MAINTENANCE AND TRANSPORTATION OF FEMALE MOSQUITOES COLLECTED IN THE FIELD[1]

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ABSTRACT. Maintenance and transportation of large numbers of female mosquitoes collected during an interval of several days may be performed easily and simply without significant mortality. Small cages used to collect were carried in large transport boxes of styrofoam. The mosquitoes, 10 to 15 females in each cage, were permitted to feed daily on blood and a 10% solution of honey. The transport boxes were kept at 21° C by use of ice in plastic bags. Important to high survival and egg production for the species of *Aedes* collected in northern Michigan was the blood-feeding in the field within a few hours of capture and the maintenance of proper humidity in the transport boxes.

Maintenance and transportation of large numbers of female mosquitoes collected during an interval of several days can be accomplished easily and simply without significant mortality. Dr. William R. Horsfall began developing this method in 1962 at the University of Illinois. Since that time numerous changes have been introduced by him and by the author. The many modifications and adaptations culminated in the following procedure used in 1975 and 1976 for some 15 species of *Aedes* collected in northern Michigan.

In 1976 about 2500 female mosquitoes were collected over a 4-day period from several areas in Michigan north of 45° latitude. These areas which had both high densities of adults and variety of species were either proximal to larval habitats or near margins of barriers to movement such as large lakes. At such sites, 10 to 15 female mosquitoes, attracted to human bait, were collected in each cage.

Cages used for collecting, transport and maintenance of mosquitoes were the *Illinois oviposition cage*, long used in this laboratory. This cage consists of 2 walls and ends of transparent Lucite® (3 mm thick) and a top of Lumite®[3] screen (mesh: 32x32 natural) and a bottom of nylon tulle (available at local fabric shops). Dimensions of each cage are 150x25x25 mm. One end has a hole 10 mm in diameter which is fitted with a cork stopper when in use.

After collecting was completed and cages labelled for later reference, they were arranged in layers above a false bottom in transport boxes made of styrofoam (size: about 200x350x250 mm). Stoppered ends of the cages were placed

[1] The opinions and use of product names contained herein are the private views of the author and are not to be construed as official or reflecting the views or endorsements of the Department of the Army and the Department of Defense.

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[3] Available from Chicopee Mfg. Co., Cornelia, Georgia 30531.

against one side of the transport box in order to minimize dislodgement of corks. Between every other layer of cages a moist strip of multilayered cheesecloth was placed. These strips were no more than 25 mm wide and long enough to be in contact with a screened side of each cage. Moisture in these strips was provided by soaking them in water and squeezing them by hand until water did not drip.

After the cages were packed, a layer of paper (such as newspaper) was placed along the exposed ends and above the cages. In the space formed between the side of the box and the paper, a container of cracked or cubed ice was placed. The ice provided means for holding the temperature in the transport box at or about 21° C. Containers used for the ice consisted of 2 to 4 plastic bags, such as commercial freezer bags, placed one inside the other. Water was drained from the bags when new ice was added.

At each stopover on the trip, all mosquitoes were offered an adequate chance to feed on blood. During these stopovers, care was taken to avoid exposing the collection to volatile toxicants such as may be used in mosquito-infested areas and rooms for transients in these areas. After all mosquitoes had fed on blood, they were repacked as described above with strips of cheesecloth moistened with a 10% solution of honey instead of water. Strips were remoistened at intervals with water. Feeding procedures were repeated each evening and morning on the trip.

Upon return to the laboratory, the cages were placed in enamel pans (300x220x 55 mm) which contained wet multilayered cellucotton pads (300x100x10 mm) saturated with deionized water. The cages were positioned on the pad in a row on the wet cellucotton. A 10% solution of honey continued to be offered using cubical plastic boxes (20x20x20 mm) packed with cellucotton and held in place with masking tape. Blood was offered daily.

Prior to oviposition, each mosquito was transferred to an individual oviposition cage. These cages were Lucite cylinders, each 10 mm high by 25 mm in diameter. The top of the cage was covered by nylon tulle. The bottom of each cage was without cover. Each cage rested on its own mat of multilayered cheesecloth squares (30x30 mm) on top of a bat of multilayered cellucotton in enamel pans (260x 200x15 mm).

For transfer to the individual oviposition cages, field-caught females were placed in an atmosphere saturated with CO_2. Anesthetized females were emptied into a small (40 mm diameter) Büchner funnel through which passed a stream of CO_2. Individual mosquitoes were placed on each square of cheesecloth in properly labelled pans and enclosed by the individual oviposition cage. The pans were then placed in an environmental chamber at 21° C, a temperature suitable for maintaining eggs of northern *Aedes*. When the females had revived after a few minutes, the cellucotton bats were saturated with deionized water to a state where they glistened. Soaking of the bats to a state of glistening was repeated daily. Small balls of cellucotton moistened with a 10% solution of honey were then placed on each cage. These balls were made by placing small pieces of dry cellucotton in a pan which contained a very shallow (1–2 mm) layer of diluted honey. The cellucotton was rolled into small balls after it had absorbed the honey. On the following days, deionized water was used to moisten the balls.

Each mosquito was given an opportunity to feed daily on blood. Feeding large numbers of mosquitoes in individual cages was accomplished by covering the layer of cages with a sheet of nylon tulle, held in place by clamps and tautly stretched over two pans. The blood source was then lightly placed on the cages and left for several minutes.

Each cage was inspected daily for eggs. Once a female had oviposited, the square of cheesecloth was replaced with a new one, and the eggs were removed to a small piece of filter paper in a petri dish containing moist cellucotton. The female and

her eggs were then assigned an identification number indicating the site of capture and sequence of deposition. Eggs were removed, stored, and conditioned in a manner similar to that shown by Horsfall and Fowler (1961).

The technique for transportation and maintenance of live mosquitoes for several days after being captured in the field has been developing over many years with increasingly better adult survival and egg production. The critical nature of blood-feeding within hours of capture and the importance of maintaining a moist, but not saturated, atmosphere in the transport boxes were recent developments. A saturated atmosphere in the transport boxes resulted in high morbidity and mortality in mosquitoes when they were held in the boxes for several days, a condition avoided by the current method of humidifying the boxes.

The beneficial nature of blood-feeding was demonstrated in 1976 when 1900 mosquitoes were collected and maintained by the techniques described above. These mosquitoes were held from 1 to 3 days in the transport boxes and 6 days in the laboratory prior to transfer to individual oviposition cages. On the day the mosquitoes were transferred, 1850 females were still alive. None of the 50 dead mosquitoes appeared to have taken a blood meal.

To demonstrate the beneficial nature of blood-feeding immediately after capture, a group of 600 mosquitoes were not allowed to feed on blood after collection or while in transit. First blood was provided 48 hours after return to the laboratory. Otherwise this group was maintained as was the other group. Only 400 of this group survived to go into the individual oviposition cages. Of the 200 dead females, 95% had died during the 1 day of transit and prior to being offered a chance to feed on blood.

Blood-feeding immediately after capture was also beneficial to egg production. Using *Aedes stimulans* (Walker) and *Ae. trichurus* (Dyar) as indicators, egg production by each female was reduced in the group not given blood immediately. Females of *Ae. stimulans* and *Ae. trichurus* deposited an average of 47 and 36 eggs, respectively, in the group which received blood immediately after capture. The females of these 2 species deposited an average of 21 and 26 eggs, respectively, in the group fed blood 2 days after capture. Thus, in regard to adult survival, egg production, and economy of effort, blood-feeding soon after collecting was obviously desirable.

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References

Horsfall, W. R. and H. W. Fowler, Jr. 1961. Eggs of floodwater mosquitoes. VII. Effect of serial temperatures on conditioning of eggs of *Aedes stimulans* Walker (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 54:664–6.

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