ARTICLES

THE DISPERSAL OF *AEDES TAENIORHYNCHUS* IV. CONTROLLED FIELD PRODUCTION[1]

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Studies of the dispersal of the salt-marsh mosquito, *Aedes taeniorhynchus,* conducted at Sanibel Island (Provost, 1952, 1957) and at Fort Pierce (Provost, 1960) in Florida, while yielding valuable information about dispersal, also made it quite clear that more precise and detailed procedures were needed in the production of the adults to be observed. Specifically needed was the ability to produce a synchronous emergence of mosquitoes in a selected nutritional state at a predetermined time and marked for lifetime recognition. These adults should also be in a location for maximum observability both before and during the departure from the emergence site.

In order to produce such a controlled population of mosquitoes, a "nursery" was established in the Entomological Research Center's salt marsh. It is this nursery which, in addition to providing excellent facilities for investigating some of the basic mechanisms of *A. taeniorhynchus* bionomics, enables us to maintain and manipulate, at our discretion, large populations of this mosquito under field conditions.

THE NURSERY. The mosquito nursery consists of two groups of artificial swales surrounded by salt marsh (Fig. 1). These swales are separated by approximately two acres of subtropical forest in which a donkey is kept to serve as an attractant and blood source for mosquitoes. Both groups of swales are surrounded by various ponds and canals. The swales are from 50 to 100 feet long by 15 feet wide and vary in depth from 2 to 3 feet. They were planted to salt-marsh grasses, with mangrove trees at their ends. Each swale is connected to a water-storage reservoir by means of a 4-inch pipe through which the swale may be drained. Swales are flooded by pumping water from the reservoir to whatever depth is desired.

As the nursery marsh is the actual "laboratory," considerable emphasis was placed upon preparing and maintaining it for use in various experiments. One of the first projects was a topographical survey of the experimental areas. Both the east and west groups of swales were cross-sectioned at 10-foot intervals and graphed. In order that all elevations throughout the nursery area be in the same plane of reference, four engineering bench marks were established in the immediate experimental area. A grid system of stakes was also set up, coinciding with the cross-section intervals in each swale. With such a network of location stakes, any point in the swales can be recorded and later relocated with accuracy.

In the center of each swale, calibrated stakes were put in place and their elevations determined (Fig. 2). These centerline stakes enabled us not only to flood the swales to any desired depth but also to make daily observations on the height of water in the swales, thus determining the amount of water loss or gain through seepage, leakage, flooding or rainfall. The various bodies of water surrounding the swales are subject to water level fluctuations associated with the water table, tide, rainfall and pumping activities. To determine what effect these outside water levels had upon the water level in the swales, and the water table in the experimental area in general, calibrated stakes of known elevation were placed in each body of water and daily recording of water level made.

To investigate what effect the ground

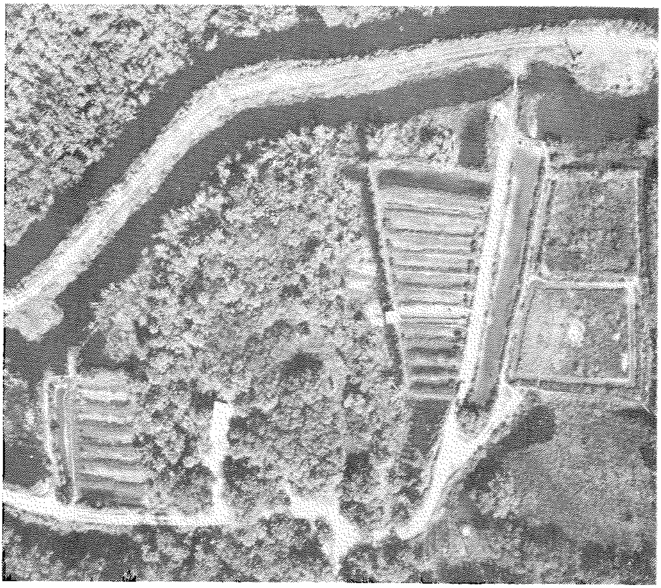

Fig. 1.—The ERC salt-marsh mosquito nursery from the air. Concrete trough is fifth swale from bottom in group on the right.

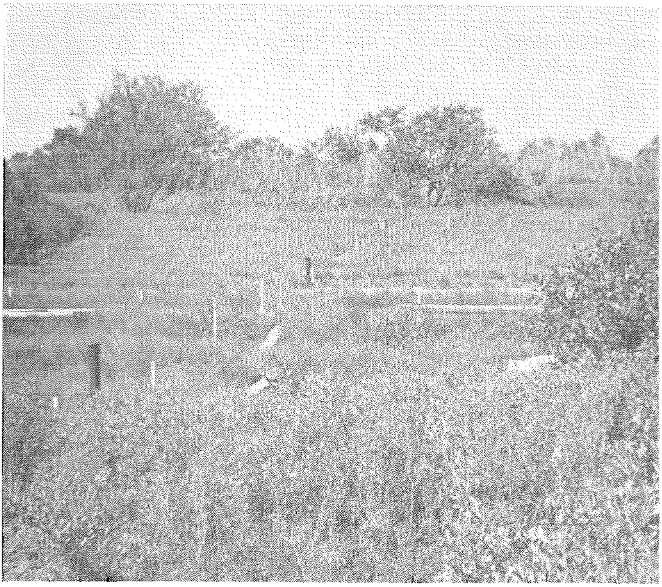

Fig. 2.—Swales showing stakes and well pipes for observation of water levels above and below ground.

water table had on the maintenance of various water levels in the swales as well as on a moist ovipositional substrate over the surface of all the swales, the elevation of the underground water table is recorded daily. Four-inch, perforated Orangeburg pipes were placed vertically in the ground at ten locations in the swale area (Fig. 2). The bottom of each tube was set well below mean sea level and the elevation of the top edge was determined. By inserting a measuring stick attached to an ordinary toilet tank float and reading its intersection with the top edge of the Orangeburg pipe, a quick water table reading could be made. This was done daily.

Two structures in the nursery area are used extensively in our studies. The first is a shed located in the woods between the two groups of swales. It is a 10 x 30-foot field laboratory, serving for storage of equipment and for processing sod samples collected in our mosquito-egg survey.

The second structure and probably our most important research tool is a 20 m x 1 m x 20 cm concrete trough with an automatically controlled heating system. This trough was constructed in the bottom of one of the swales in the east group (Fig. 3). It was designed so that "larva-proof" gates could be inserted, dividing the trough into nine separate cells, 2 x 1 meter in size. This division of the trough permits different experiments to be conducted concurrently. Embedded in the floor of the concrete trough are four rows of one-inch copper tubing through which hot water flows to heat the concrete floor and, in turn, the water. Associated with the concrete trough is a small equipment shed which houses the hot water heater, water-circulating pump and the automatic controls.

Since the ultimate purpose of the nursery, in addition to supplying mosquitoes for various other research programs being conducted at the research center, is the production of millions of adults for use in dispersal studies, an accurate and continuing picture of the production potential must be maintained. To this end we engaged in a program of periodical removal

Fig. 3.—The concrete trough with heat-controlled bottom. Separators create 1 x 2 meter cells.

of sod samples from each swale and determination of both the number and species of mosquito eggs present, using the techniques devised by Horsfall (1956) and Craig and Horsfall (1960). The numbers of eggs per sod sample, when correlated with the total ovipositional surface available, give us a close approximation of the total number of eggs in each swale. Since our samples are taken in a profile from the point of maximum flooding to the bottom of the swale, we are also able to determine the vertical elevation of the majority of the eggs and to observe belts of heaviest egg deposition. With this information, we can flood any swale to a selected depth and hatch any desired number of eggs. In addition to the sod sampling program, we sample the adult population with a New Jersey light trap and a rotating sweep-net.

Experiments and natural mortality are constantly depleting the egg supply in the nursery. In order to maintain and, if possible, increase the supply of eggs, we periodically flood swales to produce adults for oviposition purposes. Sufficient numbers of these stay in the marsh to materially supplement the immigrant mosquito population. Experience has shown an egg deposit of half a million per swale to be good. The maximum deposit measured in one swale was six million.

EXPERIMENTAL TECHNIQUES

As mentioned earlier, the primary objective of this invesigation was the synchronous production of preconditioned adult mosquitoes. Before this goal could be achieved, several fundamental difficulties had to be overcome. A discussion of these basic problems follows.

CONTROL OF WATER TEMPERATURES. Prior to the installation of the concrete trough, attempts were made to control the water temperature in several of the swales. Summer temperatures in these shallow waters commonly reach 36° to 38° while nighttime lows of 20° and 22° are frequent.[3] Mean water temperatures are about 27° in the summer and 22° to 25° in the spring and fall. For experimental purposes, constant temperatures between 20° and 34° would be desirable.

Through shading with Saran screening (Fig. 4) the daytime high temperatures in the grassed swales were reduced by about 5 to 6 degrees, but the nighttime lows remained within one degree of normal. This left a daily fluctuation of about ten degrees, so shading alone was inadequate. The addition of ice to shaded swales was not the answer, as it created more problems than could be overcome. It was therefore clear that we would have to be resigned to limited temperature control in the field.

Controlled-temperature rearing is now restricted to the concrete trough described earlier. Saran screening is used by day to reduce insolation (exposure to sunlight) when necessary. The heating mechanism, with thermostatic controls, raises the temperature at night or by day if required. This system results in a temperature control of plus or minus one degree. The summertime limits are 27° and 34°. In the spring and fall, the lower limit is about 22°.

TRANSFER OF LARVAE. The technique evolved demanded that larvae be transported to the concrete trough from the "brood" swales where they were hatched. Transferring masses of larvae without injury always poses problems.

A technique was devised where 95 percent or more of the larvae, in any instar, can be safely transferred from a brood swale to any other location. Two masonite "wings" are placed in the ground close to the drain pipe of the swale so that they form an angle the apex of which is left open and has attached to it a pocket of fine mesh nylon. With this trap in place the swale is flooded to hatch the eggs. When the maximum hatch has been obtained, the drain plug is removed and the swale is drained through the nylon pocket. Larvae, carried by the current of water, are concentrated by the wings and directed

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[3] All temperatures are expressed in degrees Centigrade.

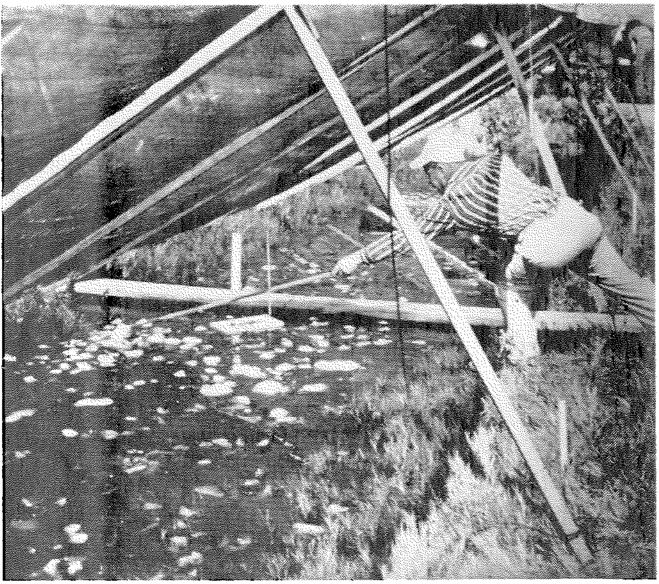

FIG. 4.—Dipping for larvae in a swale shaded by Saran screening; cooling augmented by ice.

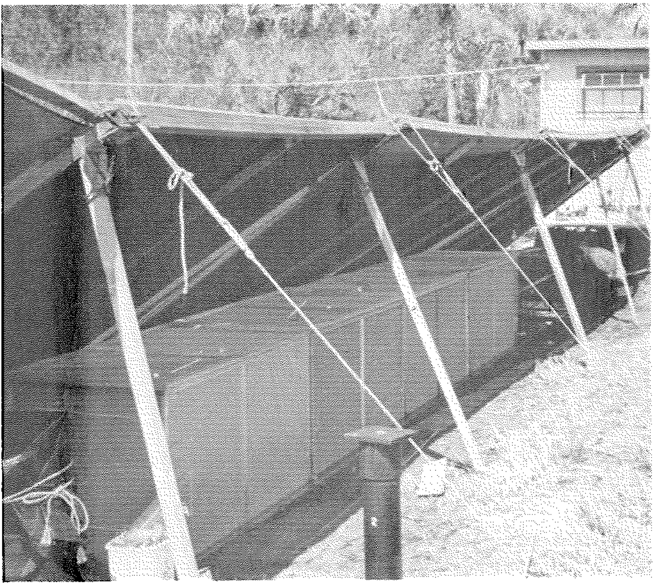

FIG. 5.—Adult emergence cages in place over the concrete trough.

into the pocket where they can be very easily scooped up with an ordinary fish net made of the same fine mesh material. The slopes of the swale are hosed down periodically so that any stranded larvae are returned to the main body of water.

This method effects the removal of essentially the entire larval population in two to four hours. However, it has been found that the transfer proceeds more quickly and easily with the later instars. The latter tend to be more in open water, and stronger streams of water can be used in the hosing process. The swale can also be drained faster when larger larvae are being trapped.

Transfer of Adults. Although the concrete trough offers a splendid situation for studies of larval development, its location is not satisfactory for observations of departing adults or for strategic placement of flight interception devices. The development of a suitable method for transfer of adults involved the construction of an emergence cage. The final design was a plastic screenwire cage, 1 x 2 x ½ meter, which fitted snugly over one section of the concrete trough (Fig. 5). Once emerged, the adults are captured by sliding a floor under the cage. They can then be moved to any desired location.

In addition to transportation, the cage serves as a device for estimating the number of adults produced. The sides and top of the cage are ruled off in a grid system enabling both visual and photographic counting of adults. A fair estimate of the total number of enclosed mosquitoes can be obtained by counting the adults on a number of squares and extrapolating to all surfaces.

Adult Marking. Although we have not as yet attempted to work with radioisotopes in the concrete trough, its adaptability for use with this type of marking medium is apparent.

Marking dusts have been used in some of our experiments. By covering the emergence cages with polyethylene and blowing clouds of marking dusts into them, we have been able to mark satisfactorily very large numbers of adults. The polyethylene covers keep the dust confined to the cages and prevent the contamination of other broods of mosquitoes and of the immediate area in general. This makes it possible to mark different fractions of a brood or different broods with different colors, an important feature of the experimentation planned for the future.

Estimation of Larval and Pupal Numbers. Before any quantitative experiments could be conducted, methods of estimating numbers of larvae and pupae had to be available. Sod sampling gives us the number of eggs per square foot, but percent hatch and percent survival remain unknown. The drop-tube sampler (Fig. 6) was developed as a tool for the estimation of larval and pupal numbers.

This device, designed primarily for use in the concrete trough, is merely a plastic tube mounted on a center shaft which can be dropped quickly into the water. The water is first stirred sufficiently to establish what appears to be a random distribution of larvae and pupae. A number of tubes are then released in rapid succession, each enclosing a measurable volume of water and a sample of the larvae and pupae. Simple extrapolation then gives a close approximation of the actual number in the section of trough involved. In test trials using a known number of larvae in a known volume of water, we were able to estimate the number of larvae within 10 percent with these drop-tube samplers.

EXAMPLES OF EXPERIMENTS

Production of a Synchronous Emergence. With water temperature in the concrete trough under control and larval feeding techniques reasonably developed, an experiment was conducted in early June of 1963 which aimed at producing a brood emergence at a predetermined time and with as much synchrony of development as possible. The technique employed was simply the progressive retardation of pupal development by cold exposure until all larvae had pupated, followed by a progressive return to higher temperature so

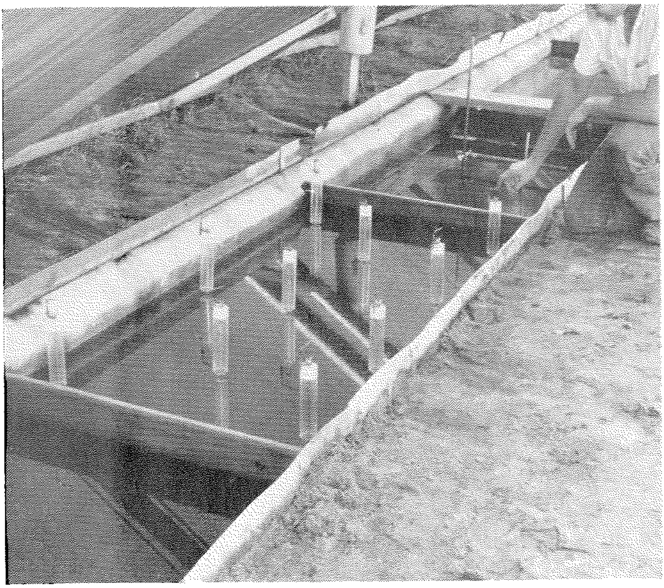

FIG. 6.—Drop-tube samplers in place for estimating numbers of larvae and pupae in the rearing trough.

that all emergences were brought into phase. The details and success of this experiment are reported in a separate publication (Pausch and Provost, 1965).

A pupation which would normally have resulted in a 40-hour emergence interval was made to result in a 12-hour emergence interval, with 53 percent compressed into 2 hours and 89 percent into 8 hours. This emergence peaked at the preselected hour and contributed valuable new information on the migratory exodus.

CONTROLLED PRODUCTION OF ENERGY RESERVES. Earlier experiments to control larval feeding in the field were very inconclusive due to the number of uncontrollable variables; a more controlled environment was needed before any positive work could be done. With the installation of the concrete trough, many of the variables which plagued previous experiments were removed.

In laboratory experiments it was found that by allowing larvae to feed for varying lengths of time, the amount of energy reserves in newly-emerged adults, in the form of triglycerides and glycogen, could be varied (Dr. P. T. M. Lum, personal communication). An attempt was then made to control this adult condition in the field.

Newly hatched larvae were transferred from a brood swale to the concrete trough set at 30° and were divided into four separate groups. Each group was given the same ample amount of food. At four different times, the larvae were transferred to foodless cells and allowed to pupate. Upon emergence, samples of females were captured for biochemical analysis in the biochemistry section of the research center. As can be seen in Table 1 we succeeded in regulating the amount of energy reserves in the adult female mosquito by this manipulation of larval feeding. The results were especially promising with glycogen, the level of which in new adults may prove to be a factor in migratory potential.

SUMMARY

In order to be better equipped to study the migratory activities of *Aedes taenior-*

TABLE 1.—Amount (in microcalories) of energy reserves per adult *Aedes taeniorhynchus* female related to the length of time the larvae were allowed to feed.

Hours larvae with food	Number of adults tested	Mean amount of reserves		
		Triglyceride	Glycogen	Total
64	10	118	440	558
68	10	124	665	789
72	10	96	870	966
76	10	274	925	1199

hynchus, a mosquito nursey has been established in the salt marsh at Vero Beach, Florida. In this experimental area, the effects of varying climatic and environmental factors on larval development can be observed. A concrete trough, which permits the regulation of water temperatures, makes it possible to conduct controlled experiments, among which is the production of synchronous emergences at predetermined times. In addition, preliminary studies indicate that adult mosquitoes can be produced of varying and selected nutritional states. The desired goal is to produce, upon demand, millions of adult mosquitoes of specified physiological conditions for release in dispersal studies at predetermined times.

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[1] This footnote refers to the two papers by Pausch and Provost—"Dispersal of *Aedes taeniorhynchus,* IV," and No. V: No. IV is Contribution No. 145, No. V, Contribution No. 146, Florida State Board of Health, Entomological Research Center, Vero Beach. The investigations were aided by grant AI04503, National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

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