AEROSOL TESTS WITH DDT AND MALATHION COMPARING FIVE SPECIES OF MOSQUITOES AND COMPARING SALT-MARSH *AEDES* FROM EIGHT LOCALITIES IN FLORIDA AND GEORGIA

ANDREW J. ROGERS AND CARLISLE B. RATHBURN, JR.

Entomological Research Center, Florida State Board of Health

Deonier and Gilbert first reported DDT resistance in salt-marsh *Aedes* in Florida in Brevard County in 1950 (Deonier and Gilbert, 1950). McWilliams and Munn (1957) more recently reported evidence of DDT resistance in salt-marsh *Aedes* at Key West. Following the report by Rogers *et al.* (1957a) of poor results with DDT in aerosol dosage tests against adult *Aedes taeniorhynchus* (Weid.) from Indian River County, the question was raised as to the possible difference in susceptibility of this species to DDT in thermal aerosols in other areas of the state, especially in counties where DDT had been used for only a few years. Consequently, in the spring of 1957 a study was initiated to demonstrate the difference in kill, if any, that might be obtained with DDT between *Aedes taeniorhynchus* adults from several areas of Florida and Georgia. Comparable tests with malathion were also conducted for comparison.

Eight areas were selected, three on the east coast and three on the west coast of Florida, plus the Florida Keys (Monroe County) and Savannah (Chatham County), Georgia. The Florida east coast areas were Dade, Indian River and St. Johns counties; the west coast areas were Lee, Sarasota and Citrus counties (see Figure 1).

In conjunction with the area studies it seemed desirable also to compare the effectiveness of DDT and malathion against several species of mosquitoes. For these tests adults of five species were used: *Aedes taeniorhynchus* (Weid.), *Aedes aegypti* (L.), *Psorophora confinnis* (Lynch-Arr.), *Culex quinquefasciatus* Say and *Anopheles quadrimaculatus* Say, plus limited studies with *Aedes sollicitans* (Walker), and *Psorophora ciliata* (Fab.).

METHODS: TEST INSECTS—The mosquitoes for the area study were collected by two methods. Originally salt-marsh sod was tested in the field for viable *A. taeniorhynchus* eggs. When productive sod was found, it was cut and transported to the Entomological Research Center at Vero Beach. In the laboratory the sods were flooded to hatch the eggs and the larvae were reared to the adult stage under identical conditions of food, temperature, light, etc. according to procedures previously described (Rogers *et al.*, 1957b). However, due to the uncertainty of obtaining sods with good numbers of viable eggs, and the problems in cutting and transporting the sods, a second method (developed by J. S. Haeger of the Entomological Research Center) was used for most of the area studies. This method consisted of collecting adult *A. taeniorhynchus* in the field, and transporting them in special jars to the laboratory where they were given blood meals. The eggs from these adults were flooded and the larvae reared in the same manner as from sods.

The mosquitoes in the species study were, however, either collected as larvae or pupae in the field or reared from laboratory colonies. TESTING—The tests were conducted in early evening hours on a level, open area with no trees or heavy underbrush to block the travel of the fog, thus allowing maximum conditions for coverage. Two cages of mosquitoes were attached to a stake (see Figure 2), one at the six-foot level and the other at two feet from the ground, similar to methods pre-

viously described (Rogers *et al.*, 1957b). The stakes were placed at intervals of 165, 330 and 660 feet, perpendicular to and downwind from the line of travel of the fogging vehicle. Each test or replicate consisted of the cages from two sets of stakes placed a block apart, a total of 12 cages. In all tests, caged *A. taeniorhynchus* from Indian River County were hung on stakes side by side with caged mosquitoes from the other areas or species under comparison (see Figure 2).

In some instances two or even three areas and/or species were compared in the same test with *A. taeniorhynchus* from Indian River County. Thus, there were as many as four stakes at each station in some tests, with each stake supporting caged adult mosquitoes from a different area.

It was felt that the inclusion of *A. taeniorhynchus* from Indian River County in all comparative tests was desirable since there are known differences in mortality due to weather factors and minor opera-

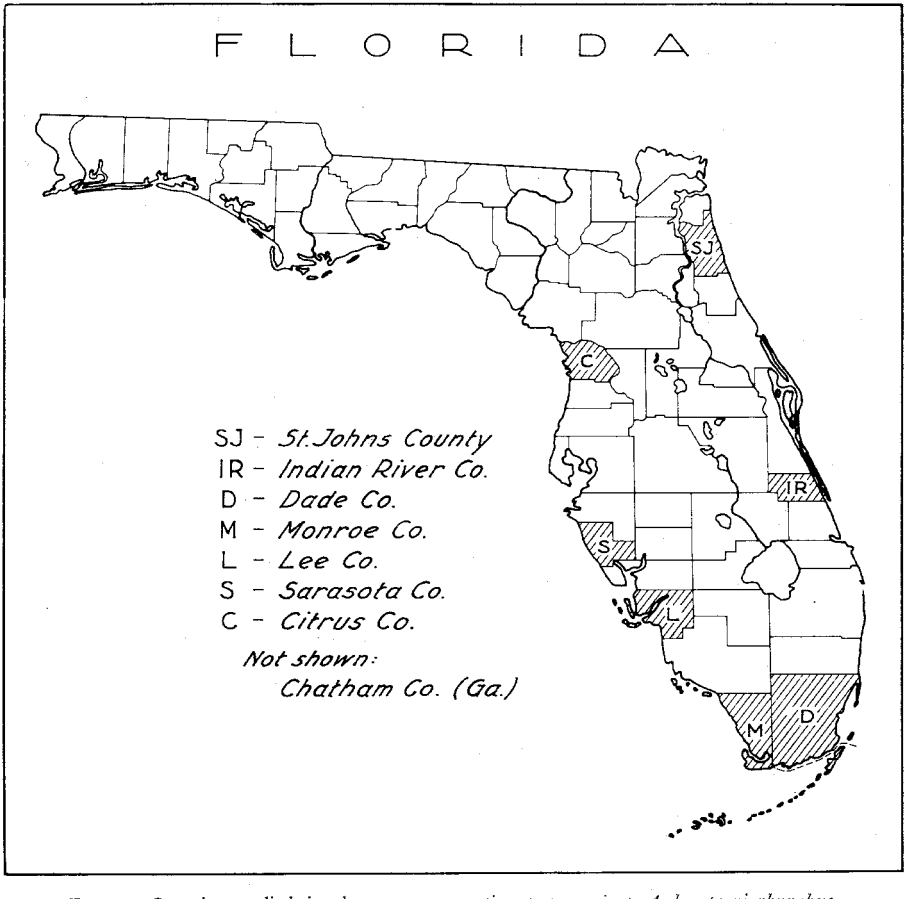

FIG. 1.—Counties studied in the area-comparative tests against *Aedes taeniorhynchus*.

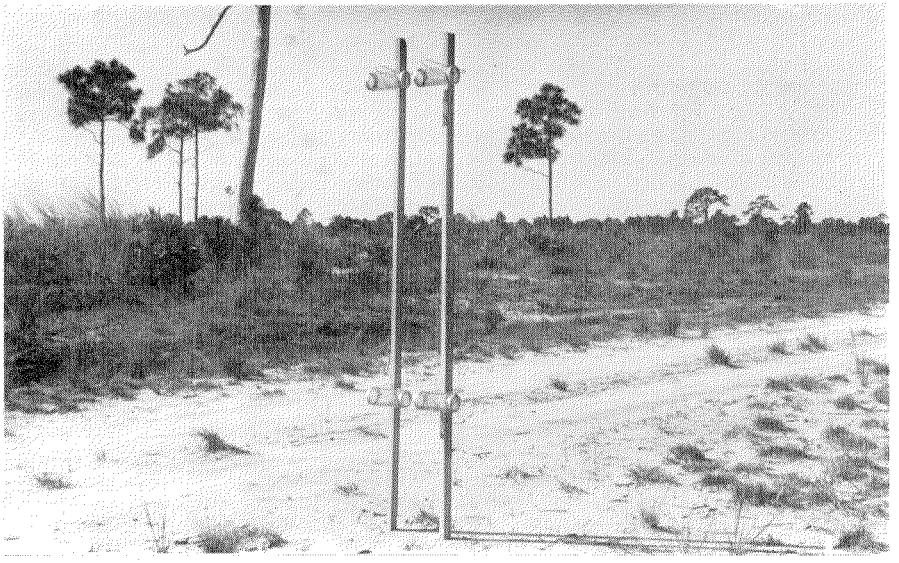

FIG. 2.—Stakes with cages attached as used in comparative tests. Cages on one stake contain *Aedes taeniorhynchus* from Indian River County; cages on the other stake hold mosquitoes to be compared.

tional differences. Thus, by including *A. taeniorhynchus* from Indian River County as a standard in all tests, it would be possible to show differences between certain areas or species.

A Jeep-mounted Tifa aerosol generator calibrated to deliver 40 gallons per hour at 1000° F. operating temperature, 25 p.s.i. formulation pressure, and driven at five miles per hour was used in all tests. Machine operations were checked constantly during tests and insecticides were measured before and after each test.

In all comparative tests, DDT was used at the rate of 10 ounces of technical DDT per gallon and malathion at 8 ounces per gallon of No. 2 diesel oil. According to the space method of calculating dosage (Rogers *et al.*, 1957a), DDT was used at approximately 0.37 lb. per acre and malathion at approximately 0.30 lb. per acre over a swath of 330 feet.

After treatment, the mosquitoes were changed into clean cages and held in a shed for 12 hours, then counted to determine mortality. Only female mosquitoes were used in determining per cent mortality. All mosquitoes used in the tests were between two and eight days old at the time of testing, and had been fed only sugar water.

RESULTS. Average kills for malathion in Tables 1 and 2 represent two replications and the DDT averages are from four replications, except where noted in the tables.

An analysis of variance, using the arcsine transformation of percentages, showed no significant differences in susceptibility to DDT between *A. taeniorhynchus* from St. Johns, Lee and Sarasota counties when compared to specimens from Indian River County. However, significantly higher kills were obtained with DDT against *A. taeniorhynchus* from Dade and Monroe (Key Largo) counties and from Chatham

County (Savannah) Georgia than against *A. taeniorhynchus* from Indian River County. In a limited number of tests with DDT against mosquitoes from Citrus County there appeared to be no difference in kill when compared to mosquitoes from Indian River County.

There appeared to be no differences in susceptibility of *A. taeniorhynchus* to malathion from any of the areas tested when compared to Indian River County specimens (see Table 1).

In the species study, considerably better kills were obtained with DDT against *A. aegypti, A. quadrimaculatus,* and *P. conn-finnis* than with *A. taeniorhynchus* or *C. quinquefasciatus*. In a limited study with DDT against *P. ciliata* there also appeared to be a difference in kill when compared to *A. taeniorhynchus*.

The kills with malathion against different species were much more uniform than with DDT (See Table 2).

In the area-comparative tests, *Aedes sollicitans* comprised seven percent of the 3824 salt-marsh *Aedes* used from all areas in the malathion replications. The overall kill (660′ swath) of *A. sollicitans* in the malathion tests was 81 percent. Six percent of the 2630 specimens within the 330-

TABLE 1.—Results with malathion and DDT comparing *Aedes taeniorhynchus* from eight geographical areas

Mosquito Origin County	Percent Kill at Indicated Swath in Feet						No. Years DDT Used Prior to Tests
	Malathion		DDT				
	330 Feet	660 Feet	330 Feet			660 Feet	
	Average	Average	Average	Range	lsd at 5% [1]	Average	
Monroe	55	47	29	19–37	6	20	6 [2]
Indian River	62	56	12	5–20		9	
Dade	85	79	29	17–58	1	20	13
Indian River	83	79	13	0–44		12	
Indian River	93	88	9	2–16	N.S.	7	11
Indian River	93	90	12	6–16		8	
St. Johns	85	76	11	3–24	N.S.	8	11
Indian River	93	83	13	5–27		10	
Lee	95	89	18	11–33	N.S.	14	11
Indian River	98	93	14	4–30		11	
Sarasota	83	73	11	1–26	N.S.	8	12
Indian River	77	70	7	2–14		6	
Citrus [3]	100	97	74	——	——	62	4
Indian River [3]	100	100	69	——		62	
Chatham (Ga.)	94	88	24	10–39	5	18	5
Indian River	85	80	6	0–10		5	

[1] Significant differences calculated only for DDT in 330′ Swaths.
[2] Key Largo only: DDT used 10 years in "lower keys," i.e. Big Pine Key to Key West.
[3] Only one replication with malathion and one with DDT.

foot swath in these tests were *A. sollicitans* and the kill at this swath was 88 percent.

A. sollicitans comprised six percent of 8337 salt-marsh *Aedes* from all areas in the comparative tests using DDT, and the overall kill (660′ swath) was 24 percent. Of 5929 specimens included in the 330-foot swath of these tests, *A. sollicitans* also comprised six percent, and the mortality with DDT at this swath for *A. sollicitans* was 30 percent.

Considering *A. taeniorhynchus* only from Indian River County in the area-comparative tests, the kill of this species in all of these tests using malathion averaged 92 percent at 330 feet and 87 percent at 660 feet. Comparable kills against *A. taeniorhynchus* with DDT in these tests were 28 percent at 330 feet and 26 percent at 660 feet.

DISCUSSION. It is felt that the low kills obtained with malathion in the Monroe vs. Indian River tests (Table 1) and in the *A. aegypti* vs. *A. taeniorhynchus* tests (Table 2) were the result of low temperatures. The temperatures at the time these tests were run were between 50 and 53° F., a temperature of approximately 50° F. being known to be the threshold of activity for *A. taeniorhynchus*. However, the low kill obtained with malathion against *P. ciliata* (Table 2) cannot be attributed to any observed factors.

The closeness of the percentage kills obtained in the comparative test of Indian River County vs. Indian River County in Table 1, or the *A. taeniorhynchus* vs. *A. taeniorhynchus* in Table 2, gives a good indication of the reliability of the tests. It appears from these data that the reproducibility of results is excellent when conducted under identical conditions of weather and machine operation. Thus, the greater differences appearing between *A. taeniorhynchus* or Indian River County results in different tests apparently is an indication of the effect of the variability of weather and operational factors when using a marginal material such as DDT against *A. taeniorhynchus* in Florida.

There is also a highly significant correlation ($r = 0.79$ for 9 df) between the percent kill obtained with DDT and that obtained with malathion when tests with

TABLE 2.—Results of tests with malathion and DDT comparing six species of mosquitoes

	Percent Kill at Indicated Swath in Feet				
	Malathion		DDT		
	330 Feet	660 Feet	330 Feet		660 Feet
Species	Average	Average	Average	Range	Average
A. taeniorhynchus	93	88	9	2–16	7
A. taeniorhynchus	93	90	12	6–16	8
A. aegypti	83	78	79	68–97	74
A. taeniorhynchus	65	55	44	6–83	34
P. confinnis [1]	100	94	61	47–74	49
A. taeniorhynchus [1]	100	96	30	15–41	22
P. ciliata [2]	65	56	81	——	77
A. taeniorhynchus [2]	88	77	5	——	3
C. quinquefasciatus	84	82	21	4–57	15
A. taeniorhynchus	90	87	31	5–83	25
A. quadrimaculatus [3]	92	81	89	80–99	83
A. taeniorhynchus [3]	91	85	33	5–60	22

[1] Only one replication with malathion.
[2] Only one replication with malathion and one with DDT.
[3] The 660′ average kill with DDT based on 3 replications.

both insecticides were conducted on the same night. This may indicate the influence of some optimum or poor fogging conditions. As yet no definite statements can be made as to the singular effects and interactions of wind, temperature, inversion, relative humidity and other factors. However, a study of this nature is being conducted, which, it is hoped, will result in a better understanding of this complex problem.

The data in Tables 1 and 2 only serve to demonstrate the differences or lack of differences that occured between mosquitoes from different areas and between different species in these tests, and it is not intended to imply that these same percent kills could be expected under different conditions of terrain, weather, equipment, etc.

By taking all tests into consideration, a definitely better kill was obtained with malathion than with DDT, even though the amount of DDT applied was considerably higher than that usually applied in control operations. It is also known that, at the dosage used, malathion is slightly sublethal to *A. taeniorhynchus* in these tests. However, this dosage was used in order to demonstrate any differences in kill that might occur with malathion between *A. taeniorhynchus* from different areas and between different species.

Data from comparable tests conducted in different years with DDT and malathion against *A. taeniorhynchus,* shown in Table 3, not only confirm the accuracy of the testing methods, but also show conclusively that DDT is not effective as a thermal aerosol against *A. taeniorhynchus* in Florida at the present time.

Widely varying factors associated with the use of chlorinated hydrocarbons in different areas studied, such as size of treated areas, number of yearly insecticide applications, dosage, etc., make a quantitative comparison of the study areas on the basis of DDT usage impractical, if not impossible. Therefore, the best available basis for attempting such a comparison seems to be in the number of years that DDT and/or other chlorinated hydrocarbons were used as the principal insecticides in each area. These records, kindly supplied by the district mosquito control directors, are shown in Table 1.

On the basis of this comparison, further supported by more detailed information not included here, it is concluded that the best kills against *A. taeniorhynchus* in these tests were not consistently associated with areas having the shortest history of DDT usage. Space will not permit a detailed area-by-area discussion of this point. The important conclusion, relating to the original objective of the research, is that at the present time DDT even in massive dosages is not an effective adulticide against *A. taeniorhynchus* in Florida, when applied as a thermal aerosol. If it is assumed that this condition is due to "acquired resistance," then it appears very doubtful that

TABLE 3.—Results of tests against *Aedes taeniorhynchus* from Indian River County with malathion and DDT in 1956 and 1957

Insecticide	Dosage [1] lb./acre	Formulation lb./gal.	Year	Replications	Percent kill at Indicated Swath 330 Feet	660 Feet
Malathion	0.30	0.5	1956	18	90	88
			1957	19	91	85
DDT	0.37	0.625	1957	33	28	26
	0.60	1.0	1956	5	41	30
			1957	2	33	25
	1.19	2.0	1956	1	11	7
			1957	3	36	27

[1] Calculated on assumed average wind velocity of 3 m.p.h. (Rogers *et al.,* 1957a.)

there are at present any areas in the state which can properly be classified as "non-resistant." It can also be concluded that the degree of tolerance by this species to DDT is not the same in all areas of the state, and that the reasons for this are not known to the authors.

The widely varying results with DDT against different species, as shown in Table 2, also present a difficult problem of interpretation. While it is true that the highest kills were obtained with *Anopheles quadrimaculatus* and *Aedes aegypti* secured from laboratory colonies, the variable results obtained with DDT against the other species, all collected from natural breeding areas, do suggest that the natural tolerance of a species to DDT should be considered. However, the malathion data do not indicate a wide difference in susceptibility to this insecticide among the species studied.

ACKNOWLEDGMENTS. The authors are indebted to J. S. Haeger of the Entomological Research Center, Florida State Board of Health for his assistance in obtaining and rearing the test mosquitoes, and to E. J. Beidler, Director of the Indian River Mosquito Control District, and his staff for furnishing and operating the equipment for most of the tests. We are also grateful to the following directors of mosquito control for their cooperation in this research: W. W. Warner, Monroe County; F. H. Stutz, Dade County; R. E. Bartnett, St. Johns County; T. W. Miller, Lee County; J. Melton Williams, Sarasota County; Paul Patterson, Citrus County; and George Carmichael, Chatham County, Georgia. David Lieux and Doyle Taylor, Entomologists, Florida State Board of Health, actively assisted by collecting and transporting adult mosquitoes from certain counties to the Research Center. Finally we wish to acknowledge with sincere appreciation the fine cooperation and generosity of the Entomology Research Branch, Agricultural Research Service, U.S.D.A., at Orlando, Florida for furnishing eggs of *Aedes aegypti* and *Anopheles quadrimaculatus* from their laboratory colonies.

Literature Cited

DEONIER, C. C. and GILBERT, I. H. 1950. Resistance of salt-marsh mosquitoes to DDT and other insecticides. Mosquito News 10(3):138–143.

MCWILLIAMS, J. G. and MUNN, B. L. 1957. Studies of mosquito resistance to insecticides at some naval activities. Mosquito News 17(4): 258–260.

ROGERS, A. J., BEIDLER, E. J. and RATHBURN, C. B., JR. 1957a. A progress report on dosage tests with mosquito adulticides. Mosquito News 17(3):190–194.

———. 1957b. A cage test for evaluating mosquito adulticides under field conditions. Mosquito News 17(3):194–198.

REPLY TO REQUEST FOR INFORMATION

Editor: In the *Mosquito News* just received, I note a query from Bob Armstrong about how to recover dusts to measure recovery at distances from the emitting machine. At Ft. Belvoir we used 6 x 6 in. glass plates coated with oil. These plates were recovered and the captured material (DDT) was analyzed quantitatively, using Dr. Haller's method, which was a relatively simple one after it was set up. I do not know if they continued this after I left or devised a better way. Perhaps members Carl Wesley or Lafe Edmunds could tell you. We tried laying the plates horizontally and hanging them up vertically and decided that our recovery was adequate either way. This measures the amount actually reaching the ground or passing through the foliage. I'd suggest a note to Carl or Dr. Edmunds might bring a response of interest since they've been at it over two years since I left and no doubt have added a lot to my primitive techniques.—(Signed) AUSTIN MORRILL, JR.