TECHNIQUES FOR STERILIZING LARGE NUMBERS OF MOSQUITOES

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ABSTRACT. Large numbers of *Culex pipiens quinquefasciatus* Say were sterilized by exposing the pupae to a water solution of 0.7 percent thiotepa for 4 hours. Competitive mating studies in outdoor cages and studies in which sterile males were released into wild populations indicated the technique was not deleterious to the males. Neither tepa nor metepa were effective as pupal sterilants for *C. p. quinquefasciatus,* but adult males emerging from holding cages through strands of polystyrene foam or polyvinyl tubes that had been dipped in methanol solutions of 5 percent tepa were sterilized. In all tests, the sterilizing doses for females were larger or the necessary periods of exposure were longer.

A simple, effective method of sterilizing large numbers of insects without seriously affecting their behavioral traits is essential for any sterile male release program. The first step is to find a satisfactory sterilizing agent. Gamma irradiation, which is effective in sterilizing the screw-worm, *Cochliomyia hominivorax* (Coquerel) (Lindquist, 1955) and the Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (Nadel and Guerrieri, 1969) had a deleterious effect at sterilizing doses on *Anopheles quadrimaculatus* Say (Davis *et al.,* 1959). Also, *Culex pipiens quinquefasciatus* Say sterilized by irradiation were not fully competitive sexually with normal males (Smittle *et al.,* 1968). However, certain species of mosquitoes can be effectively sterilized by chemicals without affecting the behavior of the insect (Weidhaas and Schmidt, 1963), and several methods have been tested. Dame *et al.* (1964) placed late third- or early fourth-instar larvae in the chemosterilant solution until pupation. White (1966) sterilized adult *Aedes aegypti* L. by exposing the pupae to a solution of thiotepa in water for 24 hours. Weidhaas *et al.* (1961) sterilized adult *Aedes aegypti* by forcing them either to rest on or feed on chemosterilant residues or solutions. Das (1967) sterilized adult *Culex pipiens fatigans* Wiedemann by dusting them with technical apholate.

Of the various methods used for inducing sterility, the treatment of pupae and the exposure of adults to residues appeared to be the simplest techniques because they combined the least amount of handling of the insects with the easiest methods of using the chemosterilants. It remained to evaluate these techniques for sterilizing large numbers of mosquitoes. Three pupal chemosterilants, tepa, metepa, and thiotepa, and one residue, tepa were used in the evaluations.

METHODS

Most of the studies were made with laboratory-reared *C. p. quinquefasciatus;* however, *Aedes aegypti* and *Anopheles quadrimaculatus* were used in a few tests. Rearing and indoor testing were done in a controlled environment (60 percent relative humidity and 80° F).

The efficiency of the treatments was determined by mating the treated sexes with each other and with normal mosquitoes of the opposite sex and then determining the number of sterile egg rafts laid. Competitive mating studies were also made to detect any detrimental effects of the procedure on the mating behavior of the males. In these studies, sterile and normal males were combined with normal females at a ratio of 9:1:1. Thus, if the sterilizing treatment was effective and did not affect the ability of the sterile male to compete for the females, 90 percent of the egg rafts would be infertile. Some initial competitive mating studies were done in the laboratory; however, most were made

outside in 1-meter cubical screen cages exposed to ambient weather.

PUPAL TREATMENTS. Although White (1966) exposed pupae to relatively low concentrations of thiotepa for long periods to achieve sterility, long exposure would be difficult to use in sterilizing large numbers of mosquitoes because some adults would emerge during the exposure period. Thus, we experimented with higher doses and shorter exposures.

Pupae of a known age were counted and placed in glass beakers containing the treatment solution, which was buffered to a pH of 8. After the allotted time they were removed from the solution, rinsed, and placed in fresh water. Then they were put into a cage containing a 2 percent sugar-water solution in a cup as food for the emerging adults; the mortality of pupae and adults was determined after 24 hours. Next, the treated mosquitoes were inactivated in a cold room (34° F), and the three possible mating crosses were made. The mated females were subsequently offered a blood meal and an oviposition site. *Culex* egg rafts were checked individually for hatch 4 days after oviposition; *A. aegypti* eggs were checked by placing 100 eggs in water and determining the number of hatched eggs after 4 hours; gravid *Anopheles* females were placed in individual vials for oviposition, and the eggs were checked for hatch after several days.

Competitive mating studies were made only with *C. p. quinquefasciatus* since it was the only species being considered for field releases.

ADULT TREATMENTS. Two methods of exposing adults to residues of tepa were used. (Tepa was the only effective residual sterilant available for these tests.)

The first procedure was a modification of the technique used by Fye *et al.* (1968) which consisted of forcing newly emerged adult houseflies, *Musca domestica* L., to crawl through a layer of polystrene strands treated with tepa. The polystyrene strands (⅛-inch in diameter and 6 or more inches long) were treated by immersing them for 5 minutes in a 5 percent solution of tepa in methanol or a mixture of acetonitrile and H_2O in a gallon container. (A screen was placed over the strands to prevent them from floating to the surface.) After exposure, the chemosterilant was poured off and the strands were spread out and dried under a hood for 8 to 12 hours. Pupae were placed in about one inch of water in a gallon container, and the water was covered with treated strands to a depth of 4 inches. (Layers of strands less than 4 inches did not cause consistent sterility.) Then the container with the pupae and treated strands was placed in a cage. Two days later, when most of the adults had emerged and migrated naturally through the strands, the container was removed, and the pupal and adult mortality was recorded. The adults were then inactivated in the cold room, and the mating crosses were set up.

The second procedure involved forcing the adults to crawl through a device made of small straight tubes resembling a honeycomb (Fig. 1) that had been treated with sterilant. The device was made of 4-inch sections of polyvinyl tubing (½-inch diameter) packed tightly and held together with a metal collar 8 inches in diameter. It was treated by dipping it in a 5 percent solution of tepa in methanol for a few minutes and then placing

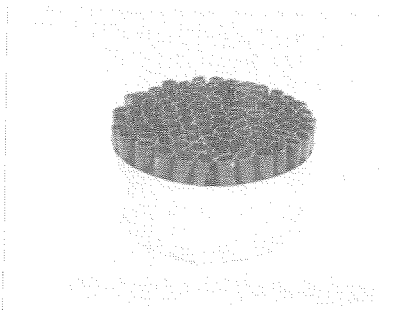

FIG. 1—Device used to expose male mosquitoes to residues of tepa. Entire device is dipped in sterilant solution, dried, and placed over container with pupae. Males are forced to crawl through the tubes before flying into the environment.

it under a hood to allow the methanol to evaporate. This treated honeycomb was placed over pupae held in a gallon container in about one inch of water so the distance between the surface of the water and the bottom of the honeycomb was about 1–2 inches. Mating crosses were set up with the adults that emerged through the tubes.

RESULTS

PUPAL TREATMENT. We found, as White has indicated, that thiotepa is an excellent pupal sterilant for male *Aedes aegypti* and is equally effective for sterilizing *C. p. quinquefasciatus* mosquitoes (Table 1). No detrimental effect on the males was noted unless high doses and/or long exposures were used. Male *Culex* pupae were completely sterilized at a concentration of 0.1 percent thiotepa when they were exposed for 24-hours; doses of 0.5 and 0.7 percent were needed if the exposures were 6 or 4 hours, respectively (see Table 1). Males exposed as pupae to 0.7 percent thiotepa for 4 hours competed favorably with normal males: at a 9:1 ratio of sterile to normal males, 91 percent of the females laid sterile egg rafts. However, females could not be completely sterilized by thiotepa without affecting both egg hatch and fecundity; for example, complete blockage of egg deposition occurred when they were exposed for 24 hours to 1.0 percent thiotepa.

Tepa and metepa were not as effective as thiotepa as pupal sterilants (see Table 2). Only 75 percent sterility of males was obtained after a 24-hour exposure to tepa at a concentration of 3 percent, and many males died. With continuous exposure (pupae exposed from 0 to 12 hours of age until adult emergence), 100 percent sterility of males and 94 percent sterility of females was obtained with a concentration of 2 percent of tepa; however, about half of the emerging adult males died within 24 hours.

Also we were unable to achieve a high degree of sterility in adults with metepa because of its toxic side effects. When the pupae were exposed continuously until emergence, most pupae treated with a 3 percent concentration died, and the few adults that did emerge died within 24 hours. Moreover, half the pupae exposed for 24–48 hours to a 2 percent metepa solution died. No pupae exposed continuously to a 1 percent solution until emergence died during the exposure, but 36

TABLE 1.—Effect of time of exposure and concentration on the sterilizing effect of tepa on pupae of *C. p. quinquefasciatus*.

Exposure time (hours)	Concentration (percent)	No. of replicates	Percentage sterility of egg rafts from mating crosses in which the indicated sex was sterilized		
			Male	Female	Male and Female
4	0.1	3	13		
	0.2	3	17		
	0.4	3	48		
	0.7	10	100	40	100
6	0.5	2	100	70	100
	0.7	2	100	90	100
	1.0	6	100	70	100
24	0.01	2	0	0	0
	0.03	6	37	0	50
	0.05	6	97	3	100
	0.1	6	100	32	100
	1.0	6	100[a]	[b]	[b]

[a] Thirty-four percent of emerging males died.
[b] No eggs laid.

TABLE 2.—Effectiveness of tepa and metepa as pupal sterilants for *C. p. quinquefasciatus*.[a]

Exposure time	Concentration (percent)	Percentage sterility of egg rafts from mating crosses in which the indicated sex was treated		
		Male	Female	Male and Female
		Tepa		
24 hours	0.05	0	0	0
	0.1	0	0	0
	0.25	0	5	0
	0.5	10	10	0
	1.0	55	10	
	3.0[b]	75		
Continuous[c]	0.1	56	3	94
	0.5	75	20	100
	1.0	76	13	99
	2.0[b]	100	94	100
	3.0[b]	100	100	100
		Metepa		
Continuous[c]	0.1	42	20	44
	0.5	48	32	42
	1.0	54	35	39
	2.0			74

[a] Average of two replicates with 100 males and 100 females in each.
[b] Over 50 percent mortality.
[c] Pupae exposed from 0 to 12 hours of age until adult emergence.

percent of the newly emerged males died within 24 hours, and about half the remaining males were sterilized. As with thiotepa and tepa, a higher dose of metepa was necessary to sterilize female *C. p. quinquefasciatus*.

Both *Aedes aegypti* and *Anopheles quadrimaculatus* (Table 3) reacted about the same as *C. p. quinquefasciatus* to pupal sterilization with tepa and thiotepa.

ADULT TREATMENTS. Residues of tepa on the polystyrene strands and the poly-

TABLE 3.—Sterility in adult *A. aegypti* and *A. quadrimaculatus* after exposure of pupae to water solutions of tepa and thiotepa.

Chemosterilant	Exposure time (hours)	Concentration (percent)	Percentage sterility of egg rafts from mating crosses in which the indicated sex was treated		
			Male	Female	Male and Female
		A. aegypti (4 replicates)			
Thiotepa	24	0.03	75	7	73
		0.05	94	12	94
		0.1	100	26	100
		0.5	100		
		1.0	99.5		
Tepa	24	0.05	6	1	8
		0.1	7	2	5
		0.5	24	3	35
		1.0	79	8	86
		A. quadrimaculatus (2 replicates)			
Thiotepa	2	0.7	82	10	82
Tepa	4	0.7	100	38	100

vinyl tubes were effective in sterilizing *C. p. quinquefasciatus* (Table 4). Male

TABLE 4.—Sterility in male or female *C. p. quinquefasciatus* after crawling over residues of tepa on two plastic surfaces.[a]

Surface treated	Treated sex	Percentage sterility of egg rafts
Polystyrene strands	Male	99.5
	Female	58
	Both	100
Polyvinyl tubes	Male	99.5
	Female	53
	Both	100

[a] Twenty replicates for each mating cross.

sterility averaged over 99 percent, but the females were not completely sterilized. Competitive mating studies indicated that the sterile males competed well with normal males; at a ratio of 9 sterile to 1 normal male, 93 percent of females laid sterile egg rafts. Also, when the treated males were released into a natural population, they competed well with the normal males (Patterson *et al.* 1970).

DISCUSSION AND SUMMARY. Our tests demonstrate that pupal exposure to thiotepa and adult exposure to residual deposits of tepa are highly effective techniques for chemosterilization of mosquitoes and that complete or essentially complete sterilization of male *C. p. quinquefasciatus* and *Aedes aegypti* can be achieved without decreasing mating competitiveness. Treatment by exposure during the pupal stage appears most promising for programs involving the release of large numbers of sterile males. The pupal stage is relatively quiescent and easily handled, and it is not necessary to anesthetize or contain the pupae as it is with adults. Also, *Culex* and *Aedes* pupae can be separated by sex by the method described by Fay and Morlan (1959). In addition, pupae can be transported easily without detrimental effects by placing them on damp filter paper and keeping them chilled. We used these methods of sterilization and transport in a field release with *C. p. quinquefasciatus;* over 25,000 males per day were processed, and the released males were able to eliminate a small indigenous population (Patterson *et al.,* 1970).

Treatment by exposure of adult males to tepa on plastic tubes is also a promising method of dealing with large numbers since handling, sexing and transporting can be done in the pupal stage, and sterilization can be accomplished at the site of release. However, we noted two problems in our field studies with this technique. First, tepa, which is hygroscopic, polymerizes in the presence of moisture and, second, it is rapidly degraded by acid catalysts (Borkovec, 1966). These properties can interfere with the life of tepa residues in the field. Usually, all adult mosquitoes emerge and are exposed to the residues within 48 hours; however, during cool weather, emergence is retarded, and the residues are exposed to a longer weathering period before all the males have contacted the residues. Consequently, the last males to emerge may not receive a complete sterilizing dose which occurred several times in field studies following rainy weather.

Second, the treated surfaces used in the exposure of adults could be a major problem in large scale tests because of the need to decontaminate or dispose of them. Polystyrene strands are very bulky and must be buried or incinerated, or the tepa must be chemically inactivated. We were successful in cleaning the polyvinyl tubes so that they could be reused; however, the process could become expensive and time consuming in a large scale program.

Nevertheless, treatment with residues, even with these disadvantages, could be very useful in sterilizing males in natural populations if they could be combined with a good attractant. The males would be attracted to a trap in which they contacted the chemosterilant before they returned to their natural habitats. However, at present, satisfactory attractants are not available.

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