OUTDOOR REARING OF *ANOPHELES QUADRIMACULATUS* SAY

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The outdoor colony of *Anopheles quadrimaculatus* Say. described in this paper was started in the spring of 1943, in conjunction with the indoor colony at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine, to augment the supply of larvae and adults for experimental purposes, and also to furnish a reserve supply in case the colony at Orlando should be destroyed.

The colony is located in a creek arising from Rock Spring in Kelly Park, 6 miles north of Apopka in Orange County, Fla., and 20 miles from the main laboratory. This spring flows from a cave beneath a large limestone cliff, at the rate of about 26,000 gallons per minute. The stream it forms is narrow and swift near the source, but widens out to about 150 feet a hundred yards below the cliff, where it is shallow enough for a person to wade with hip boots.

The water is clear and does not have the sulfur odor characteristic of many spring waters in the vicinity. It contains small amounts of dissolved solids, the most important being calcium carbonate at the rate of 46 p.p.m. The pH is 7.6.

The most important feature of the spring, in relation to the mosquito colony, is its constant temperature of 76° F. The temperature of this water apparently never varies, although air temperatures as low as 40° will lower the water temperature 1 degree at the rearing boxes. However, winter temperatures as low as this are not general in Florida, and mosquitoes can be reared the year round, although production is somewhat curtailed during the cooler months.

The stream is filled with aquatic plants. The most important of these are eelgrass (*Vallisneria americana* Michx.), waterlettuce (*Pistia stratiotes* L.), pickerelweed (*Pontederia cordata* L.), and coontail (*Ceratophyllum demersum* L.). Numerous algae are also present. With the protection of these plants natural breeding of anophelines goes on continually. Both *Anopheles quadrimaculatus* and *A. crucians* Wied. are found, the proportion of the former being greatest near the spring and gradually diminishing farther down the stream.

Description of the Rearing Boxes.—The colony was confined in a number of oblong wooden boxes suspended in the water (fig. 1). Each box was made of one-foot cypress lumber, the inside measurements being 1 by 5 feet. The bottom was covered with 30-mesh copper screen to permit free exchange of water, and the top with a removable cover of window screen. It was found necessary to use heavily galvanized nails to fasten the copper screen to the bottom; otherwise an electrolysis was set up between the copper and the iron, which destroyed the iron in a few weeks. The frame of the cover was made an inch or two larger than the box, so that the weight of the frame would press the screen down tightly enough to exclude spiders. Each box was suspended 6 or 8 inches in the water by means of four stakes driven into the sand of the stream bed. The boxes were nailed lightly to

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Fig. 1. General view of boxes for outdoor rearing of *Anopheles quadrimaculatus.*

the stakes for easy removal, since the buoyancy of the boxes made little support necessary. In the first boxes used, the screen was covered with a coat of shellac to protect it from corrosion, and also to make the openings smaller so that none of the first-instar larvae could pass through. Later experience showed that few of these larvae would dive far enough to be carried out through the screen, and the shellac was omitted to provide better circulation of water.

During December, 1943, when the air temperature was 42° F., the temperature of the water near the boxes was 75°. At the same time the temperature of the water in two old boxes with clogged screens was 71° to 73°, while in the new boxes with open screens it was the same as the water outside. This slight lowering of temperature lengthened the larval period and thereby decreased the number of pupae produced.

Growth of algae and the accumulation of food on the screens in the old boxes soon rendered them water proof and shut off all circulation of water. The resulting stagnation killed hundreds of fourth-instar larvae, especially during cold weather when the period required for development was longer.

The original colony was contained in 11 boxes, but as the period of larval development lengthened in cooler weather 9 more boxes were needed. Later, when the colony had doubled in size, a total of 40 rearing boxes were used.

Technique.—Larvae 3 days old, hatched in pans in the laboratory, were transported to Rock Springs in wide-mouth gallon jugs. The jolting 20-mile trip apparently had no deleterious effect on the larvae.

The box to be filled was removed from its stakes and scrubbed thoroughly with a brush, the screen bottom being given special attention. It was then replaced, and the surface of the water inside was covered with a layer of the ribbonlike leaves of eelgrass. The larvae were then poured into the box; if care was used, they rarely sank below the layer of grass.

Twice a day the larvae were fed brewer's yeast and finely ground dog food by shaking it over the surface from a spice can. For the first 3 days yeast only was used, because the dog food sometimes caused a scum to form which engulfed the small larvae. Part of the food sank, of course, but some of it was supported by the eelgrass within reach of the larvae (fig. 2). The larvae were observed to feed below the surface if the food was close enough so that they could reach it without losing contact with the surface. This observation has since been verified in the laboratory. Thus, except during cold weather, two feedings each day, plus whatever natural food was introduced with the eelgrass, proved to be enough to bring the larvae to the pupal stage in almost the same length of time as that required in the laboratory.

The boxes were not covered except for the window screen. The driving rains during the summer months doubtless sank much of the dog food, but evidently did not wash it from the eelgrass, since the production of pupae during these months was usually very good.

Pupae were removed from the boxes daily. To accomplish this it was found easiest to sink the eelgrass with a piece of hardware cloth cut the same size as the box, and to dip both the larvae and the pupae into a large enamel pan. The pupae were separated from the larvae by means of a medicine dropper. The eelgrass was then allowed to float to the surface and the larvae were returned to the box. The pupae were taken to the laboratory, where they were allowed to emerge in cages. The pupae were transported in the same jugs that were used for the small larvae. Again, the jolting ride seemed to have very little injurious effect, even when there were as many as 4,000 pupae in a single gallon jug.

At first a large dipper was used to remove the larvae and the pupae. Later a

Fig. 2. Fourth-instar larvae of *Anopheles quadrimaculatus* feeding on dog food supported by eelgrass.

scoop made by gluing plastic window screen to a wire frame was found to do the work more rapidly, and although the treatment would seem rough handling for larvae, no injurious effects were noticed over a long period of time. The screen scoop also had the advantage of leaving the smaller larvae in the box where they did not hinder the picking of pupae.

During the summer months the larval period was about 2 weeks. Larvae 3 days old, when placed in the boxes, began pupating after 6 or 7 days, and pupation was usually over by the 11th day. This was almost the same as the larval period in the laboratory. During the winter months the first pupae appeared 9 to 11 days after introduction and the pupation was finished after about 18 to 22 days. If any larvae were left when a box was due to be started, they were placed in a box where pupation had just begun. During June 237,667 pupae were produced, or an average of 7,922 per day.

Since there was an abundance of eggs, it was thought that doubling the number of small larvae introduced might be an economical measure even if the increase in the number of pupae was only 10 per cent. Between November 21 and December 31, 1943, 47 boxes were started, 12 with only 3,000 first instars, 27 with 6,000, and 8 with 9,000. The average numbers of larvae and pupae produced in the three groups were 1,260, 2,194 and 2,115, and the maximum numbers from any one box in the respective groups were 2,011, 3,400, and 2,830. The larvae that had not pupated in 20 days were retained in pans floating in the boxes.

The total production of pupae in 1944 is shown in table 1. Twenty boxes were in production from January to May and 30 from May to December. The pupal mortality shown approximates closely that of the laboratory-reared pupae.

It seems that about 3,000 pupae is all that can be expected from a 5-square-foot box, regardless of the number of larvae at the start. When 9,000 larvae were used they did very well until the third instar. At this stage some of them got a head start and consumed most of the food. As a result most of the larvae starved to death before they reached the fourth instar. Not only did the larger number fail to give any increase in the number of pupae, but they made picking of pupae difficult, the pupae were noticeably smaller, and the larval period was usually lengthened. Heavy mortality in the first instar was evident in the boxes started with only 3,000 larvae. The difficulty seemed to be in the feeding, as a slight excess caused a scum to form that was fatal to many of the small larvae.

Table 1.—Effect of the Introduction of Various Numbers of Larvae Into Rearing Boxes on the Production of Pupae During 1944

Month	Larvae Introduced	Pupae Collected	Average Pupae Per Day	Mortality of Pupae
	Number	*Number*	*Number*	*Per Cent*
	20 BOXES IN PRODUCTION			
January	203,000	94,255	3,040	18
February	207,000	111,385	3,840	10
March	276,000	113,550	3,660	8
April	330,000	126,030	4,210	9
	30 BOXES IN PRODUCTION			
May	468,000	206,350	6,655	6
June	492,000	208,170	6,939	5
July	474,000	209,580	6,760	8
August	540,000	193,735	6,250	6
September	588,000	161,610	5,387	6
October	660,000	205,500	6,630	4
November	666,000	167,060	5,568	8
December	564,000	92,935	2,998	7