SEXUAL COMPETITIVENESS OF *CULEX TARSALIS* MALES STERILIZED WITH THIOTEPA

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ABSTRACT. *Culex tarsalis* males were treated as pupae by immersion in 0.7% or 0.9% solutions of the chemosterilant thiotepa. Fertility rates of eggs sired by these males were 84% to 98% less than those of eggs sired by untreated controls. Sterilized colony males accomplished 41% to 52% of matings in competition against equal numbers of untreated colony males for colony females. When tested similarly against untreated wild males for wild females, sterilized colony males performed inconsistently but, overall, were responsible for 70% of all matings. Results suggest that limited field trials are appropriate.

Ever since sterile-male release was first used to control the screwworm, attention has been given to developing this technique for mosquito control. In a number of laboratory studies, male mosquitoes have been sterilized, and these males have remained more or less sexually competitive. When Ramakrishnan et al. (1962) subjected *Culex fatigans* [*pipiens quinquefasciatus* Say] to X-irradiation in the pupal stage, resulting males were sterile but were less competitive than untreated stock. Weidhaas and Schmidt (1963), working with *Aedes aegypti,* found that competitiveness was reduced after pupal treatment with gamma radiation, but not after ingestion of apholate by adults. Similar findings were reported for *Culex pipiens quinquefasciatus* by Smittle et al. (1968). Using apholate as the sterilant for *C. p. quinquefasciatus,* Murray and Bickley (1964) achieved high levels of sterility without impairment of competition. Also working with apholate and *C. p. quinquefasciatus,* which were housed in a very large outdoor cage, Patterson et al. (1968) obtained high sterility, but apparently there was substantial loss of mating proficiency. With the same apparatus, they later found (Patterson et al., 1972) that *C. p. quinquefasciatus,* when treated with tepa, became sterile and retained sexual vigor. Patterson's group (1971) also reported that when male *C. p. quinquefasciatus* were treated as pupae with thiotepa, they competed favorably as adults, and that thiotepa was superior to tepa and metepa as a pupal sterilant.

Although *Culex tarsalis* is an important disease vector, reports are lacking concerning its reaction to sterilizing procedures. The purpose of the present study was to gain information on the sexual performance of this species under cage conditions after treatment with a chemosterilant.

MATERIALS AND METHODS. Both colonized and wild mosquitoes were used. The former were of the Fort Collins strain which was established in 1958 from natural Colorado stock. There is no record of additional field-collected mosquitoes ever having been added, and the strain now differs from wild populations in behavior and virus susceptibility. Wild mosquitoes, when needed, were collected as pupae and late-instar larvae from Larimer County, Colorado. All mosquitoes were used 2 to 4 days after emergence.

The sterilizing technique was essentially that of Patterson et al. (1971) and consisted of immersing pupae of mixed sexes for 4 hours in phosphate-buffered solutions of thiotepa (pH 7.2) at concentrations of 0.7% and 0.9%. Treated pupae were removed, rinsed, and placed in holding cages. Emergent females were removed daily and destroyed, and males were retained for experimental use. Pupae for nonsterilized controls were treated with phosphate-buffered saline diluent only. The experimental design required females known to be unmated. These were obtained by isolating individual pupae in vials for emergence.

Experiments were based on comparison of 3 cages of unmated females: the 1st also containing sterilized males, the 2nd untreated males, and the 3rd equal numbers of treated and untreated males. Competitiveness was determined by observing the proportion of egg rafts from Cage 3 (mixed males) exhibiting the low hatch-rate characteristic of Cage 1 (treated males), in contrast to the normal hatch rate of Cage 2 (untreated males). Experimental mosquitoes were matched as to age and origin.

Male and female mosquitoes were caged together 4 to 8 days for mating and then were offered a host. Engorged females were then placed in individual fabric-capped cardboard cartons (1-pint ice cream cartons), each with an oviposition dish. As females oviposited, each mosquito was examined for sperm in its spermathecae. Eggs were retained to determine hatch rate. Non-hatching of eggs from inseminated females was regarded as evidence of male sterility, but rafts from spermless females were disregarded.

Experiments were done both indoors and outdoors. In the indoor insectary, the temperature was 75° F; the humidity, 60% to 75%. Cages were of plastic screen and measured 8 x 12 x 12 inches. Outdoor experiments involved both colonized and field-collected stock and were conducted during the months of June, July, and August in 3 large cages (4 x 4 x 7 feet) arranged side by side under a frame canopy. Each cage contained a pan of water near sheltered microhabitats where gradient humidities ranged from 30% to near 100%.

RESULTS. In competition trials among colony mosquitoes, average hatch rates were 78% to 95% when male mosquitoes were untreated and 2% to 15% when males had been treated with thiotepa. Occasionally a treated male sired a raft of high hatch rate. Conversely, a few rafts from untreated males were of low viability. This accounts for the overlap of hatch rate ranges between treated controls and untreated controls (Tables 1 and 2). In this circumstance it was impossible to categorize with certainty each raft originating in the "experimental" cages, in which both kinds of male were present. Each raft from those cages was therefore assigned to the category whose average it more closely resembled. At least 40% of the "experimental" egg rafts always fell in the sterile category. This is indicative of competition by sterile males. These observations are presented in Table 1, in which are summarized results of one experiment at 0.7% treatment, a second at 0.9% treatment, and a third in which these concentrations were compared in a single lot of mosquitoes. When the concentrations were tested separately, the higher concentration of thiotepa (0.9%) produced a lower hatch rate (2% vs. 15%) without a corresponding loss of competitiveness (41% vs. 50%). When compared simultaneously on a single lot of males, the higher and lower concentrations gave similar rates of fertility (2% and 3% hatch) and competitiveness (46% and 52%).

Sterilized colony males were next evaluated against untreated wild males in competition for wild females, since that situation would obtain in a field release. Results were surprising. When only one kind of male was present (control cages), that kind of male mated with the wild females. This was shown in 5 matings by sterile (colony) males and 4 by wild. However, when sterile (colony) and wild males were caged together with wild females, all of the 6 resulting matings were, on the basis of hatch rate, performed by sterile (colony) males. When tested against colony males, however (indoors, with colony females), an aliquot of these sterile males competed in the usual way, i.e., 4 of the 9 matings were by treated specimens. These results are given in Table 2.

In an attempt to confirm this observation, additional trials were made during the subsequent summer. In these, 243 egg rafts were deposited, of which only 12 were, as indicated by spermathaecal examination, from inseminated females. Of the 12 matings, only 4 took place in an "ex-

Table 1. Competitiveness of *Culex tarsalis* males treated as pupae with 0.7% or 0.9% thiotepa.

Thiotepa treatment	Experimental parameters	Control groups				Experimental groups	
		Treated males		Untreated males		Mixed treated and untreated males	
0.7%	Nos. of mosq.[a]	30:0:30		0:30:30		30:30:30	
	No. of rafts [b]	10		8		14	
	Av. % hatch (range)	15(0–49)		91(72–100)		50(13–97)	
	No. rafts sired by treated males [c]					7	
	Sterile rafts/total rafts—%					7/14–50	
0.9%	Nos. of mosq.[a]	50:0:50		0:50:50		50:50:50	
	No. of rafts [b]	21		20		17	
	Av. % hatch (range)	2(0–16) [d]		78(8–100) [e]		54(0–99)	
	No. rafts sired by treated males					7	
	Sterile rafts/total rafts—%					7/17–41	
0.7% vs. 0.9%	Thiotepa concentration	0.7%	0.9%	0.7%	0.9%	0.7%	0.9%
	Nos. of mosq.	100:0:100	100:0:100	0:100:100	0:100:100	100:100:100	100:100:100
	No. of rafts	27	28	24	26	39	27
	Av. % hatch (range)	2(0–13)	3(0–14)	95(0–99) [f]	81(16–100) [g]	45(0–100)	38(0–99)
	No. rafts sired by treated males					18	14
	Sterile rafts/total rafts—%					18/39–46	14/27–52

[a]—Numbers of treated males:untreated males:females.
[b]—Only rafts from inseminated females are included.
[c]—Resembling treated controls.
[d]—All but 1 hatched <6%.
[e]—All but 2 hatched >65%.
[f]—All but 2 hatched >75%.
[g]—All but 2 hatched >60%.

Table 2. Competitiveness of one lot of colony male *Culex tarsalis* sterilized with 0.7% thiotepa.

Kind of untreated males	Kind of females	Experimental parameters	Control groups		Experimental groups
			Treated males	Untreated males	Mixed treated and untreated males
Wild [a]	Wild	Nos. of mos. [b]	166:0:166	0:166:166	166:166:166
		Total rafts deposited	18	17	19
		No. of rafts from inseminated females	5	4	6
		% hatch individual rafts (av)	30, 22, 6, 3, 0; (12)	100, 92, 82, 76; (88)	34, 24, 15, 11, 3, 0; (14)
		No. of rafts sired by treated males [c]	5	..	6
		Sterile rafts/total rafts			6/6 (100%)
Colony	Colony	Nos. of mos.	32:0:32	0:32:32	32:32:32
		Total rafts deposited	10	10	12
		No. of rafts from inseminated females	8	9	9
		% hatch, individual rafts (av)	93, 26, 22, 22, 13, 13, 12, 5, 4; (18)	99, 99, 97, 92, 90, 88, 84, 46; (88)	100, 99, 99, 99, 88, 41, 30, 12, 6; (66)
		No. of rafts sired by treated males	8	..	4
		Sterile rafts/total rafts			4/9 (44%)

[a]—Test with wild mosquitoes was done in outdoor cages 4′ x 4′ x 7′.
[b]—Treated males:untreated males:females.
[c]—Resembling treated controls.

perimental" cage in which colony and wild males were competing; and of these 4, only one (on the basis of hatch rate) was sired by a sterile (colony) male. The results of all trials involving wild stock appear in Table 3. Of the 10 competitive matings during the 2 summers, 7 were considered to be by sterile (colony) males.

tests should be done in even larger enclosures, or in the field.

In our experiments, "competitiveness" was actually a measure not only of competitive behavior of males but also of their survival. No attempt was made to evaluate the relative effects of these factors. Obviously both must be favorable if maxi-

Table 3. Sexual competitiveness of treated colony *Culex tarsalis* males vs. untreated wild males for wild females in outdoor cages.

Trial number (Year)	Control groups: Treated colony males only Matings/rafts	Control groups: Untreated wild males only Matings/rafts	Experimental groups: Mixed males Matings/rafts	Number sterile matings	Sterile rafts/ total rafts (%)
1—(1971)	5/18	4/17	6/19	6	6/6—(100)
2—(1972)	0/59	0/45	4/63	1	1/4—(25)
3—(1972)	3/9	2/7	0/6	..	..
4—(1972)	1/21	2/13	0/20	..	..
Totals	9/107	8/82	10/108	7	7/10—(70)

DISCUSSION. It is clear that a high degree of sterility in *Culex tarsalis* can be achieved without loss of sexual competitiveness through treatment with thiotepa. This is shown by the 5 trials involving only colony stock, and is in agreement with the cited studies of others with tepa and thiotepa, as well as most of the reported studies with apholate.

The use of a behaviorally aberrant mosquito strain, like the Fort Collins colony, is not precluded for sterile male operations, provided its released males are sufficiently competitive against wild males for wild females. In our attempts to evaluate our males in this regard, we found it necessary to use large outdoor cages, because wild Colorado strains of *C. tarsalis* do not mate readily in small spaces. The tests showed that the colony mosquitoes are still anatomically and behaviorally capable of mating with wild stock. However, performance varied greatly.

The low insemination rates in outdoor cages suggest that confinement, even in such large spaces, adversely affects mating. For this reason, we feel that any further mum control is to be achieved by a release operation.

These studies show that *C. tarsalis,* like other species, can be chemosterilized without loss of vigor. They also suggest that a colony strain of this species can be highly competitive with a wild one. Patterson et al. (1970) were able to control a natural population of *C. p. quinquefasciatus* that was physically isolated from infiltration. The results reported here indicate the desirability of field trials with *C. tarsalis* to determine whether sterile male release can be developed into an effective control measure for that species.

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