STERILE MALES: THEIR EFFECT ON AN ISOLATED POPULATION OF MOSQUITOES [1]

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Scientists have recently been greatly interested in the sterility technique as a possible alternative to the use of pesticides in controlling specific insects because of the successful use of the technique to eradicate the screwworm, *Cochliomyia hominvorax* (Coqueral) from Curaçao and the southeastern United States, according to Baumhover *et al.* (1955) and Knipling (1959).

As a result, we have applied this technique to the control of *Culex pipiens quinquefasciatus* Say (*C. p. fatigans*). Prior to our work only a few laboratory studies, Ramakrishnan *et al.* (1962), Mulla (1964) and Murray *et al.* (1964) and two field tests have been reported with this species. In one test males sterilized by gamma irradiation could not compete with the normal males and thus, a low degree of sterility was obtained by Krishnamurthy *et al.* (1962). The second study by Laven (1967) demonstrated control of a field population with males sterilized by the cytoplasmic incompatibility technique. This paper reports the first successful use of males sterilized by a chemical to induce a high degree of sterility in a field population of mosquitoes.

Seahorse Key and North Key, which are two islands in the Cedar Key National Wildlife Refuge were selected as the site of the study. They are separated from other islands and the mainland by 2 miles of salt water and the prevailing winds are from the Gulf of Mexico. Thus the islands are semi-isolated and there was little chance of mosquitoes migrating from the mainland. Also neither island has any natural bodies of fresh water though both contain discarded trash that is capable of holding rain water. No open freshwater wells were found around old homesites, but on Seahorse Key, location of the University of Florida Marine Laboratory there is a well, a septic tank, and two cisterns for fresh water. The two islands

[1] Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U. S. Department of Agriculture.

are covered with dense woods, mangrove swamps, sawgrass, and sand beaches; Seahorse Key has a T-shaped clearing running from the dock and the Marine Laboratory up the hill to an old lighthouse. Bird life is abundant and there are large rookeries of white ibis, brown pelicans, cormorants, and frigate birds. In the spring, *C. p. quinquefasciatus* and *C. salinarius* Coquillett were found breeding on Seahorse Key, but no *Culex* species were found on North Key.

The tests were started on August 1 when the nesting season of the birds was over, and the populations of *C. salinarius* had almost disappeared. Thus. *C. p. quinquefasciatus* was the principal fresh-water mosquito present on the island and the larval breeding sites appeared to be limited to the area between the dock and the lighthouse and consisted of the septic tank and assorted trash. No larvae were found in the fresh-water cisterns.

Mass rearing of the mosquitoes was begun on the island a week before the first release was to take place. Each day, four to six 10-gallon plastic tubs containing 3 gallons of water to which was added a mixture of equal parts of liver powder, dried brewer's yeast, and a hog supplement were set out beneath the Marine Laboratory. Then, 2,000 first instar larvae per tub (obtained from fertile egg rafts collected on Seahorse Key) were introduced into this infusion, and when the larvae were in the second instar, fresh food was added daily at the rate of 0.5 mg per larva. When the larvae pupated (within 6–8 days), they were separated from the larvae by the ice-water technique described by Hazard (1967) and sexed in a pupae separator designed by Fay and Morlan (1959). (The males usually pupated first, so the remaining larvae, predominantly females, were either discarded or held as an additional source of eggs for stocking the rearing tubs.) Then the male pupae were counted and placed in lots of 1,000–1,500 in 2 liters of water in 1-gallon waxed-paper containers. Subsamples of about 100 pupae checked daily to evaluate the efficiency of the sexual separation showed that usually females made up less than 5 percent of the insects released.

The mosquitoes were sterilized in devices made up of 4-inch lengths of ½-inch black polyvinyl tubing packed tightly into aluminum collars 4 inches deep and 8 inches in diameter. These honeycomb-like devices were dipped in a 5 percent solution of tepa in methanol for a few minutes and then allowed to dry for at least 2 hours. When they were dry, the treated "honeycombs" were placed over the pupae in the waxed-paper containers and the containers labeled and placed behind the rearing tubs. Emerging males normally rested on the under side of the honeycombs until dusk and then crawled through the tubes where they absorbed enough tepa through their tarsi to render them sterile; females passing through the same tubes usually were not completely sterilized. Most adults emerged and flew away within 48 hours after the pupae were placed in the containers.

Seven oviposition sites, each a 10-gallon plastic tub containing 3 gallons of a liver-yeast infusion, were placed between the lighthouse and the dock area. Prior to the sterile male releases, a portion of the rafts from the ovitraps was removed, placed in individual 5-dram plastic vials containing 5 ml of water, and observed daily for 4 days for larval emergence. The remaining rafts were allowed to complete their life cycle in the trap. Following releases, all egg rafts were collected, checked for sterility, and the larvae used in the mass rearing described previously.

The releases on Seahorse Key were continued for 8 weeks and terminated September 27; however, egg rafts were collected daily until October 16 when the stormy weather caused by a hurricane made it impossible to reach the islands from the mainland.

North Key, which had no indigenous population of *Culex* was used as a check by establishing a colony of *C. p. quinquefasciatus*. The same rearing, collecting, and assaying procedures were used as on Seahorse Key.

The daily fluctuations in egg rafts de-

posited, the number of sterile males released, and the sterility of the eggs were minimized by pooling the daily counts for each 2 weeks and calculating a daily average for that period. Two weeks was chosen because a generation of *C. p. quinquefasciatus* is normally completed in about 2 weeks in the summer in Florida. The results of the study are summarized and recorded in Table 1.

TABLE 1.—The effect of releasing sterile males into a natural population of *Culex pipiens quinquefasciatus*.

Generation	Avg. no. of sterile males released per day	Avg. no. of rafts collected per day	Percentage sterility
1		22	0
2		30	0
3		104	0
4	2,600	144	31
5	2,500	44	61
6	2,750	48	75
7	2,250	58	85
8		46	51

Before the release of the sterile males, the average number of egg rafts per day increased one and one-half to threefold each generation. Then during the second generation after releases were started (fifth generation sampled), the number of egg rafts per day decreased by 70 percent; thereafter, it remained relatively constant. No sterile egg rafts were found prior to the releases on Seahorse Key, and no sterile egg rafts were ever found on North Key.

The percentage of sterile egg rafts on Seahorse Key increased steadily from 0 before releases to 85 percent for the fourth generation after releases were started (seventh generation sampled) and then dropped to 51 percent for the first generation after the releases were stopped (eighth generation sampled). However, the percentage sterility (31 percent) found in the first generation after the releases were started was not high enough to cause the observed reduction in the number of egg rafts. Therefore, the reduction must have resulted from the combined effect of the sterile males and the removal of egg masses from the population.

The leveling off of the population after the initial large decrease was, in our opinion, the result of an increase in the survival potential of the population through some such mechanism as a higher rate of survival of larvae in their breeding sites because of decreased density of population. Still, despite any such improvement in survival potential, the percentage sterility continued to increase from generation to generation. Although the experiment had to be discontinued, the theoretical models of the sterile-male release concept predict that the population could have been eliminated.

During the 8 weeks of releases, we collected a total of 4,116 egg rafts but we do not know what percentage this was of the total because we were not able to determine the total number laid. In planning the test, we had been concerned that the removal of a high proportion of egg rafts might, in itself, suppress the population. However, a large number of egg rafts continued to be produced, and the fertility was observed to increase the generation after the releases were terminated, even though we continued to remove egg rafts.

Since studies in outdoor cages indicated that the sterile *Culex* males were equally competitive with indigenous males, we were able to calculate the size of the indigenous population based on the number of sterile males released and the degree of sterility achieved. During the entire 8 weeks, 141,400 sterile males were released, an average of 35,600 per generation or 2,500 per day. Our best estimate of the initial ratio of sterile to wild males is therefore based on the percentage sterility obtained in the second generation after releases were started. Average sterility during this generation was 61 percent, which approximates an initial ratio of sterile to wild males of 2:1. Therefore, with a sex ratio of 1:1 about 1,250 wild males and 1,250 wild females per day (17,500 of each sex per generation) were entering the population initially. We then used the data on sterility for the last three

generations sampled (five through seven) and the average number of sterile males released to estimate the total number of wild males and females that entered the population during the test. (Since mated females were already present in generation 4, estimates cannot be made for this generation, but we have assumed that the number was the same as for generation 5.)

With a sex ratio of 1:1, the number of wild males emerging per day or generation equals the number of females emerging per day or generation. Then about 53,000 males and 53,000 females emerged during the 8 weeks. See Table 2.

We also found another way to use the data to estimate the total number of mosquitoes entering the population. In previous field cage tests we conducted, we noted that only about 20 percent of the females emerging over several months into the large outdoor cages were observed to oviposit, apparently because of mortality during the time required for mating, blood feeding, and egg development. In natural populations, this percentage should be even lower. If only 5 to 10 percent of the females survived to oviposit, about 40,000 to 80,000 females emerged during the 8 weeks of releases. This estimate brackets the results obtained by the original estimate of 53,000.

ual from the time releases were started through the third generation of releases because the degree of sterility increased each generation though the number of egg rafts collected per day remained relatively constant or increased only slightly.

In our experiment, the males were released at one central point on the island, and the natural breeding sites were not more than 130 yards from the point of release. Thus, the males and females did not travel more than that distance to come together to mate. It will be extremely important to determine the maximum or average distances over which males and females can come together for mating.

ABSTRACT

Chemosterilized males of *Culex pipiens quinquefasciatus* Say released into a natural population at ratios of 2–5.7 to 1 caused a consistent increase in sterility over an 8-week period. Although the experiment had to be discontinued because of unfavorable weather conditions, theoretical models predict the population could have been eliminated.

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TABLE 2.—Data showing how estimates of numbers were made.

Generation	Percent sterility	Ratio of sterile to wild males	No. of wild males emerging per day	Total no. of males emerging
4	31			17,500
5	61	2:1	1,250	17,500
6	75	3:1	833	11,600
7	85	5.7:1	440	6,200
				52,800

If we knew precisely the percentage of egg rafts removed from the total population, we could also calculate the rate of increase of the natural population from generation to generation during the releases. However, increase from one generation to the next must have been gra-

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