

MATING ABILITY OF MALE MOSQUITOES, *Aedes aegypti* (L.) STERILIZED CHEMICALLY OR BY GAMMA RADIATION

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The eradication of the screw-worm fly (*Cochliomyia hominivorax* (Coquerel)) from the southeastern United States was accomplished by releasing, into the natural population, laboratory-reared males sterilized by gamma radiation. The success of this release program has focused attention on the possibility of controlling or eradicating other insect pests through induced sterility (Knipling, 1960; Lindquist, 1961). In laboratory tests, Davis *et al.* (1959) have shown that the common malaria mosquito, *Anopheles quadrimaculatus* Say, can be sterilized with gamma radiation. Chemicals have been found that will sterilize other species of mosquitoes (Weidhaas *et al.*, 1961; Weidhaas, 1962) and house flies (*Musca domestica* L.) (LaBrecque, 1961). Furthermore, male house flies sterilized with the chemosterilant apholate (2, 2, 4, 4, 6, 6-hexahydro-2, 2, 4, 4, 6, 6-hexakis(1-aziridinyl)-1, 3, 5, 2, 4, 6-triazatriphosphorine) have been shown to be fully competitive with normal males (LaBrecque *et al.*, 1962). This report presents the results of research comparing the effectiveness of males sterilized by irradiation and those sterilized by apholate in reducing the reproductive potential among caged populations of the yellow fever mosquito, *Aedes aegypti* L.

Pupae from the laboratory colony were sexed by the difference in size. For irradiation treatment, groups of male pupae more than 24 hours old were irradiated with 8,000 r and 10,000 r of gamma rays in a cobalt-60 source having a dose rate of about 690 r per minute. Other male pupae and all female pupae were left unirradiated. Adult females were examined within 20 hours after emergence before mating to remove any males that might not have been properly sexed in the pupal stage.

For the chemosterilant treatment, groups

of unirradiated males were fed for the first 3 to 4 days after emergence on 10 percent of apholate in a 20-percent aqueous honey solution; all other mosquitoes were given untreated honey solution. After treatment, various crosses of virgin females with untreated males and males treated by both methods were made in small cages (7 inches wide, 10 inches long, and 10 inches high). In each cross, 60 females were caged with 60 normal males, 60 treated males, or 60 normal males plus different multiples of 60 treated males. Three to four days were allowed for mating and then the females were given a blood meal on a guinea pig. Two days later half the females in each cross were placed in individual glass vials which contained a small amount of water and were lined with filter paper for egg deposition.

Table 1 shows the number of eggs laid by each female, and the percent hatched. It will be noted that in three of the crosses only treated males were utilized. Half of the females from these three crosses and from the controls were dissected and examined for the presence of sperm in the spermathecae to determine the percent inseminated.

A very high degree of insemination occurred (93-100%). Therefore, neither gamma radiation nor apholate prevented the males from mating normally. Furthermore, apholate and gamma radiation were equally effective in sterilizing males, as demonstrated by the almost complete reduction of egg viability when only treated males were present. The few eggs that hatched under these conditions came from only four females. This phenomenon, the hatching of a few eggs from a cross of treated males and normal females, has occurred from time to time in other tests.

The theoretical reduction in egg viability

TABLE 1.—Fertility of untreated female *Aedes aegypti* caged with untreated males, males treated with apholate or gamma rays, or mixed populations of treated and untreated males.

Sex ratio ¹	Percent of females		Number of eggs per females		Percent of egg batches from individual females giving indicated percent hatch			Percent reduction in hatching rate ²
	Inscmi-nated	Lay-ing eggs	Average	Range	Percent of egg batches from individual females giving indicated percent hatch			
					60-100	1-59	0	
Males treated with apholate								
∞:1	97	100	107	3-154	3	7	90	98
1:1	..	93	121	0-185	36	25	39	55
1:1	..	100	138	60-177	40	10	50	60
1:1	..	100	133	78-169	14	14	72	82
1:1	..	97	129	0-160	21	10	69	78
Males treated with 8,000 r								
∞:1	100	97	119	0-161	0	3	97	99.9
1:1	..	100	134	80-154	73	6	21	18
1:1	..	93	111	74-153	89	7	4	10
Males treated with 10,000 r								
∞:1	93	100	153	122-187	0	0	100	100
1:1	..	100	115	75-160	100	0	0	0
Control								
1:1	100	100	83	9-198	96	4	0	..
1:1	..	100	119	65-165	100	0	0	..

¹ Treated males: untreated males: untreated females.

² Based on all eggs, adjusted by Abbott's formula to allow for normal failure to hatch.

as compared with the actual reduction of the various ratios of treated males: untreated males: untreated females is shown below:

Ratio	Percent reduction	
	Theoretical	Actual
apholate 1:1:1	50	55
2:1:1	67	60
4:1:1	80	80
8,000 r 4:1:1	80	14
10,000 r 4:1:1	80	0

The apholate-sterilized males reduced egg ability to near the theoretical levels. Obviously, when treated and untreated males were both present in the cages with normal females, the apholate-sterilized males were fully competitive whereas the irradiated males were not.

Males irradiated at 10,000 r were not able to compete at all with normal males, and at 8,000 r they could compete only slightly. *A. aegypti* mosquitoes copulate

frequently. Roth (1948) noted that a single female copulated 50 times with eleven males in one hour, and 1 virgin male caged with 16 virgin females copulated 30 times in 1/2 hour. Since sterile and normal males were present with the normal females in these tests, successful multiple matings by sterile and normal males might be expected to result in many egg batches giving intermediate hatches (1-59 percent). However, such a result was not observed; the majority of egg batches hatched normally or not at all.

It appears that chemosterilants offer an effective method of sterilizing males of *Aedes aegypti* without damaging their mating ability, but dosages of radiation sufficient to sterilize males prevent them from competing fully with normal males. Further studies may develop a method of administering radiation at a time in the life cycle which will not decrease the competitive ability of the males. The reason for the lack of competitiveness of males irradiated as pupae is not apparent, since

these males appeared as vigorous, lived as long, and mated as readily as normal males when they were not in competition.

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MAINTENANCE OF A LABORATORY COLONY OF *ANOPHELES MACULATUS* THEOBALD BY ARTIFICIAL MATING

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In our investigations into the susceptibility of Malayan *Anopheles* to the monkey malaria parasite *Plasmodium cynomolgi bastianellii*, *A. maculatus* was selected as the control mosquito because it was found to be highly susceptible. Originally clean adults were raised in the laboratory from eggs laid by wild caught females. This proved to be rather tedious involving much time and labour and a laboratory culture was clearly desirable.

Attempts have been made at various times to colonize *A. maculatus* in Malaya and elsewhere without success. In recent attempts the mosquitoes failed to mate in one-foot, two-foot and six-foot cube cages. However, the technique of induced copulation devised by McDaniel and Horsfall (1957) for Aedine mosquitoes has been applied with success for some anophelines. Reports of induced mating of *A. labrunchiae* by Caravaglios (1961), and of *A. punctipennis*, *A. quadrimaculatus*, *A. freeborni* and *A. albimanus* by Baker et al. (1962), suggested that our problem might be solved along similar lines.

The technique was simplified from those previously employed. The male and females are allowed to emerge separately in one-foot cube cages and maintained with 5 percent glucose solution in an insectary at 27° C. and 70-90 percent R.H. They are separated by size at the pupal stage with a reasonable amount of accuracy, as more than 95 percent of the larger pupae have been shown to be females. Two days after the emergence of the females, the glucose is removed the morning and a guinea pig is substituted the same evening, and left overnight. Fed females are collected in individual tubes the following morning. The males 3-6 days old, are caught with a fine pipette attached to a suction device. They receive no anaesthesia but are pinned laterally through the thorax using a mirror pin fixed into the end of a 6" long soft wooden stick. Several males are prepared at a time, and after removing the head and legs, are lined up ready for use. The female mosquito is anaesthetised by placing a tube, with cotton wool soaked