SOME TECHNIQUES FOR COLLECTING TREE HOLE BREEDING MOSQUITOES [1]

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In many parts of the world including the United States, there are species of mosquitoes that breed routinely in tree holes that contain water. Many of these mosquitoes apparently prefer this type of breeding environment, and certain species are of considerable medical importance.

The possible significance of mosquitoes of this type has been recognized for many years, and recently several papers have re-emphasized their potential importance (i.e. Breland, 1957; Bonnet and Chapman, 1956; Trapido and Galindo, 1956).

The writer and his associates have been collecting tree hole breeding mosquitoes intermittently for many years, and for the past year or so have concentrated upon these species. The reason for this recent concentration was the recovery in 1955 of *Haemagogus equinus* Theobald near Brownsville, Texas (Trapido and Galindo, 1956). This species, one of the so-called jungle mosquitoes, could be of considerable importance in the epidemiology of yellow fever.

In Texas, where most of our collecting has been done, seven species of mosquitoes in addition to *Haemagogus equinus,* routinely breed in tree holes. These include *Aedes thibaulti* D. and K., *A. triseriatus* Say, *A. zoosophus* D. and K., *Orthopodomyia alba* Baker, *O. signifera* (Coq.), *Toxorhynchites rutilus septentrionalis* (D. and K.), and *Anopheles barberi* Coq.

As might be expected, the species of trees with suitable cavities for mosquito breeding varies in different areas. Trees from which good collections have been recovered include live oak (*Quercus virgina*), elm (*Ulmus crassifolia*), hackberry (*Celtis* sp.), willow (*Salix* sp.), Texas ebony (*Pithecolobium flexicaule*) and a type of mimosa (*Leucaena pulverulenta*).

So far we have not discovered any of the species of mosquitoes showing selectivity in the species of tree used for breeding. *Aedes thibaulti* is reported to be more or less limited to sweet-gum and tupelo-gum trees (Carpenter and La Casse, 1955) but we have not collected a sufficient number of this species to form an opinion of its habits in this area. *Haemagogus equinus* has been collected on several occasions from Texas ebony, and there was for a time some indication that this species might prefer small cavities in this species of tree in this area. However, we recently collected this mosquito from a large cavity approximately 6 inches in diameter and 12 inches deep in a hackberry. This tree was in a region of thick vegetation which limited the amount of light which reached the cavity. At present we feel that environmental factors, such as light, are perhaps more important than the species of tree in determining the breeding habitat of this species.

The writer does not claim that the techniques to be outlined are completely original. Some of these have been mentioned in previous publications (e.g. Jenkins and Carpenter, 1946) and are considered to be standard methods of collecting tree hole mosquitoes; the new or modified procedures are a result of the experiences of many individuals. In view of the impor-

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[1] Supported by a grant, Contract No. DA-49-007-MD-744, Research and Development Division, Office of the Surgeon General, Department of the Army.

[2] Individuals who have helped in the development of the techniques herein outlined include Dr. James D. Long, Dr. Orin P. Wilkins, John G. Riemann and Fred Klaus.

tance of this ecological group of mosquitoes, however, it is believed that some of the techniques used successfully by us may have a certain value to other workers.

COLLECTING EQUIPMENT. Much of the equipment and the procedure used in transporting living specimens is the same as that outlined in a former paper (Breland, 1954) for general collecting. The collecting of immature stages rather than adults is emphasized. Living larvae are transported to the laboratory in large mouth pint, quart and half gallon fruit jars with fine mesh wire lids; the containers are partitioned boxes lined with cork.

Equipment used primarily for tree hole collecting includes an assortment of large pipettes with bulb capacities of from 25 c.c. to 8 ounces (approximately 240 c.c.); eight-ounce capacity bulbs to which rubber or plastic tubing of varying lengths is attached; small cloth bags approximately 4½ x 6½ inches with draw strings; flashlights, scout axes, and an aluminum step ladder four feet in height. Several of the half-gallon jars are filled with strained pond water at the beginning of each collecting trip, and as it is used, it is replaced from roadside ponds and streams. The uses of the equipment and water will be noted shortly.

GENERAL COLLECTING TECHNIQUES. Problems involved in collecting immature stages of tree hole breeding mosquitoes include the dark color of most tree hole water, the relatively small amount of water involved, and the fact that most tree holes are less accessible than the breeding habitats of most other species. Many tree holes occur six to ten feet above the ground, and the step ladder allows holes at this height to be more readily accessible. Tree hole water is sometimes so dark that it is difficult or impossible to determine under field conditions whether or not specimens are present in a given collection. Sometimes larvae can be seen by pouring small amounts of water into a white enamel dipper, but young instars or eggs might be present and not discovered. All water in a tree hole is routinely taken and the hole flushed with pond water previously mentioned. This water may be added to the original water, or in some cases, a separate jar is used for the flush water.

At the end of the day's collecting, each collection is examined by pouring small amounts of the water into a large white enamel pan. If larvae are present, some are preserved, using 95 percent alcohol. The larvae are first rinsed in clear water, then placed in small screw top vials. The alcohol is added to the vial with the larvae. The small amount of water that is present dilutes the preservative somewhat. A data label is placed inside the vial and a plug of absorbent cotton inserted to hold the specimens in place. The remainder of the larvae in each collection are returned to the collecting jars for transportation to the laboratory.

The methods used for removing water from tree holes vary with the situation. Sometimes openings are so small that they must be enlarged with a hand ax. If possible, pipettes are used to remove the water, but the bulb-tube combinations are employed for holes not accessible to the pipettes. After each collection, all collecting equipment is thoroughly rinsed in pond water to avoid contaminating the next collection.

It has been noted previously (Breland, 1954) that larvae and other immature stages have been transported successfully for several hundred miles in the wide mouth fruit jars with wire tops used for this purpose. When collecting with specimens in the car, care is taken to have good ventilation, and if possible to park in the shade to keep the specimens cool. When feasible, collecting jars with specimens are left at the sleeping quarters during the day while collecting.

The discussion so far applies primarily to routine collecting when rainfall has been sufficient to deposit water in many tree holes. Other methods, as noted below, have been used with some success when a high percentage of collecting sites have insufficient water. As will be mentioned, the percentage of successful collections using these methods varies with environmental conditions and with the various species of mosquitoes.

COLLECTING DRY MATERIAL. In the absence of free water, dry or moist material from tree holes is collected, put in small cloth bags and flooded later in the laboratory. In obtaining this debris, care is taken to scrape the sides of the cavity thoroughly to recover any eggs that might occur here. The percentage of recoveries using this method is apparently dependent upon climatic conditions. For example, during periods of intermittent rainfall, our recoveries have been from approximately 35 percent to more than 50 percent. In years of drought, such as occurred in Texas in 1956, successful collections have been less than 4 percent. This latter figure is based upon more than 90 collections of moist and dry material along the Texas-Mexico border with the most widely separated localities more than 500 miles apart. The absence of free water in tree holes for long periods during drought may account for this very small percentage of recovery. Several species are reported to deposit their eggs just above the water line and it may be that eggs are laid only when some water is present.

To date, the only species collected by us in this way have been *Aedes triseriatus* and *A. zoosophus.* Dunn (1926), however, reports the collecting of eight species of *Aedes* including *A. aegypti* L., from west Africa, and it seems probable that most tree hole breeding *Aedes* and other species with similar egg laying habits could be recovered by this method.

ADDITION OF WATER. Not infrequently, tree holes are moist but either have no free water or an insufficient amount for a collection. Under these circumstances, the hole is filled with water, the water removed and the hole flushed several times. Larvae of *Orthopodomyia alba* Baker were first collected in Texas by using this method (Breland, 1947) and since that time other species have been recovered.

The addition of water to moist or dry holes and leaving it for 24 to 48 hours before collecting has proven successful on several occasions. This method has been more successful within a few days after a rain rather than during periods of drought. Species that have been recovered by this method include young instars of *Haemagogus equinus, Aedes zoosophus* and *A. triseriatus.*

LABORATORY PROCEDURE. In the laboratory, large collections of larvae are placed in white enamel pans approximately 14 x 8½ x 2½ inches for rearing. Smaller pans and finger bowls are used for small collections. If the tree hole water is too dark for easy observation, it is often diluted with pond water. As a rule, compressed air is bubbled through an air stone placed in one end of each rearing pan. This air helps to prevent the formation of scum, and apparently contributes to a higher survival rate. As pupae are formed in the pans, they are transferred to small staining dishes and placed under bell jars or into cages for emergence.

Dry and moist material is flooded as rapidly as facilities permit, but until this time, it is kept in a box to prevent it from becoming too dry. As a rule, material that yields larvae will do so at the first flooding, but there are exceptions. For example, no larvae appeared in one sample until it had been dried and flooded three times. Five larvae of *A. zoosophus* appeared at the third addition of water.

The number of specimens recovered can often be materially increased by drying and flooding several times. Dr. Orin P. Wilkins, former student of the writer's, once obtained more than 800 larvae of *A. zoosophus* from a sample that was dried and flooded twelve times over a period of a year. Only about 300 larvae appeared at the first addition of water.

Collections of water or of dry or moist material that do not yield any larvae within 72 hours are assumed not to contain any eggs, and these are discarded if the rearing pans are needed for other work. We have on numerous occasions kept such samples for more than a week, and have never yet had larvae appear for the first time after 72 hours of exposure to water. Sometimes additional specimens apparently hatch after this time, but in all such instances, some larvae have appeared earlier. It is recognized that an occasional collection of water might contain freshly deposited eggs that would not hatch within 72 hours, and for this reason, collections are kept for a longer period whenever possible.

Literature Cited

Bonnet, D. D. and Chapman, H. 1956. The importance of mosquito breeding in tree holes with special reference to the problem in Tahiti. Mosquito News 16:301–305.

Breland, Osmond P. 1947. *Orthopodomyia alba* Baker in Texas with notes on biology (Diptera: Culicidae). Proc. Ent. Soc. Wash. 49: 185–187.

———. 1954. Notes on collecting mosquitoes. Mosquito News 14:173–175.

———. 1957. Some factors that might influence the reintroduction of the yellow fever virus into the United States. Texas Journal of Science 9:262–266.

Carpenter, Stanley J., and LaCasse, Walter J. 1955. Mosquitoes of North America. University of California Press, Berkeley.

Dunn, Lawrence H. 1926. Mosquitoes bred from materials taken from holes in trees. Bull. Ent. Res. 17:183–187.

Jenkins, Dale W. and Carpenter, Stanley J. 1946. Ecology of the tree hole breeding mosquitoes of Nearctic North America. Ecological Monographs 16:31–47.

Trapido, Harold and Galindo, Pedro. 1956. Genus *Haemagogus* in the United States. Science 123:634.