

SCIENTIFIC NOTES

RESEARCH NOTES ON *Aedes aegypti* FEEDING ON THE EASTERN GARTER SNAKE *Thamnophis sirtalis sirtalis*WILLIAM G. PEARSON¹ AND BRUCE A. HARRISON¹

The Entomology Branch, Third United States Army Medical Laboratory, Fort McPherson, Georgia, began research on *Aedes aegypti* feeding on animals other than man in October 1966. The initial animal used was a 27-inch Eastern Garter snake, *Thamnophis sirtalis sirtalis*. This reptile was captured on October 13, 1966, in Ashburn, a small South Georgia town. Previous attempts of feeding *Aedes triseriatus* on this snake had proven highly successful.

The present research was prompted by the finding of a remote population of *Aedes aegypti* at Alabama Army Ammunition Plant, Childersburg, Alabama. This population of *aegypti* was found approximately 3,500 feet from the proximity of the nearest human. Precipitin tests conducted by *Aedes aegypti* personnel of the U. S. Public Health Service revealed all engorged wild females tested had fed on cattle in the vicinity.

On October 13 the test colony of *Aedes aegypti* adults was started by using pupae from a colony sustained on guinea pigs. These adults were then placed in a 12" x 12" x 12" model G-1, Cornell-Gerberg cage of aluminum, wire screen and surgical stockinet. The snake was introduced into the cage on October 18 when approximately 200 adult mosquitoes were present. The snake was secured in the cage by placing it on a 1" x 3" x 11½" board and taping it down with masking tape and paper towels, leaving the middle region of the body exposed for feeding. The snake was left in the cage approximately 45 minutes for each feeding.

The initial feeding occurred on October 18 with only 12 females engorging. Subsequent feedings on October 20, 22, 24, and 26 had 10 to 20 females engorging each time. Attraction to the snake by hungry females was initially large at each feeding; however, after approximately one minute most females left the immediate vicinity of the snake and either flew around or settled on the screen. Few females remained to feed and most of these probed several times before engorging. The ratio of complete engorgement to partial engorgement was about 50:50.

It was observed that the guinea pigs were much more attractive to the *Aedes aegypti*. The colony maintained on these rodents was very active when the guinea pig was introduced for feeding. Many females arrived immediately and fed, with many others feeding during the 45 minutes. Complete engorgement on guinea pigs took about 3 to 4

minutes, while complete engorgement on the snake in the other colony always took at least 5 minutes.

An oviposition jar was present in the cage from the time of the initial feeding of adults. This consisted of a 600 ml. beaker, one-third filled with distilled water with a brown paper towel lining the inside. The towel extended below the surface of the water, thus keeping the upper portion damp.

On October 26 the oviposition jar was removed from the cage. The water in the beaker contained 97 first and second instar larvae which hatched while the beaker was in the cage. The towel was removed and 298 unhatched eggs were counted. These eggs were submerged in a white porcelain pan 11½" x 7" x 2" containing distilled water and a small amount of Liver Powder NF.

Twenty-two pupae were taken on October 30 from the pan containing the 97 larvae from the oviposition beaker. These larvae had hatched, matured and pupated in 4 to 6 days; however, the exact date of larval hatching is not known. On October 31, 277 larvae had hatched in the pan containing the 298 eggs. At this time the egg paper was removed from the water. Using these figures this gives a 92.98 percent hatching factor. On this same day 71 pupae were collected from this pan. This means that the larvae hatched, matured and pupated in 4 to 5 days or in approximately 116 hours. This differs from the colony feeding on guinea pigs which usually requires not less than 7 days to pupate. No size difference was detected between larvae and pupae from the two colonies.

A UNIT FOR FORMULATING GRANULAR INSECTICIDES

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In the course of studies on the efficacy of granular mosquito larvicides, it became necessary to develop a small unit for preparing experimental samples of granular mosquito larvicides. Such a unit was developed with low cost materials. The unit (Mulla and Axelrod 1960, 1962) proved very useful in formulating gram-quantities of experimental samples of granules used in laboratory studies. The maximum amount of impregnated granules that could be prepared in the unit was 40 grams. Therefore larger samples often needed in field evaluation work could not be formulated with this unit.

In order to formulate large quantities of granular larvicides it became necessary to develop another unit which will have a capacity of formu-

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FIG. 1.—Pilot plant for formulating granular formulations of insecticides.

lating 5- to 10-lb. quantities of granular materials. For field studies, especially aircraft usage and calibrations, these larger quantities are essential.

The unit described here is shown in Figure 1. It consists of a cement mixer frame, an electric motor, a power control unit to reduce the motor rpm and a spray gun. The specifications for these parts are:¹

1. Mixer frame—Kol Inc., St. Paul, Minnesota.
2. Electric motor—Bodine Electric Co., Chicago, 115 volts, 1.43 amps, $\frac{1}{8}$ hp, 1725 rpm. Type NSH-54. Takes power from power control unit.
3. Power control unit—Minarik Electric Co., Los Angeles, 115 volt D.C. Shunt Model SH-52. Made especially for the above motor. To be connected to 115 volt A.C. power source.
4. Spray gun—Speedy (paint sprayer), W. R. Brown Corp., Chicago, Model 131A-45 lbs. max. pressure.
5. Two sizes of tumblers made from sheet metal: large—15" high, 11½" O.D., 6" mouth

diameter and 9" bottom diameter; small—12½" high, 10¾" O.D., 4½" mouth diameter and 7" bottom diameter.

Inside the tumblers, ½" high, thin ridges of sheet metal are welded on a slant lengthwise. This facilitates rolling over of the granules during impregnation or coating.

The mixer stand or frame is rotated by means of a sprocket, pulley and belt. The tumblers containing the ingredients for formulation are placed in the frame. The quantity of granular material that can be formulated per batch is anywhere from 1 to 15 pounds. The time required for proper formulation of these quantities is anywhere from 5 to 30 minutes.

The tilt of the frame is constant. The speed of the frame can be regulated by regulating the rpm of the motor through the power control unit. The speed of the frame or the tumblers can be regulated from 3 rpm to 104 rpm. The optimum rpm, however, for formulating most granular formulations is in the range of 5-10 rpm. The power control unit has two settings at low and high and it can run the motor forward and reverse. With little experience, optimum speed can be determined for specific formulations.

The unit is suitable for formulating both impregnated and coated type granules. For the former type, the carrier is placed inside the tumblers and a solution of the larvicide containing other additives or surfactants is sprayed over the tumbling granules, from the sprayer attached to compressed air supply. For making coated type granules, the sprayer is not needed except in cases where the adhesive material or water has to be sprayed onto the core particles. In most cases of coated granules, concentrate dust or wettable powders of the larvicides or toxicants are added to the core material. The core material may be masonry sand or other cheap inert particulate material. A small amount of glue or adhesive is added to the mixture. The mixture is tumbled at the desired speed and then screened to size. Adhesives can be added as an aqueous solution or suspension by the sprayer.

The unit is not only suitable for making granular formulations of insecticides, but it is also fit for formulating contaminated diets for feeding to wildlife. In one such study (Keith and Mulla 1966), this unit was employed in preparing uniformly contaminated food of mallard ducks.

Units of this type but with larger capacity can be cheaply constructed for preparing granular mosquito larvicides for use in malaria eradication programs in various parts of the world. Gasoline engine or manually operated rotatory devices can be substituted in areas where electric power is not available. Granular insecticides can be formulated in almost any part of the world using local inert materials and pesticidal concentrates which are imported. The cost of making such a formulation would be nominal after the

¹ Mention of these products does not imply endorsement of these materials. There are products from different sources, which will perform similarly.

initial stages and technical problems of the formulating equipment are worked out.

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THE OCCURRENCE OF *Coelomomyces indicus* IYENGAR IN EGYPT, U.A.R.

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A survey of the natural enemies of the main malaria vector in Egypt, *Anopheles pharoensis*, is presently undertaken by the Research Institute of Medical Entomology, Cairo. It is hoped that this survey might lead to the consideration of one or more of the pathogens, parasites and predators as a possible agent in the integrated vector control.

Among the collected specimens of *A. pharoensis* larvae and adults, some were found packed with the sporangia of *Coelomomyces indicus* Iyengar, a fungus belonging to the order Blastocladales, family Coelomomycetaceae, which is usually fatal to its host.

The first record of the presence of *Coelomomyces* in mosquitoes dates back to 1921 in Malaya where Keilin (1921) found it in an *Aedes* larva, whereas the first known infections of anopheline larvae were found in India and the fungus *C. indicus* was first described by Iyengar (1935). Since that date about 25 *Coelomomyces* species infecting different species of *Culex*, *Aedes*, *Anopheles*, *Theobaldia*, *Uranotaenia*, *Aedomyia* and *Psorophora* have been recorded. (Couch & Umphlett 1963.)

Apart from mosquitos, there is one record by Garnham and Lewis (1959) of the occurrence of *Coelomomyces* in *Simulium* and an earlier record of its occurrence in nymphs of the aquatic bug *Notonecta* sp. by Bogoyavlensky (1922).

Although *Coelomomyces* is widely distributed in all continents (except South America), no records exist of its occurrence in the countries of the Middle East. In Africa, it is recorded in Sierra-Leone, Kenya, Liberia, Madagascar, Uganda and Zambia (formerly Northern Rhodesia).

SPECIES FOUND INFECTED IN EGYPT. *Anopheles pharoensis* and *Culex antennatus* were found in-

fecting in nature with *Coelomomyces indicus*. Infected *C. antennatus* larvae were collected from one breeding place in Kombera (Giza Governorate) while *A. pharoensis* parasitized larvae and adults were recorded in three Governorates, viz., Giza and Kalubia near Cairo and Kafr El-Sheikh, north of the Nile Delta.

None of the larvae or adults of other species examined, especially *A. coustani* and *C. pipiens*, was ever found infected with the fungus.

STAGES OF COELOMOMYCES FOUND. In all infected larvae or adults, thick-walled sporangia were always present in abundance together with less numerous thin-walled sporangia.

Dr. M. F. Madlin, who identified the *Coelomomyces* species, remarked that "the sporangia in *Culex antennatus* tend to be slightly larger than those of the fungus in the larvae of *A. pharoensis*, and commonly have a slightly larger number of wall thickening in their widest part but do not differ sufficiently from those in *A. pharoensis* to raise doubts as to their identity also as *C. indicus*" (personal communication).

In two adult *A. pharoensis* mosquitos and one *C. antennatus* larva, hyphae were observed, either simple unbranched or dichotomously branched and occasionally irregularly branched. These hyphae were found in the body cavity in abundance to the extent that a puncture with the needle to the side of the abdomen caused scores of hyphae to be liberated in the drop of saline in which the mosquito was dissected. There is no previous records of the occurrence of mycelia in the adult except that by Van Thiel (1954) who found hyphae (mycelia) in the ovaries only.

PERCENTAGE OF INFECTION. Regarding *C. antennatus*, 78 fourth stage larvae out of 284 collected from Giza Governorate (27.4 percent) were found infected. As to *A. pharoensis*, 60 infected larvae were found in Marg (Kalubia) among a collection of 1470, i.e., 4.1 percent. This percentage is rather high compared with figures of other workers (reviewed by Couch and Umphlett in Steinhaus 1963) which amount to 1.5 percent in U.S.A. Muspratt (1965) gives a much higher record of 20-50 percent infection in *Aedes aegypti* larvae breeding in several cut-off bamboos and also in a disused unglazed earthenware pot.

Larvae collected from Kombera (Giza) showed 14.7 percent infection; 127 larvae being found infected out of 864 collected and those from Kaha showed 1.1 percent infection rate (3 positives out of 270).

As to adult infection in nature, 64 female *A. pharoensis* from Marg were found packed with sporangia out of 3857 specimens dissected (1.7 percent) and 2 out of 774 from Kafr El-Sheikh (0.26 percent). Usually the infection is very heavy in larvae and adults, occurring in all parts of the body including the head, and in *C. antennatus* sporangia were even found in the siphon.

SEASONAL OCCURRENCE. In Zambia, Muspratt