STUDIES ON CHEMOSTERILIZATION OF *AEDES AEGYPTI* (L.): I. EVALUATION OF THIOTEPA AS A STERILANT IN LABORATORY AND FIELD CAGES

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ABSTRACT. Thiotepa (tris (1-aziridinyl) phosphine sulphide is known to be an effective pupal sterilant against *Ae. aegypti*. Studies were initiated to develop a method which could be adopted for sterile male releases. The present investigations have revealed that <99.6% sterility in males can be induced by immersing pupae to 0.6% thiotepa solution for 3 hr at 28±2°C. Female sterility at this dose is only 42%. The sterilized males (36–60 hr old) were almost as competitive as normal laboratory males of the same age both in laboratory cages and in a field cage experiment. Two to 10% females showed double matings when females were offered 2 types of males simultaneously, however, when both types of males were offered in sequence, the frequency of double mating was lower. The number of females inseminated by sterile males was found to be slightly lower than for normal males. Thiotepa-induced sterility in males persisted for at least 17 days. The sperms once stored in the spermathecae of females mated with sterile males, did not show any recovery of fertility during 3 gonotrophic cycles. Thiotepa did not induce inherited sterility or sex-linked recessive lethals in the progeny.

The successful eradication of an indigenous population of *Culex pipiens quinquefasciatus* (=*fatigans*) on the isolated island if Sea Horse Key by release of thiotepa-sterilized males (Patterson et al. 1970) has created interest in further evaluation of the 'sterile male technique' against this and other species of mosquitoes in the field. The WHO/ICMR Research Unit on Genetic Control of Mosquitoes was mainly engaged in the work on genetic control of *C.p. fatigans* from 1970–1974. During the first 3 years however, certain preliminary studies on *Aedes aegypti* (L.), viz. genetic control principles, basic ecology, distribution of the species in northern India and search for a site for testing the feasibility of application of a genetic technique, were also carried out. A review of this work has been presented by Rao (1974). In the 4th year, concerted efforts were devoted to develop and perfect various genetic control methods for *Ae. aegypti* in the laboratory including chemosterilization of this species.

Several laboratory studies have been previously carried out using both alkylating and non-alkylating chemosterilants tested against various life-stages of *Ae. aegypti*. A number of compounds have been found to induce high sterility in males (Weidhaas et al. 1961, Weidhaas 1962, Weidhaas and Schmidt 1963, Bertram 1963, Dame et al. 1964, Rai 1964; Glancey 1965, White 1966, George and Brown 1967, Grover and Pillai 1970, Madhukar et al. 1971 a & b).

White (1966) first reported successful sterilization of this species with thiotepa by exposure of pupae to a 0.05% solution for 24 hr. The operational advantages of pupal treatment over larval or adult treatment in mosquitoes have been described in earlier works (White 1966, Grover et al. 1967, Pillai and Grover 1969, Patterson et al. 1971). Therefore,

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this method was selected for study by the Unit. For developing a suitable procedure for sterilization for a sterile male release program, since separation and sexing of pupae more than once in a day is not practically possible, the exposure period to the chemosterilant should be short so that pupae of mixed ages (4–28 hr) could be used for treatment, still permitting time for packaging before emergence.

This paper describes laboratory and field cage studies to evaluate thiotepa (Tris (1-aziridinyl) phosphine sulphide = AI3-24916) as a potential sterilant for *Ae. aegypti*. The specific objectives were to: (1) select a dose of thiotepa which gives complete sterility in males, (2) test the effect of selected male sterilizing dose on female fertility and fecundity, (3) evaluate the effect of chemosterilization on mating ability, (4) determine the permanency of sterility in males and in females mated with sterile males, and (5) study the effect of chemosterilization on the F_1 generation.

MATERIALS AND METHODS

General Procedures

1. Strains. The studies were initiated with a Delhi strain of *Ae. aegypti* collected in 1967 and maintained initially by the procedures used at the University of Delhi (Grover and Pillai 1970). This strain was subsequently replaced by a strain from Sonepat as this town had been selected as a test site for a release experiment in 1975.

2. Sterilization. Pupae (4–28 hr old) were treated in desired concentrations of thiotepa in 0.001 molar phosphate buffer (pH 7.5) for a period of 1–3 hr. For treatment, a density of 1,000 pupae per 1000 ml. thiotepa solution was used. Specimens were exposed in an enameled bowl held within a hood maintained at a temperature of 28 ± 2°C. Pupae were rinsed twice in dechlorinated water and transferred individually to vials (10.2 cm × 2.6 cm) for emergence.

3. Adult holding, feeding, oviposition and hatchability. All the experiments in the laboratory were conducted in 30 cm cubic cages except the mating competitiveness experiments which were carried out in larger cages (60 cm × 60 cm × 70 cm). All test specimens were held in an insectory maintained at 28 ± 2°C and a R.H. of 80 ± 10%. For out-door studies, framed cages (5m × 3.5m × 2m) with double doors to guard against escape of mosquitoes were used. Inside each cage was a small thatched hut where mosquitoes could rest in the shade. They were furnished a 1% glucose solution and soaked raisins.

In the laboratory, at the end of the mating period, females were fed on mice on 2 successive days according to the requirements of the individual experiment. In field cages, females were provided with rabbits and chickens for their blood meals. Females were offered water-filled 500 ml bowls lined with filter paper for oviposition. However, when data on hatchability of individual egg batches were required, each engorged female was confined in a vial (2.6 cm × 10.2 cm). The vials were lined with filter paper, half filled with water, and covered with muslin cloth. Eggs were conditioned for 2 days on the same filter paper upon which deposited by storing the papers over water-soaked cotton. The number of eggs laid by each female was recorded and eggs were allowed to hatch separately in the vials. In other cases, all the eggs laid by a known number of females were pooled for hatching. Conditioned eggs were floated in dechlorinated water containing a pinch of brewer's yeast and after 48 hr, the hatchability was scored under a dissecting microscope. Unfertilized eggs identified by longitudinal splitting of egg shells, were excluded from counting.

Experimental Procedures. Each laboratory experiment was replicated 5 times with parallel control tests using the same number of untreated mosquitoes. Males were 36–60 hr old and females 60–76 hr old, when crosses were made unless otherwise specified.

A. *Sterilization Dosage.* To select a dose

of thiotepa which gives almost complete sterility in males and females, pupae of the Delhi strain were exposed to 0.1 to 2% concentration of thiotepa for 1 to 3 hr. A maximum exposure period of 3 hr was chosen for the sake of convenience during working hours. Subsequently sterilization dosage for males of the Sonepat strain was established by treating the pupae to 0.5, 0.6 and 0.7% thiotepa solution for a period of only 3 hr. For three successive days, 50 treated males and 50 untreated virgin females were released in each cage, and allowed to mate for 2 days. Females were given blood meals at the end of the mating period. Eggs were collected from the females in each cage and checked for percent hatch.

B. *Biological effects of chemosterilization.* A treatment of 0.6% thiotepa for 3 hr induced < 99.6% sterility in males, and this will be referred to as the 'male sterilizing dose.' Except for a study of effects on filial generations, this treatment was used in all the following studies:

1. *Effect on female fecundity and fertility.* The effect of the male sterilizing dose of thiotepa on oviposition and hatchability of eggs from females crossed to untreated males was studied. Crosses of 25 treated, mature virgin females with normal mature males were set up daily for 6 successive days and given a 2-day period for mating. Females were offered a blood meal at the end of the mating period and isolated for individual oviposition. The fecundity and fertility of the females were then assessed.

2. *Effects on biological fitness of males.* To evaluate the effect of thiotepa sterilization on the biological fitness of males, the following aspects were studied:

a) *Mating sequence.* To test the effect of mating sequence on fertilization of eggs, 100 normal virgin females were released in each of 4 cages. The females of cages 1 and 3 were allowed to mate with 200 normal males, whereas females of cages 2 and 4 were allowed to mate with 200 treated males for 24 hr. Spermathecae from 10 females from each of the 4 cages were checked by dissection for insemination after the first mating. Males in cage 3 were replaced by 200 treated males and the treated males in cage 4 were replaced by the same number of normal males. Males in cages 1 and 2 were replaced by the same type of males as in the original setting. Females were allowed to mate with the second males for next 24 hr before removal of males from all the cages. Females were then offered a blood meal and the fed females were transferred to separate vials for assessment of oviposition and egg-hatch of individual female. The sterility or percent hatch was calculated by pooling the data from individual females.

b) *Mating competitiveness of chemosterilized laboratory males vs. untreated laboratory males.* Experiments were first carried out in the laboratory. Adult thiotepa-sterilized males (36–60 hr old) combined in various ratios with normal laboratory males of same age as shown in Table 4 were allowed to compete for mating with 100 virgin laboratory females. Both treated and normal males were released into cages in the morning, so that both types of males could distribute themselves at random inside the cage before females were released in the afternoon. After a 2-day mating period, females were given a blood meal and the fed females transferred to individual vials for analysis of hatchability of their eggs. Sterility or percent hatch was calculated as noted earlier. The competitiveness[6] (e) of the sterile males was calculated using Haisch's formula (1970):

$$\frac{e-q-f}{n(f-p)}$$

(where q=fertility of untreated males, f= proportion of fertile eggs from females exposed to untreated and sterile males, n = ratio of sterile males to untreated males and p = fertility of sterile males).

[6] Average probability of each sterile male in inseminating a normal female, relative to the average probability of each normal male inseminating.

In addition, 100 older thiotepa-sterilized males (180–204 hr old) were allowed to compete with normal laboratory males of the same age for mating with normal laboratory females in a 1:1:1 ratio. Test procedures were the same as described above.

For field cage studies, 450 sterile males (36–60 hr old), 50 normal males of the same age and 50 mature virgin females were released daily in each of the two cages for 9 successive days. After 2 days, females were offered a blood meal and allowed to lay eggs in 8 black jar ovitraps provided with brown cloth paddles. Eggs were collected daily for 15 days and the percent sterility evaluated as described earlier.

(c) *Sperm production and insemination rate.* Ten sterilized males (36–60 hr old) were allowed to mate with 60 normal virgin females (60–76 hr old) each for 24 or 48 hr in separate 30 cm cube cages. After the 10 experimental periods, males were removed and transferred to another cage containing fresh virgin females and the procedure was repeated for 9 more times if males were given a mating opportunity for 24 hr, and 4 times if originally given a 48 hr opportunity. At the end of each mating period, all females were dissected in Hayes saline (Hayes 1953) and examined for sperm in the spermathecae. After the last mating series all males were dissected and checked for sperm in the seminal vesicles, vasa deferentia and testes. The number of females inseminated per male in each mating period was calculated.

(d) *Permanency of sterility in males.* One hundred thiotepa-sterilized unmated males were allowed to mate with the same number of virgin untreated females for 4 days. Males were then transferred to another cage where they were allowed to mate for an additional 4 days with 100 two-day old virgin females. Similarly these males were successively mated with 3 more batches of females. Females of each series were given a blood meal and the eggs laid were used for assessing sterility. At the end of egg-laying, half of the females of the 3rd and 5th series were dissected and spermathecae examined for the presence of sperms.

In a second experiment, 100 chemosterilized males were kept unmated for 1 or 2 weeks and then were allowed to mate with 100 two-day old virgin females in order to detect any recovery of fertility. After egg laying, half the females of this series were examined for the sperm as before.

(e) *Effect of "sperm storage" in females.* To determine if sperm stored in females show any recovery in fertility, 100 untreated females were caged with the same number of chemosterilized males for a period of 48 hr. Females were given a blood meal at the end of the mating period and allowed to oviposit in bowls. After completion of the 1st oviposition, females were maintained on 1% glucose solution for 7 days after which they were again offered blood meals and allowed to lay 2nd and 3rd egg-batches. The effect on fertility was assessed separately for the 1st, 2nd and 3rd gonotrophic cycles.

(3) *Effect on filial generation.* One hundred males that had been treated at the pupal stage with 0.4% thiotepa for 3 hr, were placed in each of 5 cages with 100 normal virgin females. The few hatched larvae that resulted from this cross were raised; the F_1 adults were crossed, and sterility was evaluated. Similarly F_2 progeny were raised, crossed, and hatchability counted. In addition, 100 F_1 adult males were mated to normal virgin females and the sterility evaluated.

RESULTS AND DISCUSSION

Studies on chemosterilization of the Delhi strain of *Ae. aegypti* revealed that males can be completely sterilized (99%) by an exposure of 1–3 hr by treating the pupae at an appropriate concentration of thiotepa (Table 1). For example, 0.5% thiotepa solution for 3 hr induced almost complete sterility in males. In females, this treatment induced only 32% sterility, and concentrations as high as 2% induced only 85% sterility. Short pupal treatment

Table 1. Induction of sterility in Delhi strain of *Ae. aegypti* exposed as 4–28 hr old pupae to thiotepa for varying concentrations and exposure periods.

Concentration of thiotepa (%)	% Sterility of N♀♀ × T♂♂ Exposure period (hr)		
	1	2	3
0 (Control)	2.1	2.7	2.1
0.1	57.0	77.2	77.0
0.2	79.5	95.9	97.0
0.3	93.0	95.9	99.1
0.4	89.1	98.4	99.3
0.5	96.0	99.6	99.9
0.6	98.0	100.0	99.9
0.7	98.3	100.0	99.9
0.8	99.9		
1.0	99.7		
2.0	100.0		

T=treated.
N=normal.

of 1–3 hr as compared with 24 hr used by White (1966) has the advantage that the thiotepa solution is not open and exposed for long periods. Hence there is (1) less risk of an accident as treatment can be continuously supervised, (2) there is less opportunity for degradation of the thiotepa and therefore a solution can be re-used several times, and (3) if pupae of mixed ages (0–24 hr) are used, emergence during treatment will not occur. Though both 0.5 and 0.6% solutions induced an average of more than 99% sterility in males of the Sonepat strain (Table 2), the latter dose gave a sterility consistently greater than 99.6% and therefore was chosen for further work.

Table 2. Sterilizing effect of thiotepa on the males of Sonepat strain of *Ae. aegypti* exposed as 4–28 hours old pupae for 3 hours based on 3 replications/concentration.

Concentration	No. eggs exam.	No. larvae hatched	% Sterility
0.5	36,316	226	99.4
0.6	52,933	143	99.8
0.7	46,999	18	100.0
0.0 (control)	32,480	31,776	2.1

Females, treated at the pupal stage with the male sterilizing dose, on the average laid slightly fewer eggs than untreated controls (Table 3). The sterility in these treated females averaged 42% but varied from 31%–60% on different days; in an earlier experiment an even lower sterility was recorded. This variation may be due to variation in the sensitivity of different ages of females to thiotepa and the age composition of different batches of pupae. White (1966) has previously reported such phenomena in *Ae. aegypti* when using this compound. The combined effect of the reduced fecundity and sterility in treated females would be to reduce their output of hatching eggs to 48% of the normal level.

The experiment involving mating se-

Table 3. Effect of thiopeta treatment* on fecundity and fertility of females of Sonepat strain of *Ae. aegypti*.

Cross	T ♀♀ × N ♂♂				N ♀♀ × N ♂♂			
	No. egg batches exam.	No. total eggs laid	No. eggs laid per ♀	% hatch	No. egg batches exam.	No. total eggs laid	No. eggs laid per ♀	% hatch
1	88	4331	49.2	69.4	72	4526	62.9	99.4
2	90	4220	46.8	63.1	68	4638	68.2	99.3
3	83	2945	35.5	40.2	46	2434	52.9	99.7
4	68	2988	43.9	54.1	88	5042	57.3	99.6
5	71	4176	58.8	65.3	66	3456	52.4	97.3
6	93	5079	54.6	50.6	52	2546	49.0	95.9
	493	23739	48.1	58.0	392	22642	57.8	98.7

T=Treated N=Normal
* 4–28 hrs. old pupae exposed to 0.6% thiotepa solution for 3 hours.

Table 4. Effect of mating sequence on the hatchability of eggs of female *Ae. aegypti* mated with thiotepa-sterilized and normal males.

Type of mating*		No. ♀♀	% egg batches with percent hatch ranges									% Sterility
First mating	Second mating	oviposited & egg batch exam.	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	(Pooled egg sterility)
N	N	312	—	—	—	—	—	—	—	2.0	2.0	96.0	4.0
T	T	353	100.0	—	—	—	—	—	—	—	—	—	99.6
N	T	234	1	0.8	—	—	—	—	0.8	—	1.2	96.2	3.4
T	N	300	99.0	—	—	0.3	—	—	—	—	—	0.7	99.4

* 100 two-day old (60–76 hrs) virgin females mated with 200 males in the first mating for 24 hrs and were replaced by 200 males for the next 24 hrs.
N = Normal Untreated } one-day old (36–60 hrs) virgin males.
T = Treated

quence (Table 4) showed that females mated with normal males first and then allowed the opportunity of re-mating with treated males laid egg-batches almost all of which were fully fertile with only 2.6% showing the influence of sterile sperms. Conversely, the females mated with treated males first and then caged with males laid egg-batches 99% of which were sterile and only 1% showed the influence of fertile sperms. The hatch range of egg batches from cages 3 and 4 indicate that in about 1% of the females, the eggs were fertilized only by the sperm from the 2nd mating. This category was explained by the fact that when 100 females were dissected after the 1st mating with each type of males, one was uninseminated, indicating that about 1% of the females were not ready to mate during the 1st mating period, though they were sexually mature. In about 1% of the females, eggs were fertilized by the sperm from both the 1st and 2nd mating, while in the great majority of the females, eggs were fertilized by the sperm of the 1st mating. This conforms with the results of Craig (1967) indicating that unless mating attempts are made within a short period, matrone in the seminal fluid from the 1st mating makes females refractory to subsequent insemination.

The results of mating competitiveness of 36–60 hr old thiotepa-sterilized males in laboratory tests are presented in Table 5. In all the tests with different ratios of thiotepa-sterilized and normal males, the actual sterility induced was similar to the expected values except in the ratio 6:1:1. In the competitive experiments, the majority of females laid egg-batches which showed a hatch range corresponding either to a normal male cross or a treated male cross but about 2–10% of the females showed egg hatchability in the range 11–80 percent indicating that these females had been fertilized by both treated and normal males. Double insemination in *Ae. aegypti* has been demonstrated by Van de Hey and Craig (1958) using genetic markers and at about the 3% level it has been observed in competi-

Table 5. Mating competitiveness of thiotepa-sterilized males of *Ae. aegypti vs.* untreated laboratory males for laboratory females.

Sex ratio			No. ♀♀ ovi posited & egg batch exam.	% Sterility (Pooled eggs sterility)		%	% egg batches with percent hatch ranges									
T♂♂	N♂♂	N♀♀		Observed	Expected*	Comp.	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
0	1	1	300	4.0	—	—	—	—	—	—	—	—	—	2.0	2.0	96.0
1	0	1	360	99.6	—	—	100.0	—	—	—	—	—	—	—	—	—
1	1	1	A 322	51.2	51.8	97.5	46.6	0.6	0.6	1.2	0.9	0.3	0.3	—	—	49.5
			B 320	52.5		102.9	45.9	0.6	0.6	2.5	1.6	0.9	1.6	1.6	0.6	44.1
3	1	1	358	74.6	75.7	94.1	70.1	0.5	1.4	0.5	1.4	0.8	1.7	1.7	1.1	20.8
1	3	1	220	26.9	27.9	94.5	21.8	1.8	0.4	—	0.9	—	0.4	—	—	74.7
6	1	1	318	79.6	85.9	63.0	75.5	0.9	1.2	—	1.6	0.3	1.6	1.2	—	17.7
9	1	1	226	90.9	90.0	111.0	89.4	—	—	0.4	0.4	—	0.8	0.8	—	8.2

T=Treated, N=Normal } > 1-day old males (36–60 hrs) and > 2-day old (60–76 hrs) females.

* Expected: Calculated from normal sterility in untreated populations of both sexes and sterility in treated male populations.

Table 6. Mating competitiveness of thiotepa-sterilized laboratory males (7–8 days old) of *Ae. aegypti vs.* untreated normal males (of the same age) for normal females in laboratory cages.

Sex ratio			No. ♀♀ ovi-posited & egg batch exam.	Pooled eggs Sterility (%)	Percent egg batch distribution									
					Percent hatch range									
T♂♂	N♂♂	N♀♀			0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
0	1	1	290	2.10	—	—	—	—	—	—	0.3	1.7	—	98.0
1	0	1	200	99.60	100	—	—	—	—	—	—	—	—	—
1	1	1	245	45.58*	38.5	0.8	0.4	2.0	1.6	2.0	2.9	1.6	4.0	46.2

T=Treated, N=Normal } > 7-day old males (180–204 hrs.) and > 2-day old females (60–76 hrs.)

* Percent competitiveness of treated males=83.8.

Table 7. Insemination rate of thiotepa-sterilized and normal males of *Ae. aegypti* mated successively with fresh batches of virgin untreated females in the ratio of 1 male to 6 females.

Mating series	No. mosquitoes used in crosses*				No. ♀♀ inseminated/male		No. mosquitoes used in crosses**				No. ♀♀ inseminated/male	
	Treated		Control		Treated	Control	Treated		Control		Treated	Control
	♂♂ :	♀♀	♂♂ :	♀♀			♂♂ :	♀♀	♂♂ :	♀♀		
I	50	300	50	300	3.4	4.0	50	300	50	300	2.9	3.3
II	44	264	47	282	0.5	0.7	47	282	45	270	0.0	0.0
III	40	240	42	252	0.0	0.0	29	174	37	222	0.0	0.0
IV	35	210	37	222	0.0	0.0	14	84	20	120	0.2	0.0
V	28	168	32	192	0.0	0.0	7***	42	7***	42	0.0	0.0
VI	15	90	32	192	0.0	0.0	—	—	—	—	—	—
VII	14	84	30	180	0.0	0.0	—	—	—	—	—	—
VIII	11	66	30	180	0.0	0.0	—	—	—	—	—	—
IX	9	54	12	72	0.0	0.0	—	—	—	—	—	—
X	9***	54	12***	72	0.0	0.0	—	—	—	—	—	—

* Mating period 24 hrs.
** Mating period 48 hrs.
*** Males had sperm in testes, vasa deferentia and seminal vesicles.

tion experiments using this method at the WHO/ICMR Research Unit (Lorimer, unpublished data).

The chemosterilized males, when 180–204 hr old, were slightly less competitive than normal males of the same age in laboratory cage tests (Table 6) as sterility induced in females was 45.6% as compared to an expected 50%.

In the field cage experiment, of the total 22,497 eggs collected from the 2 cages, 89.9% were sterile. The ratio of sterile: normal males in the cage was 9:1, which shows that thiotepa-sterilized males (36–60 hr old) were 98.4% competitive as compared to normal males in these semi-natural conditions.

The results of the study on sperm production and insemination rate are summarized in Table 7. The number of females inseminated by sterile males was slightly lower than that for normal males in the first mating series. Both treated as well as normal males could not inseminate more than 4 to 5 females in the first 10 days of their life, even though a considerable number of sperm were observed in the testes, vasa deferentia and seminal vesicles following dissections after the last mating series. These results resemble those of Jones (1973) but are quite different from those obtained by Sharma and Rai 1967, Grover and Pillai 1970, Bhasin and Pillai (personal communication) where they observed that normal *Ae. aegypti* males could inseminate about 16 females and that treated males depleted their sperm faster than normal males. The variation observed in the insemination capacity of normal *Ae. aegypti* males may be attributed to variation in strains or rearing procedures or both.

In the first experiment on permanency of sterility in males, thiotepa induced almost the same level of sterility in each of the 5 successive matings. Dissections of spermathecae of control females and of females mated with sterile males showed the presence of sperm in all instances. In the second experiment, matings of 1-week old and 2-week old unmated treated males also induced the same levels of sterility as did the corresponding matings of counterpart mated males. Thus, there was no recovery in fertility during 2-week maintenance of males as virgins.

The percent sterility of eggs laid by females mated with sterile males in the 1st, 2nd and 3rd oviposition cycles was similar showing that sperm stored in the spermathecae of the females did not show any recovery in induced dominant lethals or further impairment of the original sterilizing effect of thiotepa.

Results of the study on effect of thiotepa on filial generations are presented in Table 8. The primary crossing showed 96.8% dominant lethality due to the treatment. The fertility from crossing the F_1 was slightly lower than the parallel control, whereas the fertility from the outcross of F_1 males and incrossing the F_2 did not differ from those of the controls. The sex ratio, in the F_1 progeny

Table 8. Results of the experiment on inheritance of sterility from thiosepa-treated males* of *Ae. aegypti*.

Generation	Crosses	No. total eggs examined	Hatch (%)
P	a) N ♀♀ × T ♂♂	7990	3.2
	b) N ♀♀ × N ♂♂	4493	95.8
F_1	a) Selfed	7518	85.0
	b) N ♀♀ × F_1 ♂♂	2226	98.1
	c) N ♀♀ × N ♂♂	3842	96.6
F_2	a) Selfed	3676	93.4
	b) N ♀♀ × N ♂♂	3840	96.9

* 4–28 hrs. old pupae exposed to 0.4% thiotepa solution for 3 hrs.
N=Untreated T=Treated.

of the outcross and F_2 generations was similar to the controls. These results showed that treatment with thiotepa did not induce (1) significant sterility in filial generations or (2) sex-linked recessive lethals. Similar results were obtained by Murray and Bickley (1964) with *C. p. quinquefasciatus* using apholate.

The present study with *Ae. aegypti* has shown that thiotepa sterilized males were almost fully competitive in both laboratory and field cages and permanently sterilized. Similar results were obtained with *C. p. fatigans* males sterilized by pupal treatment with thiotepa (Sharma et al. 1973) Thus thiotepa is a promising chemosterilant for *Ae. aegypti*, and the method described for sterilization can be adopted for a sterile male release technique.

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References Cited

Bertram, D. S. 1963. Observations on chemosterilant effect of an alkylating agent, thiotepa, on a wild caught, *Anopheles gambiae* var. *melas* (Theo.) in Gambiae, West Africa and on laboratory bred *Anopheles gambiae gambiae* and *Aedes aegypti* (L.). Trans. Roy. Soc. Trop. Med. Hyg. 57:322–35.

Craig, G. B. 1967. Mosquitoes: Female monogamy induced by male accessory gland substances. Science. 156:1501.

Dame, D. A., D. B. Woodard, and H. R. Ford. 1964. Chemosterilization of *Aedes aegypti* (L.) by larval treatments. Mosquito News 24:1–6.

George, J. A. and A. W. A. Brown. 1967. Effect of chemosterilant hempa on the yellow fever mosquito and its liabiltiy to induce resistance. J. Econ. Entomol. 60:974–78.

Glancey, B. M. 1965. Hempa as a chemosterilant for the yellow-fever mosquito *Aedes aegypti* (L.). Mosquito News 25:392–96.

Grover, K. K. and M. K. K. Pillai. 1970. Mating ability and permanency of sterility in hempa-sterilized males of the yellow-fever mosquito, *Aedes aegypti* (L.). J. Med. Entomol. 7:198–204.

Grover, K. K., M. K. K. Pillai and C. M. S. Dass. 1967. Effect of certain alkylating and non-alkylating chemosterilants on *Culex fatigans* Wied. Curr. Sci. 36:625–27.

Haisch, A. 1970. Some observations on decreased viability of irradiated Mediterranean fruit fly. In: Sterile Male Technique for Control of Fruit Flies. Proceedings of a panel on the application of the sterile-male technique for control of insects with special reference to fruit flies. Organized by the joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, held in Vienna, 1–5 Sept., 1969. pp. 71–75. Vienna, International Atomic Energy Agency.

Hayes, R. O. 1953. Determination of a physiological saline solution for *Aedes aegypti* (L.). J. Econ. Entomol. 46:624–27.

Jones, J. C. 1973. A study on the fecundity of male *Aedes aegypti*. J. Insect Physiol. 19:435–39.

Madhukar, B. V. R., M. K. K. Pillai and A. B. Borkovec. 1971a. Chemosterilization of the yellow-fever mosquito. 1. Laboratory evaluation of aziridinyl compounds by larval and pupal treatments. J. Econ. Entomol. 64:1024–27. 1971b. Chemosterilization of the yellow-fever mosquito. 2. Laboratory evaluation of phosphoramides by larval and pupal treatments. J. Econ. Entomol. 64:1027–30.

Murray, W. S. and W. E. Bickley. 1964. Effect of apholate on the southern house mosquito *Culex pipiens quinquefasciatus* Say. Univ. Maryland Agr. Exptl. St. Bull. 134:1–37.

Patterson, R. S., M. D. Boston, H. R. Ford and C. S. Lofgren. 1971. Techniques for sterilizing large numbers of mosquitoes. Mosquito News 31:85–90.

Patterson, R. S., D. E. Weidhaas, H. R. Ford and C. S. Lofgren. 1970. Suppression and elimination of an island population of *Culex pipiens quinquefasciatus* with sterile males. Science 168:1368–69.

Pillai, M. K. K. and K. K. Grover. 1969. Chemosterilization of *Culex pipiens fatigans* Wiedemann by exposure of aquatic stages. I. Sterilization potential of certain aziridinyl compounds. Bull. Wld. Hlth. Org. 41:915–28.

Rai, K. S. 1964. Cytogenetic effects of

chemosterilants in mosquitoes. II. Mechanism of apholate-induced changes in fecundity and fertility of *Aedes aegypti* (L.). Biol. Bull. 127:119–31.

Rao, T. R. 1974. Research on genetic control of mosquitoes in India.: Review of the work of the WHO/ICMR Research Unit. J. Com. Dis. 6:57–72.

Sharma, V. P., R. S. Patterson, K. K. Grover and G. C. LaBrecque. 1973. Chemosterilization of the tropical house mosquito *Culex pipiens fatigans* Wied.: Laboratory and field cage studies. Bull. Wld. Hlth. Org. 48:45–48.

Sharma, V. P. and K. S. Rai. 1967. Sperm depletion in chemosterilized and normal males of the yellow-fever mosquito, *Aedes aegypti*. Can. Entomol. 99:1116–18.

Van de Hey, R. C. and G. B. Craig. 1958. Multiple fertilization demonstrated in *Aedes aegypti*. Bull. Ent. Soc. Amer. 4:102.

Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195:786–87.

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

Weidhaas, D. E., H. R. Ford, J. B. Gahan and C. N. Smith. 1961. Preliminary observations on chemosterilization of mosquitoes. New Jersey Mosquito Extermin. Assn. Proc. 48:106–9.

White, G. B. 1966. Chemosterilization of *Aedes aegypti* (L.) by pupal treatment. Nature 210:1372–73.