tion and because it attacks horses, *C. arubae* is suspect as a biological vector of VEE and as the vector responsible for the movement of VEE from South America and along the Gulf Coast from Central America into Texas. Similarly, *L. kerteszi* should also be considered as a possible biological vector of VEE.

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LABORATORY REARING OF *ANOPHELES ALBIMANUS* WIEDEMANN[1]

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ABSTRACT. For the past 2 years, a colony of *Anopheles albimanus* Wiedemann has been maintained with a rearing system that produces 8,000 to 12,000 pupae per day and requires approximately 1.5–2 man hours per day. A method for counting 2,000 1st instar larvae per tray is described as well as an egg handling technique which is suitable for reducing *Nosema* infections to a low level and should reduce other infections that are not transmitted transovarially.

Efficient and inexpensive methods of rearing mosquitos are necessary for large-scale laboratory and field studies with sterile males, pathogens, and insecticides. Thus, at the Insects Affecting Man and Animals Research Laboratory at Gainesville, Florida, we produce from 8,000 to 12,000 pupae of *Anopheles albimanus* Wiedemann per day by using the methods described herein. All stages are kept in a room maintained at 28° C and 70 percent RH. A window in the room provides natural light.

ADULTS. The adult holding cages are 76 cm wide by 61 cm deep and 61 cm high. The sides and front are made of 18-mesh wire screen; the top, bottom, and back are made of plywood covered on the inside with white Formica®. A cloth sleeve 76 cm long is attached to a 30 x 38-cm opening in the front of the cage. Cages are kept well-stocked with adults (3,000 to 5,000) of both sexes by the daily addition of 500 to 1,000 pupae. The pupal container, a 354-ml waxed cup, is covered with a paper funnel that allows emerging adults to exit but prevents gravid females from ovipositing.

Two 118-ml square bottles with cotton wicks filled with 10 percent sugar water and hung, wick down, from the ceiling of the cage are changed on Monday, Wed-

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

nesday, and Friday. The females are offered a blood meal daily by placing a rabbit with a closely shaved back in the cage for 3 hours. A wooden stanchion is used to con train the rabbit by its head and neck, and each rabbit is used once every 3rd day.

EGG COLLECTION AND HANDLING. After the females have received a blood meal, a white enameled pan 25 cm in diameter and 8 cm deep containing 1 liter of distilled water is placed in the cage. The females oviposit in this pan, usually at night. The pan is removed from the cage each morning, and the dead mosquitoes are separated from the eggs (all viable eggs float) by pouring the contents through an 18-mesh screen into another pan; then a fine jet of water from a wash bottle is used to push the eggs through the screen (the adults are left on the wire); the water in the second pan is siphoned off so that the eggs are left stranded on the sides and bottom; and a jet of water from a wash bottle is used to transfer the eggs into a 150-ml beaker. This procedure not only has the advantage of concentrating the eggs for the next step but also provides an effective cleansing technique. Moreover, the inclusion of this simple wash as a routine procedure was recently instrumental in controlling an extremely heavy infection of *Nosema* in a colony of *A. albimanus* at the Central America Malaria Research Station, CDC, San Salvador, El Salvador.

The eggs in the beaker are transferred by gently pouring them from the beaker into an 8-cm opening in a 13-cm paper ring floating on 2 liters of distilled water in a 30-cm enameled pan. The pan is then covered with a sheet of plexiglass to preclude oviposition by females that might have escaped into the room. Also evaporation of water from uncovered pans can reduce the water temperature, which could delay egg hatch and possibly reduce the synchronization of hatch. After hatching has begun (the day after the eggs are placed in the pans), 0.5 g of the larval food mix (described in next section) is soaked for 15 minutes in 25 ml of distilled water and then gently poured into the pan without disturbing the ring of eggs. This mixture provides the initial nutrient for the first-instar larvae. These larvae with the food are then held overnight before they are transferred to the rearing trays.

LARVAL REARING. We use plastic trays (51 x 38 x 8 cm) for rearing larvae. The rearing medium in each tray consists of 4 liters of distilled water infused with 2 g of a 1:1:1 mixture of liver powder (Nutritional Biochemicals, Inc.), dried brewer's yeast, and hog supplement 40 percent protein (Ralston Purina Co.); the hog supplement is ground and sifted through a 50-mesh screen. Two thousand first-instar larvae are set into each tray. Any method that provides an accurate count would be adequate. However, our method is to partially fill each of the 10 compartments of a white plastic ice tray with distilled water. Then we pick up a quantity of larvae from the hatching pan with a 2.5-cm wire loop covered with organdy material and place approximately 200 larvae into each compartment (Fig. 1). With practice, all 10 compartments can be stocked with accuracy in a few minutes. The 2,000 larvae are then poured directly into the rearing tray which has been set up as described. Also two 45 x 5-cm strips of plexiglass, slotted to form an "X", are positioned in the center of the tray to provide an additional resting surface equal in length to the perimeter of the tray.

No supplemental food is added to the tray for the next 2 days, but the third day after setting (and subsequently), a slight amount of ground hog supplement (< 50 mesh) is deposited on the surface of the water with a feeding tube as follows: The feeding tube (25-cm length of 1.27-cm polypropylene tubing covered on one end with 30-mesh screen) is filled with hog supplement and corked on the opposite end. When this tube is lightly tapped on the side of a tray, a shower of food particles falls on the surface of the

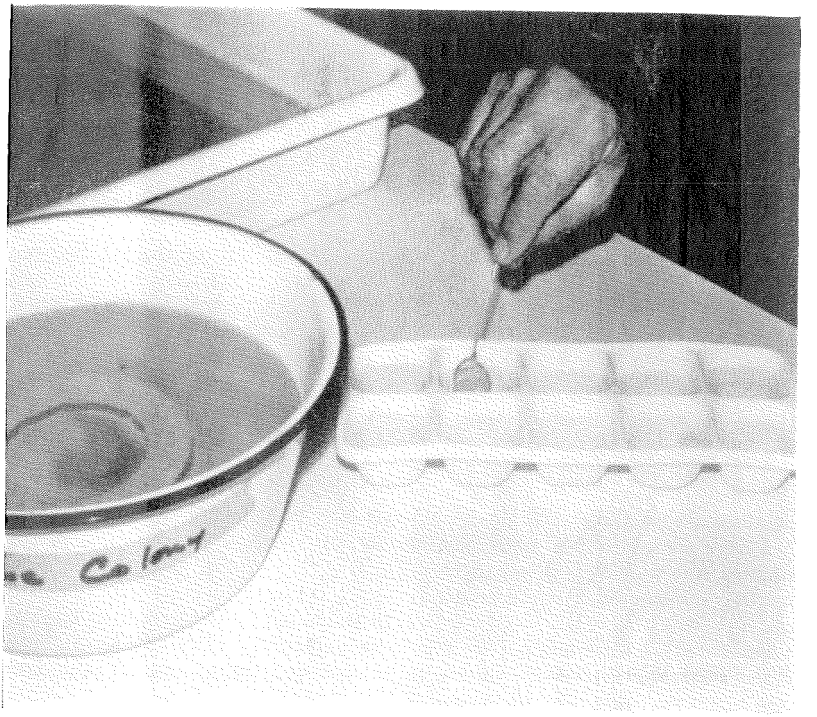

FIG. 1.—Method of estimating 2,000 larvae for setting rearing trays.

water. Food is added until the particles cease to move rapidly across the surface. On both the third and fourth days, the surface is dusted in this way in the morning and afternoon; on each subsequent day until pupation it is dusted 3 times a day. However, when pupation has started, the trays are fed once or twice daily with the feeding tube, or an alternative method that has proved quite successful is employed. Instead of using the feeding tube several times a day, a water slurry of 1 g of hog supplement is prepared and added to each tray once a day on days 4 through 7. *Anopheles albimanus* forage well on the bottom of the tray and are able to feed on the food that collects there, but if the water begins to cloud or show signs of scum (excessive growth of microorganisms), food should be withheld for a day. In that period, the larvae will normally consume the excess organisms and be ready for more food on the next day.

Pupation begins on the 8th or 9th day after setting. When approximately one-half or more of the larvae have pupated, the entire contents of the tray are poured through a screen in a 15-cm embroidery hoop. Then the pupae are separated from the larvae by the ice water method of Weathersby (1963) as modified by Hazard (1967). Usually few enough larvae are present so the larvae from 2 trays can be consolidated and returned to the rearing water of 1 tray for 1 more day. Pupae are separated from this tray the next day, and any remaining larvae discarded. (Some eclosion may occur before the time of ice water separation so we cover the trays at this time with wide-mesh nylon cloth to prevent the escape of adults into

the room. These adults are then removed with a vacuum cleaner tube inserted under the edge of the cloth.)

GENERAL MAINTENANCE. The adult holding cages are cleaned once a week by wiping the defecated blood and dead mosquitoes from the bottom and back of the cage with a damp sponge. Soap is not used in the cleaning of colony equipment. Every sixth week, a new cage is started, and the old cage is discarded when egg production in the new cage reaches an acceptable level. (If the sugar water is removed from the old cage, the adults will die in approximately 1 week; then that cage is thoroughly washed and set aside for the next cycle.)

Also, when the rearing trays are emptied, they are immediately washed with an abrasive cloth (no soap or detergents) and then rinsed in hot water several times. If the washing is done as soon as the trays are emptied, the food residue is soft and easily removed.

DISCUSSION. Every rearing system includes some procedures that require special care; thus, there are certain aspects of our method that warrant further elaboration.

The relationship between larval density, age and the amount of food required at each feeding is especially important because of the effect on the growth of microorganisms. The proper number (2,000) of first-instar larvae in the rearing tray is crucial. If too many ($>$ 2,000) are set, the majority of pupae and adults will be stunted, and the larval cycle will be extended. If too few ($<$ 1,500) are set, excess food accumulates, and the probability that scum will form is increased. However, when the correct number of larvae are present, overfeeding should not be a problem because the surface feeding limits the amount of food dust that can be introduced.

The water used in rearing is also critical. Chlorination and other treatments of municipal water supplies often make water unsuitable for rearing purposes. Moreover, most water softeners merely exchange rather than remove ions, so this type of purification may not be acceptable when used alone. If there is any doubt, it is best to use flash distilled bottled water or distilled water produced in the laboratory from the city water system.

The space required for our rearing system can be kept at a minimum. When the 8-cm deep trays are used, only 15 cm must be left between shelves; therefore, one can set 16 trays on an 8-shelf rack wide enough to hold 2 trays per shelf. Such a rack would have a minimum size of 132 x 155 x 46 cm; thus it would occupy 0.6 meter^2 of floor space. (The bottom shelf would be 30 cm from the floor.) Two of these units would provide sufficient shelf space for setting 6,000 larvae per day (three trays per day) if we assume that the larval to pupal cycle takes 10 days.

The time required to produce 5,000 to 6,000 pupae per day is minimal: the removal of the egg pan from the adult cage, the consolidation of the eggs into a 150-ml beaker, and the pouring of these eggs into a paper ring consumes about 10 minutes. Clipping and preparing the rabbit for installation in the stanchion and cage takes 5 minutes, and removal from the cage takes less time. Approximately 1 minute is consumed in adding 4 liters of water and 2 g of infusion to each tray. Counting the larvae into the compartmented tray, adding them to the rearing tray, and installing the X-shaped plexiglass separators should take about 4 minutes. Thus, the total time for setting one tray is 5 minutes. Supplemental feeding with the feeding tube is only a matter of seconds per day. Consolidating the larvae and pupae and separating the pupae by the ice water method consumes less than 5 minutes per tray. Cleanup and general maintenance will require another 15 minutes daily plus some time for equipment renovation and animal handling. Therefore, a total of 1.5–2 man hours per day is required to produce 5,000 to 6,000 pupae per day. The major requirement is that personnel must be available every day of

the week. However, should the slurry-feeding be adopted, the daily chores could be concentrated into one 2-hour period rather than extended over a period of 8 hours as with the surface-feeding method.

We have observed that a more homogeneous colony is produced by discarding the larvae that remain after 2 days of pupal separation, but this procedure may or may not be a benefit depending on the uses for which the colony was designed.

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