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SCIENTIFIC NOTE

A SIMPLE TECHNIQUE FOR RAPID COLONIZATION OF *ANOPHELES QUADRIMACULATUS* USING ADULTS ASPIRATED FROM LIVESTOCK BARNS[1]

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ABSTRACT. A technique was developed for rapid colonization of *Anopheles quadrimaculatus* larvae in an improvised insectary using blood-fed mosquitoes aspirated from livestock barns. A novel device termed the mosquito aspiration transfer and ovipositional chamber (MATOC) is described. In 2 field seasons, 14 broods were successfully mass reared, yielding more than 28,500 vigorous 3rd- and 4th-stage larvae used in rice plot and other bioassays. Crowding the females over a natural ovipositional substrate induced oviposition as early as 12 h from introduction into the MATOCs.

KEY WORDS *Anopheles quadrimaculatus,* larvae, rearing, aspirator, insectary, oviposition, crowding

During the summer of 1998, several rice plot bioassays were conducted in Arkansas using standard and experimental formulations targeted against *Anopheles quadrimaculatus* Say. Although supply logistics and field preparation were very important, obtaining the large number of *Anopheles* larvae needed for the tests became the top priority. From June 9 to July 27, approximately 3,000 larvae would be needed for installation in treatment plots. Because of the extremely hot weather conditions projected for the area, we did not want to risk heat shock mortality by using larvae obtained from eggs of established laboratory strains of *An. quadrimaculatus.*

Large numbers of blood-fed *An. quadrimaculatus* were collected from local livestock barns, and we opted to experiment with native insects and determine the easiest way to induce oviposition. In a study conducted by Bailey et al. (1980), individual field-collected female *Anopheles albimanus* Wied. oviposited at much higher rates when confined to small 5-dram plastic vials, than compared with groups of females confined to cages measuring 61 × 61 × 61 cm. On the basis of the premise of crowding, aspirated *An. quadrimaculatus* females placed into a small area with a natural ovipositional substrate would be the best way to obtain the large numbers of eggs needed to obtain cultures. We decided on this approach after we observed that *An. quadrimaculatus* placed in larger cages (measuring 1.2 × 0.6 × 0.6 m) produced very few eggs compared with mosquitoes confined to a much smaller ovipositional chamber prototype.

Aspiration equipment: Adult *Anopheles* were collected weekly from livestock barns in Arkansas County, Arkansas, using aspirators similar to those mentioned by Perdew and Meek (1990). Three aspirators were fabricated from Black and Decker® 7.2-V cordless brooms (model VP430T) each powered by a pair of Black and Decker VersaPak® rechargeable batteries. In the field, weak batteries could be removed and replaced with fresh ones, a distinct advantage over similar equipment with nonremovable internal batteries.

Modifications to each unit consisted of reversing and soldering the wires to the motor for permanence; cutting off the flattened, curved end of the removable pipe extension with a band saw; inserting and riveting the smaller end of a 5.08 × 7.62–cm PVC coupling hub to the pipe extension; taping around the resulting riveted joint to form an airtight seal; and finally placing a strip of duct tape on the interior surface of the larger portion of the coupling hub to prevent a collection container from falling out during an aspiration. The cost for each aspirator, not taking into account 2 h of fabrication time, was about $50.

Collection containers for the aspirators were fabricated from 0.236-liter unwaxed paper cartons. The carton bottoms were removed and replaced with a 8.38-cm section of 18-mesh aluminum screen and hot-glued on both the inside and outside edges. No alterations were made to the lid other than hot-gluing the top edge along the crimp to prevent pushing in the lid and accidentally releasing mosquitoes during transport.

During operation, an uncovered screened container was placed into the aspirator coupling hub, an aspiration was made, and the lid replaced before cutting off the aspirator. The number of mosquitoes collected per container ranged from 5 to 52. To prevent heat stress during transportation, collected

[1] Commercial products mentioned does not imply a recommendation for use or sale by the University of Arkansas. Approved for publication by the Director of the Arkansas Agricultural Experiment Station.

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mosquitoes were transported to the insectary in an ice chest containing a 3.78-liter bag of wet ice wrapped in newspaper. To verify that *An. quadrimaculatus* was in fact the species collected, extra aspirations were routinely made and identified.

Insectary layout and equipment: A basement room measuring approximately 9.1 × 4.5 × 3.0 m served as an improvised insectary. Double-screened doors were located at the outside entrance, and another screened door, covered with polypropylene plastic, was added to the rear of the room that adjoined a larger unused portion of the basement. Three windows along the eastern and western sides of the room allowed in sunlight during the early morning and late afternoon hours. Since southerly winds prevailed during most of the summer, the farthest window along the eastern side was screened so that warmer air flowed through the double-screened doors, across the room, and out the screened window. Three tables and 2 sets of shelves were added to the room for placement of 34 larval pans measuring approximately 35.5 × 25.4 × 5.0 cm. Three 5-gal (18.9-liter) plastic buckets were filled with tap water and allowed to dechlorinate for 2–3 days. These were maintained throughout the season to provide water for larvae. During the season, average air and water temperature in the insectary were 82.5°F and 76.5°F, respectively, with an average relative humidity of 71.8%.

Construction and use of equipment: We termed a unique and crucial piece of equipment a mosquito aspiration transfer and ovipositional chamber (MATOC). Several of these units allowed us to transfer aspirated mosquitoes to a relatively confined space that restricted movement and offered an ideal ovipositional substrate. The large quantities of eggs produced using this technique allowed us to maintain larval cultures throughout the summer.

Each MATOC was fabricated using a 2.8-qt (2.65-liter) Rubbermaid® Servin' Saver measuring 20.9 × 20.9 × 8.2 cm, four 0.236-liter waxed cartons with lids, 4 additional waxed carton lids, duct tape, and hot glue. The top of the 8 lids and the bottom of the 4 cartons were pushed out, resulting in 8 rings and 4 cylinders, and 4 evenly spaced holes measuring 3.3 in. (8.3 cm) in diameter were cut into the removable snap-on cover of the Rubbermaid container. The cylinders were inserted bottom-edge first into the holes of the Rubbermaid lid and liberally coated with silicone caulking around the bottom lip to prevent waterlogging. Four couplings were made by stacking each pair of rings so that the rounded top crimps were located at the center and duct-taping them together. Each coupling was placed on a cylinder and pushed down until contact with the Rubbermaid lid was made, and the cylinder was pushed up to make contact with the lower crimp line in the coupling. Once positioned, the coupling was hot-glued to the cylinder at the lower crimp line, and the cylinder was hot-glued to the inner surface of the Rubbermaid lid to prevent cylinder movement. The end result is a MATOC consisting of a top lid and lower reservoir. Six of these units were made and used repeatedly throughout the season to maintain larval numbers. Each unit was assembled in about 45 min at a cost of about $6 each. A complete MATOC with aspiration containers installed is presented in Fig. 1.

After mosquitoes were collected and transported back to the insectary, the reservoir of each MATOC was filled with approximately 1,500 ml of fresh Bayou La Grue water filtered through a 200-mesh sieve, the lid refastened, and the water level checked. The water level was ¼ in. (0.6 cm) or less from, but not touching, the cylinder's lip. MATOCs were placed on shelving out of direct sunlight and positioned so that mosquito transfer could be easily accomplished. Before transfer, 3 empty aspiration cups were placed in 3 of 4 MATOC couplings. A container of mosquitoes was picked up and the lid slowly removed until a thin section of cardboard could be slipped between the carton and lid. Once the cardboard was in place, the container of insects was positioned over the appropriate coupling and aligned so that sliding out the cardboard and pushing down on the container resulted in the coupling of the container of mosquitoes to the MATOC. This loading procedure was repeated for the other 3 couplings, so that 4 aspiration containers were in place for each MATOC.

Once all MATOCs were loaded, mosquitoes were offered a carbohydrate source contained in small sugar feeders taped to the screen of 2 opposing aspiration containers inserted into each MATOC. Modifications of feeders described by Porter et al. (1961) were constructed from 5-dram plastic vials with snap-on lids and 3.8 × 0.9–cm–diameter cotton rolls inserted through a 0.6-cm-diameter hole drilled in the lid's center. Vials were filled with a 10% sucrose solution, capped, shaken vigorously until the fluid dampened the wick, and taped into position so that the wick made contact with the screen.

The MATOC was left undisturbed for 48 to 72 h. Eggs were deposited and completely covered the water surface in all MATOCs. When large numbers of neonates were observed through the sides of the reservoir, the MATOC was removed, and any remaining live adults were released. The reservoir was emptied into a pan and we carefully rinsed the reservoir with a pressurized 2-gal sprayer filled with dechlorinated water to remove eggs adhering to the sides. The water level in the pan was adjusted to approximately 3.1 cm and maintained throughout larval development by adding dechlorinated water.

Larvae were fed once daily by adding 200–300 mg of powdered dog food to each of 34 rearing trays. Based on the recommendation of Gerberg et al. (1994), fat in the larval diet was kept to a minimum. Low-fat dog food was pulverized using a blender and ground manually through a 200-mesh screen sieve to yield a very fine powder (S. Bear-

Fig. 1. Two complete MATOCs with aspiration containers installed.

den, personal communication). When pans contained 2nd-stage larvae, 125–150 larvae were transferred to individual pans to reduce crowding and fed as previously described. In addition to feeding, all pans were watered daily with dechlorinated water to maintain water levels and to reflood the eggs adhering to the sides of the pans.

Water was siphoned from the pans weekly using a filter-siphon constructed from a new priming bulb type outboard motor fuel line capped on the inlet side with a 10.1-cm reinforced tube of 32-mesh screen. In operation, the screened filter tube was placed horizontally at the bottom of the pan and the outlet end placed in a bucket while squeezing the priming bulb to siphon debris from the pan. Using this method, larvae were not harmed or subjected to strong water currents, since flow was displaced over the length of the filter tube.

Groups of ten 4th-stage larvae were transferred manually from the pans to 0.236-liter paper cartons filled with approximately 1.2 cm of water, and they were immediately transported to the field for installation within treatment plots. Developmental time in the insectary was recorded daily; the average time required from egg to adult was between 13–15 days, with 3rd and 4th instars appearing on days 8 and 11, respectively.

Using the described technique, a total of 14 broods was successfully mass-reared during the 1998 and 1999 field seasons, yielding thousands of vigorous 3rd- and 4th-stage *Anopheles* larvae used in rice plot and other bioassays. An average of 15 trays loaded with about 138 larvae each generated more than 28,500 *Anopheles* larvae during 2 field seasons. The MATOC system, compared with larger cages, offered the advantages of occupying a small amount of laboratory space and providing remarkably large quantities of *An. quadrimaculatus* eggs. Crowding the blooded females over a natural ovipositional substrate induced oviposition as early as 12 h from the moment the MATOCs were loaded. There could be some type of ovipositional competition among females resulting from being confined to such a relatively small area, although this theory is conjecture. Females that oviposit early guarantee that their progeny will be able to take advantage of limited resources. Provided that blooded females and the appropriate ovipositional substrate are used, it should be possible to rapidly colonize other mosquito species in the same manner under field or semifield conditions using similar equipment.

REFERENCES CITED

Bailey DL, Lowe RE, Kaiser PE. 1980. A reliable technique for rapid colonization of *Anopheles albimanus* Wiedemann. *Mosq News* 40:410–412.

Gerberg EJ, Barnard DR, Ward RA. 1994. *Manual for mosquito rearing and experimental techniques* AMCA Bulletin No. 5 (revised) Lake Charles, LA: American Mosquito Control Association.

Perdew PE, Meek CL. 1990. An improved model of a battery-powered aspirator. *J Am Mosq Control Assoc* 6: 716–719.

Porter JE, Kozuchi G, Kuck MJ. 1961. Improved techniques for the laboratory rearing of *Aedes aegypti* (Linn.). *Mosq News* 21:340–342.