Thorsteinson, A. J., Bracken, B. G. and Tostowaryk, W. 1966. The orientation behavior of horse flies and deer flies (Tabanidae: Diptera). V. The influence of the number and inclination of reflecting surfaces on attractiveness to tabanids of glossy black polyhedra. Can. J. Zool. 44:275–279.

Townes, H. 1962. Design for a Malaise trap. Proc. Entomol. Soc. Wash. 64(4):253–262.

Wilson, B. H., Tugwell, N. P. and Burns, E. C. 1966. Attraction of tabanids to traps baited with dry ice under field conditions in Louisiana. J. Med. Entomol. 3(2):148–149.

COMPETITIVENESS OF MALE *CULEX PIPIENS QUINQUEFASCIATUS* STERILIZED BY TEPA OR APHOLATE IN FIELD CAGES[1]

R. S. PATTERSON,[2] M. D. BOSTON AND C. S. LOFGREN

Entomology Research Division, Agr. Res. Serv., USDA, Gainesville, Fla. 32601

ABSTRACT. *Culex pipiens quinquefasciatus* Say males were chemosterilized by exposure to residues of tepa on polystyrene strands or by external dusting with technical apholate and released into large outdoor cages containing untreated cycling populations of the species. Males sterilized with tepa appeared competitive with normal males after releases had been made for 4 generations, and males sterilized with apholate competed very well in one test but did not compete in a second test. Handling and rearing techniques were important factors influencing male vigor.

Sterility can be induced in *Culex pipiens quinquefasciatus* (*fatigans*) Say by irradiation or chemosterilization (Patterson and Lofgren, 1968). However, irradiated males were not as competitive as normal males in laboratory mating studies, and chemosterilized males were (Weidhaas and Schmidt, 1963; Smittle *et al.*, 1968). The first outdoor field cage studies were made by Patterson *et al.* (1968) with males sterilized with apholate by applying a technical dust to the adults (Das, 1967). The released mosquitoes did not compete as well with the normal males as expected, but over 50 percent of the egg rafts assayed were infertile, which indicated that at least some treated males were performing. We then made further field cage tests with chemosterilized males but used other methods of handling the insects.

METHOD. From May to August we sterilized the males by forcing them to crawl through a 3-inch layer of polystyrene strands which had been dipped in a 2 percent solution of tepa in a mixture of acetonitrile and water (3:1). This was accomplished by placing the male pupae in 2 inches of water in a 1 gallon waxed-paper container and packing the treated strands loosely over the water. Thus, the young adults had to crawl over the treated strands if they were to emerge into the cage and disperse. The procedure had no obvious deterimental effect on the emerging adults, and we planned to use this treatment throughout the study. However, in August, we received a new lot of tepa which for some unexplained reason, was toxic to the mosquitoes. Metepa was used for 2 weeks, but it was not as effective a sterilant as tepa when it was applied in this way. As a result, treatments with apholate dust were used during September and October. The apholate was applied as in the study by Patterson *et al.* (1968): the adults were dusted when they were 24

1 This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation by the USDA.

2 Present address: World Health Organization, Research Unit on Genetic Control of Mosquitoes, 2–3 Ring Road, Kilokri, New Delhi—14, India.

hrs old and held in the laboratory for an additional 24 hrs before they were released into one of the cages. Great care was taken in handling these insects during and after treatment in an attempt to decrease any detrimental effect of the dusting technique.

The studies were made in two cages shaped like Quonset huts 14 ft wide, 24 ft long, and 11 ft high along the ridgepole. Double-screen partitions at the middle of the cages divided them into approximately equal sections; thus, one half was used to contain the test population and the other half to contain the check colony. The cages were located on the property of the USDA laboratory in Gainesville, Florida.

In one cage, 8 plastic 20-gal tubs were placed in each half cage as oviposition and breeding sites; in the other, single small ponds were available in each half. The four populations in the two cages were maintained at a low and workable level by collecting all egg rafts each day and then returning five, each selected at random, to one of the plastic tubs and to the ponds. (If the eggs had hatched, the larvae were returned also.) The pond was covered by a screen cage to preclude oviposition in it; separate containers were provided for oviposition. In the other cage, the newly set tubs were covered with screen, so that oviposition could occur only in those with older larvae. In this way, we could be certain not to remove egg rafts set during the previous 2 days from which the larvae had not hatched. The larvae in the cages were fed a mixture of dried liver and yeast at the rate of about 0.5 mg per larva per day. Also, the plastic containers were rinsed after the adults emerged and before they were reset with eggs so larvae could not be killed by waste products accumulating through successive generations However, no effort was made to change the water in the pond so larval development there could have been limited by an accumulation of waste products or by unfavorable microflora or microfauna. Since the test populations emerged directly into the cage, young chickens, usually 2 per cage, were provided as the blood source. Also cotton pads soaked in a 50 percent honey-water solution were maintained in the cages as an additional food source for the adults.

For the first 2 months, 10–25 of the egg rafts collected daily were used to determine the percentage of sterile eggs. During the remainder of the test, all the egg rafts laid each day by the test populations were checked for sterility, but the check populations continued to be sampled as before. The production of adult mosquitoes was estimated from pupal counts, and the daily emergence of males was determined by dividing the total number of pupae by 4 (it takes about 2 days for pupae to mature and about half the emerging adults are females).

Most chemosterilized male mosquitoes released in the cages had been reared indoors with fairly constant conditions and a room temperature of about 80° F; however, about one-fourth to one-third were reared in a large cage kept outdoors in an open shed as follows: Each day 6 wooden trays (44 by 18 by 3 inches) were set up, each containing 20 egg rafts in 6 liters of water plus 2 g of a mixture of dried liver extract and dried brewer's yeast. (At 2-day intervals until pupation, more liver and yeast was added to the trays.) The developing larvae and pupae could then be separated rapidly by the ice-water technique, and the pupae were sexed with a pupae separator.

Throughout the period that releases were being made, males exposed to each treatment were mated with untreated virgin females in the laboratory, and the resulting egg rafts were checked for fertility to determine the efficiency of the residue on the polystyrene strands and of the apholate dusting, in sterilizing the males. Also, since the method of separating male from female pupae was only about 90–95 percent effective, we subsampled the released mosquitoes to obtain accurate determinations of the numbers of males released each day. This information was used to correct our figures for the

ratio of normal to sterile males in the cages.

RESULTS AND DISCUSSION. The results are given in Table 1, where the data are presented as average sterility per generation. (A generation normally developed every 2 weeks in Florida during the period of this study.) The average daily sterility in the untreated population was 3.8 percent (range from 0 to 10 percent), and all test data were adjusted by Abbott's formula for the check sterility.

Since the sterile males were released into a cycling population maintained with favorable conditions, almost two generations (a month) passed before the pretreatment (fertile) females had all died. The extended influence of the sterile males on the females as a result of this longer life was also plain when the releases were terminated: a high degree of sterility remained in the cycling population for over a month. However, from other observations, many males did not survive more than 72 to 96 hr after release. Thus, within a week after the releases were terminated, the sex ratio in the cages had returned to normal indicating that most of the sterile males had succumbed.

Our data show that males sterilized by apholate-dusting competed well with the normal males in cage 1 but not in cage 2. The average sterility for the last 4 generations in cage 2 was the same as the theoretical sterility (87%); in cage 2 the actual sterility was 67% compared to a theoretical 77%. Tepa-treated males competed very well with normal males in cage 1 during generations 6 and 7, but in cage 2, sterile males treated with tepa were released for only 2 generations so no comparison can be made. The data shown in Table 1 (from the field cages) do not indicate that metepa was ineffective as a sterilant when it was applied to the polystyrene strands, but the laboratory tests indicated that it was; therefore, its use was discontinued after 2 weeks (one gener-

TABLE 1.—Sterility produced by releases of sterile male *Culex pipiens quinquefasciatus* in large field cages *

			Cage 1			Cage 2		
			Ration	% Sterility		Ratio	% Sterility	
Month	Generation of cage population	Chemical treatment	(sterile: normal)	Expected	Actual	(sterile: normal)	Expected	Actual
May	1	None	0:1	0	6	0:1	0	4
June	2	Tepa	2:1	67	49	0:1	0	4
	3	Tepa	3:1	75	58	0:1	0	4
July	4	Tepa	7:1	88	80	0:1	0	4
	5	Tepa	3:1	75	64	0:1	0	3
Aug.	6	Tepa	10:1	91	93	6:1	87	50
	7	Tepa	6:1	87	93	7:1	88	63
	8	Metepa	7:1	88	86	12:1	92	80
Sept.	9	Apholate	29:1	97	95	15:1	94	82
	10	Apholate	8:1	89	88	6:1	87	61
Oct.	11	Apholate	6:1	87	78	1:1	50	60
	12	Apholate	3:1	75	87	3:1	75	63
Nov.	13	None	0:1	0	82	0:1	0	60
	14	None	0:1	0	78	0:1	0	38
Dec.	15	None	0:1	0	29	0:1	0	7

* The data have been corrected by Abbott's formula and arranged so the sterility shown corresponds to the date the sterile males were released.

ation). The apholate dusting still appeared to be slightly detrimental to the males based on the results in cage 2. However, care in handling the males during and after treatment appeared to increase their competitiveness compared with that reported previously by Patterson *et al.* (1968).

Males sterilized with tepa or apholate were able to compete with normal males in one test, but apholate-treated males were unable to compete in a second test. The improved sterility obtained with apholate compared with earlier studies emphasizes the need for great care in rearing, handling, and sterilizing mosquitoes.

Literature Cited

Das, M. 1967. Sterilization of *Culex pipiens fatigans* Wiedemann by apholate. Bull. Wld. Hlth. Org. 36:949–954.

Patterson, R. S. and C. S. Lofgren. 1968. The potential use of sterile males to control *Culex pipiens quinquefasciatus* Say. Proc. N. J. Mosq. Extermin. Assoc. Mtg. 52:170–175.

Patterson, R. S., C. S. Lofgren and M. D. Boston. 1968. Use of sterile males for mosquito control: a field cage study with *Culex pipiens quinquefasciatus*. Mosq. News 28(4):540–544.

Smittle, B. J., G. A. Mount, M. Das and N. Rajapaksa. 1968. Comparison of apholate and gamma irradiation for sterilization of *Culex pipiens quinquefasciatus* Say. Mosq. News 28 (2):201–204.

Weidhaas, D. E. and C. H. Schmidt. 1963. Mating ability of male mosquitoes *Aedes aegypti* (L.) sterilized chemically or by gamma radiation. Mosq. News 23(1):32–34.