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MATING COMPETITIVENESS OF CHEMOSTERILIZED MALE SOUTHERN HOUSE MOSQUITOES TREATED WITH TEPA[1]

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ABSTRACT. Male *Culex pipiens quinquefasciatus* Say sterilized as they crawled through polyvinyl tubes treated with tepa were competitive with normal males in outdoor cages; however, the data suggested that they might not survive as long as normal males. Also, the males were competitive whether, the ratio of sterile males to females was 9:1 or 1:1. Both laboratory-reared and outdoor-reared sterile males were competitive with normal males (eradication of caged population was achieved with both types of males when the ratio of sterile to normal males was 100:1). The caged populations had a rate of biotic increase greater than 29X but less than 99X.

Sterilization of mosquitoes with gamma irradiation was first demonstrated by Davis *et al.* (1959) with the common malaria mosquito, *Anopheles quadrimaculatus* Say, but these males were unable to compete sexually with normal males in the laboratory. Also, when Ramakrishnan *et al.* (1962) used irradiation to sterilize the southern house mosquito, *Culex pipiens quinquefasciatus* Say (=*C. p. fatigans* Wiedemann), they found that this method of sterilization reduced mating competitiveness. However, Murray and Bickley (1964) and Mulla (1964) found that male *C. p. quinquefasciatus* sterilized with chemicals in the larval stage were competitive with normal males in the laboratory. Also, Smittle *et al.* (1968) compared these two methods of sterilizing the southern house mosquito and confirmed that irradiation inhibited sexual activity of the males, whereas the chemosterilant apholate did not.

Except for the field release by Krishnamurthy *et al.* (1962), which was done in conjunction with the cited research by Ramakrishnan *et al.* (1962), these early studies with *Culex* were all made with controlled conditions in small cages in the laboratory. When Patterson *et al.* (1968)

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

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released laboratory-reared chemosterilized males into a cycling population of the southern house mosquito in a large outdoor cage (ambient conditions), they found that the released males did not compete with normal mosquitoes, probably because the procedures used to rear, handle, and sterilize the insects were detrimental to them. In that test, sterilization was accomplished by the method of Das (1967) which involved chilling the adults and then dusting them with technical apholate. This method had proved satisfactory for sterilizing males used in the laboratory but the males did not compete well in the mating process. [This explanation of the poor results was verified in a subsequent test in which the same researchers (Patterson *et al.* 1972) obtained better results with the apholate-treated males when they utilized improved treatment and handling procedures; they also found that males exposed similarly to residues of tepa competed favorably with normal males.]

Therefore, in 1968, we decided to conduct further experiments in outdoor cages with the southern house mosquito to evaluate the mating competitiveness of males sterilized by tarsal contact with residues of tepa and to compare the mating competitiveness of males reared indoors with controlled conditions and males reared outdoors with fluctuating environmental conditions.

METHODS AND MATERIALS. The "Quonset hut" cages used for the tests were located on the property of the Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida. Each cage was 4.2 meters wide, 7 meters long, and 3.3 meters high at the ridgepole. A partition across the middle separated the cage into two equal parts, one used for the test insects and the other for the check population. Inside each half of the cage were a chicken coop, plastic tubs for rearing and oviposition, and resting sites.

The mosquito populations in the cages were maintained as follows. The larvae were reared in 30-liter (10 gal) plastic tubs holding 11.4 liters (3 gal) of water to which a mixture of equal parts of liver powder, dried brewer's yeast, and a hog supplement were added. The larvae were fed at a rate of approximately 0.5 mg/larva/day. Larval development time ranged from 6 to 8 days. The pupae and larvae were removed from the tub by the ice water technique, and the pupae were sexed by size with a pupal separator and counted. Then, the necessary numbers of males for sterilization were removed and normal females and males were returned to the cages to produce the test ratios desired. Thus, the males to be sterilized were identical to the normal males except for exposure to the sterilant. (This procedure was used to avoid any differences that might be caused by variations in rearing procedures, strains, or environmental conditions.)

Sterilization was achieved by exposing the males to residues of tepa. Ten cm (4-inch) lengths of 1.3 cm (½-inch) black polyvinyl tubing were packed tightly into aluminum collars that were 10 cm (4 inches) deep and 20 cm (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. After the tepa-treated honeycombs dried, they were placed over the pupae in a 3.8-liter (1 gal) waxed paper container which was labeled and placed in a protected area of the cage.

Following emergence, the males normally rested on the underside of the honeycombs until dusk and then crawled through the tubes and flew into the cage. In the period of time the males rested on or crawled through the treated tubes, they absorbed enough tepa to render them sterile. (Any females passing through the same tubes usually were not completely sterilized.) Most adults emerged within 48 hours after the pupae were placed in the containers.

Females were allowed to oviposit only in tubs containing older larvae. (Tubs with young larvae were covered with

cheesecloth.) All egg rafts were removed daily and placed individually in 5-dram vials containing 3 ml of water. The rafts were held for 4 days and checked daily for sterility; any first instar larvae that resulted were returned to the rearing tubs in the cage.

In the comparison of laboratory-reared and outdoor-reared males, the laboratory males were obtained from the normal laboratory colony. These insects were fed at the same time as those outdoors, but were held at a constant 80° F with a 12-hour light-dark cycle.

RESULTS. Releases and egg collections were made daily; however, the data are summarized by generation (2 weeks) to eliminate daily fluctuations caused from varying climatic and biotic conditions. In the first set of experiments (Table 1, tests A and B), the sterile males were able to compete with the normal males when they were released simultaneously with the females (87 to 96 percent were sterile compared with a theoretical 90 to 98 percent). In both tests, the sterility was higher than expected in the first generation but then declined to less than anticipated. The reason is not known, but it might have occurred because of a shorter lifespan of the treated males. If the treated males did live a shorter time than the normal males, the actual ratio of sterile to normal males would sometimes have been a little less than 9:1, and the actual sterility would have been less than predicted.

When sterile males were released into a cycling population containing inseminated females and normal males (Table 1, test C), the degree of sterility of egg rafts was lower than anticipated (85 percent versus 90 percent) because of egg deposition by some females inseminated before the release started. However, during the second and third generations, sterility declined just as it did in tests A and B.

The number of females in a population did not affect the efficiency of the sterile males. For example, when the number of sterile males exceeded the number of normal females by 9 times (test B, first generation), 95 percent of the egg rafts collected during the first generation were infertile; when the number of sterile and normal males equalled the number of normal females (test A), 96 percent of the egg rafts collected were infertile.

TABLE 1.—Results of sterile male release experiments with *C. p. quinquefasciatus*.[a]

Generation	Avg. no. of sterile males released per day	Avg. no. of egg rafts obtained per day	Actual percentage sterility	Theoretical percentage sterility
		Test A; noncycling population[b]		
1	290	42	96	90
2	171	34	88	90
3	187	125	87	90
		Test B; noncycling population[c]		
1	345	34	95	90
2	575	23	95	98
3	2,690	46	93	98
		Test C; cycling population[d]		
1	270	51	85	90
2	127	30	80	90
3	199	144	82	90

[a] All insects reared at ambient conditions.
[b] Release ratio 9:1:10 (sterile males:normal males:normal females).
[c] Release ratio first generation 9:1:1, 2nd and 3rd generations 45:1:1 (sterile males:normal males:normal females).
[d] Release ratio 9:1:10 (sterile males:normal males:normal females).

TABLE 2.—Results of sterile male release experiment with *C. p. quinquefasciatus* in a large outdoor cage in which sterile males reared indoors and outdoors were compared.

Generation	Avg. no. of sterile males released per day	Ratio sterile males:normal males:normal females	Avg. no. of egg rafts obtained per day	Actual percentage sterility	Theoretical percentage sterility
		Sterile males reared outdoors			
1	637	19:1:2	55	93	95
2	623	19:1:2	38	93	95
3	621	29:1:2	33	91	97
4	529	29:1:2	10	97	97
5	579	29:1:2	42	89	97
6	580	100:1:2	26	95	99
7	580	100:1:2	18	97	99
8	580	100:1:2	5	99–100	99
		Sterile males reared indoors			
1	669	19:1:2	40	85	95
2	665	19:1:2	38	78	95
3	942	29:1:2	49	78	97
4	734	29:1:2	37	72	97
5	573	29:1:2	36	92	97
6	580	100:1:2	13	98	99
7	580	100:1:2	7	99	99
8	580	100:1:2	2	100	99

In the comparison of laboratory-reared and outdoor-reared males (see Table 2), eradication was achieved after 8 generations with either type of male when the ratio of sterile to normal males reached 100:1. (Initially, the laboratory-reared males did not perform as well as those reared outdoors; however, as the test progressed, the colony males performed as well as the mosquitoes reared outdoors.) When the number of sterile versus normal males remained static at either 19:1 or 29:1, the population (as indicated by the number of egg rafts laid each day) remained relatively stable, and the degree of sterility never exceeded the theoretical sterility. These data illustrate an important fact, i.e., an insect population cannot be eliminated by sterility unless the sterility induced exceeds the biotic rate of increase. Thus, our caged populations, which had controlled rearing, had a maximum rate of increase (biotic potential) more than 29X but less than 99X.

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