

IMPROVED REARING TECHNIQUES FOR LARVAL *ANOPHELES ALBIMANUS*: USE OF DRIED MOSQUITO EGGS AND ELECTRIC HEATING TAPES¹

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ABSTRACT. Improved techniques developed for mass rearing *Anopheles albimanus* Wiedemann resulted in an 80% increase in production to ca. 4200 pupae per rearing tray. The increase was brought about by (1) using thermostatically-controlled, electric heating tapes to achieve more accurate water temperature in shelved rearing trays and by (2) volumetrically measuring dried mosquito eggs so the rearing process in the trays could be ini-

tiated with greater uniformity of numbers. These procedures resulted in more uniform larval development as well as increased production. The heating tapes provide an economical means of maintaining rearing temperatures above ambient levels. These significant advances in mosquito rearing technology may be adaptable to practically any scale of rearing and to other mosquito species.

Until recently most *Anopheles* mosquitoes were reared only in small containers of water that held less than 500 larvae (Gerberg 1970; Smith 1966). Such methods are appropriate and adequate for the requirements of most research workers, but rearing for large-scale needs demands a variety of improvements in methodology and equipment. Ford and Green (1972) reported a technique for rearing ca. 2000 larvae of *Anopheles albimanus* Wiedemann per 51 × 38 × 8 cm plastic container of water and described methods of estimating the number of 1st stage larvae to be introduced into each tray and maintaining the immature and adult stocks with a minimum of manpower; they maintained the temperature of the rearing water at ca. 27°C by controlling the room temperature at ca. 28°C. Efforts to improve these techniques have been continued at our laboratory primarily because we are studying the effect of releasing sterile males into native populations of this species.

In the course of experiments designed to increase the numbers of *A. albimanus*

pupae produced per container we developed a system of using electric heating tapes with a thermistor-activated controller to maintain the temperature of the larval medium. This system provided better temperature control and allowed the use of higher temperatures without the need to overheat the ambient air. In preliminary tests at ca. 30°C, we found that increased production could be obtained but that a critical balance existed between (1) the initial number and quality of 1st-stage larvae introduced into the container, (2) the amount and type of nutrient added, and (3) the temperature.

Attempts to standardize the number of larvae introduced into a container by using a simple light transmission measurement proved unsuccessful because of difficulties in handling the small larvae and obtaining consistent measurements. Also, newly emerged larvae were sensitive to handling and overcrowding even for short periods, and in addition, variations in survival were related to the movement of the water in which the young larvae were held. Thus, our attention was focused on measurement of eggs as a means of standardizing the initial number of larvae per tray at the start of the rearing process.

¹ Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.

Since handling and measurement of small volumes of eggs that were in water or wet seemed to be difficult and impractical, we developed methods of drying the eggs and volumetrically measuring them while dry. The eggs were able to withstand a considerable amount of abuse during the drying and measurement process, and the technique seemed to be amenable to mass production.

Our overall goal of increasing the numbers of larvae per tray also required an investigation of the balance between temperature, nutrition, and numbers of larvae in relation to space requirements. Since these factors are interdependent, much time was devoted to obtaining a system balanced properly for mass rearing needs. We here describe the system developed as a result of these studies and report its efficiency at different rearing temperatures.

MATERIALS AND METHODS

REGULATION OF REARING TEMPERATURE WITH HEATING TAPES. The rearing rack

used for this study consisted of 6 shelves, 305 cm long and 15 cm apart vertically, and was capable of holding 6 trays per shelf. The rearing rack was constructed of slotted steel angles (5.7×3.1 cm) with 2 horizontal members on the outer edges (25.4 cm apart) comprising the shelf to support the trays. Electric heating tapes (Wrap-on Co., Inc., Chicago, IL) were installed on the rack (attached with friction tape) so that 2 lengths of the tape (1.25 cm wide \times 30.5 m long and 500 watt heating capacity) were in contact with each tray (Fig. 1). A proportional temperature controller (Atkins, Technical, Inc., Gainesville, FL; Oldacre Engineering, Gainesville, FL), with its thermistor sensor submerged in the water of one of the rearing trays, was used to control the voltage applied to each heating tape and maintain water temperature. One tape and controller combination was used for the top 3 shelves and another for the bottom 3 shelves. The thermistor sensor was placed in a tray in the middle shelf for the tape that it controlled and near the center of



Fig. 1. Rearing trays resting on 2 strands of heating tape.

the shelf. This arrangement of tapes and controllers was used to reduce the influence of the vertical temperature gradient of the room air on trays that did not contain the sensor. Use of more controllers (one/shelf) would further reduce the effect of the vertical temperature gradient; however, the cost of additional controllers would become excessive for most systems. The entire rack was covered with polyethylene to reduce evaporation and help minimize the influence of variations in room temperature, which ranged from 20–26°C.

During initial trials with the heating tape system, the water temperature was monitored and automatically recorded continuously with sensors placed in 1 to 2 trays at each shelf level. These measurements indicated that the overall temperature variation was ca. $\pm 0.5^\circ\text{C}$ around the controller set point (30°C). The temperature of the trays containing the controller sensors was maintained within $\pm 0.25^\circ\text{C}$ of the set point. This system provided a significant improvement in temperature control over the previous method of using room air to heat the water (in this case, the temperature variation was typically $\pm 1.0^\circ\text{C}$).

VOLUMETRIC MEASUREMENT OF DRIED EGGS. Except as mentioned herein the techniques used for handling eggs, larvae, pupae, and adults were the same as those described by Ford and Green (1972). After the eggs deposited the previous night were cleaned and concentrated, they were poured into the opening of a 13-cm polyethylene ring floating on 2 liters of water in a white enameled pan 30 cm in diameter. The eggs were allowed to incubate in this water for about 24 hr at 28°C. Then when they were 30–40 hr old, they were strained by pouring them into polyethylene cups with an organdy screen bottom (10 cm diam.). As the water drained from the cup, the eggs became more or less uniformly distributed on the screen and so could be dried by drawing ambient air through the screen and over the eggs at an arbitrary velocity of 600–700 m/sec. The drying apparatus (Fig. 2)

was constructed to handle 4 drying cups simultaneously. Each cup could hold 6–7 ml of eggs for drying. After drying for 30 min the eggs were carefully removed from the drying cups with a large camel's hair brush and transferred to a glass bottle that was capped with 100-mesh copper wire screen. The dried eggs, which at this stage could be handled much like a granular material, were then dispensed for measurement through the screen cap which served to break up any clumps. Since a very small volume of eggs was required to set an individual rearing tray, sections of a 1-ml pipette were cut, and one end was plugged so the section could be used to measure egg volumes up to 0.15 ml. The eggs were dispensed into a funnel and into the pipette section for measurement (Fig. 3). A volume of 0.085 ml of eggs, which contained 5000–6000 eggs, was adopted as the standard number to set per rearing tray since preliminary trials indicated that greater numbers of eggs did not increase production of pupae.

After measurement, the eggs were poured from the pipette section into a 90-ml cup containing 75 ml of water and

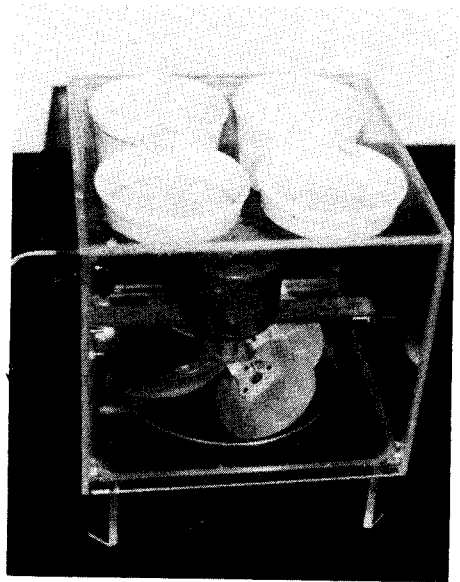


Fig. 2. Egg drying apparatus holding 4 cups.

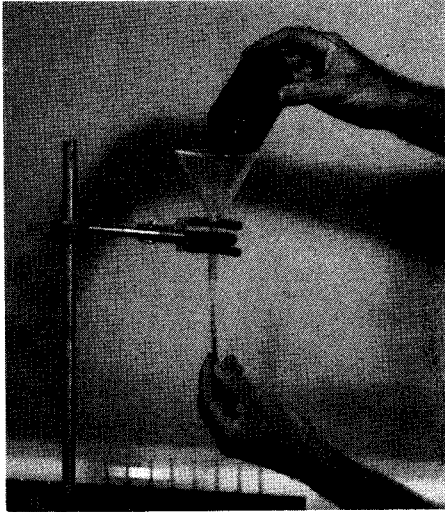


Fig. 3. Measuring dried eggs for placement in hatch cups.

1.5 ml of a nutrient suspension that provides sufficient food for the 1st-stage larvae as they hatch. The nutrient suspension was made by mixing liver powder, brewer's yeast, and water at a ratio of 1:1:100 by weight and then straining a small amount through organdy cloth to obtain a suspension of the finer nutrient particles. The cup was maintained in a tray of water heated to 30°C for 24 hr to allow the eggs to hatch.

LARVAL REARING. Tray rearing of larvae was initiated ca. 24 hr after the dried eggs were set in the cups, that is, when most of the 1st-stage larvae had eclosed. The contents of the cup were poured into a 51 × 38 × 8 cm ABS plastic tray containing 3 liters of water and 3 g of a slurry made of a 2:1:1 mixture of liver powder (Nutritional Biochemicals, Inc.), dried brewer's yeast, and hog supplement containing 40% protein (Ralston Purina Co.). Three days later (day 4) an additional 3 g of this mixture (freshly prepared) was added to the tray. On days 5 and 6 a slurry containing 3 g of hog supplement alone was added. The heated shelf system was tested at 27, 28, and 30°C; comparisons were made with

the standard method described by Ford and Green (1972) at 28°C.

Usually a few pupae would appear on day 6 in tests with the heating tapes, but unless many appeared, the 1st pupal separation was made on day 7, and the 2nd separation was made on day 8. Thereafter the remaining larvae were usually discarded. However, in these tests pupal collections were continued until pupation was complete or only a few larvae remained.

The following criteria were used individually or collectively to judge the effectiveness of the rearing technique: number and size of pupae produced, percent adult emergence, and longevity of emerged adults. The number of pupae produced was determined by making a volumetric measurement of the pupae produced daily from each test tray and calculating the number of pupae. Pupal size was assessed by counting the number of pupae in 2-3 ml from representative trays, and calculating the number per ml; this determination was made with pupae picked on day 8 when the sex ratio was usually about 1:1, so that there would be a minimum bias in size due to sexual dimorphism. Adult emergence was assessed 2 days after the pupae were collected, and survival was determined by holding up to 300 adults for 7 days in cages supplied with sugar water in cotton pads.

RESULTS AND DISCUSSION

With 0.085 ml of dried eggs, a temperature of 30°C, and the larval rearing system described, an average of 3878 pupae/tray (based on estimates of 285 pupae/ml observed in the standard trays during the period) was produced in a total of 42 trays set up on 5 occasions. This compares with an average of 2736 (individual trays set up on 5 occasions) from the standard method in which 2500 1st stage larvae were estimated by eye as described by Ford and Green (1972). This represents a 44% increase in production. The mean hatch of dried eggs observed in 100 samples was 93%. Pupation was essentially completed

in 8 days in the heating tape series; 9 days were required in the controls (temperature: water, $28 \pm 1^\circ\text{C}$; ambient, $29 \pm 1^\circ\text{C}$). Adult emergence was slightly lower in the experimental group, ranging from 70 to 92% (avg: 83%), while emergence in the standards ranged from 80 to 95% (avg: 90%). Adult survival showed a similar trend, averaging 68% in the experimental group and 73% in the standards.

Results of tests comparing rearing temperatures with the new system (using dried eggs and heat tapes) are given in Table 1. Again, pupal collection was completed in 8 days on the new system and in 9 days on the standard system. The pupae produced with the new system were slightly smaller (315/ml) than in the standards (285/ml). Pupal production was greatest at 28°C , but was also acceptable at 27°C and 30°C . At 27°C , 28°C , and 30°C pupal production exceeded the standards by 66, 80, and 72%, respectively.

dried eggs were held in 75 ml of infused water in 90-ml cups or in 300 ml of infused water in 900-ml cups the resulting pupal production was as follows (mean of 2 replications, 1 tray each):

ml H ₂ O	ml eggs		
	0.075	0.100	0.125
75	3662	3605	3883
300	3050	1881	2024

We feel that the beneficial effect of crowding is a result of the movements of the early hatching larvae, which stimulate and synchronize egg hatch. Another factor is the temporary inhibition of larval development that is caused by limited space and food. This is followed by synchronous development when the surface area is subsequently expanded and an adequate diet is provided.

Table 1. Effect of temperature on production of *Anopheles albimanus* pupae.

Rearing system	Temperature ($^\circ\text{C}$)	No. trays	No. pupae produced/tray
Tapes & 0.085 ml dried eggs	30 ± 0.5	48	3983
	28 ± 0.5	90	4178
	27 ± 0.5	48	3842
Ambient air & ca. 2500 larvae	28 ± 1	11	2321

The results show that a modest decrease in pupal size (10%) and adult emergence (7%) occurred with the new system. These reductions were more than compensated for by the 66 to 80% increase (Table 1) in production. Furthermore, adult longevity, which may be the best indicator of quality, was similar for both the experimental and standard methods.

Attempts to improve the method by putting the eggs directly into the trays rather than in cups were unsuccessful even with a variety of quantitative adjustments in the diets. Our observations generally support the conclusion that crowding during hatching is beneficial for synchronous development and therefore for the quality of the resulting insects. For example, when

Dry eggs are easy to measure volumetrically and especially useful for a mass rearing system. Our techniques were developed to provide fairly rapid egg drying and efficient measurement by hand. Many variations are possible for the drying system, even simple room air drying without a fan. Also, the basic concept should be easy to adapt to a mechanized rearing system.

Considerable effort was required to obtain a proper balance between larval density and nutrition though we are not reporting on the results of hundreds of tests in which we varied larval density and food. It is sufficient to comment that the larvae require different quantities and types of food as they develop and that determining

the optimum balance requires patient experimentation. Furthermore, any major inaccuracy in larval density on a fixed feeding regimen disturbs this balance with *An. albimanus*. This results in uneven larval development, characterized by the presence of several larval stages on days 6–8, or by fouling of the medium and total mortality in the tray by the 4th day. However, the precision of the volumetric system for egg measurement, combined with care in measuring the diet result in successful rearing in 95% of our trays. This system is now used routinely with a temperature of $28 \pm 0.5^\circ\text{C}$ in our *An. albimanus* colony in Gainesville to produce 100,000–200,000 pupae weekly.

The maximum production by the heating tape technique would be 5000–6000 pupae per tray since there are this many eggs in an 0.085 ml sample. On a few occasions, our heating tape rearing system has produced >5000 pupae per tray, though normally 4200–4600 are produced. Limited attempts to increase the production to these levels when controlling water temperature solely by regulation of the ambient temperature have been only marginally successful. This is in part due to the inherent difficulty of such control. Such a system usually requires greater energy input and causes excessive evaporative cooling at the water surface. The result is greater water loss, wider temperature fluctuation, and possibly a greater differential between surface and subsurface water temperature. Such fluctuations affect not only the rate of larval development but also the development in the medium of microorganisms that play an important (although poorly defined) role in larval nutrition. Thus, the dynamics of water temperature fluctuations probably play a significant role in rearing efficiency.

Although we designed the system to improve the efficiency of mass rearing of *An. albimanus* larvae, parts or all of the method are adaptable to other aquatic and non-aquatic organisms reared in containers. Our standard rearing rack holds up to 18 trays of the size we use but could hold many more smaller containers. Also, in

our mass rearing factory in El Salvador, ca. 3400 trays are maintained with this system on similar wooden racks at $28 \pm 0.5^\circ\text{C}$ to produce 800,000–1,000,000 pupae daily. Thus, the heating tape system is adaptable to virtually all scales of rearing activity.

In using the heating tape system, one should consider two factors. First, with this system, particularly when enclosed with the polyethylene canopy, some of the generated heat warms the air, which rises to the top by convection. Thus, a single controller may be inadequate to regulate temperatures within 0.5°C because the top shelves of the rack will bask in the convected warm air and the lower shelves will be cooler. However, two controllers and two tapes adequately regulate the water temperature within 0.5°C when one unit controls the upper 3 shelves and the other the lower 3 shelves. The second factor is the water that condenses on the inside of the polyethylene as a result of the temperature differential between the enclosed rack and the cooler ambient air. At times this condensation is quite heavy, but in our circumstances it causes no serious difficulties either in using the rack or the room in which it is housed.

For most purposes the use of the heat tape system is limited only by the fact that the desired temperature must be greater than the ambient temperature. Normally, that is an advantage since it assures that insects or other organisms can be reared with relatively inexpensive equipment (100 ft of heat tape is less than \$20 and controllers retail at about \$85–\$125) in rooms that can be maintained at comfortable working temperatures.

ACKNOWLEDGMENT

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