LARGE-SCALE REARING OF *ANOPHELES QUADRIMACULATUS* SAY AT ORLANDO, FLORIDA[1]

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Insectary rearing of *Anopheles quadrimaculatus* Say was begun at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine in October 1942 to meet the demands for biological material for use in larvicide, repellent, and adulticide studies. The colony was established from eggs obtained through the courtesy of Mark F. Boyd, of the Rockefeller Foundation for Malaria Research at Tallahassee, Fla.

The first method for the insectary rearing of *Anopheles quadrimaculatus* was developed by Boyd *et al.* (1935) and the colony at Orlando represents a continuation of this strain. Crowell (1940) worked out a simple technique in which powdered dog biscuit was used as larval food. A technique similar to Crowell's was adapted to the Orlando rearing. Subsequent expansion of the colony entailed a gradual evolution of techniques and management.

At its peak the Orlando colony was producing an average daily output of approximately 40,000 adults, 5,000 larvae, and over 100,000 eggs. The following description of equipment and operations is for a colony of that capacity.

COLONY FACILITIES AND EQUIPMENT

The colony was housed in an old concrete-floored garage and occupied four rooms totaling 975 square feet of floor space. A cabinet 6 feet wide and 8 feet long was built in a corner of one of the rearing rooms to hold emergence cages. Outside doors and windows were screened. A general view of one of the rearing rooms is shown in Figure 1. In addition to this indoor unit, an outdoor unit containing 40 rearing boxes was maintained in a stream a few hundred feet below the spring at Rock Springs, Fla. (Eide 1945.)

Temperature, Humidity, and Light.—A temperature fluctuating between 78° and 90° F. provided satisfactory rearing conditions if neither extreme was maintained for more than a day at a time; 80° to 82° was about the best temperature for larval development. Thermostatically controlled electric or oil heaters, or a combination of the two, were used to maintain these temperatures during cool weather. The cabinet holding the emergence cages was equipped with an electric heater set to maintain a temperature of 84°, in order to keep emergence on a regular 2-day schedule and provide a steady supply of mosquitoes for experimental workers. Only one room was equipped with artificial cooling. The mosquito breeding cages were kept in this room, and egg production was maintained at a high level during the summer months.

Humidity control was unnecessary because of evaporation of water from the rearing vessels and the high natural humidity in Orlando.

The larvae avoided bright sunlight, and apparently daylight was not essential to their growth. The inside room housing the breeding cages and 160 rearing pans was lighted by fluorescent lights. The pupae production in this room compared favorably with that in other rooms.

Equipment.—In the indoor colony 600 pans and 54 shallow wooden boxes were used. For general purposes a white-enameled pan, 12 inches in diameter and 4½ inches deep, was the most satisfactory. Pans used for hatching and rearing were

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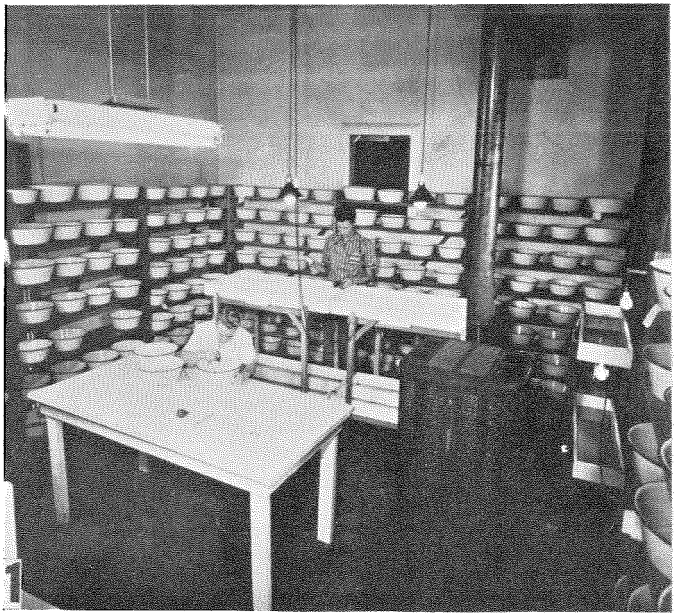
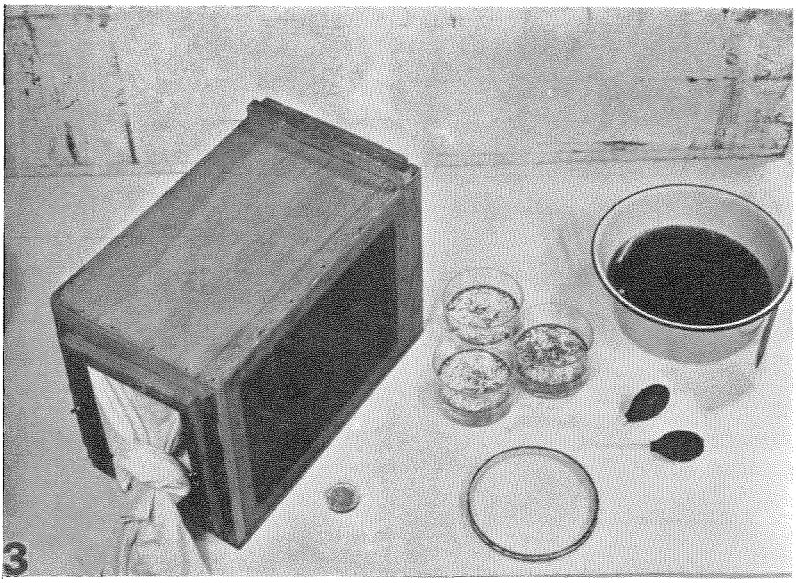


FIG. 1. One large rearing room showing arrangement of rearing pans and boxes, work table, and oil-burning heater. Note lighting over boxes. Worker at table picking pupae; worker in background feeding larvae. (Photo by R. Papen.)

FIG. 2. Feeding the larvae of *Anopheles quadrimaculatus* with dog food and yeast. Rearing troughs in foreground and rearing pans in background.



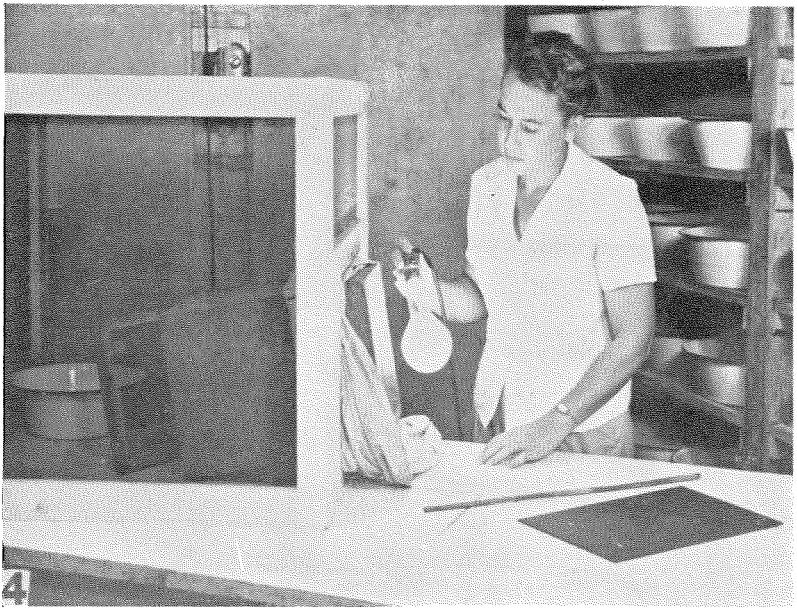


FIG. 3. Pupae and equipment for handling. Left to right: Emergence cage containing pupae, small petri dish of honey, 3 crystallization dishes containing 3,000 pupae, pupae strainer, pipettes for picking pupae, pan of pupae in dirty rearing water before being strained. (Photo by R. Papen.)

FIG. 4. Restocking a breeding cage. Cage contains oviposition pan, honey dish, and emergence cage from which mosquitoes are being driven by the light. Stick on table is used to open the door of the emergence cage inside the large cage; piece of board covered the hole in the bottom of the emergence cage until it was placed inside the large cage. Note that screen of large cage is inside the framing. (Photo by R. Papen.)

kept on level shelves 8½ inches apart, with the bottom shelf set a few inches above the floor.

The boxes were 1 foot wide, 5 feet long, and 3 inches deep. The sides were of 1-inch pine or cypress, and the bottoms of masonite or cypress. Each box was entirely covered with three coats of white-enamel marine paint. The boxes were set upon double-decked frames made of 2-inch lumber, being arranged singly or in pairs as the floor space permitted (Fig. 2), the lower boxes being 13 inches and the upper boxes 42 inches above the floor.

Most of the emergence cages used at Orlando were of the sleeve type pictured in Figure 3. They were 12 inches square by 18 inches long, with top and bottom of plywood, sides of 18-mesh window screen, and a door at the end opposite the sleeve.

The most desirable breeding cage was 30 inches square by 25 inches high, with sides of 18-mesh wire screen, and top and bottom of ¼-inch plywood (Fig. 4). The frame of the cage was constructed of 1- by 2-inch lumber, and quarter-round was used inside the corners to facilitate cleaning. The screen was on the inside of the frame. A front opening, 12 by 15 inches, was covered with a cloth sleeve 30 inches long. Larger cages were more difficult to clean and required more space.

All the rooms were equipped with work tables, running water, chairs, stools, etc. The extra equipment and glassware was stored on shelves within easy reach. All the work tables and the racks holding the emergence and breeding cages were ant-proofed.

Ten mature rabbits, caged separately, were kept for feeding the mosquitoes. Young rabbits were raised from time to time for replacement.

COLONY MANAGEMENT

Under favorable conditions a few mosquitoes developed from egg to adult in 10 days; however, others in the same lot required 2 weeks more to complete their cycle. If conditions were unfavorable, the developmental period sometimes extended over a full month, but under such circumstances a large proportion of the larvae died before pupating. The usual development and management of any given lot fell into the following time pattern:

1st day	Eggs
2nd to 4th day	Larvae in hatching pans
4th day	Transfer of larvae to rearing vessels
4th to 8th days	Larvae in rearing vessels
9th day	First pupae
9th to 15th days	Pupation period
11th to 17th days	Emergence
21st day	Oviposition

The usual daily tasks in the operation of the colony were as follows: (1) Taking up and setting eggs, (2) feeding larvae, (3) transferring young larvae from hatching pans to rearing vessels, (4) checking rearing vessels for water depth and larval populations, (5) removing pupae from rearing vessels, (6) transferring pupae from picking pans to emergence cages, (7) delivering mosquitoes to experimental workers, (8) replenishing mosquitoes in breeding cages, (9) placing rabbits in mosquito breeding cages for mosquitoes to feed upon, (10) feeding and watering rabbits and cleaning rabbitry, and (11) washing all empty rearing vessels and used glassware.

At less frequent intervals the breeding cages were cleaned, rabbits were clipped for mosquito feeding as needed, and eggs were packed for shipment to out-of-town workers.

Egg Production and Handling.—One white-enameled pan containing about an inch of water served as the oviposition pan in each breeding cage. The pans were removed from the breeding cages each morning. The eggs stranded on the side of a pan were washed down with a wash bottle, dead mosquitoes were removed, and eggs were concentrated by siphoning off the excess water. Eggs from all the oviposition pans were transferred to a glass beaker. Eggs for colony maintenance were set daily and the surplus was either stored in an ice box for future use or shipped

to other scientific workers who depended on the Orlando colony for their egg supply.

White-enameled rearing pans containing about an inch of tap water were used as hatching pans. The eggs were placed within a waxed cardboard ring, 3 to 4 inches outside diameter and ½ inch wide (Fig. 5), which floated on the water surface. The purpose of the ring was to prevent the eggs from becoming stranded on the sides of the pan. Three to five thousand eggs were sufficient for one hatching pan. The eggs were transferred with an oval loop made of fine wire, which enclosed an area of 72 sq. mm. and held about a thousand eggs in a double layer.

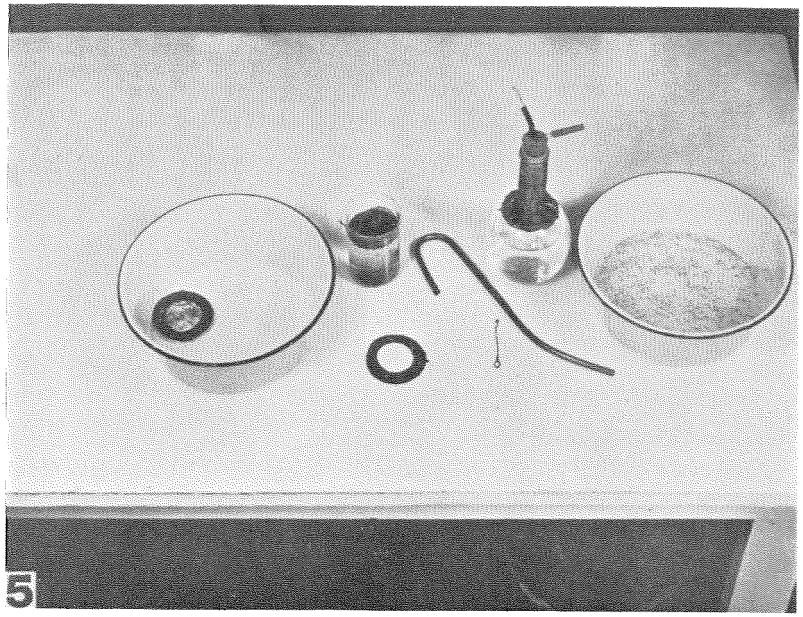
Eggs to be stored were spread in a thin layer on wet filter paper, placed in a moistened vial or small petri dish, covered, labeled with date and approximate number of eggs, and placed in an ice box. With this treatment they remained viable for 10 days. Eggs to be shipped were placed on wet filter paper and then between moistened cotton and paper pads in a paraffin-impregnated pill box. The boxes were wrapped with paper, taped, and sent by airmail special delivery in a regular letter envelope.

Handling of Larvae.—The newly hatched larvae were kept in the hatching pans until they were 3 days old. When hatching neared completion (usually 24 hours after the eggs were set), the larvae were given a sparse sprinkling of powdered yeast from a spice can. They were fed again when the surface film had been cleared away, that is, when the larvae skimmed lightly away when the surface film was blown gently. Another good indicator of whether food was needed was obtained by dropping a little yeast on the water surface; if the yeast spread quickly over the surface, food was needed. When the larvae were 2 days old, they generally took three feedings a day. Powdered dog biscuit was added to the diet after the second day. As the larvae grew, they required more food and the proportion of dog food to yeast was increased. Although Crowell (1940) had shown that a suitable dog food contained all the elements necessary for larval growth, yeast was included as a possible safeguard against the omission of some elements in the commercial product due to wartime conditions.[3] It was more satisfactory to keep a large number of newly hatched larvae concentrated in one pan during the first 3 days than to divide the eggs among the rearing dishes. The number of larvae per dish could be estimated better, and the danger of such formation, especially serious at this stage, was largely eliminated by the feeding of so many larvae.

The 3-day-old larvae were transferred to the rearing boxes and rearing pans (Fig. 2). The boxes were stocked with approximately 1,500 to 2,000 young larvae per square foot, and the pans with about 1,000 larvae each. All pans containing larvae of the same age were kept together on shelves, and were adjacent to the next older group. The pans were marked with the date the eggs were set.

Larvae were fed at 8, 11, 2, and 5 o'clock at first, and later it was found that three feedings a day (at 8, 11:30, and 4 o'clock) produced fine larvae and pupae. If larvae in any vessel were not ready for food on schedule, they were not fed until the next feeding time. Feeding required as much attention and judgment on the part of a worker as any other single task in the rearing work. Underfeeding of the young larvae resulted in stunting, or starvation, in which case dead larvae were floating in the rearing containers the next morning. Underfeeding of later instars resulted in cannibalism and uneven development among the larvae. Overfeeding also resulted in the stunting of young larvae by the formation of a surface scum to enmesh them, or in the death of larvae in a slime of excess feed on the bottom of the vessel. There were all gradations of these troubles, and it was often difficult to determine the cause. Larvae that were kept warm and well-fed developed uniformly through the

[3] Since 1946 powdered dog food alone has been employed, with entirely satisfactory results.



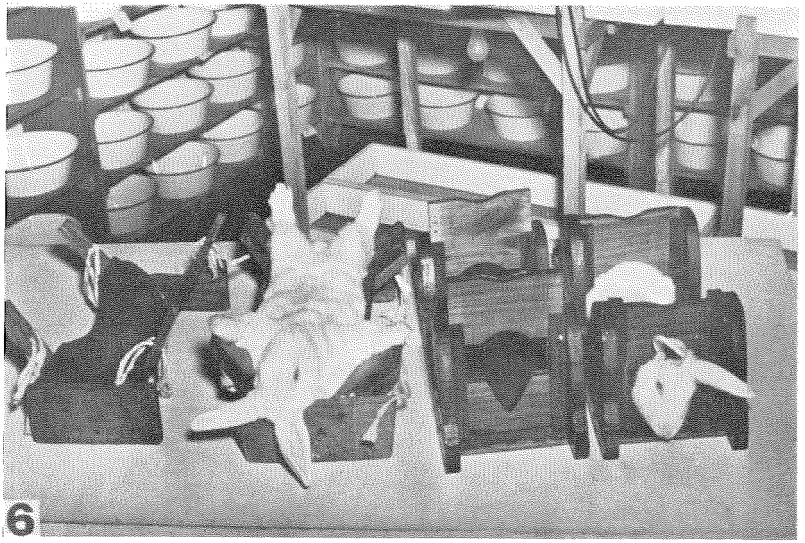


FIG. 5. Eggs and equipment for handling. Left to right: Hatching pan with eggs set in the waxed ring, beaker of eggs and dead mosquitoes, waxed cardboard ring, wire loop for setting eggs, siphon (for removing excess water in beaker), wash bottle, and oviposition pan containing eggs and dead mosquitoes just as removed from the breeding cage. (Photo by R. Papen.)

FIG. 6. Rabbit frames and rabbits ready to go into the mosquito cages. Old-type frames at left, new stock-like frames at right. (Photo by R. Papen.)

third instar, after which some individuals grew faster and pupated earlier than others even with the most careful feeding.

It was necessary to add water daily to the rearing boxes and pans to replenish that lost from evaporation. The water was kept as shallow as possible so that the larvae could feed upon the bottom. It was not necessary to change the water during the growth of a group of larvae unless an excess of food caused fermentation.

Both powdered yeast and commercial dog-food checkers were used for food. The checkers were powdered by grinding and then sieved through a 40-mesh screen. The two foods were kept in separate spice cans and were dusted alternately onto the water. At the colony's peak of production approximately 2 pounds of dog food and 1 pound of yeast were used daily.

Handling of Pupae.—The pupae were picked from the rearing pans and boxes daily. With a large medicine dropper, or pipette, a good worker easily averaged 1,000 pupae per hour without injuring either pupae or larvae, or carrying many larvae over into the pupa dish. If any pupae were missed, they would probably emerge before the next day's picking, and cause mosquito annoyance in the rearing room.

Pupae picked from the rearing vessels were strained out by pouring the water through a piece of 18-mesh plastic screen clamped tautly between two 6-inch steel embroidery hoops. The strainer was quickly inverted and dipped into a pan containing fresh water to free the pupae. They were then poured from the pan into crystallization dishes and placed in emergence cages (Fig. 3). A 5-inch crystallization dish held about 1,000 pupae; other dishes were stocked in proportion to their surface area, and enough water was added to half-fill them.

From 3,000 to 4,000 pupae were placed in each cage for emergence. A small petri dish containing a disk of screen wire over some honey was supplied each cage. Two days were allowed for the mosquitoes to emerge. Pupa dishes and those containing honey were removed through the sleeve. Jarring the dishes and blowing on their surface prevented adults from escaping.

The adults as they emerged were either used in repellent and insecticide tests, or added to the breeding stock.

Handling of Breeding Stock.—Initial stocking of a breeding cage with about 5,000 mosquitoes and subsequent replenishment at weekly intervals with 2,000 adults gave an average daily egg production of 15,000 to 20,000 eggs. Six of these cages were maintained to supply the eggs for the colony's needs and requests from workers. Figure 4 shows a cage being restocked.

The first eggs were laid the fourth day after the initial stocking and feeding of the females on a blood meal obtained from a shaved rabbit. Full production was reached by the end of the first week. Each weekday one breeding cage had approximately 2,000 mosquitoes added to its population and by this arrangement it was possible to keep the egg supply fairly uniform and at the same time avoid using too many mosquitoes from 1 day's crop. Each breeding cage was provided with undiluted honey, water, and an oviposition pan; and once daily, except on Sundays and holidays, a rabbit was put in for 1 to 2 hours. The racks used to hold the rabbits (Fig. 6) were patterned from a holder designed by Laug (1944). A constant supply of water was required, and its omission from the cages for more than a day in warm weather resulted in as much as 90% mortality. The mosquitoes required protection from ants and spiders, and from winds, strong lights, and extremes in temperature.

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