MASS REARING OF *ANOPHELES ALBIMANUS*[1]

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ABSTRACT. A system developed for mass rearing *Anopheles albimanus* Wiedemann was used to produce ca. 3.97 X10^5 male pupae/day for a period of 26 weeks. Innovations for drying eggs. use of thermostatically controlled heat tapes, and feeding of defibrinated beef blood in prophylactics made from sheep intestine increased efficiency over that achieved previously.

Until recently, the production of large numbers of sterile male anopheline mosquitoes has been one of the major problems preventing the use of the sterile-male technique for control of malaria vectors. Gerberg et al. (1968) described a method for rearing large numbers of *Anopheles stephensi* Liston, and Dame et al. (1974) used the methods of Ford and Green (1972) to produce ca. 50,000 sterile male *Anopheles albimanus* Wiedemann per day in a successful demonstration of the sterile-male technique at Lake Apastepeque, El Salvador, Central America.

As a result of the success at Lake Apastepeque, a large-scale field trial requiring the production of ca. 500,000 sterile male *An. albimanus* per day was undertaken in 1975 on the coast of El Salvador. During the initial phase of this experiment, a warehouse located in the city of Santa Tecla was renovated for the mass production of the mosquito. The preliminary design and techniques were based on the methods of Ford and Green (1972), but as new, more efficient rearing techniques were reported (Bailey et al. 1978, 1979, and Dame et al. 1978), they were modified and incorporated into the rearing system.

DESCRIPTION OF PROCEDURES

COLONIZATION. A colony of *An. albimanus* was established by collecting ca. 30,000 females throughout the proposed release area over a 3-wk period. Eggs from these females and also from the 1st and 2nd generations of laboratory stock were reared to adults, but each generation was maintained separately until the 3rd generation. Egg production from the F_1 and F_2 generations was very low, but by the 6th or 7th generation the colony was producing in excess of 10^6 eggs/day.

EGG HANDLING. Eggs were collected on water in plastic bowls, 15–20 cm in diameter, that had been placed in the adult cages overnight. The eggs were washed through a fine mesh screen with a jet of water from a spray nozzle to separate them from the dead adults, concentrated on a fine cloth stretched over an embroidery hoop, washed thoroughly, and transferred to plastic trays (51 x 38 x 8 cm) containing 2 liters of water. A plastic, rectangular frame (50.5 x 37.5 cm) that floated on the surface prevented the eggs from moving up the miniscus at the sides of the tray. The eggs were distributed in a monolayer by gently blowing them across the surface of the water as they returned to the surface after being poured into the water. The surface area of 1 tray was sufficient to accommodate ca. 25 ml of eggs (2 x 10^6) distributed in a monolayer. The eggs in the trays were held at 27.5±0.5°C for 24 hr and then transferred to 46 x 46 cm pieces of cloth for drying (Dame et al. 1978).

To facilitate drying, the eggs were distributed evenly over a 900-cm^2 area in the center of the cloth. To accomplish this, the piece of cloth was draped inside a 25 cm diameter funnel equipped with a shut-off valve. With the valve closed, the eggs were poured into the funnel and

[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.

water was drawn out until the level of the water was within the 900 cm^2 area marked on the cloth. The eggs were then washed back into the water, which was drained from the funnel. As the water level dropped, the eggs were drawn up the miniscus and deposited on the cloth.

The cloth was removed from the funnel, placed on sponges to remove as much water as possible, and sandwiched between 2 screened, aluminum frames that supported the cloth during the drying process and prevented the edges of the cloth from falling on top of the eggs. The drying apparatus consisted of a wooden frame that supported the cloth in a flow of air generated by an electric blower. Eggs prepared as described were usually dry in 35–45 min.

When the eggs were dry, the cloth was first stretched on the bias and then gathered up to form a bag that was shaken vigorously to dislodge the eggs. Because many eggs stick together during the drying process, they must be separated to insure accurate volumetric measurement.

The device used for this purpose consisted of 2 screen cylinders mounted one inside the other; the inner cylinder was made of 35 mesh screen and the outer of 80 mesh screen. The eggs were poured into the inner cylinder and the entire apparatus was mounted in a ⅜ in. (1.91 cm) electric drill. As the apparatus was rotated the separated eggs passed through the screens into a plastic tray.

The eggs were measured volumetrically with a manually operated gunpowder measurer that could be adjusted to deliver the desired quantity of eggs. By counting known volumes of eggs, it was determined that there was an average of 93.94 eggs per 0.001 cc. Therefore, based on the work of Dame et al. (1978), who reported that 5000–6000 eggs/tray resulted in the most efficient production of pupae, a volume of 0.06 cc of eggs was chosen.

The eggs were dispensed into 5 dr plastic shell vials, the inner surfaces of which were scored to prevent the buildup of static electricity, and then poured into styrofoam cups containing 75 ml of a liver-yeast suspension. The eggs slowly dispersed over the entire surface of the suspension (29.2 cm^2) but did not become trapped on the sides of the cups due to the negative miniscus formed at the interface of the styrofoam and the suspension. The eggs were maintained at 29±0.5°C for 24 hr during which time hatching occurred. The contents of the cups were then poured directly into the rearing trays (1 cup/tray).

LARVAL HANDLING. The larval diet was the same as that used by Dame et al. (1978). The brewer's yeast and desiccated hog liver (Nutritional Biochemicals, Inc.) were sifted through a 30-mesh screen to obtain the desired particle size. The hog supplement (Ralston Purina Co.) was cooked at ca. 200°C for 2 hr, ground and sifted through a No. 50 USAS standard sieve.

Stocks of larval food were prepared by mixing the appropriate quantities (w/w) of each dry component in a 3.8-liter plastic jar to obtain the liver:yeast diet for freshly hatched larvae and the 2:1:1 diet of liver:yeast:hog supplement used to rear the larvae in trays.

Each day the required quantities of larval diet were mixed with water at a ratio of 1 g of diet:50 ml of water. A small amount of the total volume of water was mixed with the dry diet forming a paste that was then mixed with the remainder of the water. The resulting slurry of the liver:yeast diet was strained through organdy and dispensed into the hatch cups, 1.5 ml/cup with a syringe. However, due to the tendency of the heavier food particiles to settle out of larval diet suspensions prepared in large volumes, it was necessary to design special equipment for mixing the 2:1:1 and hog supplement slurries. A 55 gal (208 liter) drum equipped with a 2-in. (5.08 cm) outlet and an external, self-leveling, metering tube was used to rapidly dispense water into a washing machine capable of holding 60 liters of larval diet, and modified to mix and agitate slurries. The slurries were drawn off into 10-liter buckets and dis-

pensed into the trays with plastic cups calibrated to deliver the desired quantity of diet.

The larvae were reared in plastic trays (51 x 38 x 8 cm), which were maintained at 29.5±1.5°C by the use of electric heat tapes (Dame et al. 1978). On the same day the eggs were placed in the hatch cups, the trays were placed on the shelves and 3 liters of water were dispensed into each tray so that the water would have time to stabilize at 29±1.5°C and any chlorine in the water would have time to evaporate. The following day, 150 ml of 2:1:1 slurry containing 3 g of diet and the 1st instar larvae were added to each tray. Three days later (day 4) an additional 150 ml of 2:1:1 slurry (3 g of diet) was added to each tray. Then on days 5 and 6, 150 ml (3 g) of a slurry containing only hog supplement was added to each tray.

Pupae were harvested on the 7th and 8th days utilizing the cold water technique of Weathersby (1963) as modified by Hazard (1967). After the 1st larval-pupal separation, the larvae were returned to their respective trays, but after the 2nd separation, the remaining larvae were discarded as they represented only ca. 10% of the total production and were predominantly female.

The majority of the pupae were sexed with a mechanical separator adapted from Fay & Morlan (1959). The male fraction was sterilized for release, and a portion of the female fraction was mixed with unsexed pupae from the 2nd larval-pupal separation to obtain a sex ratio of 1 male to 2 females for stocking the colony adult cages.

ADULT HANDLING. The cages for adult mosquitoes were constructed by covering a wooden frame (76 x 76 x 61 cm) with aluminum or fiberglass screen. Tubular sleeving attached to an opening 30 x 30 cm) in the front allowed access to the interior for the introduction and removal of pupal cups and egg deposition pans; 4 feeding ports (Bailey et al. 1978) located in the top of the cage made it possible to provide sugar and blood without entering the cage. The interior bottom and top of the cage were covered with white Formica® to facilitate cleaning.

The room for holding the adults was maintained at 27.8±2°C and 70–85% RH. During working hours, 7:30 am to 4:30 pm, it was illuminated by 4 fluorescent lamps; the remainder of the time it was illuminated by natural light that entered through 5 fiberglass panels in the ceiling and a 7.5-watt yellow, incandescent bulb mounted ca. 1.5 m above the panels.

The room was designed to hold 45 cages. Each day, 1 or 2 of the cages were removed for cleaning and repair and were replaced with an equal number of new cages each stocked with 6000 pupae plus the adults from the old cages that had been removed that day; all other cages were stocked with 3000 pupae each day. Thus it was possible to obtain eggs from 42–45 of the cages every night.

The adult diet consisted of a 10% sugar solution on cotton that was available at all times plus defibrinated beef blood that was fed daily in natural lambskin membrane prophylactics (Youngs Rubber Co.) as described by Bailey et al. (1978). This blood was obtained from a local slaughter house twice weekly and stored at 4–6°C. At the beginning of each day, refrigerated blood was heated to 40–44°C in glass beakers and poured into 20 prophylactics that were then placed in 10 cages, 2/cage. Throughout the remainder of the day, these membranes were removed from the cages at 20 to 30-min intervals, reheated in a water bath and placed in another 10 cages until each cage had been offered blood 3 times. The blood was then discarded, and the membranes were rinsed with water and stored in water at 4–5°C until the following day. Prophylactics subjected to this regime were reusable an average of 5 days before they deteriorated and had to be replaced.

HYGIENE AND DISEASE CONTROL. *Nosema algerae* Vavra and Undeen, a microsporidian pathogen of mosquitoes capable of decimating colonies of anophelines (Hazard 1970), was isolated from *An. albimanus* collected in El Sal-

vador in 1971 (Anthony et al. 1978). This pathogen was also detected in field-collected females during and shortly after the period when our colony was established. Fortunately, the simple procedure of washing the mosquito eggs with water is an effective method of controlling *N. algerae* (Alger and Undeen 1970, Ford and Green 1972). Periodic examination of the colony failed to reveal any infection.

The use of electric heat tapes with the larval rearing trays resulted in a very high RH (90%+) that favored the growth of mold and mildew. To prevent the growth of these organisms, we periodically washed the wooden shelves, plastic canopies, and walls thoroughly with a 3% solution of chlorine bleach. The larval rearing trays were also scrubbed, rinsed, and dipped in a 3% solution of bleach.

MONITORING PRODUCTION AND INSECT VIABILITY. At various points throughout the rearing cycle, the performance of personnel, the level of production, and the viability of the insects were evaluated to determine the level of proficiency that had been achieved and where improvement was needed. Three individuals who had demonstrated an exceptional ability and interest in the work were given additional training in the more technical aspects of colony maintenance and assigned the responsibility for data collection and supervision of personnel. The remainder of the work force was divided into teams that were rotated among the following major assignments: (1) egg collection and processing; (2) preparation of larval rearing trays; (3) preparation and feeding of larval diet; (4) larval-pupal separation; (5) washing trays; and (6) feeding of adults and cleaning cages. Individual workers were observed at random to determine whether they were performing their duties by the prescribed methods.

The maintenance of the correct temperatures throughout the facility and RH in the adult room were checked daily. Each electric heat tape and controller was tested periodically.

To monitor the quality and quantity of the mosquitoes produced, we obtained data as follows:

(1) A daily record was maintained of the number of cages producing eggs, the total volume of eggs produced, the percentage hatch of eggs that were transferred directly from the egg deposition bowls to hatch cups and held at 29±0.5°C for 48 hr, the percentage hatch of eggs that were exposed to the drying process in the routine rearing system, and the number of trays with high larval mortality.

(2) Twice each week the pupae harvested from 40 trays of each of the 2 larval-pupal separations were measured volumetrically. The number of pupae in a 3-cc sample of each separation were counted and the sex ratio and percentage emergence were recorded. Adults that emerged from pupae harvested at the 2nd separation were maintained on a 10% sugar solution for 8 days at which time the number of survivors of each sex was recorded.

(3) One to 3 times/week representative samples of the 2:1:1 and hog supplement slurries were collected from the food mixer and the delivery cups at the time of feeding and compared with prepared standards on the basis of liquid and sediment volume.

RESULTS

During the 25-week period in which this rearing system was in effect, an average of 3.97×10^5 male pupae/day was produced. The status of the colony and the production levels achieved during the 5 consecutive weeks of highest pupal production are reported in Table 1. The average daily production of 1.97 ml of eggs/cage is equivalent to ca. 1.85×10^5 eggs; the average total daily yield of 6.85×10^6 exceeded the regular requirements for setting larval trays. Since ca. 1067 female pupae were placed in each cage

Table 1. Weekly summary of daily averages of laboratory production of *Anopheles albimanus* in El Salvador during a 5-wk period of peak production in 1977.

Week	No. producing cages	ml eggs/ cage	% Hatch[a] Not dried	% Hatch[a] Dried	No. trays of larvae set	% of trays not producing pupae	No. pupae/ cc	No. pupae/ tray	No. ♂ pupae/ tray	Emergence (%)	Survival of adults (%)[b] ♂	Survival of adults (%)[b] ♀	No. ♂ (X 10^3) Produced	No. ♂ (X 10^3) Released
10	32.6	1.21	85.5	77.3	358.0	10.3	314.5	3243.1	1672.8	92.1	91.5	89.9	532.5	144.3
11	37.1	2.30	85.0	69.0	403.7	9.3	320.9	2758.4	1490.8	93.8			481.6	164.7
12	37.6	1.83	85.5	69.7	425.0	14.0	292.9	2584.7	1345.1	94.2	87.3	54.9	499.2	190.1
13	37.6	2.37	88.0	61.9	425.0	13.1	297.3	2072.3	1002.3	91.2	81.1	64.3	286.4	197.9
14	37.4	2.06		64.4	425.0	29.6	299.3	2545.4	1237.4	94.5	90.1	75.1	321.4	162.3
Avg.	36.5	1.97	85.8	68.5	407.4	15.5	305.7	2640.8	1349.6	93.2	87.2	66.7	464.3	171.9

[a] At 48 hr of age.
[b] Fed only a 10% solution of sugar on cotton for 8 days.

daily, an average of 173 eggs/female pupae was obtained. The averages given for percent hatch indicate that processing of the eggs by the dry egg technique reduced egg viability. However, the percent hatch of dried eggs after 72 hr was comparable to that of eggs that were not dried, indicating that the embryos had not been killed but that their development had been retarded. These results are in contrast to the findings of Dame et al. (1978), who reported that dried eggs of *An. albimanus* had a mean hatch of 93% after 48 hr. However, when we compared the data for larval rearing with that reported by Dame et al. (1978), who had used essentially the same larval rearing system, it was seen that we had a much higher percent of trays not producing pupae (16 vs. 5%), that the pupae were ca. the same size (306 vs. 315/ml), that the number of pupae per tray was significantly lower (2641 vs. ca. 4400), and that the percent emergence was slightly greater (93 vs. 83%). The higher percentage of trays failing to produce pupae and the lower production of pupae per tray were probably the result of an improper balance between larval diet and larval density resulting from the delayed hatching of the eggs.

During the 5-week period, an average of 464,300 male pupae/day was produced, but the average number of sterile males released was only 171,900 (37%). In accounting for the difference between the number of males produced and the number released, ca. 7% were utilized to stock the adult cages and 7% failed to emerge, but the major loss was a combination of 2 factors: (1) the technique for sexually separating the pupae resulted in a male fraction that was ca. 12% female and a female fraction that contained ca. 11% of the total males; and (2) a self-imposed limit of 25,000 females released/day. Taking into account the mortality of males during transport to the release site, the release of ca. 170–175 x 10^3 males/day was the maximum that could be achieved.

During the development of our mass

rearing system, we determined that consistent production of large numbers of mosquitoes could only be achieved by close monitoring of insect production and quality, environmental factors, food quality, and the day to day performance of personnel.

A labor force of 14 men was normally adequate to complete all phases of the daily work schedule. However, when pupal production exceeded ca. 800,000, an additional 2 men, each working 4 hr, were utilized to sex the pupae mechanically. At peak production (1.25×10^6 pupae), an additional 2 men would have been required to mechanically sex all the available pupae had the equipment been available. However, it was possible to complete all phases of the work except sexual separation of the pupae, with a crew of 16 men. Thus, it was possible to produce ca. 76,900 pupae/man-day, which is 3.1X the production/man-day reported by Ford and Green (1972).

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