95% C.L.	LC_{90}	95% C.L.	Slope	Std. error
			Malathion–1974					
Laboratory strain		28	0.019	0.019–0.020	0.038	0.036–0.040	4.27	0.18
Dade	Cutler Ridge	11	0.053	0.046–0.062	0.371	0.290–0.475	1.52	0.12
Indian River	Indian R. Shores	6	0.131	0.117–0.146	0.439	0.364–0.528	2.44	0.18
Indian River	Vero Bch.	4	0.147	0.131–0.165	0.398	0.321–0.495	2.96	0.28
			Malathion–1975					
Laboratory strain		44	0.025	0.025–0.026	0.055	0.052–0.058	3.82	0.15
St. Lucie	Ft. Pierce	7	0.092	0.084–0.101	0.319	0.268–0.380	2.38	0.16
Volusia	Tomoka	16	0.138	0.128–0.148	0.531	0.451–0.625	2.19	0.12
Volusia	Oak Hill	4	0.167	0.146–0.189	0.594	0.441–0.800	2.32	0.22
Hillsborough	Ruskin	14	0.180	0.162–0.199	0.932	0.730–1.191	1.79	0.10
Hillsborough	Port Tampa	10	0.186	0.170–0.205	0.530	0.461–0.611	2.82	0.20
Charlotte	Punta Gorda	4	0.322	0.278–0.373	1.290	0.909–1.820	2.13	0.22
			Malathion–1976					
Laboratory strain		64	0.013	0.013–0.014	0.034	0.033–0.036	3.15	0.07
Hillsborough	Ruskin	14	0.046	0.042–0.050	0.159	0.138–0.182	2.38	0.17
Hillsborough	Port Tampa	12	0.068	0.062–0.074	0.237	0.210–0.268	2.36	0.11
Flagler	Flagler Bch.	13	0.079	0.074–0.085	0.339	0.284–0.404	2.03	0.11
Broward	Dania	24	0.090	0.084–0.096	0.518	0.427–0.627	1.68	0.08
Sarasota	Longboat Key	8	0.098	0.088–0.109	0.315	0.272–0.366	2.53	0.15
Manatee	Perrico Is.	10	0.119	0.105–0.133	0.638	0.505–0.805	1.76	0.10
Monroe	No Name Key	19	0.143	0.132–0.155	0.708	0.605–0.828	1.84	0.09
Sarasota	Manasota Bch.	9	0.209	0.189–0.230	0.740	0.583–0.940	2.33	0.17
Monroe	Sugarloaf Key	26	0.210	0.193–0.228	1.301	1.031–1.665	1.61	0.09
			Naled–1974					
Indian River	Indian R. Shores	3	0.032	0.025–0.040	0.091	0.072–0.116	2.81	0.50
Indian River	Wabasso	8	0.048	0.046–0.049	0.079	0.074–0.085	5.75	0.34
Laboratory strain		40	0.057	0.056–0.058	0.127	0.120–0.135	3.67	0.13
Dade	Cutler Ridge	7	0.060	0.056–0.065	0.132	0.113–0.154	3.79	0.42

Table 1. (continued)

County	Area	Reps.	Lethal concentration in μg/ml.				Slope	Std. error
			LC_{50}	95% C.L.	LC_{90}	95% C.L.		
			Naled–1975					
Hillsborough	Port Tampa	7	0.043	0.041–0.046	0.077	0.070–0.084	5.14	0.45
Volusia	Tomoka	2	0.046	0.041–0.051	0.105	0.082–0.135	3.54	0.49
Volusia	Oak Hill	3	0.066	0.055–0.079	0.210	0.121–0.365	2.55	0.47
Laboratory strain		9	0.070	0.067–0.073	0.123	0.112–0.136	5.20	0.38
			Naled–1976					
Monroe	Sugarloaf Key	12	0.032	0.030–0.034	0.068	0.063–0.073	3.97	0.22
Flagler	Flagler Bch.	16	0.034	0.032–0.036	0.080	0.075–0.085	3.43	0.18
Hillsborough	Port Tampa	8	0.038	0.036–0.040	0.079	0.073–0.085	4.04	0.22
Monroe	No Name Key	14	0.040	0.038–0.042	0.094	0.086–0.104	3.39	0.20
Laboratory strain		48	0.041	0.040–0.042	0.092	0.088–0.096	3.65	0.13
Broward	Dania	16	0.044	0.042–0.046	0.086	0.080–0.092	4.42	0.24
Sarasota	Manasota Bch.	8	0.068	0.064–0.071	0.133	0.116–0.152	4.39	0.38
			Fenthion–1974					
Laboratory strain		16	0.00091	0.00088–0.00094	0.00169	0.00160–0.00179	4.78	0.20
Indian River	Wabasso	16	0.00124	0.00120–0.00129	0.00230	0.00213–0.00247	4.81	0.25
			Fenthion–1976					
Broward	Dania	11	0.00093	0.00089–0.00097	0.00160	0.00150–0.00171	5.41	0.34
Laboratory strain		25	0.00095	0.00093–0.00098	0.00180	0.00171–0.00188	4.66	0.15
Monroe	No Name Key	10	0.00101	0.00096–0.00107	0.00216	0.00194–0.00240	3.91	0.27
Sarasota	Manasota Bch.	8	0.00114	0.00107–0.00121	0.00239	0.00209–0.00275	3.96	0.38
Monroe	Sugarloaf Key	16	0.00201	0.00193–0.00210	0.00366	0.00332–0.00404	4.94	0.34

Table 2. Susceptibility of *Culex nigripalpus* Theob. larvae to malathion and naled, 1974–1976.

County	Area	Reps.	Lethal concentration in μg/ml.				Slope	Std. error
			LC_{50}	95% C.L.	LC_{90}	95% C.L.		
			Malathion–1974					
Laboratory strain		40	0.023	0.022–0.024	0.047	0.045–0.049	4.15	0.16
Pasco	New Pt. Richey	20	0.033	0.032–0.034	0.055	0.053–0.057	5.89	0.30
Bay	St. Andrew St. Pk.	7	0.040	0.039–0.041	0.057	0.055–0.060	8.43	0.48
Bay	Panama City Bch.	21	0.053	0.052–0.054	0.076	0.074–0.079	8.16	0.30
			Malathion–1975					
Laboratory strain		14	0.029	0.028–0.030	0.047	0.044–0.051	6.01	0.38
Polk	Lakeland	6	0.035	0.034–0.037	0.051	0.047–0.056	7.80	0.86
Volusia	Oak Hill	15	0.048	0.047–0.049	0.072	0.068–0.076	7.34	0.35
			Malathion–1976					
Laboratory strain		16	0.034	0.033–0.035	0.049	0.047–0.050	8.29	0.38
Manatee	Perrico Is.	8	0.045	0.044–0.047	0.067	0.063–0.071	7.44	0.46
Sarasota	Longboat Key	8	0.046	0.045–0.048	0.074	0.070–0.079	6.13	0.36
Broward	Dania	16	0.052	0.051–0.053	0.073	0.070–0.076	8.78	0.43
Sarasota	Manasota Bch.	8	0.055	0.054–0.057	0.082	0.077–0.088	7.40	0.45
Polk	Bartow	4	0.060	0.057–0.063	0.095	0.085–0.106	6.36	0.52
			Naled–1974					
Laboratory strain		12	0.037	0.036–0.038	0.054	0.052–0.056	8.03	0.45
Pasco	New Pt. Richey	16	0.068	0.067–0.070	0.091	0.088–0.094	10.30	0.52
			Naled–1975					
Laboratory strain		4	0.054	0.052–0.057	0.073	0.066–0.080	10.16	1.16
Volusia	Oak Hill	6	0.061	0.059–0.063	0.082	0.078–0.086	10.16	0.83
			Naled–1976					
Laboratory strain		34	0.052	0.051–0.053	0.071	0.069–0.074	9.40	0.33
Manatee	Perrico Is.	8	0.059	0.058–0.061	0.075	0.072–0.078	12.63	0.71
Sarasota	Longboat Key	8	0.064	0.063–0.065	0.084	0.081–0.087	11.08	0.67
Sarasota	Manasota Bch.	4	0.066	0.064–0.069	0.093	0.087–0.099	8.85	0.69
Broward	Dania	8	0.074	0.073–0.075	0.089	0.087–0.091	15.85	0.99

Table 3. Susceptibility of *Aedes taeniorhynchus* (Wied.) adults to malathion and naled, 1974–76.

County	Area	Reps.	Lethal concentration in mg/ml.				Slope	Std. error
			LC_{50}	95% C.L.	LC_{90}	95% C.L.		
			Malathion–1974					
Laboratory strain		25	0.130	0.124–0.137	0.358	0.331–0.338	2.91	0.12
Indian River	Indian R. Shores	4	0.367	0.231–0.582	2.144	0.957–4.806	1.67	0.38
Dade	Cutler Ridge	5	3.201	2.215–4.625	28.589	9.183–88.982	1.35	0.27
Indian River	Wabasso	6	5.041	3.463–7.338	37.256	13.999–99.129	1.48	0.25
			Malathion–1975					
Laboratory strain		20	0.160	0.152–0.168	0.429	0.390–0.472	2.98	0.12
Hillsborough	Ruskin	5	0.331	0.281–0.391	1.810	1.329–2.464	1.74	0.17
Volusia	Tomoka	3	1.531	1.299–1.806	9.927	6.654–14.182	1.58	0.15
Hillsborough	Port Tampa	3	1.577	1.247–1.993	4.152	3.021–5.706	3.05	0.50
St. Lucie	Ft. Pierce	3	2.473	1.735–3.526	12.078	5.102–28.596	1.86	0.45
			Malathion–1976					
Laboratory strain		34	0.220	0.211–0.229	0.763	0.705–0.826	2.37	0.06
Sarasota	Longboat Key	4	1.159	0.919–1.462	7.357	5.110–10.593	1.60	0.15
Broward	Dania	10	2.276	1.998–2.592	21.612	14.942–31.261	1.31	0.10
Hillsborough	Port Tampa	8	2.834	2.482–3.236	17.366	11.337–26.595	1.63	0.19
Monroe	No Name Key	8	4.740	3.912–5.744	39.337	21.772–71.072	1.39	0.17
Sarasota	Manasota Bch.	6	5.732	5.105–6.437	20.663	15.646–27.290	2.30	0.27
Monroe	Sugarloaf Key	8	10.280	7.695–13.734	181.447	73.181–451.752	1.03	0.12
Flagler	Flagler Bch.	4	10.445	4.167–26.182	95.104	10.612–852.118	1.34	0.40
			Naled–1976					
Broward	Dania	4	0.085	0.077–0.093	0.182	0.158–0.210	3.87	0.34
Laboratory strain		14	0.161	0.154–0.168	0.380	0.343–0.421	3.43	0.16
Monroe	Sugarloaf Key	6	0.190	0.178–0.203	0.317	0.282–0.356	5.77	0.56
Monroe	No Name Key	3	0.196	0.162–0.237	0.427	0.281–0.648	3.78	0.69
Sarasota	Manasota Bch.	2	0.212	0.184–0.244	0.354	0.260–0.481	5.77	1.14

Table 4. Susceptibility of *Culex nigripalpus* Theob. adults to malathion and naled 1974–76.

County	Area	Reps.	Lethal concentration in mg./ml.				Slope	Std. error
			LC_{50}	95% C.L.	LC_{90}	95% C.L.		
			Malathion–1974					
Laboratory strain		34	0.673	0.645–0.703	2.058	1.905–2.224	2.64	0.08
Pasco	New Pt. Richey	9	0.928	0.826–1.043	3.107	2.576–3.748	2.44	0.19
Bay	Panama City Bch.	10	1.251	1.132–1.382	3.280	2.756–3.905	3.06	0.24
Bay	St. Andrews St. Pk.	6	1.338	1.177–1.521	3.229	2.562–4.070	3.35	0.37
Dade	Cutler Ridge	2	1.445	1.233–1.692	2.974	2.294–3.858	4.09	0.68
			Malathion–1975					
Laboratory strain		4	0.207	0.166–0.258	0.823	0.635–1.067	2.14	0.28
Volusia	Oak Hill	7	0.914	0.822–1.017	3.185	2.669–3.800	2.36	0.16
Polk	Lakeland	2	1.072	0.748–1.536	2.639	1.519–4.586	3.27	0.71
			Malathion–1976					
Laboratory strain		22	0.451	0.425–0.479	1.740	1.573–1.923	2.19	0.08
Broward	Dania	9	1.071	0.939–1.221	9.482	6.607–13.766	1.35	0.11
Manatee	Perrico Is.	8	1.370	1.226–1.532	5.518	4.396–6.925	2.12	0.15
Sarasota	Longboat Key	10	1.443	1.313–1.586	5.673	4.642–6.933	2.16	0.13
Sarasota	Manasota Bch.	6	1.466	1.297–1.657	6.361	4.860–8.325	2.01	0.15
Orange	Orlando	4	2.075	1.780–2.419	6.294	4.378–9.045	2.66	0.35
Polk	Bartow	2	2.157	1.436–3.241	10.537	3.200–34.690	1.86	0.52
			Naled–1976					
Laboratory strain		10	0.090	0.085–0.096	0.247	0.217–0.279	2.93	0.15
Broward	Dania	4	0.097	0.088–0.108	0.187	0.163–0.214	4.54	0.51
Sarasota	Longboat Key	3	0.138	0.128–0.149	0.253	0.221–0.291	4.86	0.48
Manatee	Perrico Is.	4	0.189	0.140–0.254	0.976	0.300–3.174	1.80	0.53

Perrico Island and Manasota Beach) and the Florida Keys (No Name Key and Sugarloaf Key) were 6X to 13X less susceptible than the laboratory strain at the LC_{50} level and 8X to 31X at the LC_{90} level. During 1974, F_1 adults from Dade and Indian River counties were 25X and 38X more resistant respectively at the LC_{50} level when compared to the laboratory strain. During 1976, F_1 adults from the Florida Keys (No Name Key and Sugarloaf Key) were as much as 16X and 40X respectively less susceptible than the laboratory strain at the LC_{50} level.

NALED vs *Ae. taeniorhynchus*. F_1 larvae from all areas tested during the 3-year period were as susceptible (<2X) to naled as to the laboratory strain (Table 1). When adults from malathion resistant areas in 1976 were tested for susceptibility to naled, very little difference was noted when compared to the susceptible laboratory strain (Table 3).

FENTHION vs *Ae. taeniorhynchus*. A limited number of fenthion tests were performed mainly during 1976 (Table 1). F_1 larvae from 4 areas sampled during this period were found to be as susceptible (<2X) as the laboratory strain.

MALATHION vs *Cx. nigripalpus*. First generation larvae sampled during 1974–76 were almost as susceptible to malathion as those of the laboratory strain (Table 2). Adults tested from some areas during the same period were 2X to 5X less susceptible than the laboratory strain at the LC_{50} level and from 2X to 6X at the LC_{90} level (Table 4).

NALED vs *Cx. nigripalpus*. Larvae from all 6 areas sampled during 1974–76 were as tolerant to naled as the laboratory strain (Table 2). Adults were tested from areas sampled during 1976 only and were found to be 2X and 4X less tolerant than the laboratory strain at the LC_{50} and LC_{90} levels respectively.

DISCUSSION

Previous results on the susceptibility of Florida mosquitoes to insecticides have been reported by Rathburn and Boike (1967) and Boike and Rathburn (1968, 1969, 1972) and included mostly larval data, while a recent report (Boike and Rathburn 1975) contained mainly larval data and some adult tests. The data reported herein contain substantially more adult testing and in many instances both larval and adult results can be compared for a given area. The data also indicate that *Ae. taeniorhynchus* continues to be resistant to malathion in the state, with apparently the highest degree of tolerance noted in adults from the lower Florida Keys. Although no resistance to malathion was noted in larval samples of *Cx. nigripalpus*, some adult populations were 6X less susceptible to malathion than the susceptible laboratory strain. This could be significant; however, it probably represents vigor tolerance in the adult stage.

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LABORATORY OBSERVATIONS ON THE MATING BEHAVIOR OF *CULEX TRITAENIORHYNCHUS* GILES

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ABSTRACT. Male insemination capacity and the incidence of multiple insemination was studied using a wild-type, laboratory strain and a recessive eye color mutant of *Cx. tritaeniorhynchus*. Sexually mature, wild-type males inseminated an average of 5.1 mature females during a 4-day test period with most inseminations occurring the 2nd night. Multiple insemination was detected in 8 of 389 fertile egg rafts and occurred most frequently in small cages having high pair densities. No preferential mating was observed among the mutant males and females, and progressively more wild-type matings occurred at lower pair densities and in larger cages.

Studies of *Culex tritaeniorhynchus* Giles mating behavior have recently been initiated to provide background information as a prelude to genetical control experiments in nature. Information on phenomena such as male insemination performance, the incidence of multiple insemination and preferential mating are critical in planning releases. Observations on *Cx. tritaeniorhynchus* mating behavior in nature (Kawai et al. 1967, Reisen et al. 1977) and during colonization attempts (Newson and Blakeslee 1957, Sasa et al. 1967; Shirasaka et al. 1968, Baker and Sakai 1974) have been restricted to the time and place of mating and the incidence of successful mating under laboratory conditions. In fact, there are relatively few detailed studies of many aspects of the mating behavior of *Culex* mosquitoes, in general, with the exception of *Cx. p. fatigans* (e.g. Kitzmiller and Laven 1958, Sebastian and de Meillon 1967) and to a lesser extent *Cx. tarsalis* (Asman 1975) and *Cx. nigripalpus* (Lea and Edman 1972).

The present paper describes male insemination performance, the incidence of multiple insemination, and preferential mating in *Cx. tritaeniorhynchus* under laboratory conditions.

METHODS AND MATERIALS

STRAINS. The Balloki, Pakistan, strain of *Cx. tritaeniorhynchus* was used throughout and has been under continuous culture at our laboratories for the past 6 years (Baker and Sakai 1974). A recessive, sex-linked, heat sensitive, eye color mutant, rose eye (Baker and Sakai 1973), was used in the multiple insemination and preferential mating experiments. This mutant, originally isolated from the Balloki strain, expresses itself well in the larval stage as red eye color at 28°C, becoming paler to

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