CONTAINER FOR IRRADIATION AND MASS TRANSPORT OF ADULT MOSQUITOES [1]

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ABSTRACT. A container made by molding urethane foam was used to hold adult *Culex pipiens quinquefasciatus* Say for irradiation and transport to the field for a sterile-male release study. The adults were immobilized with cold and placed in lots of 1000 to 2000 in individual 4-oz plastic foam cups. Seven of these cups containing mosquitoes were stacked together, placed in a plastic bag and fitted into the container. The mosquitoes were confined in these containers for 2.5 hours. Of the 1.2 million adults handled in this manner, there was <1 percent mortality. This same container was used to transport adult *Aedes aegypti* (L.) for field biolgical and genetic studies with <1 percent mortality.

INTRODUCTION. The successful use of chemosterilized males to control *Culex pipiens quinquefasciatus* Say (Patterson *et al.* 1970) and *Anopheles albimanus* Wiedemann (Lofgren *et al.* 1974) has increased interest in the genetic approach to mosquito control. One important aspect of the genetic approach involves the release of genetically-altered and mass-produced insects; therefore, suitable methods of transporting and releasing these insects in the field in large numbers are needed. Such methods must have little or no adverse effect on the survival and behavior of the released insects and must allow large numbers to be handled readily with a minimum of space and equipment. Obviously, the methods used must depend on the stage in which the insect is sterilized and released. For example, Patterson *et al.* (1970) and Lofgren *et al.* (1974) in their studies released chemosterilized males in the pupal stage, which were easily transported to the release site in trays. We planned to irradiate adults to reduce the detrimental effects of pupal irradiation (Smittle *et al.* 1968). Therefore, a technique for irradiation and transport of adult mosquitoes became necessary.

The present paper describes the design and successful use of a container for irradiating and transporting sterile adult mosquitoes to field sites. It permits irradiation and transport in the same container, which reduces the handling of the adults, and it allows the packaging of large numbers in small volumes. We have used the method with *C. p. quinquefasciatus* and *Aedes aegypti* (L.) in field studies that involved the release of sterile insects and in small-scale trials related to the development of genetic methods of control.

MATERIALS AND METHODS. Several requirements had to be met in the design and development of the container. If we were to eliminate extra handling, the container would have to be used for irradiation and transport. Thus it could not exceed a diameter of 11 cm and a height of 25 cm since it had to fit into the irradiation chamber of the ^{60}Co source used in these studies. The container had to confine the adults during irradiation without injury and without preventing the passage of the gamma rays used for irradiation. In addition, it would have to serve as an insulated holding device for prolonged periods without affecting the normal behavior of the mosquitoes.

In preliminary trials, cold, nitrogen, and CO_2 were tested as methods of immobilizing the mosquitoes, and several types of containers were evaluated for holding different quantities of mosquitoes. These trials led to the containers and techniques described herein. It was found that adults could be immobilized best by cold and could be kept in a precooled ice chest for 3 hours with very little mortailty.

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

Molded urethane was selected for the insulated container since it is structurally strong, an excellent insulating material and because gamma rays pass through it readily. The containers were made by using 1/2 gallon cardboard cartons 11 cm in diameter and 21 cm high. A mold, an 8-cm metal cylinder wrapped in teflon, was placed in the center of the carton and raised 1.5 cm from the bottom. Then the liquid urethane foam (Fas-Foam®) was mixed and poured into the carton, and allowed to expand. After it hardened, the excess was trimmed off and the center mold was removed. This provided a cavity 8 cm in diameter and 19.5 cm high surrounded by 1.5 cm of insulating foam. A lid was made of molded plastic foam for insulation at the top of the container.

We found it necessary to confine the adults in small lots to prevent injury due to compaction and abrasion during holding and transport. To accomplish this, we used 4-oz (120 ml) plastic foam cups that could be stacked so a 1.5 cm space was left between the cups. These cups are 5.2 cm high with an outside diameter of 5.7 cm at the bottom and 7.3 cm at the top. The container and the cups used to hold the mosquitoes are shown in Figure 1.

The mosquitoes were prepared for irradiation and transport by placing the caged adult mosquitoes in a cold room at 2° C for 5 to 10 minutes to immobilize them. Then they were measured volumetrically (1000 immobilized *C. p. quinquefasciatus* males equaled 12.5 ml in a 50-ml plastic beaker) to estimate the number present and 1000 to 2000 were placed in each precooled cup. Seven cups containing mosquitoes were stacked together, an empty cup was placed on top as a cover, and the stack of cups was placed in a small plastic bag to facilitate handling. After the bags had been inserted into the precooled insulated container, the container was placed in a plastic foam ice chest with 2 quart-size cans of frozen "canned ice." Each container was removed from the ice chest for about 12 minutes for irradiation, then placed back in the ice chest.

We used a Rubicon® potentiometer with a copper vs. constantan thermocouple to measure the temperature inside the cups of mosquitoes while they were in the insulated container inside the ice chest. A study was made of the relationship between temperature, mortality, and recovery time by placing ice in alternating cups, or in the top and bottom cups only, or by eliminating ice completely.

Also we tested the efficiency of the urethane container by counting mosquitoes, placing them in their containers, irradiating them, returning them to the ice chest, and holding the chests for 2.5 hours in the laboratory. A the same time, a similar group of mosquitoes was transported to the field, a process involving a 2.5-hour car and boat trip to the test site on an island, where the mosquitoes were placed in large screen cages. Then, after allowing a few minutes for recovery, the number of mosquitoes dead or unable to fly was counted to determine percentage mortality.

In addition, in a field study with *C. p.*

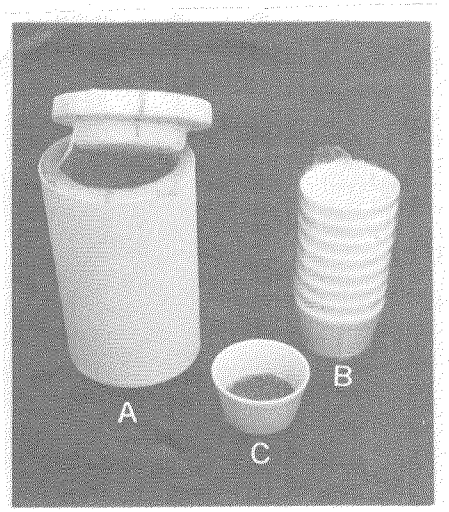

FIG. 1. A. Insulated container. B. Molded plastic foam cups in plastic bag. C. Cup with mosquitoes.

quinquefasciatus, the adults were irradiated, transported to the island, and placed in large white plastic pans for recovery and dispersal. After a few minutes, the mosquitoes unable to leave the pan were counted to determine the percentage mortality.

Likewise adult *A. aegypti* were immobilized in the cold room, placed into the same type of container in the same way, transported to the field, and released for biological and genetic studies.

RESULTS AND DISCUSSION. In the preliminary evaluation of temperatures, we found that *C. p. quinquefasciatus* recovered faster and had better survival when no ice was placed inside the insulated container. When ice was placed in alternating cups or in the top and bottom cups, recovery was slower and more mosquitoes died, apparently because of the lower temperatures. Temperature measurements indicated that in a cup of mosquitoes between 2 cups of ice the temperature did not reach 4° C in 2.5 hours; when there was no ice in the containers, the temperature rose to almost 15° C. Thereafter in the laboratory and field release studies, no ice was placed inside the insulated container.

In the laboratory tests in which the *C. p. quinquefasciatus* adults were held in the container in an ice chest in the laboratory for 2.5 hours after irradiation, mortality averaged 0.8 percent. When the irradiated adults were carried to the island and released in cages, mortality averaged 0.4 percent.

In the field study over 1.2 million *C. p. quinquefasciatus* adults were irradiated, transported to the island, and released, the daily mortality averaged 0.9 percent (range 0.2–1.2%) over the 90-day period. Within 5 minutes after the adults were placed in the trays, most had flown away. The sterility data from the field evaluations (unpublished data) indicated that these males performed well in the field.

When the urethane containers were used to transport and hold *A. aegypti* for 2.5 hours in laboratory tests, mortality was less than 1 percent. Also, when Jack A. Seawright of this laboratory (personal communication) used the container and technique to transport adult *A. aegypti* for a field study of the mating competitiveness of translocation heterozygotes, mortality averaged less than 0.6 percent.

In addition to the uses of this container and the associated techniques described herein, this container should prove very useful in many other studies involving the transport of adult mosquitoes or other small fragile Diptera.

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