

## ARTICLES

USE OF RADIOSTERILIZED MALES TO CONTROL  
INDIGENOUS POPULATIONS OF *CULEX PIPIENS*  
*QUINQUEFASCIATUS* SAY: LABORATORY  
AND FIELD STUDIES

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**ABSTRACT.** Gamma irradiation was used as an effective means of sterilizing male *Culex pipiens quinquefasciatus* Say, since it possesses certain qualities that cannot be duplicated by other genetic techniques. Males were sterilized when exposed as pupae or adults to 6 kR. There was less inhibition in adult vigor when older pupae or young adults, rather than young pupae, were exposed. When adults more than 24 hours old

were irradiated, there was no reduction in mating competitiveness. At 5 kR females were rendered completely sterile since they failed to oviposit. Two short term field studies strongly indicated that irradiated sterile males were able to function favorably in the wild. Sterility in one of the indigenous populations within a 4-week period rose to a high of 72% just prior to termination of the release.

With the present trend towards urbanization throughout the world, *Culex pipiens quinquefasciatus* Say, a breeder in polluted water, is becoming a serious public health problem, both as a biting nuisance and as a vector of filariasis and encephalitis. Although mosquito numbers can be reduced by the use of insecticides, Graham and Bradley (personal communication) found that such treatments did not necessarily decrease the incidence of disease transmission. Theoretically an indigenous mosquito population could be eliminated and the transmission cycle broken if sterile male releases were integrated with conventional control techniques. The sterile male principle has been used successfully to control two indigenous populations of *C. p. quinquefasciatus*, one in a small vil-

lage in Burma using a cytoplasmic-incompatible strain (Laven 1967) and the other on a small island in the Gulf of Mexico (Patterson et al. 1970) using chemosterilized males.

Early laboratory studies on the use of irradiation to sterilize *C. p. quinquefasciatus* had indicated that radiosterilization of *Culex* mosquitoes in the pupal stage markedly impaired the vigor of the adults (Ramakrishnan et al. 1962, Smittle et al. 1968). Nevertheless, radiosterilization does have certain advantages over other genetic techniques such as chemosterilization, translocation, or cytoplasmic incompatibility. For example, there is no problem of chemical residues, no need to rear a strain that has a high degree of sterility and no concern that the accidental release of an incompatible female along with males might result in the establishment of a nonindigenous strain. The objectives of these studies were to improve the practicability of the radiosterilization technique as a means of controlling the Indian strain of *C. p. quinquefasciatus* under field conditions. This research was divided roughly into 3 parts.

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1. Basic laboratory studies to determine

the optimum methods of achieving the lowest sterilizing dose ( $99 \pm 1\%$  sterilization) that would not impair the vigor or behavioral characteristics of adult males.

2. The effect of the male sterilizing dose on females that are inadvertently irradiated and released with the sterile males.
3. The release of sterile males irradiated as pupae or adults into indigenous field populations to determine their survival, dispersal, and mating competitiveness.

## MATERIALS AND METHODS

All mosquitoes used in these studies were reared in the laboratory by the methods of Singh et al. (1974). The  $^{60}\text{Co}$  radiation source was located at the Indian Agricultural Research Institute and consisted of a Gamma Cell 200® with an exposure chamber measuring  $9 \times 19$  cm, and a dose rate of 1.7 kR/min.

**LABORATORY STUDIES.** For laboratory studies pupae to be irradiated were placed in plastic petri dishes either on damp cloth or in a small volume of water; adults were placed in dry plastic petri dishes after having been immobilized with cold. Following irradiation, mosquitoes were held in the laboratory in cages,  $20 \times 20 \times 30$  cm. For studies of sterility and mating competitiveness, irradiated and normal insects in various ratios of the same sex were allowed to cohabit for 3–4 days with normal insects of the opposite sex, and were allowed to feed on a 1% glucose solution. The 4th day food was removed and insects were starved for 1 day after which they were offered a young chick overnight for a blood meal. The following morning, chicks were removed, and the mosquitoes were again offered a fresh glucose solution. Three days later, a container of 1% fermented yeast infusion was placed in the cages for oviposition. Resultant egg rafts were set up individually in ca. 5 ml of water in plastic vials and were held in the laboratory for 48 hours, then the per-

cent hatch in egg rafts was checked microscopically to determine sterility.<sup>5</sup>

In the mating ability studies, adult males were irradiated and caged with 6 times their number of normal females. Normal males were likewise caged with a similar number of females as a control. Five days later, spermathecae of females were dissected and examined for presence of sperm to determine whether irradiation had affected the mating ability of the males.

An experiment was also run to determine whether monogamy in females was affected when they were inseminated initially by irradiated sterile males. Normal virgin females were caged with irradiated males for 5 days, then removed and recaged with normal males. Fertility of eggs would suggest that females had been inseminated more than once.

Since the survival of released insects is important to any sterile male release program, 3 studies were set up to determine whether irradiation seriously affected the survival of adult males. In the 1st experiment, males that had been exposed to doses ranging from 4 to 8 kR as 4 to 28-hour-old pupae, were caged with virgin females and checked for mortality at 5-day intervals for 30 days. A 6 kR dosage appeared to be most favorable; and in the 2nd experiment 2 groups of pupae, one more and the other less than 24 hours old, were exposed, and the adult males, when placed together with females, were checked for mortality every 5 days for 40 days. In the 3rd test 2 lots of 2-day-old adults exposed to 6 kR

<sup>5</sup> Sterility was based on the total number of rafts laid compared with those rafts that had less than 4% hatch. An average *Culex* raft in India consisted of 150 eggs because of variation in response of individuals to radiation. It is not unusual for a female which had been inseminated by an irradiated male to deposit a raft with a few viable eggs. Most of the resulting larvae would die prior to pupation. In nature, we found that less than 2% of the rafts laid had such low hatch. In the laboratory we found that 5% of virgin females which took a blood meal would oviposit a sterile raft, thus in nature some eggs which fail to hatch might have been laid by females which were technically uninseminated or virgin.

were checked for mortality every 5 days. One lot was caged with females, and the other alone to determine if mating activity shortened their survival. Untreated control lots were maintained in all the survival studies.

A test was made to determine whether the irradiated males remained sterile throughout their normal life span, thus 2-day-old males were exposed to 6 kR and placed with normal virgin females, removed, held alone for 14 days and then placed with a 2nd lot of normal virgin females. The eggs from both lots of females were checked for sterility. Also, any recovery of fertility in the 1st group of females that had been inseminated by irradiated males was checked by holding them for 14 days after their 1st oviposition, giving them a 2nd blood meal, and holding them for a 2nd oviposition. Any loss of sterility in the 2nd lot of eggs was interpreted as indicative of recovery in the females.

**FIELD TESTS.** The 1st field release was conducted in Sultanpur, a small village containing 200 houses and a human population of 1700. It was selected because of its high natural *C. p. quinquefasciatus* population. Numerous egg rafts for evaluation were readily obtained in 2 cement drains, that supplied about two-thirds of all the known larval breeding sites in the village. Starting March 4, 1971, and continuing daily until March 11, we released a total of about 74,500 males (averaging approximately 9,000/day) irradiated as 4 to 28-hour-old male pupae at 6 kR in a  $^{60}\text{Co}$  source. The procedure was as follows: Pupae were placed on damp netting in a plastic petri dish (1000 per dish), and the dishes were stacked in a holder for insertion into the exposure chamber; in this manner, the entire lot was exposed at one time so as to avoid prolonged use of the source. The irradiated pupae in the petri dishes were then packed in an ice chest and transported directly to the village. This method was satisfactory, and no excessive pupal mortality was observed during the packing, irradiation, transportation, or release. On the 1st day the

pupae were placed in clay pots positioned alongside the 2 main drains, but as these were destroyed by the villagers the pupae were subsequently placed directly into the main drains. From the number of dead pupae observed in the drains, mortality was estimated to be at least 10% before eclosion.

On September 14 a 2nd release was initiated for 1 month in the village of Pochanpur with adult males having been radiosterilized. This village with its 210 structures and surrounding fields covers an area of approximately 130 ha. There were about 1100 persons and 500 cattle housed in this complex. Within a 0.5 km radius of the village, there were 44 wells capable of breeding mosquitoes. However, most of the breeding was confined to 2 abandoned wells at the edge of the village and a few receptacles within the village. Based on hand catches in 15 houses, the estimated size of the indigenous population in this village at the start of the experiment was 14,860.

To evaluate sterility, 40 ovitraps (clay pots half filled with a fresh 1% yeast infusion) were distributed at random in cattle sheds throughout the village. These were set up on Monday and egg rafts were collected until Friday when they were overturned. Pretreatment sterility was 7.0%.

Following emergence males were held in the laboratory for at least 24 hours, etherized, then packed in small wire cylinders (7.5 x 13 cm) in lots of 10,000. These cylinders were then placed in a refrigerator to immobilize the adults. The cylinders were then transferred to prechilled ice chests for transport to the  $^{60}\text{Co}$  source. Following exposure to gamma irradiation (6 kR), mosquitoes in cylinders were returned to the ice chest for transport to the village and for release at 10 sites. Within 4 hours after irradiation, the mosquitoes had arrived at the release site and were beginning to revive.

## RESULTS

### LABORATORY STUDIES. *A. Optimum male*

*sterilizing dose.* Initially, male pupae (4-28 hours old) were exposed to various dosages from 1 to 10 kR of gamma radiation. The results are given below:

Dosage kR	1	2	3	4	5	6	7	8	9	10	0
Sterility %	8	19	30	48	96	99	99	100	100	100	3

Six kR was therefore considered the lowest suitable dose for sterilizing young male pupae. In subsequent studies 6 kR was equally effective against older pupae (>24 hours old).

Competitive mating studies were also carried out to determine whether there was some definite period for irradiation during the pupal or adult stage that would produce more vigorous males. As indicated below, those males exposed as 4 to 28-hour-old pupae to 6 kR, although less competitive sexually than normal males, were the least affected.

Ratio (males) sterile:normal	Sterility % in egg rafts when male pupae were exposed to			
	Theo- retical	6 kR	7 kR	8 kR
1:1	50	30	24	19
3:1	75	52	45	33
6:1	86	66	59	34
9:1	90	69	57	42

A 2nd experiment indicated that in the laboratory exposure of adults of both ages to gamma irradiation had little or no adverse effect on sexual competitiveness. When males were exposed as older pupae (>24 hours old) they were more competitive than when they were exposed as young pupae (<24 hours).

Ratio (males) sterile: normal	Theo- retical	Sterility (%) in egg rafts when males exposed as			
		Pupae		Adults	
		<24 hours	>24 hours	<24 hours	>24 hours
1:1	50	30	33	46	52
3:1	75	37	59	71	71
6:1	86	58	78	81	86
9:1	90	67	79	87	91

Also competitive mating studies were conducted in large cages (5.5 x 3.5 x 2.4 m) in the field with males exposed as pupae less than 24 hours old (Group 1), more

than 24 hours (Group 2) and as young adults less than 24 hours old (Group 3). The ratio of sterile to normal males was 4.5 to 1. Releases of approximately 2000 sterile males were made daily for 26 days. Results showed percentage sterility in egg rafts of 68, 70 and 72 compared with the expected 82 for groups 1, 2, and 3, respectively.

Thus competitiveness was more reduced than in the previous studies; possibly the reduction can be attributed to crowding and improper handling of the insects prior to, during, and after the exposure to gamma irradiation as well as to the larger cage and the ambient conditions in the field.

*B. Effect of gamma irradiation on female fecundity.* Because sexing of individuals in the pupal stage is seldom 100% accurate, some females are invariably irradiated and released with the males. Fecundity in females exposed as pupae to various doses of gamma irradiation was as follows:

Dose (kR)	Rafts collected	Average no. eggs/raft	Sterility (%)
0	34	246	2
1	35	205	8
2	38	199	8
3	27	97	27
4	12	47	56
5-10	0	..	..

Thus, the females failed to oviposit following an exposure to 5 kR or more in the pupal stage. Even at 3 and 4 kR there was a reduction in the number of eggs per raft as well as 27 and 56% sterility, respectively. In a subsequent test, virgin females were exposed with males to 6 kR in procedures identical to those developed for field releases. Then shortly after ex-

posure the females were removed and caged with normal males again. None oviposited. Therefore, sexing for an experimental release need not necessarily be precise, because females exposed to the male sterilizing dose are incapable of oviposition and would not bias the results. (All sterile eggs would result from sterile males mated with indigenous females.)

*C. Side effects of radiosterilization.* Additional studies of the effect of irradiation on the mating ability of male mosquitoes exposed to 6 kR as 1-day-old adults verified previous results. No significant difference was apparent in the mating ability of irradiated versus normal males. In both groups insemination averaged 4 females in 5 days. Also males irradiated with 6 kR appeared to produce female monogamy.

Male sequence	No. rafts with viable eggs	No. rafts with sterile eggs
Sterile followed by normal	6	55
Normal followed by sterile	48	8

Thus radiosterilization apparently did not interfere with the production of the accessory gland substance "matrone" (Craig 1967). The few viable eggs ob-

ing from 4 to 8 kR are given in Table 1. Survival of males treated with 4 kR compared favorably with that in the untreated check, but as the dose increased, longevity decreased.

When 2-day-old males were irradiated at 6 kR and released into cages and held with males only or with an equal number of females (Table 2), there was little difference in overall survival. However, these survived slightly longer than males irradiated as pupae.

The permanence of the sterility in males exposed to 6 kR and in the females crossed with them was excellent. In 2 weeks neither sex recovered fertility.

*FIELD RELEASES.* The 1st test involved releases over 8 consecutive days in the spring with males irradiated as pupae with a dose of 6 kR. Ten days after the initial release, the 1st observable sterility was noted. It increased steadily to 25% on the 14th day; thereafter it declined gradually (Table 4). From calculations similar to those used by Weidhaas et al. (1971) to determine the bionomics of a *Culex* population, this rapidly increasing population numbered approximately 15,000. Then with the estimated 10% mortality of the irradiated pupae observed in the drains and the 50 to 75% loss of competitiveness<sup>6</sup> we documented for the irradiated males, this ratio of sterile to

Table 1. Mortality of *C. p. quinquefasciatus* adult males exposed to various doses of gamma irradiation as 4 to 24-hour old pupae (100 males per test, average of 8-9 replicates).

Dose in kR	Cumulative % mortality at indicated days after treatment					
	1-5	6-10	11-15	16-20	21-25	26-30
None	5	7	7	8	9	81
4	5	6	8	11	15	96
5	6	11	24	45	62	100
6	5	10	45	68	75	100
7	7	21	63	82	90	100
8	8	21	65	81	86	100

tained in the sterile-normal sequence resulted because some females were not inseminated during the initial 5-day holding period.

Results of the survival study of males irradiated as young pupae at dosages rang-

<sup>6</sup> The estimated 10% mortality was based on the number of dead pupae counted in various sections of the drain as compared to number of live pupae released. The loss in competitiveness was based on laboratory and field cage studies where sterile to normal ratios of males to virgin females were known.

Table 2. Mortality of *C. p. quinquefasciatus* males irradiated when they were 2 days old with 6 kR and released into cages with and without females.

Treatment	Cumulative % mortality at indicated days after treatment						
	1-5	6-10	11-15	16-20	21-25	26-30	31-35
	100 males alone						
Control	2	2	4	8	14	22	95
Treated	3	12	14	17	20	27	96
	100 males and 100 females						
Control	2	4	5	7	8	15	99
Treated	0	3	5	7	11	25	97

fertile males in the release area should have approached 1:3. At this ratio, the theoretical sterility in the population should have been and was 25 percent. The natural sterility in the *Culex* population of the control village ranged from 0 to 3 percent during the test and averaged 1.1 percent. Therefore, for the first time irradiated males had been used successfully to incorporate a significant degree of sterility into a natural field population of any species of mosquitoes.

The 2nd test involved releases over 4 weeks in the fall with males irradiated as adults. A reduction in the hand catches and egg rafts in the study village and in an adjoining control village during the

latter part of the release was attributed to the cool weather.

An average of 38,500 radiosterilized adults were released daily at the various sites in the village of Pochanpur. The hand catch indices increased during the weeks of releases and showed a preponderance of males. During the first 3 weeks of the releases 6 times more males than females were estimated in the population, an indication of a sterile:normal male ratio of 5:1. Then 83 percent sterility should have been observed in the natural population since Rajagopalan (personal communication) stated that radiosterilized males from the same colony survived and dispersed as well as normal males. In fact sterility did increase significantly in the natural population, increasing as high as 72% on one occasion; however, it leveled out at about 60% (Table 4) which indicated that a significant number of radiosterilized males were mating with the indigenous virgin females.

Two days after the releases were terminated, males were collected from the village and brought to the laboratory for evaluation of sterility. The results (single pair crosses) indicated that there were about equal numbers of sterile and normal males in the village 2 days after the final release.

Table 3. Effect of releasing an average 9,000 *C. p. quinquefasciatus* males per day for 8 consecutive days (sterilized as pupae with 6kR) into a natural population of *Culex* in the village of Sultanpur.

Week <sup>a</sup>	Total no. egg rafts scored <sup>b</sup>	Avg. sterility (%)
Pre-release	72	0
1	878	1.5
2	5,398	16.3 <sup>c</sup>
3	12,218	13.0
4	3,600	9.7

<sup>a</sup> Release dates March 4 to 11, 1971. On March 4, the release pots were broken, and almost 1/2 of the pupae were spilled on the ground and died.

<sup>b</sup> The total number of egg rafts in the two main drains were scored in the second and third week. Subsamples were scored before and after this period.

<sup>c</sup> Highest sterility (25%) was observed in the natural population the 6th day following termination of releases.

## DISCUSSION AND SUMMARY

Although male *C. p. quinquefasciatus* have been sterilized by exposure to gamma irradiation in earlier studies, such treat-

Table 4. Effect of releasing an average 38,500 *C. p. quinquefasciatus* males per day for 4 weeks (sterilized as adults with 6 kR) into a natural population in the village of Pochanpur.

Week	House index <sup>a</sup>	Sex ratio M:F	Avg. no. of males released/day x 1000	Total no. rafts collected per week	Sterility (%)
Pre-release	1.4	....	0	160	6.8
1	6.2	1:0.17	30.7	209	7.6
2	13.6	1:0.16	39.0	95	26.3
3 <sup>b</sup>	16.5	1:0.17	44.4	43	63.0
4	9.7	1:0.04	40.0	48	60.4
5 <sup>c</sup>	...	....	0	18	50.0
6	2.0	1:0.22	0	..	...

<sup>a</sup> Average number of mosquitoes captured in a 15-minute period in a room based on 15 houses checked.

<sup>b</sup> A sterility of 72% was observed on one day during this period.

<sup>c</sup> During the 5th week, the house index and sex ratio data were lost.

ments have always been accompanied by a significant reduction in adult vigor. This same reduction occurred in the present study, but it was less when older pupae or young adults were treated and did not occur when adults more than 24 hours old were treated and held in the laboratory. Male vigor can also be reduced by improper handling of mosquitoes prior to, during, and after irradiation.

Irradiation appears to reduce the life span of male mosquitoes regardless of the stage at exposure, but it is not significant the 1st week or 10 days after treatment. Since the average life expectancy of the male mosquito in the field is only about 5-7 days, this shortened life span should be of little consequence. Indeed, our field releases with irradiated males have confirmed this assumption. Sterility in males exposed to 6 kR was permanent for 14 days, and exposure of males to irradiation did not affect their ability to produce monogamy in female *Culex* mosquitoes.

Females were completely sterilized and failed to oviposit when exposed to 5 kR. Smittle (1968) reported that females failed to oviposit after an exposure to 7 kR.

In 2 field studies, releases of moderate numbers of radiosterilized males caused a high degree of sterility in the indigenous *C. p. quinquefasciatus* population. The sterile males were therefore competing

favorably in nature. This method of sterilization, if done properly, could be used in large scale tests in isolated areas against *Culex*.

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