extended patterns which would be necessary to obtain control or eradication in a given area. Attempts to find larval sterilants which are effective at lower concentrations have been unsuccessful to date.

Summary. Apholate and tepa in solution were effective sexual sterilants of *Aedes aegypti* (L.) larvae in the laboratory at 10 parts per million either alone or in combination. Fifteen parts per million of apholate coated on pyrophyllite gave similar results, even when used over soil. In the field, tepa solutions became ineffective after 3 days. When in competition with untreated males, males treated with apholate reduced the viability of eggs from untreated females by approximately 66 percent of the theoretically expected reduction. Multiple matings severely reduced the effectiveness of individual sterile matings.

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FIELD BEHAVIOR OF SEXUALLY STERILE *ANOPHELES QUADRIMACULATUS* MALES

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Field experiments on the use of sterile males to control *Anopheles quadrimaculatus* Say were first attempted in 1959–60 at Lake Okeechobee and subsequently at Lake Panasoffkee, Florida (Weidhaas *et al.*, 1962). Although trials with radiosterilized males apparently failed to reduce the natural populations, they did stimulate an awareness of the need for increased knowledge of the biology of *quadrimaculatus* in relation to the sterilization approach. Dame and Schmidt (1962) discussed the possible factors influencing the ability of sterile *quadrimaculatus* males to inseminate females in the field. Release of sufficient numbers of males to overwhelm the natural male population, vigor of treated males, male sexual compatibility with females in the natural population, and a knowledge of the behavioral characteristics which guide both sexes to potential mates are all prerequisites to the success of the sterile-male technique.

Since the termination of the 1959–60 sterile-male release programs, intensive studies have been conducted in the Panasoffkee release area to gain insight on the mating behavior, physiological age, and natural fertility of the local *quadrimaculatus* population. In conducting these investigations Woodard *et al.* (1962) de-

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veloped a suitable technique for field evaluation of mating behavior. In this method virgin females are released, recaptured, and their insemination and fertility evaluated. With this technique it was possible to study the mating behavior of sterilized *quadrimaculatus* males. Results of these investigations, conducted during 1962 and 1963, and their implications are presented herein.

Methods and Techniques. The study area, the same as that used by Weidhaas *et al.* (1962), encompassed a small portion of a large breeding area along Panasoffkee Creek which flows into Lake Panasoffkee. The release area, extending along the south bank of Panasoffkee Creek for about 400 yards, included a swath of wooded swampland approximately 150 yards wide. On the north the area was bounded by the creek, on the west by a railroad track, on the south by open fields, and on the east by extended wooded swamplands. Thus, the area was by no means isolated from other breeding areas but was in fact an integral part of a much larger ecosystem. During these studies, we did not anticipate control or eradication of *quadrimaculatus*. By confining our efforts to this area we were able to study the mosquito in a familiar breeding place with the hope of being able to detect small changes in the natural population brought about by short-term sterile-male releases.

Adult *quadrimaculatus* from two different sources were utilized: (1) the long-established Orlando colony and (2) the progeny of field-collected adult females. The former, henceforth referred to as colony males or colony females, were collected in the pupal stage from the colony. The latter, henceforth referred to as laboratory-reared wild mosquitoes, were the progeny of females captured in the Panasoffkee area. These females were given a human blood meal in the laboratory, if not already engorged, and oviposition was then forced by holding each gravid female individually in a 10-dram glass vial containing ½ to 1 inch of water. Resulting larvae were reared in enamel pans by standard techniques and pupae were collected as they became available. Adult males and females from both the colony and laboratory-reared wild strains were separated during the first 24 hours after emergence to insure virginity. Individual handling was facilitated by rapidly immobilizing the adults in a cold room at $34° \pm 2°$ F. The separated adults were maintained in 12-inch cylindrical wire-screen cages 18 inches long, closed with a stockinette sleeve at one end and plywood at the other. Unless otherwise stipulated, they were fed a 20 percent honey-water solution in a saturated cotton pad, and water was supplied by placing a water-soaked cotton pad atop the cage.

Males were sexually sterilized by three methods. In the first method, they were contact-sterilized by exposure to residues of 7 mg./sq. ft. of tepa on glass. The insides of quart jars and their glass covers were coated with a measured quantity of a 0.1 percent solution of tepa in methanol, the jars were rolled until they appeared dry and then allowed to dry further overnight; 50 to 80 one- or two-day-old males were held in the jar for 2 hours. In the second method, other males were sterilized by feeding for the first 3 to 4 days after emergence on 1 percent apholate in 20 percent honey solution. In the third method, males were sterilized by the techniques used in the 1959–60 Okeechobee and Panasoffkee sterile-male release programs. Pupae from 0 to 24 hours old were exposed to 12,000r of gamma radiation from a cobalt-60 source (660 ± 14r/min.)

After each treatment the insects were held under standard laboratory conditions prior to release as 1- to 4-day-old males. Control groups of 50 treated males and 50 untreated females were held in a cage in the laboratory to confirm the actual degree of sterility caused by the treatments. Since wild males do not mate readily in the laboratory, the effectiveness of their exposure was assayed with colony males treated similarly. Although an occasional egg hatched in these controls, virtually all of the egg batches were completely

sterile. In evaluating sterility during releases, only egg batches in which no eggs hatched were considered sterile.

The virgin females were held in the laboratory until they were 2 to 4 days old, immobilized prior to release, and sprinkled with either aluminum or bronze powder to facilitate positive identification upon recapture. Before each release the spermathecae of 20 or more females were examined for the presence of sperm. Since no sperm was found in any of these routine inspections, all released females were assumed to be virgins.

The insects were transported from the laboratory to the field in the cylindrical holding cages swathed with moist cotton pads, or in quart cardboard cartons inside a cooled, insulated box. Insect loss during transportation and subsequent handling was seldom greater than 5 percent. All releases were made between 4 and 5 p.m. in the woods either along the edge of the creek or a few yards from the edge of the field. At the release site the cover of the holding container was removed and the insects were lightly shaken out or allowed to fly out at their leisure. The personnel then left the area and returned at dusk for approximately 2 hours to make the evening collection. Additional collections were made on subsequent days.

Scattered throughout the release area were resting boxes and various natural resting sites which served as regular daytime collection stations. Occasionally other daytime collections were made from a pig shed ¼ mile south of the release area. Evening collections were made by stationing three to four vehicles and men at regular intervals in the field along the south side of the release area. As the mosquitoes left the woods at dusk, they alighted on the automobiles and were easily captured alive with a hand or battery-powered aspirator. Occasionally evening collections were similarly made at the northwest corner of the area underneath a railroad trestle upon which the mosquitoes rested.

Mosquitoes collected in the field were put directly into holding cages and later offered a human blood meal prior to confinement in individual oviposition vials. It was possible with the vial technique to determine accurately the hatchability of eggs from individual females. Since *quadrimaculatus* eggs hatch approximately 2 days after deposition, a waiting period of 5 days after oviposition was considered sufficient; furthermore, uninseminated *quadrimaculatus* females oviposit only on extremely rare occasions. These factors precluded any false interpretation stemming from either incomplete embryonation or lack of insemination. Although a low incidence of multiple mating with *quadrimaculatus* females has been demonstrated under laboratory conditions (French and Kitzmiller, 1962), our studies have produced no evidence that multiple matings occur extensively in the field, since we have seen no increase in egg batches with intermediate hatch such as would occur if females mated with both sterile and fertile males. A classifying system capable of detecting such a phenomenon was used in gathering the data, but for the purpose of this report, egg batches are referred to as either fertile or sterile—a sterile batch being defined as a floating egg batch having no viable eggs.

Detinova's (1959) method of ovarian dissection was used to determine the physiological age of samples of the field-collected wild females. The technique was altered according to Schmidt and Williams (1962) by utilizing a dilute solution of alkyl-aryl-sulfonate to loosen the ovarian sheath and facilitate the separation of individual ovarioles. The numbers of follicular relics (dilatations on ovarian tube) were observed by phase microscopy to determine the physiological age.

Releases of Sterile Colony Males and Virgin Colony Females. Since previous evidence indicated that sterile colony males did not seek out and mate with wild females, the colony males were re-

leased in conjunction with virgin colony females to determine colony male behavior. The releases were conducted on mild evenings between January 31 and May 1, 1962, with enough time elapsing between releases to insure that one release would not affect another. Half (500 to 700) of the males and females were released along the creek and the other half near the field on the opposite side of the release area. The males had been sterilized either by gamma radiation, or by tepa or apholate. Results are given in table 1.

Recapture of released females varied from 0.7 percent to 12.6 percent, averaging 4.7 percent. The colony males effectively sterilized 44 percent to 69 percent of the colony females recaptured, but were ineffective against the wild females. The high sterility resulting in each of these releases indicated that the colony male mating activities were not adversely affected by any of the treatments.

Extended Release of Sterile Colony Males. Having found the colony males effective with colony females in the field, the tepa treatment was selected for use in a sterile colony male release lasting 2 weeks to investigate further the colony males' ability to mate with wild females. Prerelease sterility information was derived directly from concurrent survey studies and from the control releases cited in table 1. Between 900 and 1,400 sterile colony males were released daily along the creek bordering the release area from May 16 to May 29. On the 7th day of the release program, marked virgin colony females were released near the field on the opposite side of the release area, 150 yards from the male release sites, to gain direct information on the activity, whereabouts, and effectiveness of the released males. During and following the release program, daily collections were made of resting and biting mosquitoes. The daily releases and results are tabulated under the heading for 1962 in table 2; the summarized results are given in table 3).

Natural sterility in wild females was not increased by sterile colony males. However, when virgin colony females were released on the 7th day of the sterile-male release program, more than 90 percent of those recaptured deposited sterile egg batches. Fifty percent of the virgin colony females released 1 week after the termination of the sterile-male releases exhibited complete sterility. Thus, although the sterile colony males were effective with colony females, they apparently were not able to seek out wild females in sufficient numbers to induce detectable sterility.

Release of Laboratory-Reared Wild Females, Colony Females, and Sterile Colony Males. On June 26 and 27, 1962, sterile colony males were released in conjunction with colony females and laboratory-reared wild females to see whether the colony male could mate with the wild female if he did not have to search for her. The three groups were divided equally and released at two

Table 1.—Effect of released sterile colony males of *Anopheles quadrimaculatus* on the fertility of virgin colony females released at the same time and recaptured, and on wild females captured in the release area.

Treatment	Number released		Number of females ovipositing		Percent of egg batches sterile	
	Colony males	Colony females	Wild	Colony	Wild	Colony
Apholate	1000	1000	13	29	8	45
	1000	1000	33	18	6	44
Tepa	1050	1200	11	26	9	69
Radiation	1400	1000	15	36	13	58
Control	0	1200	14	19	0	0
	0	1450	24	9	4	11

TABLE 2.—Sterile *Anopheles quadrimaculatus* male releases and resulting sterility in captured wild females.

Day	Time	Number of males released		Number of females ovipositing		Number of egg batches sterile	
		Colony 1962	Wild 1963	1962	1963	1962	1963
1	Day	1000	450	..	..	..	..
	Night	0	0	34	14	0	0
2	Day	1000	400	6	266	0	0
	Night	0	0	12	12	0	0
3	Day	950	600	22	31	1	0
	Night	0	0	15	27	2	1
4	Day	1000	1200	18	58	1	0
	Night	0	0	26	52	0	0
5	Day	1000	850	12	52	2	1
	Night	0	0	24	41	0	0
6	Day	1000	450	6	78	1	1
	Night	0	0	11	92	0	0
7	Day	1400	350	5	81	1	0
	Night	0	0	14	18	0	0
8	Day	1200	400	3	58	1	1
	Night	0	0	20	73	2	2
9	Day	1150	900	6	29	0	2
	Night	0	0	15	50	0	0
10	Day	1150	850	10	28	1	1
	Night	0	0	25	25	1	0
11	Day	1300	1450	8	40	0	1
	Night	0	0	35	122	0	6
12	Day	1300	1500	11	45	0	0
	Night	0	0	23	23	0	0
13	Day	1200	1800	7	28	0	1
	Night	0	0	55	70	0	1
14	Day	900	2600	8	37	0	0
	Night	0	0	10	68	0	1
15	Day	0	3300	..	34	0	1
	Night	0	0	..	14	0	0
16	Day	0	1700	5	54	0	1
	Night	0	0	15	26	0	0
17	Day	0	1700	2	37	0	0
	Night	0	0	3	25	0	0
18	Day	0	0	..	19	..	0
	Night	0	0	..	26	..	0
19	Day	0	0	..	43	..	0
	Night	0	0	..	16	..	0
20	Day	0	0	25	27	0	1
	Night	0	0	92	196	0	1
21	Day	0	0	31	48	0	0
	Night	0	0	78	46	0	0
22	Day	0	0	..	40	..	1
	Night	0	0	..	16	..	0
23	Day	0	0	..	46	..	0
	Night	0	0	..	42	..	1
Total		15,550	20,500	692	2,273	13	25

points. Thus, 1,000 males and 500 females from each source were released at one spot in the woods and a similar release was made simultaneously approximately 50 yards away. Results of this study are given in table 4. The colony males were capable of copulating with the laboratory-reared wild females, as indicated by the 50 percent egg batch sterility. In spite of the small number of these females observed to oviposit, it was obvious that there was no physical barrier to prevent mating of a colony male with a wild female. The reciprocal wild male x colony female cross, of course, was demonstrated repeatedly in the control releases and in the work of Woodard *et al.* (1962). Colony males conferred sterility on more than 90 percent of the colony females collected, as was demonstrated in the previous extended release.

Extended Release of Sterile Laboratory-Reared Wild Males. The final phase of these investigations was initiated early in April, 1963, with intensive daily collections of adult wild females from the release area. The male progeny of these females were sterilized with tepa and released in the same manner as the sterile colony males in the extended 1962 release. Between 350 and 3,300 sterile laboratory-reared wild males were released daily from April 24 to May 10, 1963. Wild females were evaluated for sterility during the 19-day pre-release period, and the 23-day release and post-release period. One week prior to the release program, and again on the tenth day, virgin colony and laboratory-reared wild females were released in the study area, as in the extended release of the colony males, to gain direct information on the activity, whereabouts, and effectiveness of the released males.

The daily release and resulting sterility data are tabulated under the heading for 1963 in table 2; summarized results are in table 3. Based on individual evaluation of 3,068 egg batches during the pre-release period and 2,273 egg batches thereafter, the percentage of sterile egg batches in wild females was increased by 4.8 times (from 0.23 percent to 1.10 percent) following the sterile wild male release. Virgin laboratory-reared wild females and colony females released during the program produced egg batches of which 15 percent and 23 percent, respectively, were sterile, whereas no sterility was evident in similar females evaluated during the pre-release period.

Discussion. The failure of the 1959–60 sterile *quadrimaculatus* male-release programs might possibly have been due to any or all of the following reasons:

(1) Lack of sufficient sexual vigor on the part of the released males due to radiation damage; (2) sexual incompatibility between the released strain and the wild population; (3) behavioral deficiencies in the released males which resulted in their failure to locate the females in the natural populations; and (4) inadequate numbers of released males to overwhelm the natural

Table 3.—Sterility in *Anopheles quadrimaculatus* females collected in conjunction with sterile colony male (1962) and sterile laboratory-reared wild male (1963) releases.

Period of capture	Type of female	Number of females ovipositing		Percent of egg batches sterile	
		1962	1963	1962	1963
Pre-release	Wild	38	3068	2.63	0.23
	Laboratory-reared wild	..	5	..	0.00
	Colony	28	8	3.57	0.00
Release and post-release	Wild	692	2273	1.88	1.10
	Laboratory-reared wild	..	20	..	15.00
	Colony	126	30	92.9	23.33

male population. We offer critical evaluation of the results presented above, supplemented by the additional observations to delineate the effects of mating vigor, sexual compatibility, and behavioral deficiencies, as follows:

The ability of sterile colony males to induce a high degree of sterility in colony females in the field (table 1) demonstrated that mating activity of these males was not seriously affected by either radiosterilization or chemosterilization. Although the sexual vigor of the colony male may be reduced by radiation (Davis *et al.*, 1959), our results showed that when sufficient numbers of either radiosterilized or chemosterilized colony males were present in a population of their own kind, they could effectively reduce the fertility of the female.

Successful induction of sterility in laboratory-reared wild females in the field by the colony male when it did not have to seek out the female (table 4) indicated that the two strains were sexually compatible. Furthermore, the ability of wild males to mate with colony females in the field confirmed that there were no physical barriers to a wild x colony cross.

Although laboratory-reared wild males increased the natural sterility by 480 percent, the inability of the colony males to induce sterility in the wild females of the natural population indicated that severe behavioral deficiencies existed in these males (table 3). A close inspection of the amounts of sterility induced in the virgin females released during the male-release programs (table 3) showed that in 1962, 93 percent of the colony females were sterilized by colony males but in 1963 only 23 percent of the colony females and 15 percent of the laboratory-reared wild females were sterilized by laboratory-reared wild males. This difference occurred in spite of the fact that these females were released at a time when comparable cumulative numbers of sterile males had been released in each series—7,350 in 1962, and 6,400 in 1963.

The high degree of sterility in released colony females in 1962 indicated that overwhelming numbers of colony males (9 sterile: 1 wild) were in the immediate release area—or to phrase it another way, many of the released colony males had failed to leave the release area. The high sterility induced in colony females released 1 week after the end of the 1962 colony male release showed that many of the released males were still in the immediate release area. On the other hand, the more modest degree of sterility induced in the released colony virgins in 1963 indicated that many of the laboratory-reared wild males had flown from the release area, leaving a ratio of only 1 sterile to 4 or 6 wild males in the release area. Furthermore, the data show that these laboratory-reared wild males were sufficiently mobile to successfully locate and mate with the wild females in the natural population.

Incomplete insemination checks of the captured colony females showed the degree of insemination to be high in 1962 and low in 1963. This finding indicated that even in 1963, when the general wild population was 2 to 3 times larger than in 1962, relatively few males were present in the release area. Thus, the sterility, density, and insemination evidence pointed to an extremely immobile colony male and to a highly mobile laboratory-reared wild male.

Table 4.—Sterility resulting in wild, laboratory-reared wild, and colony females following release of sterile *Anopheles quadrimaculatus* colony males.

Type of female	Numbers of females collected	Numbers of females ovipositing	Percent of egg batches sterile
Colony	129	96	93
Laboratory-reared wild	20	6	50
Wild	696	31	4

Behavioral differences were also apparent in released females. Most significant was the low recapture rate of laboratory-reared wild females as compared with that of colony females (table 4). The recapture rate of laboratory-reared wild females, less than a sixth that of colony females, was typical of casual observations made during the 2-year study. This phenomenon was probably due both to a superior natural mobility of the wild females and their observed lack of attraction to human hosts for feeding. The latter observation was confirmed by Col. R. M. Altman (personal communication), who assayed the feeding behavior of this population and out of 184 positive-precipitin tests found none of the females to have fed on humans. Although these findings did not necessarily indicate preference rather than host availability, the personnel on this project were seldom bitten in the field by wild *quadrimaculatus* females but often by marked laboratory females.

Thus, it appeared that, under field conditions, the released sterile colony males were sexually vigorous and competitive with wild males for females of their own kind (colony females) and also were sexually compatible with wild females. However, their lack of mobility, and possibly other factors, prevented successful completion of their reproductive responsibilities in the field. It is unnecessary to speculate on the fourth factor—whether the wild male populations were sufficiently overwhelmed in the 1959–60 sterile-male release programs—since it should be evident that a significant degree of intermating would not have occurred anyway.

Although this project was not designed to control or eradicate *quadrimaculatus*, it was quite evident that the release of 20,500 sterile males in a small sector of a large breeding area had very little practical effect on the total population. This result was due to many factors, two of which are worthy of comment. First, 36 percent to 53 percent of the natural population sampled was made up of females which had already completed 2 or more gonotrophic cycles (table 5) and thus may not have been available for sterilization at any time during the extended release programs. Only by a prolonged release program could one hope eventually to reach all the individuals of a population at a time when they were susceptible to induced sterility. Secondly, because of the mobility of the female population, there was a massive dilution factor involved in the capture of sterilized females. This factor was evident in the recapture of laboratory-reared wild females both in 1962 and 1963. Probably most of the sterile females leave the immediate area and their effect is thus diluted, and further dilution would occur because of the

Table 5.—Physiological age of wild *Anopheles quadrimaculatus* females captured during extended sterile-male release programs.

Night collected after release of males	Number of females evaluated	Percentage of females having completed ovarian cycle				
		0	1	2	3	4
1962						
4th	2	0	50	50	0	0
7th	8	0	63	25	12	0
9th	7	0	29	57	14	0
Total	17	0	47	41	12	0
1963						
13th	25	32	40	20	8	0
14th	15	13	47	40	0	0
17th	23	35	22	35	0	8
Total	63	29	35	30	3	3

rapid movement of unsterilized females into the release area.

The original colonization of the species by Boyd (1930) was achieved only after years of concentrated effort. It is significant that the major barrier to colonization was the inability of the species to mate in confinement. It is now evident that successful colonization created a strain no longer able to move afield and find mating partners in the wild. It is doubtful whether the phenomenon of significant behavioral change resulting from colonization is unique to *quadrimaculatus*.

One can speculate that other species which prove particularly difficult to colonize because of flight or mating behavior may not be suitable for the sterile-male release approach to control. Furthermore, the methods described herein may provide a suitable technique for evaluating the sexual prowess of the sterile male.

SUMMARY. Through the concurrent release of sterile males and virgin females, it was demonstrated that sterilized males of the colonized Orlando strain of *Anopheles quadrimaculatus* were sexually vigorous and compatible with females in nature. However, the colony males were unable to induce sterility in the natural population because of behavioral deficiencies brought on ostensibly by the colonization process. The first-generation male progeny of females from the natural population successfully induced sterility in the natural population.

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