Temperatures of the lower and upper halves of the back wall of the box were recorded on the time-phase film. All species began to move to the lower half of the box, which was cooler when the temperature of the upper half rose above approximately 80° F.

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MASS PRODUCTION OF STERILIZED MALE *AEDES AEGYPTI*[1]

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The effectiveness of released sterilized male *Aedes aegypti* in reducing field populations of the species was studied at Pensacola, Florida, in 1960 and 1961 (Morlan *et al.*, 1962). Sterile males for these tests were reared at the Technical Development Laboratories, Savannah, Georgia, and air-shipped each week during the study periods.

Initial production methods used in 1960 were patterned closely after those used in 1955 to produce *Ae. aegypti* for previous biological activity studies (Morlan *et al.*, 1963). The present paper describes modifications both in the rearing methods and the irradiation procedure (McCray *et al.*, 1961).

Colony cages, as described by McCray (in press, 1963), were used to minimize the escape of adult mosquitoes during colony maintenance operations. Six colonies produced the eggs required. Each colony was stocked initially with 10,000 male and 10,000 female pupae and supplemented with 5,000 female and 1,000 male pupae per week for four weeks. A rabbit was used as a blood meal source for 3 hours every second day and a 1:1 mixture of 40 percent honey-water:raisin juice (from stewed raisins) was added twice weekly as supplemental liquid food.

A 3-inch-high stainless steel oviposition strip was covered on each side with a single layer of wet paper toweling from the top to within ½ inch of the bottom.

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When the strip was placed vertically in 1¼ inches of water, over 90 percent of the eggs were deposited on the toweling within ½ inch above the water level. Strips containing eggs less than 24 hours old were removed daily and placed for an additional 24 hours in a second tray holding 1 inch of water to insure embryonic development of the eggs (if held for 48 hours, some hatch occurred). The toweling and eggs were air-dried at 80 percent RH for one day and then stored in a plastic box at humidity above 80 percent. Over 80 percent hatch was obtained from eggs held in this manner for up to two months.

For larval production, 156 trays on 13 steel racks, were used. Each tray of galvanized metal, coated with paraffin, was 72″ long x 10″ wide x 2″ deep. Each held 6 to 12 liters of water that could be drained through a ¾-inch diameter pipe at one end when the tray was tilted and a rubber stopper removed. To allow time for temperature stabilization, 6 liters of water were added to each tray 24 hours before the larvae were introduced.

Egg strips were placed on the bottom of one rearing tray, dampened with water for a 45-minute period, and then flooded by 8 liters of a 24-hour-old hatching medium consisting of 100 mg. of laboratory chow and 50 mg. of powdered brewers' yeast per liter of water. Hatching commenced immediately. After 2 hours, the newly hatched larvae were drained into an agitator-type washing machine, the tray was flushed clean with a stream of water, and the water in the washer was brought to the desired volume of 20, 40, 60, or 80 liters depending on the number of trays to be filled. With water agitation the larvae were uniformly distributed. Based on counts of forty 1-ml. samples, a volume containing 8,000 larvae was then added to each rearing tray. Laboratory chow, ground to pass through a 40-mesh screen, was fed at 0.2, 0.3, and 0.4 mg. per larva on the day of hatching, day 0, day 1, and day 2, respectively, and at 0.6 mg. on days 3 through 6.

Prior to feeding on day 6, sets of five rearing trays were drained and the larvae, trapped in 20-mesh screen containers, were combined in a sixth tray. On day 7, about 4 hours before pupae were collected, 2.8 grams of paris green were added to each combined tray of mixed larvae and pupae. The slower-developing larvae ingested the paris green and were killed, while larvae nearing pupation did not feed.

Pupae and moribund or dead larvae were drained from the trays and placed in buckets of water. The dead and moribund larvae sank and the pupae were skimmed from the water surface, placed on a mechanical separator (McCray 1961), and washed with a gentle stream of water (Fig. 1). Male pupae dropped through

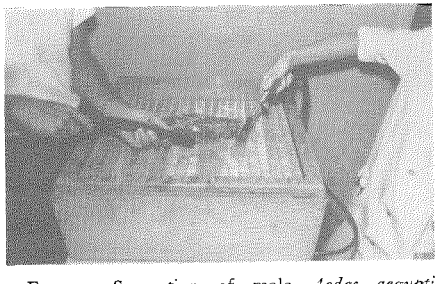

FIG. 1.—Separation of male *Aedes aegypti* pupae by washing through 0.039″ louvered slits.

the slits of the separator and the female pupae were retained on a screen. The accuracy of separation was checked periodically using an adjustable separator (Fay and Morlan, 1959).

The male pupae were then placed on nylon tulle stretched between two concentric plastic rings and irradiated with Cobalt-60 from a point source (McCray *et al.*, 1961. The sterilized pupae, in ½-pint cardboard containers holding 1 inch of water, were air-shipped in insulated boxes containing chemical refrigerants and arrived at the field area within 24 hours.

The rearing procedure was designed to

provide pupae for irradiation on a weekly basis. Initially, insectary conditions of 80° F. and 80 percent relative humidity were used. Fluctuating the temperature, however, showed: (a) that rearing at a mean temperature of 76° F. reduced the yield of pupae and retarded their development to 8 days; (b) that an accumulation of 600 or more degree-hours over 80° F. over the 7-day period produced emergence of considerable numbers of adults on day 7; (c) that approximately 500 degree-hours over 80° F. gave the best developmental pattern in 7 days; and (d) that a gradual increase of temperature gave an average increase of 20 percent in pupal production. The ambient temperature schedule finally adopted was recorded as 81, 82, 83, 83, 84, 84, and 85° F. on days 1 through 7 respectively.

The effect of 6, 8, or 10 liters of water per tray on the yield of pupae from 8,000 larvae initially showed little difference and gave average 2-day yields, respectively, of 6,900, 7,000, and 6,600 pupae of both sexes. Checks were made on the effect of crowding upon the percentage of male pupae obtained on days 6 and 7. Using 15,000 and 8,000 larvae per tray, the numbers of pupae collected during a 2-day period were in proportion to the original larvae and the males represented 57 percent and 53 percent, respectively. Since adult survival tests showed the pupae from the 15,000 trays to be equal in vitality to those from the 8,000 trays, it was apparent that the higher number of larvae per tray could be used in routine rearing.

Further improved techniques were carried to pilot production but not introduced into standard methods, as field releases were terminated. While aiding in the separation of pupae, paris green reduced potential production by eliminating many mature larvae and by restricting collection of pupae to a single day. From single trays with paris green treatment, an average of only 5,840 pupae was obtained as compared to 7,270 pupae without it.

Bar-Zeev and Galun, (1961) described a method for the separation of mosquito larvae and pupae by which the larvae, made magnetic through the ingestion of iron dust placed in the rearing medium, were held by an electromagnet while the non-magnetic pupae were drained off with the water. Following this general technique, magnetic iron oxide dust was placed in the larval rearing trays on day 2 at a concentration of 100 p.p.m. Larvae of all instars became magnetic within a few hours and did not appear adversely affected. About 1 to 1½ hours before pupating, the larvae became non-magnetic apparently through evacuation of the iron dust from the gut.

On day 6, larvae and pupae in the mixed culture were forced toward one end of the rearing tray by a screen barrier. An electromagnet was then placed across the tray directly behind the barrier with the magnetic surface in the water approximately ½ inch from the floor of the tray. The barrier was removed and a beam of bright light was directed into the water. To avoid the light, the larvae and pupae moved under the magnet. The larvae were trapped, while the pupae passed freely to the area beyond the magnet where they were removed. Some larvae passed under the magnet but these were non-magnetic and pupated within 1 to 1½ hours. After removal of the pupae and prepupae, the larvae were released from the magnet and allowed to continue development. On day 7, the process was repeated. Using 8,000 larvae per tray, 7,080 pupae per tray were obtained with the magnetic-iron technique (12 trays) as compared to 5,840 pupae per tray with the paris-green technique (12 trays).

The irradiation procedure for male pupae followed that described by McCray *et al.* (1961). Pupae were placed on nylon tulle stretched between two concentric plastic rings, ⅛-inch-high and with diameters of 4 and 6 inches, respectively. Each ring had a tulle surface of approximately 10 square inches, sufficient to hold approximately 3,000 pupae. In making a 3-inch stack, empty rings were placed at the top and bottom and between them

were 21 rings holding 60,000 pupae. The Cobalt-60, a point source, was passed upward through the center of the ring stack starting ½ inch below the bottom and moving at ½-inch steps for 3.5 minutes at each of seven steps. This method resulted in a considerable variation in dosage, ranging from 9,400 R per pupa for those on the outer periphery of the top ring to 18,750 R per pupa for those on the inner side of the ring at the center of the stack.

Production checks showed that sterilization was produced even by a dosage of 8,400 R per pupa. Doses above 12,000 R affected the emergence of the adults and their subsequent longevity. Pupae irradiated in the inner, middle, and outer portions of the rings showed adult emergence of 77, 88, and 94 percent, respectively, and survival for one week of 66, 84, and 88 percent of the emerging adults.

In view of the adverse effect on some pupae, the physical arrangement and the timing for irradiation were modified to narrow the range of the dosage administered. To reduce the difference in dosage received by pupae on the inner edge of the bottom and top holders in a stack, the Cobalt-60 source was started 3 inches below the stack and moved upward in ½-inch steps to 3 inches above the stack, using 1½ minutes at each step. To overcome the differential irradiation of pupae on the inside and outside edges of the stationary ring holders, a series of 2-inch-diameter discs was substituted. The center of each disc (Fig. 2) was located 3 inches from the path of the Cobalt-60 source or approximately the same distance as the outer edge of a ring holder. These discs were then slowly rotated so that pupae in the center of each disc remained at 3 inches while pupae on the periphery of the disc varied from 2⅛ to 3⅞ inches from the path of the Cobalt-60 source. The overall range of dosage with the above modifications was then 8,800 to 9,500 R per pupa, and 94 percent of the pupae survived. By using 9 discs at each level, an area of approximately 19 square inches was available for pupae, or an increase of approximately 9 square inches per level in the stacks. This would make possible the irradiation of 110,000 pupae at one time.

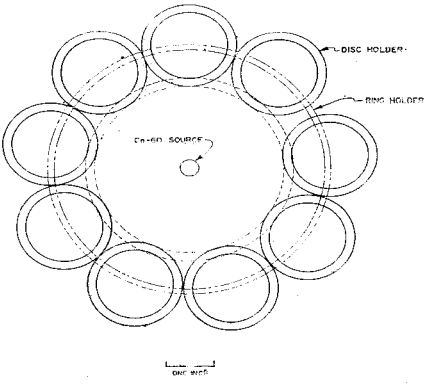

FIG. 2.—Relationship of Cobalt-60 source to stationary ring and rotating disc type holders for pupae during sterilization. Diagram shows both types of holders superimposed.

With the magnetic iron technique, pupae were collected on both days 6 and 7. Day-6 pupae were held at 80° F. or 50° F. for 24 hours and then irradiated along with day-7 pupae to see if all irradiation could be done at one time. The resultant mortalities (Table 1) showed the younger day-7 pupae and the day-6 pupae held at 50° F. to be more sensitive to radiation damage.

TABLE 1.—Effect of age and prior holding temperature on mortality of *Aedes aegypti* pupae subjected to Cobalt-60 irradiation

Age of pupae (hrs.)	Holding temp. (° F.)	Co-60 treatment	Mortality of pupae (%)
24–48 (Day 6)	80	None	4.5
	..	Irradiated	6.1
	..	..	..
24–48 (Day 6)	..	..	..
	50	None	1.9
	..	Irradiated	25.9
0–24 .. (Day 7)	80	None	0.8
	..	Irradiated	23.0
	..	..	..

Using 500 to 600 individuals at each test condition, 5 replicate tests were made on the percent survival of normal and sterile males under laboratory temperatures of 80° F. and gave values of 75 percent and 58 percent for one week, and 30 percent and 34 percent for two weeks, respectively. Similarly, in an outdoor cage at variable temperatures, the percent survival was 87 percent and 45 percent for one week and 53 percent and 19 percent for two weeks for the normal and sterile males, respectively.

Since considerable numbers of sterile males survived in the field cage for one week, the mating competitiveness of different aged males was determined using 2- to 7-day-old sterile and normal males at ratios of 20:1:1 and 5:1:1 sterile males: normal males:normal 2-day-old females with 25 females in each test. The loss of competitiveness with age appeared greater with sterilized males than with normal males (Table 2). In all cases, the hatch of eggs exceeded the theoretical values based on the proportions of sterile: normal males.

SUMMARY. For mass production of sterilized male *Aedes aegypti* pupae, modifications have been made (a) in rearing large numbers of mosquitoes, (b) in obtaining higher production of male pupae from the rearing trays, (c) in separating the pupae from the larvae through the use of paris green or magnetic iron oxide dust, and (d) in reducing the range of the irradiation dosage during the sterilizing process. Studies were made also on the mating competitiveness of sterile and normal adult males 2 to 7 days of age.

TABLE 2.—Percent hatch of 14-day-old eggs obtained from 25 *Aedes aegypti* females combined with sterile and normal males of different ages

Age of males (Days)		Percent hatch of eggs from combination ratios of (sterile males: normal males females)	
Sterile	Normal	20:1:1	5:1:1
7	2	48	69
6	2	34	68
5	2	30	50
4	2	21	40
3	2	36	46
2	2	17	43
2	3	23	45
2	4	24	44
2	5	19	24
2	6	17	31
2	7	16	25

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