# PRODUCTION EFFICIENCY AND RATE OF INCREASE OF A MASS-REARED LABORATORY COLONY OF *ANOPHELES ALBIMANUS* WIEDEMANN

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ABSTRACT. A study was conducted in El Salvador, Central America, to determine the rearing efficiency and rate of increase of a laboratory colony of *Anopheles albimanus* Wiedemann. An average of 6779 eggs per larval rearing tray produced 6250 larvae, 3822 pupae, and 3502 adults (1873 males and 1629 females). From each rearing tray, an average of 874 mosquitoes died as larvae and 1554 larvae were discarded after the third pupal separation. Adult females laid an average of 145 eggs each; therefore, 1629 females (the number produced per rearing tray) should produce a total of 236,205 eggs. A 34.8X rate of increase per generation occurred with this colony.

## INTRODUCTION

Many papers have been published describing techniques for estimating natural populations of insects, particularly in anticipation of controlling those populations with chemicals, parasites, predators, pathogens, sterile insects or other methods. Specifically, many studies of the dynamics of natural populations have been conducted with *Anopheles albimanus* Wiedemann (Breeland 1972, Breeland 1974, Breeland et al. 1974, Hobbs 1973, Weidhaas et al. 1974). Weidhaas et al. (1972) published a technique for estimating rates of increase of populations of *Culex pipiens quinquefasciatus* Say, *Musca domestica* L., and *Stomoxys calcitrans* (L.), all of which were under the influence of released sterile insects. Later, Weidhaas et al. (1974) used that technique to estimate rates of increase of a population of *An. albimanus* at Lake Apastepeque in El Salvador, Central America, during a project involving control of that species by the release of sterile males. During the 15-month period of their study the rate of increase of the natural population ranged from a low of 0.4X to a high of 4.8X.

Although much work has been done on rates of increase in natural populations, little has been published on rates of increase in laboratory colonies. Rates of increase become important when estimating the size of a rearing facility and the necessary equipment for mass producing a given species for the release of sterile insects, or for other research needs. Thus, a laboratory study was conducted in El Salvador to determine the rate of increase of mass-reared *An. albimanus*.

## MATERIALS AND METHODS

Two 0.085-ml samples of dried *An. albimanus* eggs (Dame et al. 1978) were measured, and all the eggs in each sample were counted with the aid of a microscope. The samples were then transferred to separate cups containing water and held for 24 hr to allow eclosion. The percentage hatch was determined by microscopic examination of 3 samples of 100 eggs each, then the larvae were transferred to plastic rearing trays (56 × 43 × 7.5 cm) containing larval food. The handling of eggs, larval rearing, pupal separation, and maintenance of adults in these experiments followed the system of Bailey et al. (1979). On days 6, 7 and 8 after the newly-hatched larvae were placed in the trays, pupae were separated from the larvae. On days 6 and 7 the larvae were returned to the rearing trays, the harvested pupae from each tray were counted, and the sex ratios of those pupae were determined by microscopic

examination of a sample of 100 pupae from each tray. On day 8 the pupae were again separated and counted, and the sex ratio was determined. The remaining larvae were counted in order to determine the production loss due to the failure of some of the larvae to pupate by the 8th day, then the larvae were discarded. After each pupal separation the pupae were placed in water in a plastic cup in a cage, the adults were allowed to emerge for 48 hr, and the number of adults that emerged was recorded. The experiment was repeated for a total of 4 replicates involving a total of 8 trays.

To determine the longevity and egg production of the adults, we placed ca. 10,000 pupae (measured volumetrically) in water in plastic cups in each of 2 colony cages. After 48 hr the cups were removed and the percent adult emergence was determined by counting the dead pupae. Cotton soaked with sugar water was provided continuously, and warm defibrinated bovine blood in prophylactics made of sheep intestinal membranes was offered 3 times per day after the third day (Bailey et al. 1978). Cages were held in a room maintained at a temperature of 26±2°C with a relative humidity of over 70%. Each day dead adults were removed from the cages and the numbers of each sex were recorded. Also, a container of water was provided in the cages each night as an oviposition site, and each morning the eggs were removed, dried and measured. The experiment, which consisted of 4 replicates involving a total of 8 cages was continued until all adults in the cages were dead. We were able to follow closely the production and survival of each life stage of this species while it was being mass-reared. The 4 replicates, for both the immature stages and for the adults, were conducted at different times; thus, we were evaluating a different laboratory population involving a complete generation with each replicate.

## RESULTS AND DISCUSSION

Table 1 presents the average production of *An. albimanus* from each rearing tray throughout the various life stages. The average number of eggs set for each rearing tray in our mass-production system was 6779. This represents 0.085 ml of eggs, which had been established as the optimal quantity through prior research (Dame et al. 1978). The number of eggs in the 8 samples used in the experiment ranged from 5473 to 7826 (±15% variation from the mean). Part of this variation can be attributed to the degree of settling of the eggs in the measuring tube caused by a vibrating apparatus which was used to dispense the eggs. Also, the eggs probably varied slightly in size from day to day, depending on a variety of environmental factors.

Table 1. Average production of *Anopheles albimanus* per rearing tray (eggs to emerged adults).

| Stage of production | Number per rearing tray | | | Percent of previous stage |
|---|---|---|---|---|
| | Males | Females | Total | |
| Eggs set (0.085 ml) | | | 6779±618 | 100.0 |
| Larvae hatched | | | 6250±595 | 92.2 |
| Pupae harvested | | | | |
| 1st separation | 704 | 301 | 1005±652 | |
| 2nd separation | 1060 | 1059 | 2119±563 | |
| 3rd separation | 265 | 433 | 698±363 | |
| Total pupae | 2029 | 1793 | 3822±867 | 61.2 |
| Adults emerged | | | | |
| 1st separation | 661 | 283 | 944±614 | |
| 2nd separation | 997 | 996 | 1993±614 | |
| 3rd separation | 215 | 350 | 565±344 | |
| Total adults | 1873 | 1629 | 3502±897 | 91.6 |

The percentage hatch was more constant, ranging only from 91 to 96%. Therefore, from the average of 6779 eggs set for each tray, 6250 larvae hatched (92.2%). From those larvae that hatched, 3822 pupae were harvested (61.2% of the larvae) and from those, 3502 adults emerged (91.6% of the pupae). The largest number of pupae was collected in the 2nd separation, which accounted for more than the 1st and 3rd separations combined for both males and females. The 1st separation was predominantly males (70%), the 2nd was equally divided between the 2 sexes, and the 3rd was predominantly females (62%). Because of the larger numbers of male pupae in the 1st separation (704) than females in the 3rd separation (433), more males than females were collected from each rearing tray, 2029 and 1793, respectively (53.1% males). If only 2 pupal separations had been made, the difference would have been greater: 1764 males and 1360 females (56.5% males). In a program involving the release of sterile males, the higher male ratio is an advantage when males have to be separated from females by conventional means in either the pupal or adult stage. The higher the female ratio, the more difficult and less complete the separation of the males becomes. An average of 1873 adult males and 1629 adult females emerged from the pupae produced in each tray.

It is obvious from the data in Table 1 that the greatest loss of mosquitoes occurred in the larval stage, since only 3822 pupae were harvested from the 6250 larvae that hatched (61.2%). A better breakdown of the losses of immature forms and where they occurred, based on the original 6779 eggs that were set per rearing tray is shown in Table 2. An average of 529 eggs (7.8%) from each 0.085-ml sample set was lost because of failure to hatch. Because we counted all pupae that were harvested, and also all larvae that were discarded after the 3rd pupal separation, we were able to subtract these 2 figures from the number of larvae that hatched, and to calculate the larval mortality. An average of 874 of the original number died as larvae (12.9%), but the major loss actually occurred as a result of the number of larvae that were discarded (22.9%), representing almost half the total loss of 48.3%. However, it is not practical to hold the rearing trays for additional days, since the number of additional pupae harvested even in the 3rd separation is minimal. Only 4.7% of the total loss occurred in the pupal stage.

Table 3 summarizes in 3-day periods the adult mortality and the egg production from the average 1629 females that emerged from each rearing tray, based on the observations from the cages set up with 10,000 pupae. Complete mortality occurred in both sexes within 27 days; mortality of the males however, was more accelerated than that of the females during the first few days. An average of 17.8% of the females died during the first 3 days, before any eggs were collected, but 26.6% of the males died during the same period. However, it is assumed that most of the mating had occurred by that time, and that the males were of no further use. More than 90% mortality occurred in the males within 12 days, but it was 18 days before the mortality of the females reached this level.

The period of greatest egg production occurred between 7 and 9 days (46.5% of total production); 70.3% of the eggs had been deposited by the end of 9 days. Although 17.3% of the females were still alive after 15 days (82.7% mortality), only 4.5% of the total eggs were deposited

Table 2. Stage in which losses of *Anopheles albimanus* occurred during mass rearing of the immature stages (based on original 6779 eggs).

| Stage in which losses occurred | Number lost | Percent loss |
|---|---|---|
| Eggs | 529 | 7.8 |
| Larvae (mortality) | 874 | 12.9 |
| Larvae (discarded) | 1554 | 22.9 |
| Pupae | 320 | 4.7 |
| Total losses of immature insects | 3277 | 48.3 |

Table 3: Adult mortality and egg production of *Anopheles albimanus* throughout the adult stage (based on an average of 1873 males and 1629 females from each rearing tray).

| Days | Accumulative percent mortality | | Egg production | | |
|---|---|---|---|---|---|
| | Males | Females | Estimated number from 1629 ♀[a] | Percent of total | |
| | | | | Actual | Accumulative |
| 1–3 | 26.6 | 17.8 | 0 | 0 | 0 |
| 4–6 | 62.2 | 44.4 | 56,217 | 23.8 | 23.8 |
| 7–9 | 82.0 | 59.8 | 109,835 | 46.5 | 70.3 |
| 10–12 | 91.8 | 72.3 | 41,572 | 17.6 | 87.9 |
| 13–15 | 95.4 | 82.7 | 17,715 | 7.5 | 95.4 |
| 16–18 | 97.2 | 91.1 | 8,267 | 3.5 | 98.9 |
| 19–21 | 99.1 | 96.3 | 1,890 | 0.8 | 99.7 |
| 22–24 | 99.7 | 98.5 | 709 | 0.3 | 100.0 |
| 25–27 | 100.0 | 100.0 | 0 | 0 | |
| Total | | | 236,205 | 100.0 | |

[a] Based on egg production resulting from a single stocking of 10,000 pupae.

after that time, and no eggs were laid after 24 days. We estimate that 236,205 eggs were produced by the 1629 females during their adult life (145 eggs/female).

Figure 1 shows that the rate of increase of *An. albimanus* during this study averaged 34.8X. When compared with the maximum rate of increase of 4.8X that Weidhaas et al. (1974) found in a natural population of *An. albimanus*, the efficiency of the laboratory system was at least 7.25X as great as the natural system that they described. Considering that these workers reported the rate of increase in the field to be as low as 0.4X at times, then at those times the laboratory production was as much as 87X as efficient as field production.

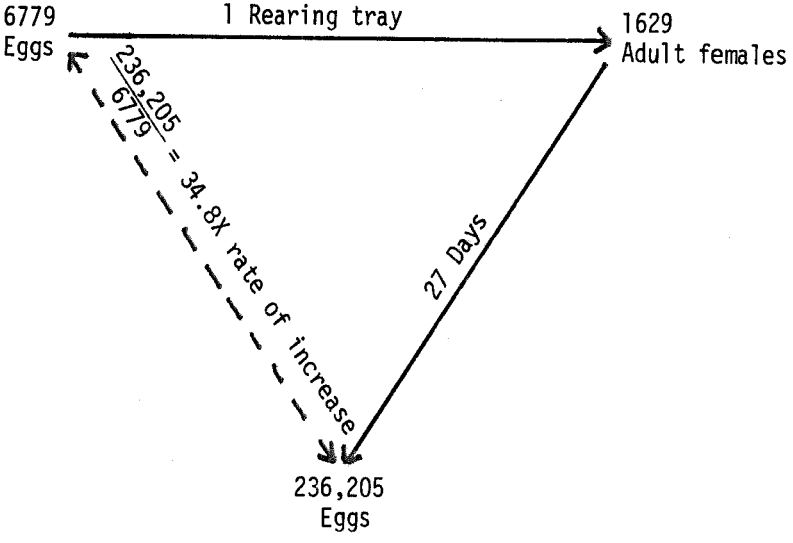


Fig. 1. Rate of increase per generation of a laboratory population of *An. albimanus*.

This laboratory rearing efficiency has been developed over several years and has required considerable experimentation to obtain effective environmental conditions and diets for the different life stages and the development of techniques for precisely controlling those conditions. In programs requiring large numbers of insects for field release, especially if only one sex is to be used, the greater the rate of increase in the laboratory colony, the more efficient the production in the rearing program will be. A 1X rate of increase is all that is necessary to maintain a laboratory colony. Any increases above this level will provide the numbers needed for releases.

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