ARTICLES

STERILIZATION OF MALE *CULEX QUINQUEFASCIATUS*: EVALUATION OF FIVE INSECT CHEMOSTERILANTS AND GAMMA IRRADIATION[1]

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ABSTRACT. When male pupae of *Culex quinquefasciatus* Say were exposed to 5 chemosterilants, 3 compounds caused over 95% sterility. Males exposed to 9 or 10 kR of gamma irradiation as pupae were over 94% sterile. The chemosterilants and the irradiation reduced mating competitiveness in most tests. Male pupae irradiated 22 to 26 hr after pupation had higher levels of sterility and less mortality than those irradiated 4 to 8 hr after pupation. The importance of both sterility and mating competitiveness in a sterile male release program was emphasized by theoretical calculations showing that fully sterile and competitive males released into a population of one million males and one million females at a 19:1 ratio would reduce the population to less than one individual in 5 generations. However, when males were 98% sterile but only 50% competitive, it would require 8 generations, and when males are 89% sterile and 90% competitive, it would take 22 generations to reduce the population to less than 1 individual.

In recent years there has been considerable interest in the use of sterile males to control *Culex quinquefasciatus* Say (=*fatigans* Wiedemann). Patterson et al. (1970) were successful in suppressing an indigenous mosquito population on a small island by releasing males sterilized with tris (1-aziridinyl) phosphine sulfide (thiotepa). More recently, Patterson et al. (1975) were able to induce sterility in the indigenous mosquito population of 2 small villages in India by releasing males sterilized with gamma radiation. The present study was conducted to obtain more information concerning the usefulness of both chemosterilants and ionizing radiation applied to pupae in producing effective sterile male *Cx. quinquefasciatus* for field release.

MATERIALS AND METHODS. The chemosterilants used in this study were selected from compounds shown by Lofgren et al. (1973) to produce sterility in male *Anopheles albimanus* Wiedemann treated as pupae. To evaluate these compounds against *Cx. quinquefasciatus*, male pupae were obtained from the laboratory colony by using a Fay and Morlan (1959) type pupal separator. The pupae (200 per treatment) were removed from water with a small strainer, placed momentarily on paper toweling to remove excess water, and 200 pupae were transferred from the strainer onto filter paper (9 cm, Whatman No. 2) in plastic petri dishes of the same size. Then 6 ml of an aqueous solution (buffered to pH 7) of one of the chemicals (concentrations of 0.025 to 0.2%) were pipetted over the pupae and filter paper. The control was treated with 6 ml of pH 7 buffer solution. The petri dishes were then partly covered with the lid to retard evaporation but left sufficiently ajar so emerging males could escape into the aluminum screen cage (15.2 x 25.4 x 25.4 cm) where the dishes were placed. Each cage was provided with

[1] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.

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3% sugar water solution. Mortality of pupae and adults that did not complete emergence was determined after 48 hr. Adults that did emerge were immobilized in a cold room (1–2°C) within 24 hr of emergence to facilitate the handling and counting of males. Then groups of 25 treated males were placed with 50 virgin, untreated females in an aluminum cage as previously described containing 3% sugar water as a source of nutrient. Three days later, a 2- to 3-day-old-chicken was placed in each cage to serve as the blood source for the females. Three to four days later paper cups containing an infusion of larval diet (1:1:1 ratio of dried brewer's yeast, liver powder and a hog supplement) were placed in the cages to serve as the oviposition medium. After oviposition the cups were removed and 25 egg rafts from a treatment were picked at random, and each raft was placed in a plastic vial containing ca. 10 ml of infusion. Subsequent to hatching, the percentage sterility was determined by comparing the number of unhatched eggs with the total number of eggs.

For the tests of sterility induced by gamma irradiation, male pupae of mixed ages were placed in plastic petri dishes with ca. 10 ml of water and exposed to doses of gamma rays ranging from 5 to 10 kR in a cobalt 60 source similar to that described by Jefferson (1960). The dose rate ranged from 1224 to 1258 R per minute. In the tests to compare the effects of irradiation on pupae of different ages, the pupae were separated at pupation; then one group was irradiated 4 to 8 hr after pupation ("young" pupae) and another group was irradiated 22 to 26 hr after pupation ("old" pupae). After irradiation the pupae were placed in paper cups containing water, and these cups were placed in screen cages for the adults to emerge. Thereafter the mosquitoes were handled as in the chemosterilant tests.

For the tests of mating competitiveness, pupae were either treated with one of the three chemosterilants that produced over 95% sterility at the 0.1% concentration or with gamma irradiation. The treated and untreated males were taken from the same batch of insects. Exposures to the chemosterilants or gamma rays followed the procedures previously described. The 1:1:1 ratio (sterile male:normal male:normal female) tests were conducted in 15.2 x 25.4 x 25.4 cm cages while the 4:1:1 ratio studies were conducted in 41 x 41 x 41 cm cages. The females were placed in the cages 2 hr after the treated and untreated males. This gave the males sufficient time to recover from the effects of the low temperature inactivation. The sterility was assayed as described above. All tests were replicated 3 to 9 times.

RESULTS AND DISCUSSION. The data obtained when male pupae were exposed to various concentrations of the 5 chemosterilants are shown in Table 1. Thiotepa was the most effective of the chemicals tested as all concentrations produced over 95% sterility. P,P - bis(1 - aziridinyl) - N - ethylphosphinothioic amide produced 83 to 100% sterility and P,P - bis(1 - aziridinyl) - N - methylphosphinothioic amide produced 90 to 99% sterility; bis(1 - aziridinyl) ethylphosphine sulfide and P,P - bis(1 - aziridinyl phosphinothioic amide produced only 61 and 51% sterility, respectively, at 0.025%. There was some mortality in all tests, but the 95% mortality observed after treatment with 0.2% bis(1 - aziridinyl) ethylphosphine sulfide precluded its use as a sterilant. The control had low mortality indicating that the treatment method itself had little adverse effect.

The data obtained by exposing pupae of mixed ages to varying doses of gamma irradiation are summarized in Table 2. Mortality was low in all tests.

Table 1. Effect of chemosterilants on the mortality and sterility of male *Cx. quinquefasciatus* treated as pupae on filter paper.

Sterilant	Concentration (%)	Mortality of pupae and adults at eclosion (%)	Sterility (%)
Thiotepa	0.025	7.3	100.0
	0.05	8.3	95.7
	0.1	11.0	96.5
	0.2	12.5	100.0
P,P-bis(1-aziridinyl)-*N*-ethylphosphinothioic amide	0.025	8.2	83.0
	0.05	8.8	98.0
	0.1	9.0	99.5
	0.2	18.8	100.0
P,P-bis(1-aziridinyl)-*N*-ethylphosphinothioic amide	0.025	7.8	90.0
	0.05	11.3	90.0
	0.1	11.2	99.0
	0.2	11.7	96.0
Bis(1-aziridinyl)-ethylphosphine sulfide	0.025	23.0	61.5
	0.05	22.1	81.0
	0.1	28.0	91.0
	0.2	95.0	100.0
P,P-bis(1-aziridinyl)phosphinothioic amide	0.025	19.0	51.0
	0.05	21.0	70.5
	0.1	22.5	76.5
	0.2	7.0	100.0
Control	pH 7 buffer solution	2.8	1.8

Pupae exposed to 5 to 7 kR were less than 90% sterile; 8 to 10 kR produced over 90% sterility. Complete sterility (100%) was obtained in some tests with 10 kR, but this effect was not consistent.

The results of exposing young and old pupae to gamma irradiation are shown in Table 3. Adults from young pupae exposed to 6 kR had sterility of 40.3%; those exposed to 10 kR were 89% sterile; and the same exposures produced 55 and 93.5% sterility, respectively, in adults irradiated as old pupae. The sterility data indicated an SD_{50} for the young pupae of 6.6 kR compared to 5.9 kR for the old pupae. Also, the SD_{90} was higher for young pupae, 9.3 kR, compared with 8.9 kR for old pupae. Also, the difference was significant ($t = 8.4 < t_{0.05}) = 2.14$) between the observed mortality of young

Table 2. Effects of gamma irradiation of male pupae of mixed ages of *Cx. quinquefasciatus*.

Exposure dose (kR)	Pupal and adult mortality (%)	Sterility (%)
5	4.0	56.0
6	4.9	77.5
7	5.0	88.0
8	4.8	91.0
9	8.0	94.8
10	7.8	98.5
Control	2.0	1.6

Table 3. Effect of gamma radiation on "young" and "old" male pupae of *Cx. quinquefasciatus*.

	Young pupae (4–8 hr)		Old pupae (22–28 hr)	
Dosage (kR)	Mortality (%)	Sterility (%)	Mortality (%)	Sterility (%)
6	14.0	40.3	2.0	55.0
7	13.6	51.6	3.0	65.0
8	14.6	77.7	3.0	75.0
9	20.6	79.0	3.0	84.0
10	20.3	89.0	7.0	93.5
Control	2.6	0.33	1.6	0

and old pupae, which indicated that irradiation may have caused more somatic damage in young pupae. When the mortality and sterility of the young and old pupae were examined together, it was apparent that irradiation of old pupae produced higher levels of sterility with lower levels of mortality. It is therefore advantageous to irradiate older pupae to produce sterile males.

The results of the competitive mating experiments with male mosquitoes exposed to chemosterilants or gamma irradiation are shown in Table 4. Males exposed to thiotepa showed increased competitiveness at the 1:1:1 ratio, but all other chemosterilized males showed decreased competitiveness. Males exposed to irradiation as pupae showed a general decrease in competitiveness with increase in dose. This decrease was most evident at the 9 and 10 kR level. Both the chemosterilized and the irradiated males appeared to be less competitive at the 4:1:1 ratio than at the 1:1:1 ratio. The reason for this is unknown. The males exposed to 9 and 10 kR were less competitive at both ratios than were the males exposed to the three chemosterilants.

Table 4. Mating competitiveness of *Cx. quinquefasciatus* males treated with chemosterilants or gamma irradiation.

Treatment	Mating ratio[a]	% Expected sterility[b]	% Observed sterility
Thiotepa (0.1%)	1:0:1		98
	1:1:1	49	62
	4:1:1	78.4	70.2
P,P-bis(1-aziridinyl)-*N*-ethylphosphinothioic amide (0.01%)	1:0:1		98
	1:1:1	49	49.0
	4:1:1	78.4	55.0
P,P-bis(1-aziridinyl)-*N*-ethylphosphinothioic amide (0.01%)	1:0:1		100
	1:1:1	50	43.7
	4:1:1	80	59.5
5 kR	1:0:1		58.5
	1:1:1	29.3	29.0
	4:1:1	46.8	49.6
6 kR	1:0:1		65.0
	1:1:1	32.5	30.0
	4:1:1	52.0	51.5
7 kR	1:0:1		89.5
	1:1:1	44.8	39.0
	4:1:1	71.2	69.5
8 kR	1:0:1		89.0
	1:1:1	44.5	40.1
	4:1:1	71.0	60.8
9 kR	1:0:1		98.0
	1:1:1	49.0	28.0
	4:1:1	78.4	46.0
10 kR	1:0:1		99.0
	1:1:1	49.5	24.5
	4:1:1	79.2	40.0
Control	1:1	0	1.0

[a] Treated ♂:Normal ♂:Normal ♀.
[b] Corrected from observed sterility.

Since irradiation reduced competitiveness before complete sterility of males was obtained, we were interested in comparing theoretically the degree of population control with different levels of sterility and competitiveness. In our tests 9 or 10 kR caused ca. 98% sterility but reduced competitiveness by ca. 50%. Reducing the dose to 7 or 8 kR increased competitiveness to ca. 90% of normal but only caused ca. 89% sterility of males. We can determine which dose level would be more effective by the following theoretical calculations:

$$F_1 = P(1-S)R$$

where P is the number of insects in the parent generation; S is the degree of sterility introduced by the sterile males into that generation; R is the population growth rate per generation; and F_1 is the number of insects expected in the next generation. The degree of sterility (S) is determined by the number (N) of sterile males released (S = N/N+P). N must be adjusted for competitiveness and N and P must be adjusted for the release of fertile males. These calculations assume that no fertile females are released. If fully sterile and competitive males are released into a population of one million females and one million males at a ratio of 19:1, the population is reduced to less than 1 individual in 5 generations. When males are 98% sterile, but only half competitive, it requires 8 generations to reduce to less than one individual; however, the population is reduced by 99.5% after 5 generations. When males are only 89% sterile but 90% competitive, it requires 22 generations to reduce to less than 1 individual, and there is only 73% reduction of the population in 5 generations. In this case using the higher dose (higher sterility and lower competitiveness) would be most effective.

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POSITION ANNOUNCEMENT

A medical/veterinary entomologist is being sought to fill an academic position in the Department of Entomology, University of Missouri, Columbia, MO 65201. Additional information may be obtained from Dr. M. L. Fairchild, Department Chairman.