A RELIABLE TECHNIQUE FOR RAPID COLONIZATION OF *ANOPHELES ALBIMANUS* WIEDEMANN

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ABSTRACT. A simple, reliable technique was developed for rapid colonization of *Anopheles albimanus* Wiedemann. Field-collected adult females were placed in plastic vials containing water to induce oviposition. Subsequent generations of adults were then placed in colony cages for mating and oviposition. The 500 parental females produced 9009 F_1 females, which laid >200,000 eggs. An equal number of field-collected females placed in a cage for oviposition produced only 62 F_1 females, which failed to oviposit.

Most laboratory research on insects is conducted with strains that have been maintained in the laboratory for many years. One of the main advantages of using laboratory-reared insects is that laboratory rearing usually becomes more efficient with each succeeding generation after colonization. Many species are difficult to colonize, thus it is much simpler and economical to maintain a strain that has been adapted and selected for laboratory rearing. However, at times the performance of laboratory strains is questioned, and their performance needs to be compared with individuals less removed from the field condition.

Rozeboom (1936) described techniques that he used to colonize *Anopheles albimanus* Wiedemann. His colony was established from more than 1500 field-collected larvae, and was supplemented with field-collected adults from stables. He cited high adult mortality and low oviposition rates as two of the major difficulties in colonizing that species.

Dame et al. (1974) also described techniques that they used to establish a colony of *An. albimanus* from ca. 11,000 adult females collected from stables in El Salvador, Central America. The slow rate of colonization in their study was attributed to a low oviposition rate due to a low level of insemination of females in filial generations.

During a recent sterile-male release pilot study in El Salvador, Central America, with *An. albimanus* we needed to periodically assess the performance (mating competitiveness, migration, and attraction to calf-baited traps) of mass-reared, sterile males (initially colonized in 1975) by comparing them with a recently colonized field strain. Because of limited rearing space, limited personnel, and a need for proper timing, we needed a simple, reliable technique for colonizing a field strain repeatedly and in numbers sufficient for field use (several thousand) after 1 or 2 generations in the laboratory.

Lofgren et al. (1974) described techniques for assaying sterility in field-collected *An. albimanus* by placing females in 5-dram plastic vials for oviposition. This technique was also used in the later pilot study in El Salvador, and oviposition rate of the females in the vials was usually high. We thought that this source of field-collected material could provide large quantities of stock for colonization of *An. albimanus*. Thus, 2 methods of colonization of this species were evaluated and are described in this paper.

METHODS AND MATERIALS

We designed an experiment to compare the ease of establishing colonies of *An. albimanus* by placing field-collected females in vials or in cages. We collected adult females from a stable on the Pacific Coast of El Salvador for 10 days. Each day 50 females were placed in a screen cage (61 x 61 x 61 cm) and 50 females were placed individually in 5-dram plastic vials containing 5 ml of water infused with a small amount of liver powder and dried

yeast (1:1). Females in both groups were held in a room with a temperature of 26±2°C, relative humidity (RH) above 70% and with a photoperiod of 10 hr light (supplied by 4 40-watt fluorescent lamps) and 14 hr darkness.

In the cage system the females were provided with cotton pads soaked with a 10% sugar-water solution and were fed defibrinated bovine blood 3 times daily through natural membranes (Bailey et al. 1978). A plastic pan containing water was placed in the cage each evening as an oviposition site and removed each morning. Eggs of filial generations were counted and reared to the pupal stage (Bailey et al., 1980), and the pupae were counted and placed in a clean colony cage for adult emergence. A record was kept of the number of eggs collected from those F_1 adults.

In the vial system, females were held in the vials for 4 days then the number of vials containing eggs were counted. To determine the approximate average number of eggs laid per female and the percentage hatch, we counted the total number of eggs in 5 randomly selected vials/day and the number of eggs that hatched. Larvae were then reared to the pupal stage, counted, and placed in a colony cage similar to that used in the cage system. When adults emerged, they were provided with sugar water and blood as in the cage system. Eggs were collected from the colony cage daily, held for 24 hr on water, then dried and measured. The number of eggs produced was calculated with the system of Bailey et al. (1979) (ca. 6779 eggs/0.085 ml). The dried eggs were then placed on water for an additional 24 hr. Percentage hatch was determined by microscopic examination of a 100-egg sample. With these techniques we were able to determine the rates of increase for both systems from the original 500 adult females to the F_2 generation eggs.

RESULTS AND DISCUSSION

The results of the cage and vial systems for colonization of *An. albimanus* are shown in Table 1. The attempt to colonize this species with the cage system was unsuccessful. The 500 adult females collected from the stable produced only 334 eggs. These eggs developed into 62 F_1 females (0.125X rate of increase), but no F_2 eggs were collected, apparently because there were too few adults for successful mating and oviposition. However, the vial system was quite successful. The number of F_1 adult females produced showed an 18X rate of increase over the original 500 females.

The F_2 eggs and larvae increased at a lower rate (5.5 and 5.2, respectively), probably because the F_1 females were not individually placed in vials for oviposition. Lack of selection for mating could also have contributed. The high rate of increase of F_1 adults was undoubtedly a result of the large number of eggs produced by the parent generation females in the vials (74.9 eggs/female), which provided the potential for high numbers of F_1 adults. This is the basis for the success of the vial system: field-collected females oviposit at a much higher rate when confined in small vials than when placed in cages.

Table 1. Comparison of 2 systems for the colonization of *Anopheles albimanus*.

	Cage system		Vial system	
Life stage	Number	Rate of increase	Number	Rate of increase
Adults (parent generation)	500		500	
Eggs produced (F_1)	334		37,438	
Larvae hatched (F_1)	205		25,110	
Pupae produced (F_1)	149		18,900	
Females emerged (F_1)	62	0.125	9,009	18.0
Eggs produced (F_2)	0	0	207,359	5.5
Larvae hatched (F_2)	0	0	130,198	5.2

The F_1 females laid an average of 23 eggs each. Bailey et al. (1979) reported that an average of 145 eggs/female was produced by a laboratory strain of *An. albimanus* that had been in colonization for ca. 4 years. That strain showed a 34.8X rate of increase between generations in the laboratory. The 5X rate of increase with the vial system is considerably lower than that demonstrated with the laboratory strain, but even a 5X rate of increase represents more than 1 million eggs that could be produced in the F_3 generation from an original collection of 500 adult females.

On the several occasions we used the vial method to establish new colonies of mosquitoes from field-collected females, we had similar success. This method therefore proved to be a reliable technique for rapid colonization of *An. albimanus*.

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