FIELD STUDIES ON THE RELEASE OF STERILE MALES FOR THE CONTROL OF *ANOPHELES QUADRIMACULATUS*[1]

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INTRODUCTION. As early as 1937 Knipling (1960) suggested the possibility of controlling insects by inducing sterility in the males and elucidated the theories and applications of this important concept. Later, the availability of gamma radiation to sterilize large numbers of insects at a reasonable cost made the application of this approach practical. The screw-worm fly (*Cochliomyia hominivorax* Coq.)) proved to be ideally suited to the application of the sterile-male technique, and it has been successfully eradicated from the southeastern United States (Knipling, 1960; Lindquist, 1961). The tremendous success of this technique with the screw-worm fly suggested its promise for the control of other species, among which could be certain anopheline mosquitoes. The large numbers of some species of mosquitoes in wild populations and their high biotic potential might prevent this method from being used successfully but, even if not suitable for use alone, it might be useful in combination with other control methods or at a time when breeding conditions are unfavorable to the insects.

Davis *et al.* (1959), in preliminary studies on the development of the sterile-male technique with *Anopheles quadrimaculatus* Say, showed that dosages of 8,865 to 12,900 roentgens of gamma radiation applied in the pupal or adult stage produced sterility in both sexes. Sterilized females mated to normal males produced no eggs, whereas normal females mated with sterilized males produced a normal number of eggs, but none hatched. When sterilized males were introduced into caged populations of normal males and normal females at ratios of 4:1:1 or less, no reduction in the total number of viable eggs was produced, but at ratios of 6:1:1 and 10:1:1 there was a reduction of about 80 percent. Because of these promising laboratory results, the research program on the sterile-male technique with *quadrimaculatus* was expanded to include the field tests described in this paper.

LAKE OKEECHOBEE EXPERIMENT. Preliminary surveys were conducted during the summer of 1959 to locate a suitable area to study the possibility of controlling or eradicating *quadrimaculatus* by the release of sterilized males. Two factors of prime importance in selecting the site were that it should afford isolation from outside infestation and that it should contain a relatively small population of mosquitoes so the experiment would remain within the limited capabilities of laboratory-rearing facilities. An area about 5½ miles long and about 1 mile wide was selected in the southeast part of Lake Okeechobee near Chosen and Belle Glade, Florida. This area comprised two islands known as Torrey and Kreamer, which appear to form a peninsula that juts into Lake Okeechobee. They are separated from each other and the mainland only by narrow strips of water. Torrey Island forms the basal half and Kreamer the apical half. Except near the base of Torrey Island, the sides of the test area are at least 2 miles from the nearest land.

Both islands were well stocked with cattle. Systems of dikes, ditches, and pumps for controlling the water level were

[1] The authors express their appreciation to the following: in the Entomology Research Division, Carroll N. Smith for experimental supervision, H. R. Ford who assisted in all phases of the experiment, E. Nottingham who was responsible for rearing the mosquitoes; and in the Palm Beach County (Fla.) Mosquito Control Department, to R. C. Ridgeway and S. V. Knowles who assisted in making adult counts.

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present on both islands. The water level in this area fluctuates a great deal because of rain, wind, and the draining or flooding operations of cattlemen and farmers. During the summer months some of the farm lands are allowed to become completely flooded but in the winter these same areas are pumped dry for the production of vegeable crops. On both islands, the land is about equally divided between pasture for cows and vegetable-producing farm land.

Before initiating the release of sterile males in this area, methods of evaluation of the experiment were developed and some prerelease data were accumulated by determining the density of adult *quadrimaculatus* and the viability of eggs laid by them. Six adult counting stations were established on Kreamer Island, seven on Torrey Island, and five in a check area approximately 6 miles away near Lake Harbor, Florida. Counting stations were either buildings in the areas or artificial "privy-type" resting stations. One month after the release of sterile males had been started five additional check stations were established along the edge of Lake Okeechobee north of Canal Point, Florida; three were approximately 15 miles and two were 25 to 30 miles from Kreamer Island. Wild females were collected to assay egg viability in a natural population. These females were placed individually in small (2-oz.) glass vials (1" in diameter and 3" tall) containing approximately ½ to 1 inch of water. A majority of the females laid eggs under these conditions. The eggs laid by each female were counted and a subsample of these eggs was set for determination of percent hatch. However, it was evident later in the experiment that with the personnel available this method was too time-consuming and a scheme for classifying viability by estimating within 50 the number of eggs laid per female was devised.

The percent hatch was also estimated as falling within one of the following classes:

Class I —60 to 100 percent hatched
Class II —10 to 59 percent hatched
Class III— 1 to 9 percent hatched
Class IV— 0 percent hatched

With the establishment of adult counting stations and egg viability determinations, two methods were available to study the effect of sterile males on a natural population. Larval surveys were considered as a third method of evaluation; however, larvae were so widely scattered and at such a low level that this method of surveying was impractical to use. Dipping produced mostly first and second-stage larvae and microscopic identification between *quadrimaculatus* and *Anopheles crucians* Wied. can only be made on the late third and fourth stages. Six hours of dipping provided only 21 larvae of this size, of which only 4 were *quadrimaculatus*. Consequently, larval dipping was discontinued.

For 1 month prior to the first release weekly counts were made of the adults of *quadrimaculatus* in resting stations on Kreamer and Torrey Islands and in the Lake Harbor check area. Fifty-six females were collected on Kreamer and Torrey Islands for egg-viability determinations, 35 of which laid single batches of eggs ranging from 15 to 300 per batch (average of 150 per female). The viability of the eggs from all these females except one fell into Class I. One female laid 15 eggs all of which sank and did not hatch.

Before beginning releases, a barrier strip 4 miles long and ½ mile deep along the edge of the mainland adjacent to Torrey Island, was sprayed with ½ pound of DDT per acre to increase the isolation of these two islands from the mainland. This treatment was repeated half way through the experiment.

Mosquitoes for release were reared according to the customary method but on an expanded scale. Pupae, collected daily from the laboratory colony so that none were over 24 hours old, were exposed immediately to 12,000 r of gamma radiation in a cobalt-60 source. Within 24 to 48 hours after the adults emerged, they were inactivated in a cold room (35° to 40° F.) and the males transferred to cages

for release in the test area. Females were not released, since their bites would have caused annoyance and their presence invalidated the weekly adult-female counts made in resting stations as well as the egg-viability determinations.

All releases were made on Kreamer Island, the first on October 23, 1959. Nine release stations were set up so that the only spot on the 2-square-mile island that was more than ¼ mile from one of them was a flooded section on the east side of the island, which was inaccessible when the experiment was begun. In January 1960 a 10th release point was added to cover this area after it had been drained. Males were released every Monday, Wednesday, and Friday, generally once a week at each station. During weeks when production was unusually high or low, releases at a given station were made more or less than once a week. Sterile males were transported from Orlando to the release area by car in cylindrical screen cages (12" in diameter and 18" high) covered with a wet cloth sleeve.

Table 1 gives a summary of the number of sterile-male mosquitoes released during the course of the experiment. The number varied considerably from one month to another depending on the production level of the colony. From December 7 to December 27, 1959, no males were available because of rearing difficulties. A total of 328,900 males was released during the 11 months the experiment was in operation, or an average of 3,700 males per square mile per week. The study was concluded in September 1960, following a hurricane.

TABLE 1.—Summary of sterile male *Anopheles quadrimaculatus* mosquitoes released on Kreamer Island

Month	Number released	Average number per square mile per week
1959:		
October	11,600	4,350
November	11,000	1,400
December [a]	9,800	1,850
1960:		
January	34,600	3,950
February	35,200	3,900
March	40,100	4,500
April	11,200	1,400
May	22,000	2,300
June	47,400	5,500
July	37,600	4,200
August	60,800	6,100
September	7,600	3,800

[a] No release made from December 7 to December 27, 1959.

The monthly abundance of female *quadrimaculatus* on Kreamer and Torrey Islands and in both check areas is summarized in Table 2. There was a gradual decrease in the number of females in all areas during the fall and winter months. On Kreamer Island this decrease started at the end of October and only an occasional female was present from February through May 1960. The trend was the same on Torrey Island, with the exception that it started a month later. *Anopheles quadrimaculatus* abundance in the Lake Harbor check area showed a decrease in October and November 1959, a temporary increase in January 1960, then a further decrease, with only an occasional female being found from March through June. In the Lake Okeechobee check area populations decreased in December 1959, and January 1960, increased again in February and May, but were very low during June and July. Populations began to increase during June on Kreamer Island, July on Torrey Island and Lake Harbor, and August in the Lake Okeechobee check area, and in the following months reached or exceeded the numbers found before releases were initiated.

The presence of sterile males may have affected the abundance of *quadrimaculatus* on Kreamer Island from October 23, 1959, through May of 1960, but not afterwards, and the downward trend of female *quadrimaculatus* in all four areas definitely showed that most of the reduction on Kreamer Island was seasonal. However, it appears significant that the decline of *quadrimaculatus* females appeared first and most strikingly on Kreamer, then spread to Torrey Island immediately after

TABLE 2.—Monthly abundance of female *quadrimaculatus* in resting stations. (Average and range of total weekly counts in all resting stations in each area.)

Month	Kreamer Island		Torrey Island		Lake Harbor check area		Okeechobee check area[a]	
	Average	Range	Average	Range	Average	Range	Average	Range
1959:								
September	29	7–49	651	303–1,297	80	51–112	..	..
October	68	37–110	441	299–621	55	44–81	..	..
November	18	13–23	836	556–1,139	19	7–31	168	..
December	7	2–12	88	47–132	22	3–29	87	41–179
1960:								
January	4	1–7	27	2–89	29	10–51	69	35–104
February	2	0–4	26	5–28	21	1–49	133	60–236
March	1	0–3	4	0–10	2	1–8	35	15–90
April	0	0–1	0	0–0	1	0–3	23	7–49
May	1	0–4	0	0–0	1	0–2	46	8–131
June	60	1–124	3	0–5	4	0–9	8	1–15
July	366	233–486	49	14–70	42	17–75	8	4–9
August	276	153–537	65	15–91	61	36–116	79	42–113
September	223	3–465	444	74–1,219	207	86–336	289	166–1,155
November	..	..	193	135–250	223	187–258	1,252	1,064–1,440

[a] This check area brought into the study the last week of November, 1959.

he initiation of the release program. Furhermore, populations in the two check reas showed one (Lake Harbor) or two Lake Okeechobee) substantial increases n abundance of females between January nd May, whereas those on Kreamer and Torrey Islands decreased to the vanishing oint through May.

Table 3 summarizes data accumulated on the viability of eggs from wild females collected from Kreamer and Torrey Islands and the Lake Okeechobee check area. A few females laid eggs with no floats which sank and did not hatch or laid some floating and some sinking eggs. The classification of egg masses according to percent hatch is based only on eggs which floated and consequently could

TABLE 3.—Monthly summary (November 1959 through November 1960) of female *quadrimaculatus* collected from Kreamer and Torrey Islands and the Okeechobee check area and the percent viability of the eggs laid by these mosquitoes

Month	Number of females		Percent of egg collections in class			
	Collected	Laying eggs	I	II	III	IV
			Kreamer Island			
959:						
November	28	16	62	6	12	19
December	17	13	85	8	0	8
960:						
January	23	17	76	18	0	6
June	12	11	100	0	0	0
July	102	90	80	2	1	18
August	106	67	95	3	0	3
September	63	40	95	3	0	3
November	..	..	..	..	..	..
			Torrey Island			
959:						
November	46	31	58	13	10	16
December	27	16	94	0	0	6
960:						
January	22	12	100	0	0	0
February	12	7	100	0	0	0
July	21	13	86	0	0	14
August	26	18	88	0	0	12
September	21	9	78	11	0	11
November	36	25	92	8	0	0
			Okeechobee Check Area			
959:						
November	20	16	53	0	20	27
December	36	22	95	5	0	0
960:						
January	41	25	94	0	6	0
February	31	19	100	0	0	0
March	13	7	100	0	0	0
June	6	5	100	0	0	0
July	15	10	100	0	0	0
August	26	15	100	0	0	0
September	29	19	98	2	0	0
November	65	40	91	3	3	3

hatch. A small degree of sterility occurred during November 1959 and July 1960 on Kreamer Island, during November 1959 and July, August, and September 1960, on Torrey Island, and during November 1959 in the Lake Okeechobee check area.

On September 10, 1960, a hurricane (Donna) moved through the State of Florida, passing to the west of the test area. The winds from this storm had little effect on the *quadrimaculatus* populations; however, all cattle were removed from Kreamer Island during the week preceding the storm. A survey the week following the hurricane showed *quadrimaculatus* populations to be normal on Torrey Island and in the two check areas, but only three unengorged females were found on Kreamer Island. Flood waters from central Florida moved south into Lake Okeechobee and flooded both islands, and counting stations were inaccessible except by boat and wading. The effects of the hurricane, following the summer months during which no reduction in the numbers of *quadrimaculatus* in the test area had been shown, led to the termination of the experiment.

LAKE PANASOFFKEE EXPERIMENT. In June, 1960, surveys of *quadrimaculatus* populations were initiated near Lake Panasoffkee in Sumter County, Florida. An extensive breeding area was located at the south end of this lake in a tree-covered swamp flooded by water from springs and spring-fed streams. Two counting stations for adult abundance were established in the breeding area. Another counting station was established ¼ mile away from the breeding area, three 1 mile away, three 2 miles away, and four 5 miles away in concentric circles around the two stations within the breeding area. From June to November 1960 adult counts were taken at all stations and wild females were collected and brought to the laboratory for egg viability studies.

A total of 104,700 sterile males were released from September 23 through December 2, 1960, at the two counting stations in the breeding area, which were approximately ⅛ mile apart. The weekly releases were as follows: September 23—6,500; September 30—12,700; October —11,000; October 14—14,600; Octobe 21—7,800; October 28—12,200; Novembe 4—7,300; November 11—7,800; Novem ber 18—8,800; November 25—8,500; De cember 2—7,500.

This release of an average of 9,50 sterile males per week had no observ able effect on the population level as show in Table 4. Data on egg viability (Tabl 5) did not conclusively indicate any in duced sterility in the wild females. Th results obtained suggest either that th number of sterilized males released wa insufficient, or that the males were inad quate in other ways to show a significan effect on the wild population.

DISCUSSION. Attempts to induce a sig nificant degree of sterility into natur populations of *quadrimaculatus* have bee unsuccessful in two locations. In the Lak Okeechobee test males were not release at known breeding sites; in the Lak Panasoffkee experiment males were r leased in only a small portion of an exte sive breeding area. Further studies o the biology and behavior of this specie are being undertaken to discover biolog ical information which will lead to th successful application of this techniqu Factors such as the interbreeding of th laboratory colony and wild individual the time and location for release of male the extent and location of mating activit the composition of the natural populatio with respect to inseminated and older f males, and the total numbers of indivi uals and their dispersion must be know to develop this method.

SUMMARY. Two experiments in whic *Anopheles quadrimaculatus* males steri ized in the pupal stage with 12,00 roentgens of gamma radiation were r leased into natural populations of *qua rimaculatus* have been conducted. In on of these, a total of 328,900 males were r leased during an 11-month period (a average of 3,700 males per square mile p week) at 9 to 10 release points on small semi-isolated island in Lake Oke chobee, Fla. In the other, a total of 104

TABLE 4.—Total number of *A. quadrimaculatus* and percentage of females in resting stations in Lake Panasoffkee area at various distances from a point where sterile males were released within a breeding area

Date		Release site		¼ Mile		1 Mile		2 Miles		5 Miles	
		Total	Percent females	Total	Percent females	Total	Percent females	Total	Percent females	Total	Percent females
June	28	208	52	1,000	100	656	99	71	97	409	99
July	5	10	67	2,000+	100	48	98	29	93	68	98
	12	500	52	2,000+	100	143	100	157	100	39	95
	19	800	40	2,000+	98	527	99	71	97	155	93
	26	558	51	1,000+	99	360	99	73	99	119	97
August	2	52	43	150	100	77	98	35	97	135	99
	9	150	45	150	100	61	100	36	97	90	95
	16	125	46	250	100	262	100	73	93	191	91
	23	145	41	175	100	103	99	88	67	216	88
	30	700	44	105	100	133	89	184	88	213	86
September	6	80	25	300	100	620	99	156	85	618	90
	13	460	50	173	100	417	98	275	100	851	93
	21	700	34	72	100	426	99	63	95	512	96
	28[a]	310	28	102	96	424	99	161	89	643	89
October	5	525	45	47	89	319	99	92	88	500	98
	12	400	49	200	94	300	94	61	74	568	97
	19	25	24	450	98	495	95	102	88	688	98
	26	956	41	1,100	96	631	98	120	88	838	98
November	2	85	84	2,000+	99	601	97	199	100	487	98
	9[b]	323	19	1,000+	97	712	93	46	85	..	..
	16	154	66	500	87	250	57	..	..	350	99
	23	187	17	210	95	70	50	..	..	25	100
	28	650	16	500	100	550	73	..	..	150	100

[a] Releases of sterile males begun on September 23, 1960.
[b] The number of stations at 1, 2, and 5 miles was decreased.

TABLE 5.—Viability classification of eggs laid by female *A. quadrimaculatus* collected from the Panasoffkee area

Month (1960)	Location of resting station	Number of females collected	Number laying eggs		Percent laying floating eggs	Percent of egg collections in class			
			Floating	Sinking		I	II	III	IV
June	Breeding area	2	1	0	50	100	..	..	..
	¼ mile	1	1	0	100	100	..	..	..
	1 mile	6	6	0	100	83	17	..	..
	2 mile	8	4	2	50	75	25	..	..
	5 mile	8	5	0	63	80	20	..	..
July	Breeding area	18	9	0	50	100	..	..	..
	¼ mile	9	8	0	89	88	12	..	..
	1 mile	26	16	0	62	94	..	6	..
	2 mile	32	20	0	62	100	..	..	..
	5 mile	32	23	0	72	96	..	4	..
August	Breeding area	33	19	0	58	95	5	..	..
	¼ mile	29	15	1	52	100	..	..	..
	1 mile	42	26	1	62	92	..	..	8
	2 mile	36	30	0	83	93	..	..	7
	5 mile	47	27	3	57	93	..	..	7
September	Breeding area	20	11	1	55	100	..	..	..
	¼ mile	49	38	5	78	97	..	3	..
	1 mile	37	20	2	54	100	..	..	..
	2 mile	24	18	3	75	100	..	..	..
	5 mile	46	27	2	59	96	..	..	4
October	Breeding area	27	13	0	48	84	8	..	8
	¼ mile	59	37	2	63	84	5	8	3
	1 mile	39	22	7	56	70	..	..	30
	2 mile	25	21	0	84	100	..	..	..
	5 mile	45	24	2	53	93	..	3	4
November	Breeding area	54	8	0	15	75	25	..	..
	¼ mile	157	78	9	50	96	4	..	..
	1 mile	82	50	6	61	93	5	2	..
	2 mile	30	18	5	60	94	..	..	6
	5 mile	63	39	7	62	98	..	..	2

700 males were released during an 11-week period (average of 9,500 males per week) at two stations about ⅛ mile apart within an extensive breeding area in a tree-covered swamp flooded by rain water and spring-fed streams, located at the south end of Lake Panasoffkee, Sumter County, Fla. To determine if these sterile males caused any reduction of, or sterility in, the natural populations, the number of adult *quadrimaculatus* in resting stations and the viability of eggs from females collected from these resting stations were followed in the release and check areas.

In the Lake Okeechobee experiment the release of sterile males may have influenced the abundance of *quadrimaculatus* during the first half of the experiment when the natural population was in a seasonal decline, but it had no effect when the natural population increased during the second half of the test. Release of sterile males in the Lake Panasoffkee area did not conclusively demonstrate any induced sterility in wild females.

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NULLIPARITY IN SUMMER AND FALL POPULATIONS OF *CULEX TARSALIS* COQ.[1]

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INTRODUCTION. A practical technique for the separation of nulliparous and parous female mosquitoes was originated by Detinova (1945, 1949). Its basis is the simple fact that the ovarian tracheoles retain an original coiled state until stretched, irreversibly, by the development of eggs. The method of Detinova was first applied to the study of *Culex tarsalis* Coquillett by Kardos and Bellamy (1961). It has been used further to analyze the special collections of *C. tarsalis* which are discussed in the present paper.

All of these special collections were made in the summer and fall of 1960 in Weld and Boulder Counties, Colorado. The ovaries were dissected from all females that were neither engorged nor gravid. Females with ovaries retaining only a few mature eggs were called parous. The tracheoles of the mounted ovaries were not always readable and though, in separate collections, the state of the ovaries in as many as one-fourth of the deplete females could not be determined, the occurrence of these unknowns is believed not to compromise any statement made in this paper.

PROPORTION OF NULLIPAROUS FEMALES AS AN INDICATOR OF POPULATION AGE. In a study of western and St. Louis encephalitis under enzootic conditions in Weld County, Colorado (Blackmore *et al.*, 1962), it was found that the transmission rates in two different areas were not related to the corresponding prevalence data

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