

this dosage also, applications of TDE at intervals of 1 mile over a 5-mile section of one stream gave 98.6 per cent control.

TDE was apparently much less toxic than DDT to rainbow and brook trout, having no noticeable effect on those exposed for short periods to applications in emulsion form up to 5 p.p.m. With DDT at this dosage, 4 of 16 trout were killed and the others were severely affected. Severe temporary effects on fish exposed

to dosages of 2 and 3 p.p.m. were also observed.

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REARING OF *ANOPHELES QUADRIMACULATUS* SAY AND *Aedes Aegypti* (L.) IN THE LABORATORY¹

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The importance of *Anopheles quadrimaculatus* Say as a common vector of malaria has caused many workers to try to rear this species. The purpose of this paper is to give a simple practical method of rearing this species under laboratory conditions. Directions are also given for rearing *Aedes aegypti* (L.).

Anopheles Quadrimaculatus

The original culture of *Anopheles quadrimaculatus* maintained at the Bureau of Entomology and Plant Quarantine laboratory, Agricultural Research Center, Beltsville, Md., was obtained through the courtesy of Walter Reed Medical Center, Washington, D. C. This culture was producing a maximum of 100 adults a day, but as more work was to be done with this species it was necessary to increase the culture to produce a maximum of 5,000 adults a day. Several shipments of eggs

were made from the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine. Three shipments were made from the Tennessee Valley Authority, one lot was received from the United States Public Health Service, and numerous shipments extending over a period of three months were received from a commercial concern. As a result of this increase there was always a surplus of eggs for experimental use.

More than a hundred methods and techniques² using various foods and media were tested in this laboratory. The method that was evolved as a result of these tests produces from 3,000 to 5,000 pupae a day. This method has been used for 3 years and has proved very efficient.

TREATMENT OF EGGS

White-enamel pans 8 inches in diameter are used for oviposition. They are filled two-thirds full of pond water,³ and on the

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² The authors wish to thank M. F. Schott and Norman Moskowitz for their help in making many of these tests.

³ The pond is an abandoned iron mine that is fed by springs and has a slight run-off. It has about 1 acre of surface.

inside of each is a 2-inch strip of paper toweling placed near the top and extending 1 inch below the water surface. This paper prevents the eggs from sticking to the side of the pan and also keeps them moist if water evaporates.

Pans for oviposition are put in the stock cages every day. The pans containing eggs are removed and kept in a constant temperature room at 25° C. until the eggs hatch, which takes about 48 hours.

TREATMENT OF LARVAE

The larvae are reared in white-enamel photographic trays 17 by 14 by 3 inches, which are kept on a rack (fig. 1). As larvae seem to prefer darkness, the trays are kept covered with pieces of masonite. These covers also eliminate drafts, which tend to disturb the larvae, keep out dust, and provide support where it is necessary to stack the trays.

Each tray is cleaned with a solution of

sulfuric acid and sodium dichromate and then thoroughly rinsed with water before it is used.

By maintaining a temperature of 27° C. in the rearing room it is possible to keep the temperature of the rearing medium at not less than 25°, which seems to be favorable to growth.

The newly hatched larvae are transferred by means of a medicine dropper to the rearing trays, approximately 500 larvae to a tray. Each tray contains 2.5 liters of distilled water, 50 mg. of brain-heart infusion, and 50 mg. of pure dry brewers' yeast. The trays are then placed in the rack and the contents allowed to settle before the larvae are fed. The larvae are fed a high-grade dog food, which is ground through a corn grinder and then further pulverized in a ball mill for 36 hours. They are fed once the first day and twice each day thereafter except Sundays. The amount of dog food to be given each tray

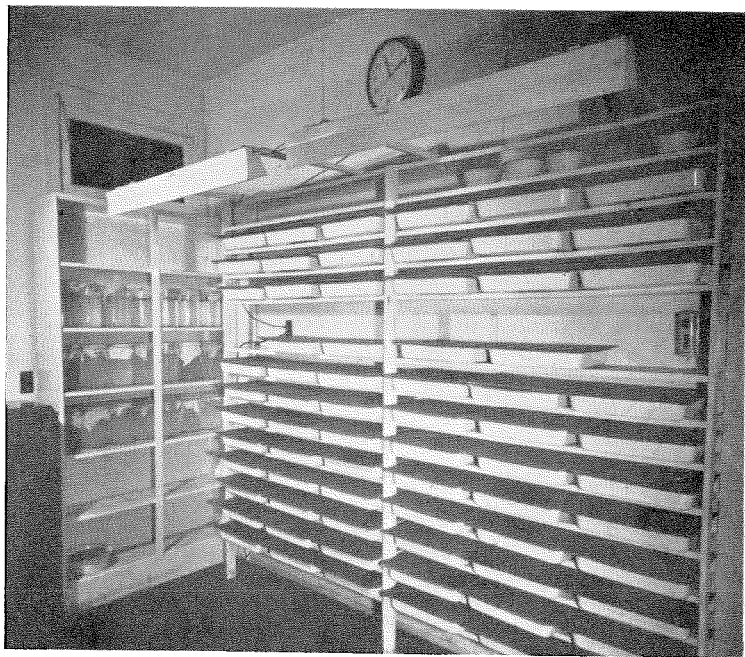


FIG. 1.—Rack holding photographic trays and oviposition pans. Shelves to the left hold battery jars containing *Aedes aegypti*.

of larvae varies with the number and size of the larvae. A tray of 500 first-instar larvae takes about 40 to 50 mg. at each feeding when the temperature of the medium is 25° C., whereas 500 fourth-instar larvae under the same conditions take about 1.1 to 1.2 grams. A series of experiments showed that feeding the larvae twice a day and omitting Sunday feedings gave satisfactory results.

Before each feeding the surface of the medium is washed with distilled water to remove any old food or dust and also to replace the water lost by evaporation. The medium tends to darken, and by the time larvae have reached the fourth instar is

of a dark brown color although there is no trace of scum. There is a slightly sweet odor to the medium. By this method 64 per cent of the larvae pupated in 11 days and 96 per cent in 15 days.

TREATMENT OF PUPAE

The pupae are collected every day except Sunday. To collect the pupae in trays in which the larvae have just started to pupate, or where there are only a few pupae and many larvae, a 25-ml. pipette with a rubber tube is used. In trays in which about two-thirds of the larvae have pupated, the contents are poured through a strainer lined with gauze, and the pupae

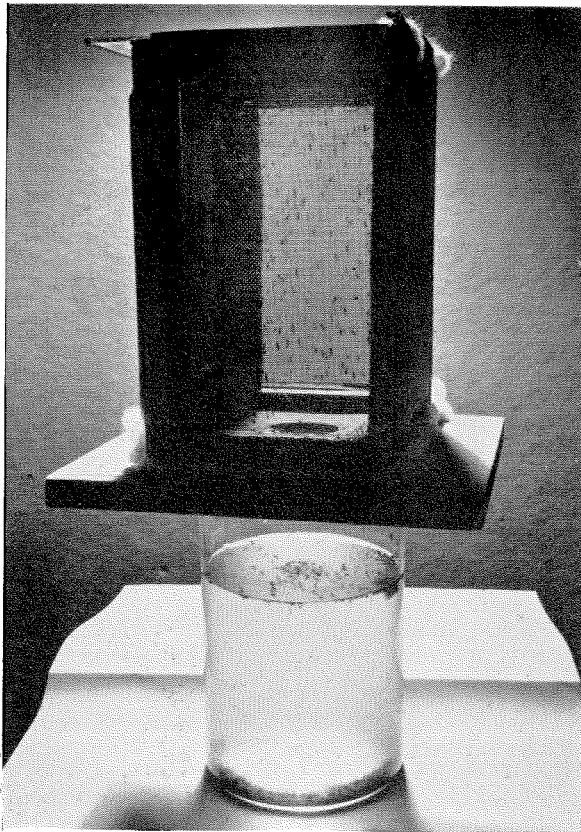


FIG. 2.—Pupae in battery jar, and cage containing adult mosquitoes that have just emerged.

and larvae then washed from the gauze into clean water.

The pupae to be used for testing are put in 6-inch round battery jars, about 1,500 to 2,000 to a jar, and allowed to emerge into small cages which are placed over the jars (fig. 2). The pupae to be used for stock are kept in small pans, which are placed in the stock cage.

It is important that the pupae be in clean water to prevent emerging adults from getting caught in the media and drowning.

The adults emerge in about 36 hours when the temperature is between 25° and

27° C. The emergence has been between 95 and 100 per cent of the pupae.

TREATMENT OF ADULTS

The adults are kept in a room where the temperature is constant at 27° C. and the relative humidity is between 80 and 85 per cent. The room is darkened for a part of the day, as mosquitoes feed better in the dark.

Wooden cages 22 by 36 by 24 inches, covered with cheesecloth and having two sleeve openings in front with a celluloid window between the openings, are used for breeding cages (fig. 3).

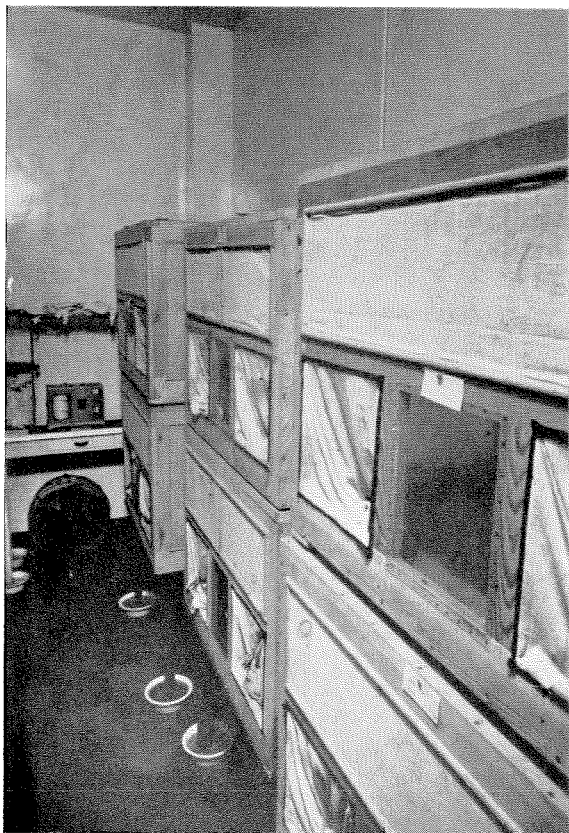


FIG. 3.—Cages for breeding *Anopheles quadrimaculatus* and *Aedes aegypti* mosquitoes. *A. aegypti* eggs are being dried on rack and stored in jar on table.

Three cages are stocked in rotation, one each week, with from 3,000 to 5,000 pupae. No pupae are added to a cage for 3 weeks. At the end of this time the cage is cleaned thoroughly and then restocked. By having three cages and stocking one each week, two cages of mosquitoes are always producing well.

Raisins and a slice of apple are placed in petri dishes on the floor of the cage, and cotton saturated in a 10-per cent sugar solution hung from the top are kept in the cage at all times.

About the third day and every day thereafter a blood meal is offered the mosquitoes. Guinea pigs are used for this purpose. They are held in small metal cages with removable trays and bands small enough to prevent the pigs from turning around (fig. 4). Each pig is left in a cage from 2 to 3 hours. The backs of the guinea pigs are shaved about once a week.

Aedes aegypti

Aedes aegypti is a satisfactory insect to rear in the laboratory because it requires a minimum of care. The supply of these mosquitoes can be controlled to meet the demand, as the eggs may be kept at least 6 months if stored at a constant temperature of 25° C. and a relative humidity of about 85 per cent, and where there is good ventilation.

A rack with 6 shelves holding 42 battery jars is used for rearing *aegypti* larvae.

TREATMENT OF EGGS

The eggs of this species will not hatch until they have dried for several days and are then soaked for 24 hours.

An 8-inch pan is covered with a paper towel that has been dipped in pond water. Enough water is placed in the pan to prevent the paper from becoming dry. One pan is placed in each stock cage, and after 24 hours it is replaced by another pan.

The towel on which the eggs have been deposited is taken from the pan and placed on a dry towel or a rack to dry. When the eggs are dry, they are stored in a jar in the constant-temperature room. As larvae are needed, these towels containing the eggs are soaked in pond water for 24 hours (about half of the eggs should hatch) and then removed, dried, and again stored pending further use (fig. 5).

The larvae are fed whole-wheat bread from which the crust has been removed. It is sliced and each slice is cut into 16 pieces, dried in an oven, and stored until

FEEDING

Approximately 2,000 young larvae are put in a battery jar containing 2 liters of pond water. About 50 mg. of brain-heart infusion, 50 mg. of powdered brewers' yeast, and a piece of whole-wheat bread are added. The jar is then covered with cheesecloth. The amount of food is increased as the larvae grow older. The jars are inspected each day; if any food is left, no more is added, as it is important not to leave food in the jar long enough to decay.

The larvae pupate in from 6 to 10 days. When most of the larvae have pupated, the contents of the jar are strained through gauze and the pupae put in distilled water to emerge.

The emergence cages are similar to those used for *Anopheles quadrimaculatus* (fig. 3).

Aedes aegypti adults are handled and fed in the manner described for *Anopheles quadrimaculatus*.

COLLECTING MOSQUITOES IN THE LABORATORY

Each day a hand vacuum cleaner is used to collect any loose mosquitoes in the laboratory, as well as adults that may have emerged in trays or jars.

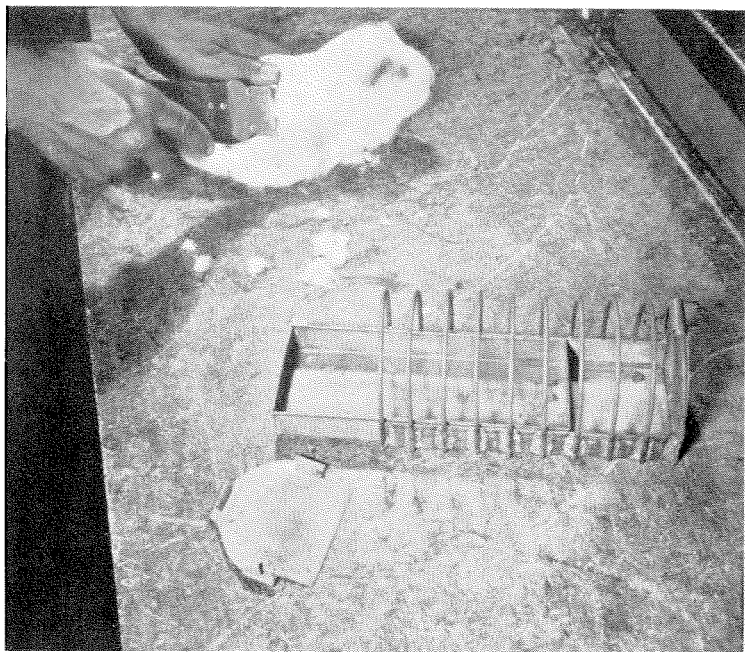


FIG. 4.—Shaving the guinea pig, and cage used to hold it.



FIG. 5.—Removing the egg papers which have been soaked.