

FIELD TESTS WITH SEXUALLY STERILE MALES FOR CONTROL OF *Aedes aegypti*¹

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INTRODUCTION. Eradication of the screw-worm fly, *Callitroga hominivorax* (Cqrl.), through release of males sexually sterilized by gamma radiation, was reported by Lindquist, 1955; Baumhaver *et al.*, 1955; and Knipling, 1960. Observations on sterilization of mosquitoes were published by Davis *et al.*, 1959 and Weidhaas *et al.*, 1961. In laboratory tests, production of viable eggs of *Aedes aegypti* (L.) was greatly reduced when normal females were caged with normal:sterile males at a ratio of 1:20 (McCray *et al.*, 1961). The present report of tests in Escambia County, Florida, compares natural populations of *A. aegypti* in two areas of sterile-male release and in two untreated check areas.

MATERIALS AND METHODS. Sterile males for the field tests were obtained from the Technical Development Laboratories of the Communicable Disease Center in Savannah, Georgia. A laboratory strain of *A. aegypti* was used in 1960 and a Pensacola-derived strain in 1961. The male pupae were sterilized by exposure to 11,000 to 18,000 roentgens of gamma radiation from cobalt 60. Batches of 1,100 or 2,200 irradiated pupae were placed in water held in one-half-pint or one-pint cartons for transportation to Pensacola by commercial air express. To reduce loss in transit, cartons were shipped with chemical refrigerants that maintained the insulated boxes at temperatures of about 60° to 70° F.

Usually the pupae were distributed to release sites within 24 hours after irradiation.

Field releases of males were calculated from records of laboratory emergence. Two or more cartons of pupae from each shipment were retained in the Pensacola laboratory in one-gallon cartons with bobbinet covers. Each day the live adults were removed and the numbers of each sex were recorded. Emergence records were obtained from 2.6 percent of the 3,912,000 pupae received in 1960 (96 percent males) and from 2.4 percent of the 6,708,600 pupae received in 1961 (97 percent males).

Release Area 1, located about 5 miles west of downtown Pensacola, consisted of nearly 600 premises of about 238 acres. From July 18 to October 3, 1960, pupae were distributed twice weekly to 42 release sites in 37 blocks. At each release site, pupae were placed in a 6-quart carton that was protected from direct sun and possible spillage by a shelter of plywood and hardware cloth. After October 10, 1960, the release area was reduced to 24 centrally located blocks, and pupae were placed in 152 old automobile tires located on alternate intersections of a 150-foot grid. Evaluation of the 1960 releases was limited to 114 acres containing 300 premises in 19 blocks. In this area, an average of 83,348 males was released in each of the 16 weeks at the rate of 731 per acre.

In 1961, irradiated pupae were distributed weekly in release Area 1 from April 3 to October 2. Releases and evaluation were in an area of 139 acres with 446 premises in 24 blocks. In the first 14 weeks, when pupae were equally distributed to the 152 release sites, the calculated release averaged 131,040 males per week at the rate of 943 per acre. In the last 13 weeks, releases were reduced to an aver-

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age of 24,211 males per week (174 per acre), but release sites near natural infestations were supplied with up to 6 times more pupae than other sites.

Release Area 2, located about 7 miles from downtown Pensacola, was a rural settlement of about 48 acres with 48 houses along 3 parallel country roads. Irradiated pupae were distributed to old tires once each week from July 10 to October 2, 1961. The single release site at each premise was located near the center of actual or potential breeding places. Uninfested premises received a minimum of 1,100 pupae each week, while 2 to 6 times this number were distributed to premises with *A. aegypti* infestations. An average of 99,014 males was released in each of the 13 weeks at the rate of 2,063 per acre.

Untreated check Area A, located about 2 miles northwest of downtown Pensacola and 4 miles northeast of Release Area 1, contained 10 blocks with 148 premises on about 26 acres.

Untreated check Area B, located about 43 miles north of downtown Pensacola, contained 105 premises along six roads and lanes on about 96 acres.

There were no control operations in any of the four study areas during the test period. Blocks adjoining Area A were sprayed with a 2.5 percent DDT emulsion applied with powered equipment during the period of February-April, 1961. There were no control operations near any other study area.

Estimation of natural populations of *A. aegypti* was based on actual counts of larvae every second week. Except for samples collected for identification, larvae were counted and returned to the original breeding places. Normal males were assumed to be one-half of the number of larvae.

Field collected eggs, obtained by lining formerly infested receptacles with paper towels, were counted and submerged in a 24-hour-old mixture of 0.1 g. brewers' yeast and 0.1 g. ground dog chow in one liter of water. Each hatching test was completed in the week following egg col-

lection. Larvae were counted 24 hours after eggs were submerged and then held for identification.

RESULTS AND CONCLUSIONS. Estimates of sterile males released and normal males

TABLE 1.—Estimated numbers of sterile and normal males in *Aedes aegypti* Release Area 1, Pensacola, Florida

Week of	Sterile	Normal	Ratio
1960			
July 11	0	343	..
18	20,152	428	47:1
25	42,108
Aug. 1	32,686	490	67:1
8	46,062
15	70,862	684	104:1
22	105,630
29	102,938	326	316:1
Sept. 1	77,091
12	83,600	439	190:1
19	80,014
26	88,352	97	910:1
Oct. 3	87,758
10	97,757	580	169:1
17	171,703
24	182,614	194	941:1
31	44,240	124	357:1
Nov. 7	..	125	..
1961			
Apr. 3	168,514	7	24,073:1
10	44,744
17	189,600	6	31,600:1
24	56,406
May 1	100,320	45	2,229:1
8	223,136
15	107,692	3	35,897:1
22	175,408
29	159,051	0	..
June 5	97,900
12	115,893	0	..
19	88,770
26	170,568	1	170,568:1
July 3	136,552
10	45,068	0	..
17	25,688
24	34,800	4	8,700:1
31	18,895
Aug. 7	19,800	0	..
14	16,500
21	26,244	21	1,250:1
28	20,368
Sept. 4	22,226	66	332:1
11	23,256
18	20,404	3	6,801:1
25	20,064
Oct. 2	21,436	16	1,340:1

present were used to calculate ratios. In Area 1, ratios of sterile to normal males ranged from 47:1 to 941:1 in 1960; during 1961, normal males were apparently absent in 4 different weeks and the lowest ratio was 332:1 (Table 1). In Area 2, ratios ranged from 25:1 to 148:1 during the 1961 releases (Table 2).

TABLE 2.—Estimated numbers of sterile and normal males in *Aedes aegypti* Release Area 2, Pensacola, Florida

Week of	Sterile	Normal	Ratio
1961			
July 3	0	1,319	..
10	72,939
17	75,764	2,853	27:1
24	75,570
31	82,399	2,264	36:1
Aug. 7	126,294
14	87,528	3,435	25:1
21	122,973
28	105,616	1,732	61:1
Sept. 4	99,990
11	59,551	1,621	37:1
18	123,200
25	150,696	1,015	148:1
Oct. 2	104,664
9	..	967	..

In Area 1, the releases may have reduced but failed to eradicate natural populations of *A. aegypti*. Comparative data on 1960 populations of *A. aegypti* (Table 3) failed to demonstrate any conclusive differences between the area of sterile male release and the untreated area (A). In July, the percentage of premises with larvae was slightly higher in the release area than in the untreated area but the reverse was true during the period from August to November. The highest percentage of infested premises in the untreated area was 15.1 in the week of August 22 compared to a release-area high of 9.7 percent in the week of September 12.

In 1961, the percentage of premises with larvae in release Area 1 remained below one percent; from June to October it did not exceed 0.4 percent (Table 3). The 10 blocks of untreated Area A were inspected 8 times in the period from April

10 to July 17, 1961 without finding *A. aegypti*. In the week of July 31, 37 larvae of *A. aegypti* were found in two containers on a single premise. No further infestations were found in the five inspections from August 14 to October 9, 1961. Populations of *A. aegypti* in check Area B showed a progressive increase from June to the last of August (Table 4).

In Area 2 (Table 4) the release of sterile males failed to reduce the natural population of *A. aegypti*.

In release Area 1, 25 percent of 2,566 eggs collected hatched compared to 42 percent of the 2,440 eggs collected in check Area A (Table 5). Because of the low population of *A. aegypti*, eggs were not obtained from release Area 1 in 1961. In release Area 2, 64 percent of 1,002 eggs hatched compared to 57 percent of the 808 eggs from check Area B.

In 1960, release of sterile males of the laboratory strain was not started until July 18. Release sites may have been too far apart to provide a uniform distribution of males. Moreover, it was certain that viable eggs had been deposited before releases were begun. These 3 possible disadvantages were largely eliminated in 1961 when release of sterile males of the Pensacola strain was started in April and the release sites were located on alternate intersections of a 150-foot grid.

In 1960, the population of *A. aegypti* in Area 1 did not show a marked reduction during the release period, but in 1961 a definite decrease occurred in the number of premises infested and in the number of larvae. The reduced 1961 population may have resulted from cumulative effects of releases in two successive breeding seasons and failure to obtain eradication may be due to reinfestation of the relatively small area. However, the low 1961 population in untreated Area A raises questions of other causal factors and precludes a conclusive statement on the effect of the sterile males released.

Paucity of *A. aegypti* in check Area A during 1961 may have resulted from: (1) the observed reduction in the number of water containers through voluntary action

TABLE 3.—Populations of *Aedes aegypti* in area of sterile-male release and in an untreated check area, Pensacola, Florida

Week of	Percent of premises with larvae		Number of larvae	
	Release area I	Check area A	Release area I	Check area A
1960				
July 11	1.8	..	687	..
18	4.6	..	859	..
25	..	3.2	..	191
Aug. 1	4.9	..	980	..
8	..	8.1	..	373
15	4.3	..	1,368	..
22	..	15.1	..	982
29	8.5	..	653	..
Sept. 5	..	10.8	..	858
12	9.7	..	878	..
19	..	14.8	..	1,020
26	4.6	..	195	..
Oct. 3	..	12.5	..	753
10	9.0	..	1,160	..
17	..	12.1	..	610
24	4.3	10.0	389	600
31	4.7	7.5	249	441
Nov. 7	3.6	5.6	250	197
1961				
Apr. 3	0.9	..	14	..
10	..	0	..	0
17	0.7	..	12	..
24	..	0	..	0
May 1	0.7	..	91	..
8	..	0	..	0
15	0.2	..	7	..
22	..	0	..	0
29	0	..	0	..
June 5	..	0	..	0
12	0	..	0	..
19	..	0	..	0
26	0.2	..	1	..
July 3	..	0	..	0
10	0	..	0	..
17	..	0	..	0
24	0.2	..	9	..
31	..	0.7	..	37
Aug. 7	0	..	0	..
14	..	0	..	0
21	0.4	..	42	..
28	..	0	..	0
Sept. 4	0.4	..	133	..
11	..	0	..	0
18	0.2	..	6	..
25	..	0	..	0
Oct. 2	0.4	..	32	..
9	..	0	..	0

TABLE 4.—Populations of *Aedes aegypti* in area of sterile-male release and in an untreated check area, Pensacola, Florida

Week of	Percent of premises with larvae		Number of larvae	
	Release area 2	Check area B	Release area 2	Check area B
961				
June 5	..	5.7	..	203
12
19	..	13.3	..	738
26
July 3	64.6	19.0	2,639	610
10
17	58.3	26.7	5,797	1,003
24
31	58.3	32.4	4,528	1,929
Aug. 7
14	70.8	38.1	6,871	2,530
21
28	58.3	40.0	3,464	2,207
Sept. 4
11	68.8	20.0	3,242	1,254
18
25	56.2	20.0	2,031	712
Oct. 2
9	51.1	18.1	1,934	724

TABLE 5.—Hatching of eggs of *Aedes aegypti* collected from areas of sterile-male releases and from untreated check area, Pensacola, Florida

	Release Area 1		Check Area A	
	No. of eggs	Percent hatched	No. of eggs	Percent hatched
960				
July	42	67
Aug.	362	32	375	54
Sept.	563	49	699	73
Oct.	1,335	11	1,240	21
Nov.	264	27	126	32
Total	2,566	25	2,440	42

	Release Area 2		Check Area B	
	No. of eggs	Percent hatched	No. of eggs	Percent hatched
961				
July	508	61	401	69
Aug.	131	62	123	27
Sept.	309	75	184	80
Oct.	54	39	100	2
Total	1,002	64	808	57

of residents; and/or (2) DDT spraying in adjacent blocks, which eliminated the possibility of introductions from nearby areas.

In a few instances, it was shown that the infestation of *A. aegypti* in the release area was introduced by the movement of receptacles into the area. The frequency of such introductions and the extent of their influence on observed populations is not known.

The estimated ratios of sterile males to normal males (Tables 1 and 2) were obtained from averages applied to the entire release area but do not necessarily represent actual relative numbers at any specific location. The extent of dispersion of sterile males from a limited distribution of irradiated pupae was unknown. Since completion of the field releases, laboratory studies have indicated that a few days difference in age of males favors mating success of the younger mosquitoes. Thus, the 1961 releases of only once each week, while desirable from the viewpoint of logistics, were probably less effective for *A. aegypti* control than the twice-weekly

releases of 1960. Adaptation of the sterile-male method for mosquito control requires additional investigation of the biology, especially the dispersion of males under field conditions.

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