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COLONIZATION OF *CULEX (MELANOCONION) AIKENII* (AIKEN AND ROWLAND, 1906) IN PANAMA [1]

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INTRODUCTION

Culex mosquitoes of the subgenus *Melanoconion* have been recently incriminated as vectors of arboviruses, pointing to the need of laboratory colonies of one or more species of this group of culicines for use in transmission experiments.

There have been three laboratory colonies of *Culex* (*Melanoconion*) mosquitoes reported in the literature. There was one colony of *C. portesi* at the Trinidad Regional Virus Laboratory (Takahashi, 1968), a second one of *C. cedecei* Stone and Hair, 1968 at the Communicable Disease Center (Hair, 1968), and a third of *C. peccator* Dyar and Knab, 1909 at Lake Charles, La. (Chapman and Barr, 1969). None of these colonies has reached a high enough population level to permit its use in experimental transmission work with arboviruses.

Culex (*Melanoconion*) *aikenii* came under suspicion as a potential vector of Venezuelan equine encephalitis (VEE) in the Middle Chagres river basin in the Panama Canal Zone, during studies conducted there by S. Srihongse and P. Galindo of Gorgas Memorial Laboratory, Panama (unpublished). Recently, Galindo and Grayson (1971) successfully transmitted VEE to laboratory hamsters

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from naturally infected mosquitoes of this species collected in that area.

Attempts to colonize *C. aikenii* were initiated early in 1970 at the Rand Insectary of Gorgas Memorial Laboratory (Duran, unpublished). Successful colonization was accomplished a year later only after repeated trials in which constant modifications of methods were introduced. The authors wish to express their appreciation to Mr. Eduardo Duran, technician at Gorgas Memorial Laboratory, whose original ideas and development of various techniques contributed materially to the success of this project.

MATERIALS AND METHODS

Colonization of the species was achieved by the introduction into an outdoor stock cage of a constant supply of adults reared from larvae collected from water-lettuce plants (*Pistia stratiotes* Linn.) in the area of Juan Mina, in the Middle Chagres river basin. Collections which averaged 800 larvae each were brought from the field twice a week until the colony became established.

Stock cages were 2 cubic feet in capacity, and were aluminum-screened (Fig. 1). They were held outside the insectary, as adults require natural lighting for mating. Humidity inside the cage was obtained from several sources. The top was covered with moistened absorbent cotton. Two sides of the cage were covered with wet hand-towels hanging from enamel pans completely filled with cotton soaked in water and covered with a gauze cloth. An inverted clay pot placed standing on a white enamel dish with water served as source of humidity as well as a diurnal resting place for the adults.

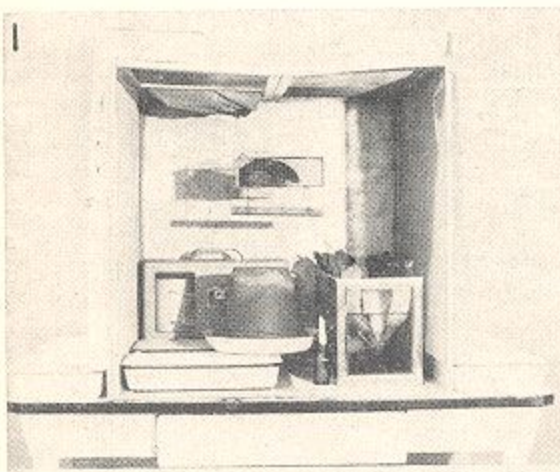
To record relative humidity and temperature a hygrothermograph was kept inside the cage during 4 months. The minimum and maximum mean weekly temperatures and relative humidities recorded were 73 °F and 85.6 °F, and 59.5 percent and 92.4 percent RH, respectively. Minimum and maximum temperatures and relative humidities recorded were 70° and 90° F and 50 percent and 98 percent RH. These figures refer to general ambient conditions inside the cage which differ from those of the microhabitat of the mosquitoes while at rest.

A 10 percent sucrose solution was made available to adult mosquitoes by means of a dental wick hanging out of a small bottle containing the solution. A blood source was offered 5 nights a week by hanging from the top of the cage two small rectangular, wide-meshed wire cages, one containing a golden hamster *(Mesocricetus auratus)* and the other a cotton rat *(Sigmodon hispidus)*.

An aquarium 18"x 6½" x 8¼" half-filled with constantly aerated water and packed with thoroughly washed fresh wild *Pistia* plants with the roots cut about 3" below the crown was also placed inside the cage. It served the following purposes: oviposition, adult emergence, diurnal resting places for the adults and additional source of humidity. The aquarium was taken out of the cage once a week and water containing *Pistia* plants was transferred to large enamel pans 16¼" x 10⅛" x 2⅞". Early instar larvae were changed from these pans in groups of 100 into similar smaller pans 11¾" x 7½" x 2⅛" containing 900 ml of water from natural breeding places and lined with a sheet of filter paper. One or two large fresh *Pistia* leaves were floated on the water in each pan and constant mechanical aeration was provided by means of an airstone attached by plastic tubing to an air pump. (Fig. 2 and 4). One 0.44 gram finely-ground tablet of emulsified Squibb's yeast was suspended in the water to serve as food, adding one-half additional tablet every 3 days.

When almost all the larvae in a pan had pupated, those remaining were grouped together with larvae from other containers. If the rearing medium became badly polluted larvae were changed to pans containing fresh water. When all early instar larvae were removed from

the large pans containing water and *Pistia* plants from the aquarium, leaves of *Pistia* with unhatched eggs were detached from the plants and floated on the water in the large pans to allow hatching of all eggs. Larvae were searched for in these containers for 6 consecutive days. After this period, a new cycle of larvae rearing was begun. Pupae were removed from the rearing pans every day and transferred to the aquarium inside the cage to permit emergence of



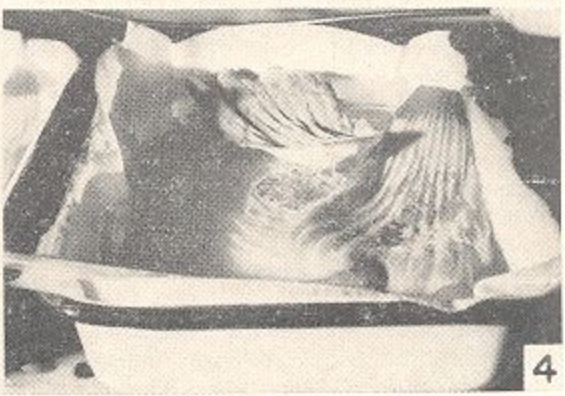


FIG. 1.—Adult colony cage.
FIG. 2.—Rack for holding larval rearing pans.
FIG. 3.—Eggs of *Culex aikeni* laid singly on *Pistia* leaf.
FIG. 4.—Individual rearing pan showing floating *Pistia* leaves.

adults. Maximum, minimum and mean temperature recorded during the four months of colonization in the Insectary rearing rooms were 86 ° F, 72 ° F and 78 ° F, respectively.

RESULTS

MATING. Copulation begins to take place 60–73 hours after emergence of the adults. The act of mating has not been closely observed, but it appears to occur during the short twilight period both at dusk and at dawn.

BLOOD FEEDING. Females begin taking blood meals with the onset of sexual activity, but blood-feeding may occur before or after mating. Of the two vertebrate hosts used as a source of blood, the golden hamster and the cotton rat, the latter seems to be preferred.

LONGEVITY. As observations on longevity of adults are still in progress, we do not have definitive data on mean life span. However, females have been maintained alive for over 3 months.

OVIPOSITION AND EGGS. During the first gonotrophic cycle, oviposition begins about 7 days after the blood meal. Eggs are laid singly, and loosely attached to both sides of the *Pistia* leaves, usually close to the surface of the water (Fig. 3). Many eggs become detached from the leaves and drop to the surface of the water where the larvae emerge. Others hatch *in situ*. Upon being laid, fertilized eggs are whitish in color changing to black within several hours; infertile eggs become grayish in color. Hatching of eggs takes place from 2 to 5 days after oviposition and there is no evidence of resistance to desiccation or of a diapause period subsequent to embryonic development.

LARVAL AND PUPAL PERIOD. Soon after the establishment of the colony, duration of the larval period was approximately 14 days from larval hatching to pupation. In recent generations this period has shortened to about 10 days although rearing conditions have not been changed. Duration of the pupal period is approximately 2 days.

BEHAVIOR OF COLONY

ADULTS. During the daylight hours the majority of males and females rest on the crowns of *Pistia* leaves inside the aquarium. A few adults also rest inside the inverted clay pot. Adults emerge from these resting places at dusk when mating and blood feeding activity begins. Females will feed on blood throughout the night.

LARVAE AND PUPAE. The immature stages in this colony are found closely associated with the water-lettuce plants. Larvae and pupae remain motionless for x hours under the leaves of the plant in the rearing pans. Both stages attach their external respiratory structure to air pockets present on the lower side of the leaf. When pupae are transferred to the aquarium in the stock cage they always congregate under the leaves until adult emergence. Attempts to rear the larvae in the absence of *Pistia* have failed.

PRODUCTION. Productivity of the colony has been very high, but irregular. During peak production as many as 2,300 pupae are produced daily. Irregular production may be due to the system of removing larvae from the aquarium only once a week, which probably induces losses of many first instar larvae that emerge early in the week.

DISCUSSION

The following discussion is presented as a plausible explanation for the high production rapidly attained by the colony when compared with other *Melanoconion* colonies. This heavy production seems to reflect a very high reproductive potential of *C. aikenii*, a species with highly gregarious immature stages, which in the absence of some environmental stresses present in its breeding place attains very high population levels. As a result of these stresses *C. aikenii* appears to have

developed a number of survival mechanisms. The immature stages, as mentioned previously, tend to congregate motionless under the leaves of the *Pistia* plants. The larvae are usually greenish in color, matching the coloration of the plants, which helps to conceal them. The behavior displayed by the immature stages noted above, as well as the larval coloration, seem to act as protective mechanisms against the many predators (dragonfly and mayfly naiads, dytiscid larvae, small frogs and fishes, etc.), that inhabit the ecosystem of which the water-lettuce communities form a part. The shortness of the larval cycle, in contrast to that reported for other *Melanoconion* species, also appears to be a survival mechanism since it shortens the period of exposure of the larvae to the environmental pressures present in the breeding places. Finally, females show a short gonotrophic cycle and an extended life span which would seem to enhance the possibility of several oviposition cycles during the life span of a given female. The short larval and female gonotrophic cycles would also seem to favor a rapid production of fresh populations resulting in enough survivors to be able to cope with the pressures of the environment. In summary, it appears that the very high production shown by the colony could be the result of the suppression of the very heavy predation pressures to which the species is subjected in its natural environment.

SUMMARY

The colonization of *Culex (Melanoconion) aikenii* (Aiken and Rowland) is reported. The immature stages of this colony are bred in close association with the water-lettuce plant (*Pistia stratiotes* Linn.) Eggs are laid on the leaves of this plant. The larval period lasts 10 days. Adults emerge 2 days after pupation. Mating occurs during the twilight period at dawn and dusk. Females are fed on rodent blood and have been maintained alive for over 3 months. As many as 2,300 pupae per day have been produced by this colony.

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