SCIENTIFIC NOTES

MODIFICATION OF THE ICE WATER METHOD FOR HARVESTING *Anopheles* AND *Culex* PUPAE [1]

E. I. HAZARD

Entomology Research Division, Agr. Res. Serv., U.S.D.A., Gainesville, Fla.

Methods of rapidly separating mosquito pupae and larvae in the insectary have been reported by Lewis (1933), McKiel (1957), and McCray (1961). Also, Fay and Morlan (1959) used a mechanical device with two slightly separated vertical glass plates arranged so the amount of separation was adjustable; by widening the distance between the plates at the lower end, they were able to catch the pupae while permitting the larvae (which are thinner) to pass through. They also could separate male and female pupae because of the difference in size. Machines of this type are in wide use today. Another method is that of Bar-Zeev and Galun (1961) who added iron dust to the rearing medium; the larvae ingested these particles and were held to the bottom of the rearing pan by a magnetic field; the free-swimming pupae were poured from the container. However, the most satisfactory method of harvesting pupae of *Anopheles quadrimaculatus* Say and *Culex pipiens quinquefasciatus* Say is the ice water technique described by Weathersby (1963). It gives rapid and complete separation of pupae and larvae and has no adverse effect on the insect. The procedure he used is satisfactory but has several time-consuming steps which we were able to eliminate by using the apparatus described here.

Using Weathersby's cold water principle, we constructed a device (Figs. 1–3) mounted in a ring stand that consists of a 200mm. x 150 mm. Kimax® funnel heat fused to a Pyrex® high vacuum stopcock having a 15-mm. bore. The funnel is filled with ice water, and larvae and pupae from 5 to 10 pans (depending on the number of insects per pan) are poured onto a 16-mesh screen mounted in a 15-cm. embroidery hoop. The hoop is inverted over the funnel to bring the larvae and pupae into contact with the ice water. The cold water inactivates the larvae, which instantly sink to the bottom of the funnel (Fig. 1). The pupae also become inactive but they float. When all the larvae have come to rest at the bottom of the funnel (Fig. 2), the stopcock is opened and the larvae are drained out onto the embroidery hoop. The stopcock is closed as soon as all larvae have been removed from the funnel and before any pupae have been drained out (Fig. 3). Then the larvae are washed from the embroidery hoop, divided

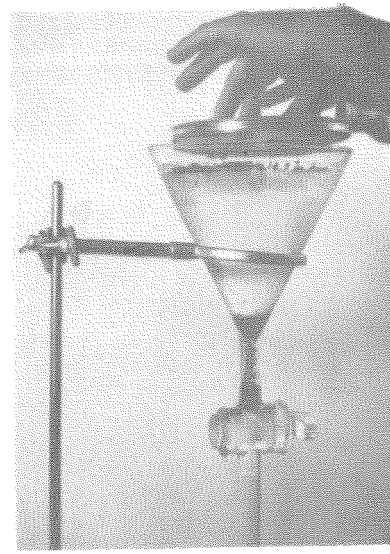

FIG. 1.—Larvae and pupae being placed on ice water.

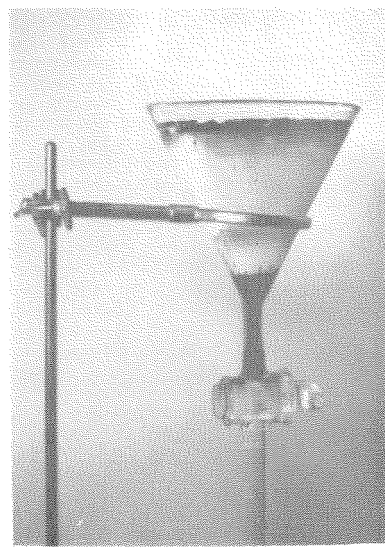

FIG. 2.—Separation of larvae and pupae in funnel of ice water. The cold water inactivates the larvae which fall to the bottom of the funnel.

[1] Mention of a proprietary product does not necessarily imply endorsement of this product by the U.S.D.A.

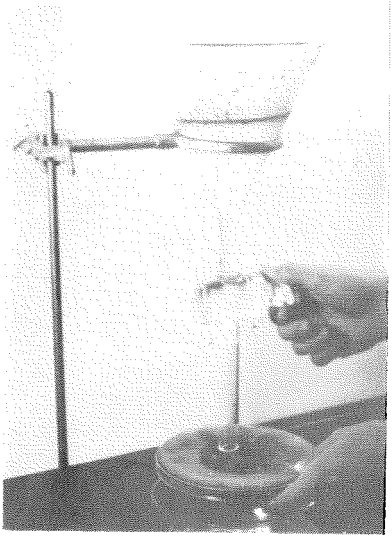

FIG. 3.—The stopcock is opened to drain larvae from the funnel. After larvae are removed, the pupae are drained into a separate container.

into groups of equal numbers, and returned to the rearing pans. The pupae on the water surface are now removed by allowing the remaining water to drain onto the embroidery hoop; they are then placed in a separate container.

The water taken from the funnel is rechilled by the addition of ice cubes for a few seconds and then returned to the funnel. The process is repeated until the pupae from all pans have been collected. Using this instrument, two men can harvest 20,000 pupae in less than 1 hour, whereas the time needed for 2 men to pick this number of pupae with the old bulb pipette method is about 8 hours.

The cold water method of separating pupae from larvae was tested on *Aedes aegypti* (L). and *Aedes taeniorhynchus* (Wied.) and the separation was not complete. Some of the pupae do not float after they become inactive in the cold water, therefore, the mechanical pupae separation method of Fay and Morlan (1959) is recommended for these two species.

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OCCURRENCE OF *Aedes abserratus* (FELT AND YOUNG) AND *Culiseta morsitans* (THEOBALD) IN INDIANA

R. E. SIVERLY
Ball State University, Muncie, Indiana

Larvae of *Aedes abserratus* (Felt and Young) were collected in three localities in northern Indiana during the spring of 1966. First and second instar larvae of this species were taken near Mongo, in Lagrange County, and near Fremont, in Steuben County, on March 26. Third and fourth instar larvae of *A. abserratus* were collected in sphagnum pools in Pinhook Bog, in La Porte County, on April 9. Evidently *A. abserratus* is distributed across the northernmost tier of counties in Indiana.

Larvae of *Culiseta morsitans* (Theobald) were collected at Pinhook Bog on May 1, 1966. These larvae were taken in dark, flooded recesses at the bases of tamarack scattered throughout the bog. Some of these recesses were too small to accommodate a pint-sized dipper, and collections were made with a rubber syringe.

On the April 9 collection, larvae of *Aedes canadensis* (Theobald) and *Aedes excrucians* (Walker) were associated with *A. abserratus*. On the May 1 collection, larvae of *Culiseta melanura* (Coquillett) were associated with *C. morsitans,* and outnumbered *C. morsitans* approximately ten to one. Larvae of both species of *Culiseta* were fourth instar and evidently had overwintered in the larval stage.

All larvae collected were transported to the laboratory, where rearings were completed and identifications confirmed in the adult stage. So far as it is known, there are no previously published accounts of the occurrences of *A. abserratus* and *C. morsitans* in Indiana. This brings the present total of recorded species of mosquitoes in Indiana to 44 (Siverly, 1966a, 1966b).

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