



## THE EFFECT OF GAMMA IRRADIATION ON REPRODUCTION AND LIFE SPAN OF THE MOSQUITO *CULEX TARSALIS* COQUILLET

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The successful pilot attempt to eradicate the screw-worm fly, *Cochliomyia hominivorax* (Coquerel), from the island of Curaçao (Baumhauer *et al.*, 1955) by releasing gamma-irradiated sterilized adults encouraged the more elaborate eradication program in the southeastern United States, (Lindquist 1959, Knipling 1960). The result was that many workers began to consider the use of similar techniques against other pests including mosquitoes (Davis *et al.*, 1959, Weidhaas *et al.*, 1963).

The present paper is a report of studies conducted in the laboratory at Corvallis, Oregon to determine the effects of gamma irradiation on different stages of the mosquito, *Culex tarsalis* (Coquillett), and the effects of adding large numbers of irradiated males to caged populations of normal males and females.

**MATERIALS AND METHODS.** The mosquitoes were an insecticide-susceptible strain of *C. tarsalis* maintained at this laboratory. All stages of the insects were irradiated with gamma rays from a cobalt-60 source. The dose was about 0.460 kiloroentgens (kr.) per minute though lower rates were used with no apparent difference in effect. All stages were irradiated by placing them in small polyethylene containers that were lowered into the well of the unit. After a predetermined time, the containers were removed, and the contents were transferred to holding cages for observation of the complete life cycle. Egg masses and pupae were exposed on damp filter paper; larvae were left in water. Adults were first anesthetized with carbon dioxide for ease of handling and then placed in dry containers and allowed to recover before irradiation. Adult diet consisted of a 5 percent sugar water solution.

**RESULTS. Preliminary Experiments.** Radiation of Adults. When 1- to 2-day-old adults were irradiated, doses of 50 kr. and above caused 100 percent mortality within 7 days; also 100 kr. caused total mortality in 24 hours. At 25 kr. and lower doses, the exposed adults lived as long or longer than the controls. Normal males mated with females irradiated at 10 kr. or more produced no eggs. Males irradiated at 10 kr. or more mated with untreated females produced infertile egg masses, an indication of sterility.

**Radiation of Pupae.** Preliminary experiments had indicated that females irradiated as pupae were 100 percent sterilized at 5 kr. and 94 percent sterilized at 4 kr. Males, however, were 100 percent sterile only when they were treated at more than double this amount of radiation (about 12.5 kr.).

**Radiation of Eggs and Larvae.** When eggs of *C. tarsalis* were irradiated at 0.2, 0.4, and 0.8 kr., the eggs were severely affected at the two lower doses, and all were killed at the higher dose. Larvae and adults resulting from the few eggs that hatched after irradiation at 0.2 and 0.4 kr. were normal.

When third and fourth instar larvae were irradiated at 1, 2, 4, and 8 kr., all died in the larval or pupal stage at 4 and 8 kr. At 2 kr., only 15 of 500 attained adulthood, and of these only 3 of the 7 surviving females produced fertile egg masses. At 1 kr., 29 of 500 reached the adult stage, and the 12 surviving females produced 8 fertile egg masses. No sterile egg masses were produced.

**Radiation of Pupae to Produce Sterile Adults.** Pupae irradiated when they were only 1-6 hours old had low survival rates when they were treated at sterilizing dos-

ages; better than 50 percent mortality occurred during ecdysis at doses of 10 kr. and above. Adults that did emerge died within 7 days after irradiation without reproducing. Pupae irradiated when they were 36 hours old or more had emerging and survival rates equalling or surpassing those of the untreated controls, and larger doses were necessary to sterilize older pupae.

**MATING EXPERIMENTS.** Data from crosses of irradiated males and females with untreated males and females are presented in Table 1. Eggs were pro-

The effects of gamma irradiation on the feeding response and lifespan of adults irradiated as pupae are shown in Table 2. Pupae irradiated when they were 6 or more hours old lived about as long as the untreated controls, an indication of little or no adverse effect on the lifespan of the mosquito at these dose levels. However, irradiation did affect the feeding response of the females; those given the higher doses did not feed as readily as the controls or as those treated at the lower doses.

#### Effect of Flooding a Normal Population

TABLE 1.—Egg production and viability after adult *C. tarsalis* were irradiated as pupae (25 males and 25 females/cage; average of 2 replications).

Dose, kr.		Total no. egg masses	No. larvae produced	Estimated no. eggs	Estimated % viable eggs	% Comparative fertility $\frac{X}{\text{Control}} \cdot 100$
Male	Female					
Pupae 6-36 hours old when exposed						
10	0	24	625	4800	13	14.7
12.5	0	15	101	3000	3.4	2.5
15	0	15	69	3000	2.3	1.7
0	0	12	0	2400	0	0
10	10	1	3	30	10	0.075
12.5	12.5	0	0	0	0	0
15	15	0	0	0	0	0
0	10	0	0	0	0	0
0	12.5	0	0	0	0	0
0	15	0	0	0	0	0
Pupae 36-72 hours old when exposed						
12.5	0	25	25	5000	0.5	0.62
0	12.5	0	0	0	0	0
12.5	12.5	0	0	0	0	0
15	0	19	320	3800	8.4	8
0	15	0	0	0	0	0
15	15	0	0	0	0	0
Control						
0	0	21	3990	4200	95	100

duced by all untreated females, regardless whether or not they were mated with treated males. Also, some larvae developed from each group of egg masses, an indication of low fertility except in the virgin female checks which produced 12 egg masses but no larvae. (These check females had been separated from the males 24 hours or less after ecdysis to insure virginity.)

with Irradiated Males. One- to two-day-old pupae were irradiated at 10 or 15 kr. and then transferred to 400-ml beakers of distilled water which were placed in cages for emergence. The emerging adults were separated by sex before they were 18 hours old to prevent mating. The untreated males and females were also separated by this method. Adequacy of the procedure was checked by maintaining a group of

TABLE 2.—Effects of gamma irradiation on feeding and longevity of adult *C. tarsalis* irradiated as pupae (25 males and 25 females/cage; 2 replications).

Dose (kr.)	% Surviving females feeding	Average lifespan (days)	
		Males	Females
0	89	17	30
10	80	14	18
12.5	77	18	31
15	40	19	26

25 supposedly unmated normal females; these females laid seven egg masses, but no larvae hatched.

Groups of 25 normal females were put into small cages within 24 hours after emergence, a similar number or more irradiated males were introduced, and then immediately 25 normal males were introduced. The test room was maintained at  $25^{\circ} \pm 4^{\circ}$  C. and 60 percent  $\pm 5$  R. H. The females were given four opportunities to feed on young white mice over a period of 12 days after emergence; consequently, more than one egg mass per female was obtained in one case. Table 3 shows re-

tility is given based on the production of larvae by controls that had only normal males present.

Suppression of fertility in the group with a ratio of 250:25:25 (10 kr. dose) was 12 percent of the production of larvae by the controls, and normal females mated with irradiated males (10 kr.) at a 1:1 ratio produced only 125 larvae, which is 7 percent of the production of the controls. Thus not all the males were sterilized at this dose.

A dose of 15 kr. gave somewhat similar results except that normal females mated to irradiated males with no normal males present produced six egg masses and no hatching occurred. Thus mating behavior may have been altered by the larger dose of irradiation given to the males, which may explain the larger number of larvae produced by this group compared with the 250:25:25 group treated with 10 kr.

DISCUSSION. At 100 kr., 100 percent mortality of adult mosquitoes occurred within 24 hours, a result similar to that obtained by Cole *et al.* (1959) who reported that the amounts of irradiation necessary to cause 100 percent mortality

TABLE 3.—Typical results when confined populations of *C. tarsalis* consisted of various ratios of irradiated (R) males and normal (N) males and females.

Dose (kr.)	Population components			No. egg masses	No. larvae produced	% Relative fertility <sup>a</sup>
	R ♂	N ♂	N ♀			
10	25	25	25	13	890	48.8
10	50	25	25	14	1005	55.1
10	250	25	25	9	222	12.1
10	25	..	25	8	125	6.8
15	25	25	25	16	594	32.5
15	50	25	25	17	618	33.8
15	250	25	25	24	300	16.4
15	25	..	25	6	0	0
0 Control	..	25	25	27	1825	100
0	..	..	25	7	0	0

<sup>a</sup> Controls=100%

sults of typical experiments made at different times; doses are shown in kr, population components, number of egg masses, and number of larvae produced are shown; and the percentage relative fer-

in 24 hours ranged from 67.5 kr. for the American cockroach, *Periplaneta americana* (L.), to as much as 225 kr. for the adult Pharaoh ant, *Monomorium pharaonis* (L.).

The dose of irradiation necessary to cause 100 percent mortality in eggs also varied greatly with species. I found that a dose of 0.8 kr. caused 100 percent mortality of eggs of *C. tarsalis*. Cole *et al.* (1959) found that only 0.6 kr. was required to cause complete kill of eggs of the house fly, *Musca domestica* L., but 50 kr. was required to cause 100 percent kill of 0- to 2-day-old eggs of the body louse, *Pediculus humanus humanus* L.

The amount of irradiation necessary to sterilize the pupal stages of several species of mosquitoes was nearly the same. *Aedes aegypti* (L.) required doses ranging from 8 to 10 kr. to completely sterilize the males (Weidhaas, 1963). *Anopheles quadrimaculatus* Say required doses ranging from 8.8 to 12.9 (Davis, 1959). Females and males of *C. tarsalis* required about 5 and 12.5 kr., respectively, for sterilization in the pupal stage, a dose that is in direct contrast to that required for screw-worm flies whose males need only about

half the dose necessary for the females (Baumhover *et al.*, 1955).

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