

GAMMA IRRADIATION OF EGGS, LARVAE, PUPAE, AND ADULTS OF *CULEX PIFIENS QUINQUEFASCIATUS* SAY

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ABSTRACT. When eggs, larvae, pupae, and adults of *Culex pipiens quinquefasciatus* Say were exposed to gamma rays from a cobalt-60 source, eggs proved to be the most sensitive to gamma irradiation. At a dosage of 1,000 R, over 95 percent of the eggs hatched but only 1 percent emerged as adults. The larvae were not as sensitive, but mortality was high at a dose of 4,000 R. Most of the mortality occurred either in the pupal stage or in the emerging adults that were so weak they died on the water.

However, adult males obtained from larvae exposed to 3,000 R were able to induce only 21 percent sterility in the eggs from unirradiated females. Mortality was not a problem when pupae and adults were irradiated, and dosages of 10,000 R produced about 99 percent sterility in males irradiated either as pupae or adults. However, dosages as high as 12,000 R to pupae and 20,000 R to adults did not produce 100 percent sterility in all tests.

Eggs, larvae, pupae, and adults of *Culex pipiens quinquefasciatus* Say were irradiated to determine whether sterile males could be produced more advantageously by irradiating a particular stage.

only males were irradiated. Controls for all stages were handled in exactly the same manner except for the exposure to irradiation.

METHODS AND MATERIALS

After irradiation, the egg rafts and larvae were placed in 30 x 19 x 5-cm enamel pans containing 1,000 ml of an infusion of desiccated hog liver and dried brewer's yeast in distilled water; the liver-yeast powder was added to the pans each day as food for the larvae. The pupae and adults were placed in cages made of 16 x 24 x 25-cm aluminum frames covered with tubular gauze and provided with 10 percent sugar water. Adults were allowed 3 days for mating. Then the females were offered a blood meal (1 to 14-day-old chicken); and 3 or 4 days later, paper cups containing liver-yeast infusion were placed in the cages to serve as the oviposition medium. Each egg raft deposited was placed in a separate cup and held for 3 days to allow for hatching, before a determination of percent sterility was made by comparing the number of unhatched eggs with the total number of eggs. The rearing rooms were maintained at 27° ± 1° C.

The mosquitoes used in the study were taken from a colony that was started from egg rafts collected at Gainesville, Florida, and had been reared in the laboratory for five generations. Irradiation exposures were made in a cobalt-60 source similar to the one described by Jefferson (1960). During this study, the dose rate ranged from 410 R per minute at the start to 370 R per minute at the termination. During irradiation, the eggs, larvae, and pupae were held in plastic containers in 15-50 ml of distilled water; the adults were held in cages constructed of ½-pint cardboard cartons and screen wire. The irradiation was done when the eggs were 12-18 hours old, when the larvae were in the fourth instar, when the pupae were less than 24 hours old, or when the adults were less than 10 hours old. In the tests with eggs and larvae, both sexes were irradiated; in the pupal and adult tests,

RESULTS AND DISCUSSION

IRRADIATION OF EGGS. The results of the egg irradiation tests are reported in Table

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TABLE 1.—Effect of gamma irradiation on eggs of *C. p. quinquefasciatus*.

Dose (R)	No. of eggs	Percentage of hatch	Percentage of pupation	Percentage of adult emergence ^a
500	3,178	95.7	31.5	15.5
1,000	4,541	95.5	4.6	1.2
1,500	3,141	66.2	0	
2,000	2,836	60.8	0.3	0
3,000	835	43.7	0	
0 (Control)	4,327	97.0	92.8	91.8

^a Includes only those adults able to leave the water surface.

1. The eggs were extremely sensitive to gamma irradiation, and mortality was high. As the dosage increased, the percentage of unhatched eggs increased. Exposures of 500 and 1,000 R had little effect on egg hatch but caused high mortality in the larval stage. Exposures of 1,500 R or more resulted in over 99 percent mortality by the end of the larval stage. Many emerging adults were weak and died on the surface of the water.

The results were therefore in agreement with those of other investigators. Willard (1965) reported that the egg stage of *Aedes aegypti* (L.) was the most susceptible of the stages to X-rays and that only a few reproducing adults were produced from eggs exposed to 1,600 R. Davis *et al.* (1959) found that 2,600 R of gamma irradiation caused a 50 percent reduction in the hatch of eggs of *Anopheles quadrimaculatus* Say. Terzian and Stahler (1966) reported that only 8 percent of eggs of *Aedes aegypti* exposed to 2,000 R of gamma irradiation became adults; how-

ever, after selection for over 30 generations, almost 60 percent became adults.

An attempt was made to evaluate the sterility of the adults resulting from eggs exposed to 500 and 1,000 R; however, the small number becoming adults prevented adequate evaluation. Thus, it appears that high mortality would preclude the irradiation of eggs of *C. p. quinquefasciatus* to produce sterile males.

IRRADIATION OF LARVAE. Larval and ensuing pupal mortality, the number of adults that emerged from irradiated fourth-instar larvae, and the sterility of the emerging adults are reported in Table 2. Exposure of the fourth-instar larvae to 500, 1,000, and 2,000 R had little effect on mortality or sterility, but exposures of 3,000, 4,000, and 5,000 R reduced the number of adults leaving the water by 32, 84, and 96 percent, respectively. Also, exposures of 4,000 and 5,000 R failed to supply sufficient adults for evaluation of sterility. Exposures of 3,000 R resulted in 21 percent sterility, but the sterility ob-

TABLE 2.—Effect of gamma irradiation on fourth-instar larvae of *C. p. quinquefasciatus*.

Dose (R)	Percent mortality in larval stage	Percent mortality in pupal stage	Percent mortality as adults on water surface	Percent surviving as adults and leaving water	Percent sterile eggs produced by surviving adults
500	0	5.1	2.7	92.2	4.3
1,000	5.6	1.2	1.2	92.0	2.2
2,000	5.0	2.7	3.8	88.5	5.5
3,000	6.5	6.2	20.0	67.3	21.4
4,000	7.3	34.3	42.5	15.9	
5,000	21.8	46.3	28.0	3.9	
0 (Control)	1.4	3.9	1.6	93.1	3.4

tained from exposure to 500, 1,000, and 2,000 R approximated the control sterility of 3.4 percent.

In contrast, Rai (1963) reported that *Aedes aegypti* fourth-instar larvae exposed to 2,000 R or more of X-rays died before becoming adults. Willard (1965) reported similar results when the same species was exposed to 1,600 R. Davis *et al.* (1959) reported that the LD₅₀ for the fourth-instar *Anopheles quadrimaculatus* was 125,000 R when the treatment was evaluated at a 24-hour posttreatment but only 32,000 R when it was evaluated at a 48-hr posttreatment. This almost four-fold difference in the dose required to produce 50 percent mortality at 24 and 48 hours after treatment indicated that larval response to irradiation was not immediate. In our tests, at the higher dosages, many irradiated larvae of *C. p. quinquefasciatus* died during the ecdysis to pupae; likewise, there was high mortality during the ecdysis to adults. However, Rai (1963) reported that most of the *Aedes aegypti* larvae exposed to 2,000 to 4,000 R died as untanned pupae; we had only 34 percent mortality in the pupal stage when larvae were exposed to 4,000 R.

The high mortality in larvae exposed to 4,000 and 5,000 R and the low sterility resulting with exposures of 3,000 R or less made the irradiation of larvae unsatisfactory as a method of producing sterile males.

IRRADIATION OF PUPAE. Table 3 reports the sterility of eggs from unirradiated fe-

TABLE 3.—Sterility of eggs of untreated female *C. p. quinquefasciatus* mating with males irradiated as pupae.

Dose (R)	No. of rafts examined	Percent sterility of eggs
6,000	42	46.3
7,000	47	92.3
8,000	35	86.6
8,500	63	94.9
9,000	84	97.1
10,000	192	99.6
11,000	32	99.7
12,000	33	99.7
0 (Control)	125	4.2

males mating with males exposed to 6,000 to 12,000 R as 1-day-old pupae. Mortality was not a problem in the irradiation of pupae. Exposure to 6,000 R produced only 46.3 percent sterility; exposure to 7,000 and 8,000 R gave 86.6 and 92.3 percent sterility, respectively. However, a dosage of 8,500 R gave 94.9 percent sterility, exposure to 9,000 R produced 97.1 percent sterility, and exposures to 10,000 to 12,000 R gave over 99 percent sterility. Thus, high levels of sterility can be produced in males by exposing pupae to gamma irradiation; however, 100 percent sterility was not obtained with dosages as high as 12,000 R. This result does not agree with that of Ramakrishnan *et al.* (1962). They reported that irradiation of *Culex fatigans* Wiedemann pupae with 11,550 R of gamma rays gave complete sterility and that exposure to 7,700 R produced almost complete sterility (98 percent). Also, they found that 50 percent of the egg rafts exposed to 7,700 R had no hatch but that from 1 to 6 eggs hatched from the remainder. In contrast, we found that at 8,500 R an average of 9.4 larvae hatched per raft; and at 10,000 R an average of 0.86 larvae hatched.

Thus, males irradiated as pupae can be sterilized. However, Smittle *et al.* (1968) reported that irradiated males are less competitive than normal males. Therefore, larger numbers of irradiated males would have to be released to produce the sterility expected if they were equally competitive.

IRRADIATION OF ADULTS. Table 4 reports the sterility of eggs from unirradiated females mating with males exposed to 5,000 to 20,000 R less than 10 hours after emergence. Mortality was not a problem in the irradiation of adults. Dosages of 5,000 and 6,000 R produced less than 90 percent sterility; dosages of 7,000 to 10,000 R gave 93.64 to 98.87 percent sterility. All dosages above 10,000 R gave over 99 percent sterility. However, with doses of 16,000 and 18,000 R all replications produced 100 percent sterility, but in some replications of doses 17,000, 19,000, and 20,000 R, from 1 to 3 eggs hatched.

TABLE 4.—Sterility of eggs of untreated female *C. p. quinquefasciatus* mating with males irradiated less than 10 hours after emergence.

Dose (R)	No. of rafts examined	Percent sterility of eggs
5,000	59	81.23
6,000	55	87.49
7,000	57	93.64
8,000	35	95.29
9,000	57	97.45
10,000	22	98.87
12,000	78	99.65
13,000	68	99.70
14,000	70	99.82
15,000	86	99.92
16,000	40	100.00
17,000	57	99.97
18,000	20	100.00
19,000	59	99.99
20,000	43	99.99
0 (Control)	231	1.94

The results of irradiating adults are therefore comparable with the results obtained when pupae were irradiated, which indicates that either adults or pupae could be irradiated for use in a sterile male release program. However, the preliminary results again indicate that males irradiated as adults exhibit reduced mating competitiveness as did males irradiated as pupae. Moreover, the preparation of adults for irradiation is more time-consuming than the preparation of pupae, and more pupae than adults can be irradiated in a given volume. Thus irradiation of pupae would

be most advantageous in a large-scale sterile male release program unless it is necessary to release males only. Then irradiation of adults should be considered because large numbers of pupae are very difficult to sex without including at least 1 percent females in with the males. Also, when mechanical means are used to separate pupae, only 50 to 60 percent of the males are utilized; all males would be utilized if adults were irradiated.

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