STERILE MALES FOR MOSQUITO CONTROL: A FIELD CAGE STUDY WITH *CULEX PIPIENS QUINQUEFASCIATUS*

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Ramakrishnan *et al.* (1962) were able to suppress populations of *Culex pipiens fatigans* Wiedemann (called *quinquefasciatus* Say in the United States) that were breeding in laboratory cages by introducing males sterilized by gamma irradiation; however, this technique was not successful in field tests. Krishnamurthy *et al.* (1962) could not reduce larval or adult populations of *Culex* in field tests when they released sterile males, even though the number of infertile egg rafts increased slightly.

LaBrecque (1961) and Weidhaas *et al.* (1961) reported the effectiveness of chemosterilants for the suppression of house flies, *Musca domestica* L., and mosquitoes, *Anopheles quadrimaculatus* Say and *Aedes aegypti* L., in the laboratory. Murray and Bickley (1964) conducted a series of basic studies of the effect of apholate on *Culex pipiens quinquefasciatus* and showed that sterile males exposed to 10–15 p.p.m. of apholate as fourth instar larvae were sexually more competitive than normal males. Mulla (1964) found that adult mosquitoes exposed continuously as fourth instar larvae to 10 p.p.m. apholate laid eggs which were 96 percent infertile; mosquitoes exposed to the same concentration of tepa and metepa laid eggs that were 16 and 5 percent infertile, respectively. Also, adult males and females fed for 24 hr. on a concentration of 0.01 per-

cent apholate, tepa, or metepa in solutions of sugar-water produced no live progeny. Sakakibara (1966) found that ad libitum feeding on a 0.01 percent metepa-sugar solution caused sterility in adult *Culex pipiens pallens* (Coquillett).

Lewallen *et al.* (1965) made three applications of apholate (75 p.p.m. each) at 10-day intervals to a small desert pool infested with *Culex tarsalis* Coquillett. Most of the egg rafts collected were sterile at the end of the experiment; however, the population returned to normal within a month after termination of the test. They inferred that the population built up so rapidily because the three treatments did not reach all the overlapping generations.

The objective of this present study was to attempt control outdoors of a limited caged population of a wild strain of *C. p. quinquefasciatus* with sterile males. The use of a large outdoor cage permitted us to obtain seminatural breeding and activity; the confinement of the mosquitoes made it possible for us to make detailed observations on mating activity and biology.

METHODS. The cage had a redwood frame that was covered by 20-mesh nylon screening; it was shaped like a Quonset hut. Its base dimensions were 20 x 14 ft., and its maximum height was 11 ft. A double-screen partition at about the middle divided the cage so that one-half could be used for the test population and the other half for the check population. The facilities were basically the same in each half of the cage and consisted of a 4 x 2 ft. x 8 in. pond lined with plastic and sod, a small metal cage for chickens, and a wooden resting box. Most mosquitoes actually rested during the day behind 4 x 8 ft. pieces of plywood that leaned against the screen partition and were intended as covers for the ponds to prevent overflow during heavy rains.

Chickens, usually two per cage about a month old, were the only source of blood for the mosquitoes. Cotton pads soaked in a 50 percent honey solution were placed periodically in the cages to provide an additional source of nutrition for the adult mosquitoes. About every 2 weeks, a handful of chick starter mash was thrown into the ponds. The resulting infusion acted as a rich medium for larval production.

The females readily oviposited in the ponds, and larval production was good. Overproduction was such a problem that the population in the cage had to be limited by removal of egg rafts to keep it workable. Unfortunately, throughout the time sterile males were being released, both control and test mosquitoes had an infection of *Entomophthora coronata* (Cost.) Kevorkian. Just what effect this disease had on the population is unknown, but it should have been about the same in both populations. A more troublesome effect was the manner of death of diseased adults; they die in a normal resting posture and are held to the surface by the fungus mycelia. Thus, meaningful counts of adults were hard to obtain, and the biotic potential of the populations had to be based on counts of pupae. Ten samples were taken daily from each pond with a standard water dipper after it was determined that the number of pupae picked up in 10 dips from each pond represented about one-third the total number present. Then, on the basis of the number of pupae, the daily emergence of males was estimated by dividing the number of pupae by four (since it takes about 2 days for pupae to mature and approximately half the emerging adults were females).

The mosquitoes that were to be sterilized and released were reared in the laboratory at a constant temperature of 80° F. and 60 percent R.H. Each day throughout the test, a 44 x 18 x 3 in. wooden tray was set up with 20–30 egg rafts, 8 liters of water, and 2 grams of a mixture of liver extract and dried brewer's yeast. After 3 days, more liver and yeast and some finely ground laboratory chow were added. By this method, development to the pupal stage took from 6 to 8 days. Rapid separation of larvae and pupae was accomplished by the ice water technique (Haz-

ard, 1967), and the pupae were sexed with a pupae separator (Fay and Morlan, 1959). Cages of male pupae containing 2 percent sugar solution on cotton were placed in the rearing room and held until 2 days after emergence. After this 48-hr. period, the males were inactivated in the cold room, counted, placed in a waxed paper cup, dusted with apholate, and then returned to the rearing room and held another 24 hrs. before release. The daily release of sterile males that began September 6 usually took place in the late afternoon near to the breeding pond. There was normally very little male mortality from the apholate treatment prior to release.

Periodically, during the releases, checks were made of the efficiency of sterilization by placing treated males with virgin females. The egg rafts from this cross were then checked for percentage hatch.

The mating effectiveness of the sterile males was determined by collecting egg rafts from the ponds and determining their fertility. The rafts were set up individually in 3-oz. waxed paper cups containing tap water. A week later, the eggs from each raft were checked for hatch under the microscope. For easier counting and checking, each raft was placed in a 1 percent solution of KOH for a few hours to separate the individual eggs.

It was noted that many of the sterile males survived for only a day or two after release. Due to the lateness in the season and lack of available facilities, actual survival time was not checked until the following summer. These survival tests consisted of exposing groups of 100 3-day-old sterile males and normal females for 3 days in twelve 4 x 8 x 6 ft. outdoor cages, each containing a black resting box, a cup with cotton soaked in 2.5 percent sugar solution, and a pan of water. After 24, 48, and 72 hrs., all the live mosquitoes in four cages were collected with a small battery-powered aspirator, and the numbers of males and females were recorded. Also, the number of inseminated females was determined by examining the spermathecae for sperm.

RESULTS. Table 1 shows the results. No natural sterility was found in the five

TABLE 1.—Population, release, and sterility after releases of sterile male *C. p. quinquefasciatus* in large screen cages. 1966.

Date (week starting)	Release cage					Check cage		
	Approx. no. of new males emerging per day	No. of sterile males released per day	Theoretical ratio normal: sterile males	No. of egg rafts collected	% Sterile egg rafts	Approx. no. new males emerging per day	No. egg rafts collected	% Sterile egg rafts
July 31	...	....	...	4	0	...	4	0
August 8	...	....	...	5	0	...	5	0
14	...	....	...	5	0	...	5	0
21	...	....	...	5	0	...	5	0
28	...	....	...	10	0	...	10	0
September 4[a]	61	1,000	1:15	10	0	109	10	0
11	53	400	1:10	10	40	69	10	0
18	38	250	1:5	20	15	41	10	10
25	15	500	1:30	20	25	38	10	0
October 2	125	1,000	1:10	16	50	109	10	10
9	76	1,000	1:15	27	52	60	12	0
16	34	1,000	1:30	20	55	22	14	7
23	51	1,000	1:20	23	48	81	10	10
30	25	1,000	1:40	27	56	56	10	10
November 6[b]	25	....	...	29	21	33	20	10
13	44	....	...	17	47	65	12	0

[a] Sterile male release began.
[b] Sterile male release ended.

samples from each population checked before the first release was made September 6. About 2 weeks passed before the first sterile egg rafts were detected. For the first month, while the ratio of sterile to normal males averaged about 15:1, the sterile egg rafts averaged only 27 percent of the total. In the natural population, one egg raft of 40 was sterile during this same period. During the next month, the ratio of sterile to normal males increased to about 23:1, and the average percentage of sterile egg rafts increased to 52 percent; also, during this time, about 7 percent of the egg rafts from the natural population were sterile. Two weeks after the last release of sterile males, sterile egg rafts were still being picked. Obviously, the sterile males reared in the laboratory were not competing favorably with normal males in the seminatural environment since, theoretically, the sterility during the last month of the test should have approached 95 percent. No positive explanation can be given for the lack of competitiveness. However, on the basis of the subsequent survival tests, behavior and lack of hardiness of the sterile male were probably the primary reasons for the low degree of competition demonstrated.

The survival tests showed that after 24 hrs. in the cages, only 44 percent of the sterile males were alive, and only 25 percent of the females were inseminated. After 48 hrs., 30 percent of the males were alive, and 80 percent of the surviving females were inseminated; at the end of the test (72 hr.), 25 percent of the sterile males were alive, and 97 percent of the females were inseminated. Thus, mortality of released males accounted for some of the discrepancy between the theoretical and actual sterility obtained. Another important factor was the difficulty in determining the number of normal males emerging each day. Dipping and counting pupae accurately is difficult, especially since the pupae are never evenly distributed over the surface of the pond and dive under the water quickly when they are disturbed. Thus, we probably underestimated the normal male emergence.

All egg rafts obtained from the laboratory cross of sterile males and virgin females were sterile. These results substantiated the effectiveness of the sterilizing technique.

Although we did not obtain proof of the practicality of sterile male releases for control of *C. p. quinquefasciatus* in the field, we were able to refine techniques and uncover some problem areas that must be resolved before success can be achieved. The most obvious necessity is a good, standardized mass rearing technique that will produce hardy male mosquitoes. However, information on the dispersion and survival of naturally breeding mosquitoes is also a serious lack. It appears to us that research must be concentrated on these problems before control with sterile males can be achieved.

SUMMARY. Sterile males were released in an attempt to control a population of *Culex pipiens quinquefasciatus* reproducing naturally in a large outdoor cage. During the second month of release, the ratio of sterile to normal males was 23:1, and the average sterility of egg rafts was 52 percent. The test was discontinued after 9 weeks because of cold weather, even though complete sterility was not achieved. The lack of normal competitiveness of the sterile males was probably caused by poor survival after release based on survival tests conducted the following summer in which only 44 percent of the sterile males survived outdoors after 24 hr. of exposure in 4 x 8 x 6 ft. cages.

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