PRELIMINARY RESULTS ON EXPERIMENTAL DETECTION OF MANSONIA UNIFORMIS (THEOB.) MOSQUITO IMMATURES ¹

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Mansonia uniformis is an important vector of filariasis in several Asian countries. This mosquito is also present in Taiwan and Vietnam where filariasis is not a major disease; however, it does pose an omnipresent potential for transmission of this disease. Even in areas where this mosquito is not important in the transmission of filariasis it plays a very significant role as a serious pest insect.

Control of this mosquito is decidedly different from that of other genera of mosquitoes, due to the way in which *Mansonia* immatures breathe. Other mosquitoes accomplish exchange of gases by rising to the surface of their aquatic environment and breathing by means of a siphon attached to the water surface. This behavioral activity lends itself to control of culicine mosquitoes by applying either insecticide or oil to the water surface.

The larvae and pupae of *Mansonia* carry out respiration by attachment to plants which contain internal air spaces and penetration of these by their siphon. Although *Mansonia* aquatic forms may attach to floating leaves, typically they attach to the roots of these plants. The larvae will also readily attach to submerged and partly submerged brown paper which also has air pockets (Laurence and Smith, 1958).

Attempts to control mosquitoes of this genus have been confined largely to destroying the adult mosquitoes as they emerge from the aquatic stages. In a few instances success has been realized through applications of herbicides to host plants required by the mosquito.

Particular emphasis should be placed on source reduction. It is essential to elucidate where the *Mansonia* pass their immature stages so that selective control measures can be carried out.

Since the aquatic forms are usually attached to the roots of plants, the conventional aquatic mosquito survey technique of skimming larvae and pupae samples from the water surface is useless.

As observed by several investigators (Hodgkin, 1939, and Chow. Mansonia larvae can be collected from roots of water plants such as water hyacinth, Eichornia, but, as pointed out by Laurence (1960), once the larvae become fixed to a plant they do not readily detach. As a result, surveying for these mosquito immatures not only depends on examining the correct type of plant but also on extremely close observation of the roots. Various techniques have been devised to help overcome this problem. Surveys are conducted by extracting suspected host plants from their habitat and rapidly immersing the roots into a pail of water. The plants are then roughtly shaken in an effort to dislodge any attached immatures. Unfortunately, the roots of many plants such as Eichornia, are often covered with mud, and the water in the pail is muddied for several minutes before settling and clearing take place. The few larvae which are dislodged by this action are not readily

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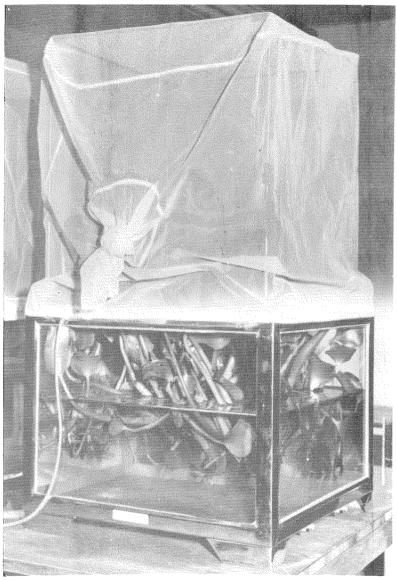


Fig. 1.—Aquarium equipped with a flight cage.

noticed because, as pointed out by Ingram-(1912), they go to the bottom of the pond or in this case the container and burrow into the sediment. While working with Mansonia perturbans (Wlk.) Bidlingmayer (1954) was able to survey for this species using a sheet metal cylinder. However, this method, though effective for that species, is very time-consuming.

All factors considered, surveying for

Mansonia larvae was a long, arduous and often fruitless process. With these thoughts in mind, we set out to devise a simple technique for detecting the presence of Mansonia larvae and pupae. A procedure that could cause the attached mosquito immatures to detach from their host plants and rise to the surface of the water where they could easily be seen was devised.

MATERIALS AND METHODS. Several colonies of *Mansonia uniformis* were established in the laboratory to furnish a continuous supply of mosquito larvae and pupae. Aquaria were especially constructed, 44 cm. on each side and 30 cm. high. In addition to providing a simulated pond these glass-sided aquaria permitted close observation of the plants. Fitted to the top of each aquarium was a net flight cage 40cm.³ which enclosed the tops of

plants to prevent escape of the adult mosquitoes. This flight cage also permitted feeding and mating of adults (Fig. 1). Air was bubbled into the water tanks to prevent scum layers on the water. Duckweed, *Lemna minor* and water hyacinth *Eichornia speciosa* were used, the former for egg deposition and the latter for the attachment of larvae and pupae. Strips of brown file folders were provided as additional sites for attachment of immatures.

Preliminary screenings of solutions examined to determine if detachment and rise of the specimens could be effected, were made using larvae attached to 1" strips of brown paper file folders. Various solutions of different concentrations were tested. In all cases ordinary tap water warmed to room temperature was used as a control liquid.

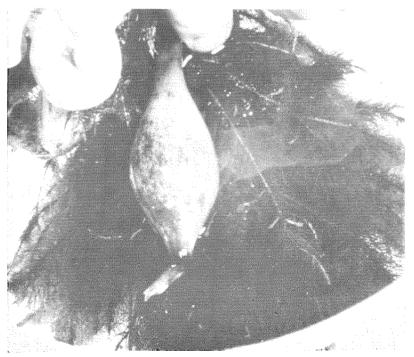


Fig. 2.—A typical Eichornia plant with the mosquito larvae firmly attached to the roots.



Fig. 3.—Position of the larvae after the plant has been transferred to a different battery jar and the plant shaken vigorously.

RESULTS AND CONCLUSIONS. Tap water, ice water, hot water, r percent hydrochloric acid, 5 percent hydrochloric acid, 5 percent sodium chloride, and r percent sodium hydroxide all gave negative results. However, 5 percent sodium hydroxide and 50 percent sucrose solution produced the desired result. Five percent KMnO₄ caused opacity which precluded any observations of the mosquito immatures.

Since both 5 percent sodium hydroxide and 50 percent sucrose solutions are rather viscous solutions, tests were made using other chemical solutions of similar viscosity. Sucrose solutions are expensive and difficult to work with. Using 1 percent sodium hydroxide, mixtures of 1 percent sodium chloride and 1 percent sodium chloride and 1 percent sodium hydroxide, 5 percent sodium chloride and 1 percent sodium hydroxide, and

5 percent sodium bicarbonate also gave negative results. On the other hand, solutions of 3 percent sodium hydroxide, 5 percent sodium chloride, 10 percent sodium chloride, and 10 percent sodium bicarbonate all gave partially desirable results but not as good as 5 percent sodium hydroxide. With 5 percent sodium hydroxide all immatures detached and rose to the surface immediately.

Further exploration of still other solutions resulted in the following findings. Five percent zinc sulfate in combination with 1 percent sodium hydroxide gave negative results as did the 1 percent sodium chloride and 1 percent sodium hydroxide solution. The 4 percent sodium hydroxide solution gave the desired result rather slowly. The 5 percent zinc sulfate and 5 percent sodium hydroxide gave the same result as a 5 percent sodium hydrox-

ide solution. Both the 15 percent and 20 percent sodium chloride solutions gave excellent results.

Additional screenings of salt and sodium hydroxide solution at various concentrations confirmed that 15 percent (+) salt water solutions and 5 percent sodium hydroxide solutions gave optional results.

Demonstration of the effectiveness of these solutions as tools for surveying for the presence of *M. uniformis* aquatic forms can be seen in the accompanying plates. Figure 2 shows a typical *Eichornia* plant with the mosquito larvae firmly attached to the roots. Figure 3 shows the position of the larvae after the plant had been transferred to a different battery jar and the plant shaken vigorously to simulate the standard survey techniques presently utilized in the field. In this case only 4

or 5 of approximately 100 larvae were dislodged by the action. Two larvae rose near the surface of the water then swam to lower depths with the other three. However, when *Eichornia* plants were dipped into the 5 percent sodium hydroxide solution (Fig. 4), without shaking, apparently all the larvae detached from the roots and rose to the surface of the solution. Somewhat similar but less dramatic results were noted when the mosquito-bearing plants were dipped in sodium chloride solutions in excess of 15 percent.

SUMMARY. A technique of surveying for *Mansonia uniformis* immatures under laboratory conditions has been developed. When the mosquito-laden roots of the host plant are dipped into a solution of either 5 percent sodium hydroxide or 15 percent



Fig. 4.—Eichornia plant dipped in 5 percent sodium hydroxide solution where all larvae have detached and risen to the surface.

sodium chloride, the larvae and pupae detach immediately and rise to the surface of the solution where they are easily seen.

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MALARIA ERADICATION IN THE USSR

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According to the Soviets, malaria as a disease of the masses was eradicated in 1952 in the USSR. In the same year, however, malaria was fairly widespread in some of their southern Republics. Cases numbered over 180,000 in 1952—a tremendous drop from the nearly 800,000 reported in 1950 (Sergiev et al., 1959). Complete eradication on a national scale was discussed and approved in 1952. Another drastic reduction in cases occurred in 1958 when only 2,504 were reported.

When I was in Moscow in 1959, I was told that total eradication of the disease was expected in 1960, despite foci which remained in several of the southern

Republics.

From 1966 to 1968 almost all malaria transmission had stopped except for a few areas in the south, mainly in Georgia and Azerbaidzhan (Sergiev et al., 1969). Bruce-Chwatt (1970), a well-known malariologist in England, reported that malaria had been eliminated from the whole of Europe and stated that as an indigenous disease, it had disappeared from most of

the USSR. This indicated that the disease had not been eradicated from all parts of the Asian Republics of the Soviet Union.

Early history of malaria and its control in the USSR, and details on eradication up to 1959, have been published by Bruce-Chwatt (1959).

Some of the scientists involved in the malaria eradication campaign included V. N. Beklemishev, T. S. Detinova, H. H. Dukhanina, V. A. Nabokov and P. G.

Sergiev.

Dr. Beklemishev, a member of the USSR Academy of Sciences, was head of the Entomology Division of the Malaria Institute (now called Martsinovsky Institute of Medical Parasitology and Tropical Medicine). He was instrumental in organizing the country-wide malaria control program. Beklemishev and collaborators introduced the principle of age-grading of female *Anopheles*. The part that this system played in eradication will be discussed under Detinova's work.

Dr. Detinova, entomologist and senior scientific worker at the same institute, has lectured on physiological age-grading of

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