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THE STERILE-MALE TECHNIQUE FOR CONTROL OF MOSQUITOES: A FIELD CAGE STUDY WITH *ANOPHELES QUADRIMACULATUS*

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ABSTRACT

The use of sterile males to control a cage population of a laboratory strain of *Anopheles quadrimaculatus* was not successful, even though 80 to 90% of the females laid sterile egg clutches. At the ratio of sterile males to normal males being used, complete sterility should have been quickly achieved. Subsequent studies of the survival and sexual vigor of these sterile males indicated that only a small number, about 5%, of the released males were actually competing with normal males for the uninseminated females. Thus, until a more hardy and sexually vigorous male *A. quadrimaculatus* can be reared in the laboratory, the use of the sterile-male technique to control this insect seems impractical.

Research was begun several years ago to adapt the technique of releasing sterile males to the control or eradication of mosquitoes. Davis et al. (1959) showed that doses of 8,865 to 12,000 R applied in the adult or pupal stage caused complete sterility in males and females of *Anopheles quadrimaculatus* Say. However, in the laboratory, males sterilized by gamma irradiation were not as competitive as normal males in inseminating females, although at high ratios of sterile to fertile males (6:1 and 10:1) production of viable eggs decreased more than 80%. In contrast, Weidhaas et al. (1962) were unsuccessful in either suppressing natural populations of *A. quadrimaculatus* or in causing a significant degree of sterility in wild females by the release of males sterilized by gamma irradiation. Also, when Dame et al. (1964) studied the behavior of released sterile *A. quadrimaculatus* from the laboratory colony and that of wild mosquitoes in the field, they concluded that the behavioral deficiencies of colony males prevented them from effective sexual competition for wild females though they were capable of mating effectively with released colony females.

To gain a better insight into the problems associated with the release of sterile male mosquitoes, we decided to release sterile males into a population of *A. quadrimaculatus* breeding in a large outdoor cage. By using the caged population, we could study both the efficacy of the induced sterility and also the behavior and survival of a strain exposed to semi-natural conditions. The regular Gainesville laboratory colony of *A. quadrimaculatus* was used as a source of the sterile males and to start the cage population since it was difficult to establish a wild strain in the cage (several attempts had little success).

METHODS AND MATERIALS

The cage used for the experiment was 40 x 16 ft, and 12 ft high, and consisted of an aluminum frame covered by 20-mesh plastic screen. It

was located in a wooded area near the Insects Affecting Man Laboratory, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, Florida. A wooden shed, 12 x 8 ft by 7 ft high, was built within the cage, and a calf stabled in this shed throughout the experiment as a blood source for the female mosquitoes. The shed also served as the main resting area for engorged females, which made it relatively easy to estimate the adult female population. Cotton pads soaked in honey and water were placed in the cage and changed weekly to provide nutrient for the males. Also, 2 plastic-lined ponds (5 x 6 x 2/3 ft), a stainless steel tank (4 x 3 x 2/3 ft), and three wooden trays (3 2/3 x 1 1/2 x 1/4 ft) filled with water were installed to serve as oviposition and breeding sites. In addition, about 2 inches of soil was put on the bottom of each of the two plastic-lined ponds, and about five clumps of grasses and reeds obtained from a nearby swamp were planted in the soil. A small amount of soil and leaves was added periodically to the stainless steel tank to act as infusion media in the tap water. Although the females readily laid eggs in these containers, larval development was poor. Therefore, periodically throughout the experiment, a mixture of dried liver extract and brewer's yeast was added to the ponds with the daily supplement of finely ground laboratory chow. Despite this, larval production remained so poor that only about 1% of the progeny from these eggs ever reached adulthood. It became apparent after 2 months that the colony strain of *A. quadrimaculatus* could not maintain itself at a high level under these conditions. Moreover, the number of larvae decreased after the first few generations, indicating that something was being depleted from the water or that unfavorable elements were accumulating that prevented development.

To increase production, we set the three wooden trays on a rack under a leanto on the back side of the calf shed and added distilled water infused with liver extract and yeast. The female mosquitoes rarely oviposited in these trays, but larvae placed in them developed readily with little mortality. As the larvae matured, finely ground laboratory chow was sprinkled on the surface of the water as a supplemental food source. The trays had several advantages over the ponds, among them the increased production of adult mosquitoes and the ease with which pupae could be counted; also, these pupal counts permitted us to make a more accurate estimate of the daily adult emergence. The trays could be easily washed, and, since they were set up at staggered intervals, they could be cleaned after each generation and set up again without greatly interfering with overall adult production. Therefore, the best method of achieving a workable population of *A. quadrimaculatus* for this study was to use the two plastic-lined ponds and the stainless steel tank primarily for egg deposition and the trays for rearing the larvae to adults. We gathered the eggs daily by using a common water dipper (running it along the rim of the ponds and tank) and then transferred them to the trays.

As noted, the laboratory colony of *A. quadrimaculatus* also served as the source of sterile males for release in the cage. These larvae were reared on a diet of dried liver extract, brewer's yeast, and finely ground Purina laboratory chow. The pupae were held in waxed paper cups in cages, and cotton soaked in a 2.5% sugar solution provided food for the

emerging adults. Two days after emergence, these mosquitoes were inactivated in a cold room and sexed, and the males were sterilized by dusting with apholate. The dusting was accomplished by placing about 100 males in an 8-oz waxed paper cup and sprinkling about 1 g technical apholate over them. The cup was gently rotated at an angle to insure an even distribution of the material on all the insects, and then removed from the cold room and placed in a small cage in a room at 85°F and 60° relative humidity. Once the males had recovered, a cup containing a 2.5% sugar solution on cotton was placed in the cage. The sterilized males were held in the laboratory for 24 hr to check for any excessive mortality. Normally little mortality occurred.

The releases of the sterilized males were usually made daily in the late afternoon. First the cloth sleeve of the small cage was turned back, and the waxed paper cups containing the technical apholate and the sugar solution were removed. Then the cage was placed just above the rearing trays under the leanto. By morning, all the live males had left the smaller cage and migrated to resting areas around the calf shed. General observations on total numbers of males present at any given time in the cage indicated that the majority problably did not survive more than 2 to 3 days. Since these sterile males were 3 days old at the time of release and were released near newly emerging females, they should have had ample opportunity to mate.

Population estimates were made by daily counts of all engorged resting females and pupae present in the rearing trays. The three ponds normally averaged 20 pupae per day throughout the experiment; however, pupae were extremely difficult to locate in the ponds. The ratio of sterile to nonsterile males was established each day from the count of pupae divided by four (about 48 hr is required for pupae to mature and half the newly emerging adults are females).

Weekly, as many as 3 dozen engorged females were taken from the calf shed with an aspirator and placed individually in 10-dram vials containing 5 ml of distilled water. A waxed paper ring about 1/8 in. wide and the same diameter as the vial was floated on the water surface to prevent surface tension from pulling the eggs up the sides of the vial where they would dessicate. After a week, the percentage of hatched eggs was determined. As a check on the handling procedures, engorged females obtained from the laboratory colony were placed in vials, and the viability of their eggs was determined in the same manner. Also, periodically throughout the program, sterile males were removed from the holding cage and placed with control virgin females. After 4 days, these females were blooded on a guinea pig, and their eggs were also assayed for sterility.

As noted previously, most males survived only a few days, but no facilities were available to evaluate the effect of this factor on the results. In addition, the experiment had to be discontinued before it was completed because of cold weather. We therefore found it advisable to develop a technique for determining the actual longevity of the sterilized, released males at comparable conditions. Twelve outdoor cages, 4 x 8 x 6 ft, were each stocked with 100 three-day-old sterile males and 50 three-day-old virgin females from the laboratory colony of *A. quadrimaculatus*.

Each cage contained a black resting box, 2.5% sugar solution, and a pan of water. After 24, 48, and 72 hr, all the live mosquitoes were collected with a small hand battery aspirator from four cages, the number was recorded, and the females were dissected and their spermathecae were checked for sperm, indication of successful insemination.

RESULTS

Table 1 summarizes the data. So many outside factors obviously affected the population that it was almost impossible to evaluate suppression of the population (short of almost complete eradication). Any variation in the care and handling, for example, the number of eggs transferred to the trays or the amount of food used, influenced the number of larvae that survived. Also, the test was conducted during September and October, and the cool weather undoubtedly affected the survival of the adults. The removal of as many as 3 dozen females each week from a fairly small population may have influenced the size of the total population, especially since complete sterility was never nearly achieved for any length of time. Thus, the number of females that laid sterile egg batches was the only true indication of the effectiveness of the sterile males, but because the method of inducing sterility was not always 100% effective, the percentage of sterile females could only be based on the number of females laying normal free-floating egg clutches in which less than 10% of the eggs hatched. Many clutches from sterile females contained fewer eggs than the nonsterile clutches.

Between the second and fifth weeks of the test while the ratio of sterile to nonsterile males ranged from 10:1 to as much as 40:1, the percentage of females that laid sterile eggs ranged from 40 to 65. For the first 2 weeks after the ratio was increased to 100:1, the percentage remained about the same (62 to 67%), but the last 3 weeks of the test, it increased to better than 80%. At the release rate of 100:1, the theoretical number of sterile females should have been 99% if the sterile males had been competing favorably. Obviously they were not, even though they were sexually mature at the time of release (3 days old). Indeed, at this rate, even if only half the released males were competing adequately, almost all the egg clutches should have been sterile.

No positive explanation of the results can be offered, but the male survival tests conducted after the release experiment indicate that the lack of hardiness of the colony-reared males is the logical cause. In these survival tests, only 50% of the males and 78% of females survived 24 hr, 7% of the males and 48% of the females survived 48 hr, and less than 1% of the males and 4% of the females survived 72 hr. After 24 hr. only 3% of the surviving females were inseminated. Then since only 7% of the males were surviving after 48 hr and less than 1% after 72 hr, the number of additional females that could have been inseminated was low. If we assume a value of 5% insemination, only 5% of the males released in the cage could have been competitive with normal males. The theoretical ratios of sterile:nonsterile males based on this figure have been computed and are reported in the table. The degree of sterility obtained (based on these figures) was close to the theoretical value (83% theoretical vs. 80 to

Week starting	Population data for cage: Avg. No. Females or pupae observed each day: Females	Population data for cage: Avg. No. Females or pupae observed each day: Pupae	Population data for cage: Approx. No. new males emerging per day	Release data: No. sterile males released per day	Release data: Theoretical ratio non-sterile to sterile males based on— Actual No. released	Release data: Theoretical ratio non-sterile to sterile males based on— Probable No. sexually active	Sterility data for females from— Cage: No. of ♀♀	Cage: No. of egg clutches	Cage: % Laying sterile clutches	Lab Colony: No. of ♀♀	Lab Colony: No. of egg clutches	Lab Colony: % Laying sterile eggs
July												
6							12	9	11	11	11	9
13							12	7	0	12	12	8
20							12	11	9	12	10	0
27							12	9	16	12	12	25
August												
10							12	7	0	12	10	0
17										12	6	0
27							5	3	0	15	12	8
					Sterile male release begun							
31	49	33	8	100	1:10	1:0.6	12	8	8	12	11	0
September												
7	63	132	33	250	1:10	1:0.4	12	8	50	12	8	13
14	109	48	12	500	1:40	1:2	18	10	40	12	7	0
21	118	119	30	500	1:20	1:1	19	14	64	20	13	15
28	163	93	23	500	1:20	1:2	26	20	65	15	9	22
October												
5	159	44	11	1,000	1:100	1:5	29	21	62	20	13	0
12	174	33	8	1,000	1:100	1:5	20	18	67	20	17	6
19	199	35	9	1,000	1:100	1:5	36	19	90	20	14	14
26	156	32	8	1,000	1:100	1:5	32	20	80	20	16	0
November												
2	81	38	9	1,000	1:100	1:5	21	12	83	20	18	11
					Sterile male release terminated							
9	59	81	20				30	4	50	20	12	33

*Calculated on the basis that only 5% of the males released were sexually active. See discussion on survival tests for further explanation.

90% actual during the last 3 weeks of the experiment when the ratio of sterile to nonsterile males was 5:1).

A check cage of sterile males and nonsterile female mosquitoes was held in the laboratory for 3 days while the survival tests were made. Of these, 69% of the sterile males survived for 3 days and 82% of the females. Thus, the poor survival in the outdoor cages was undoubtedly caused by the rearing conditions for the larvae in the laboratory and the handling of the adults before they were placed in the outdoor environment.

Tests made during the experiment to verify the effectiveness of the apholate treatment showed that more than 97% of the females mated to apholate-dusted males laid sterile egg masses. Natural sterility in females from the laboratory colony was checked each week and found to range from 0 to 33% with an average of 8%.

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