negative nature. Although these studies have unearthed no evidence favoring the concept of EEE virus overwintering in hibernating mosquitoes, admittedly they do not eliminate the possibility of this occurring.

SUMMARY. During the winter of 1956–57 hibernating mosquitoes were collected from seven localities in Connecticut where there had been history of eastern equine encephalomyelitis (EEE) virus activity. The 2,569 specimens collected represented six species: *Culex pipiens* 1,337, *Culex salinarius* 910, *Culex restuans* 115, *Culex territans* 33, *Anopheles quadrimaculatus* 132, and *Anopheles punctipennis* 42.

The mosquitoes were currently pooled according to species, triturated and inoculated into chick embryo tissue cultures for detection of virus. Thirty-one of the 73 pools were also inoculated into infant mice. No virus was isolated.

The implication of these observations upon the overwintering of EEE virus in hibernating mosquitoes in Connecticut is discussed.

References

1. BLACKMORE, J. S., and WINN, J. F. 1956. A winter isolation of western equine encephalomyelitis virus from hibernating *Culex tarsalis* Coquillett. Proc. Soc. Exper. Biol. and Med., 91, 146.

2. WALLIS, R. C., JUNGHERR, E. L., LUGINBUHL, R. E., HELMBOLDT, C. E., SATRIANO, S. F., WILLIAMSON, L. A., and LAMSON, A. L. 1958. Investigation of Eastern Equine Encephalomyelitis. V. Entomologic and Ecologic Field Studies. Amer. Jour. Hygiene 67(1):35–45.

3. MELNICK, J. L. 1955. Tissue culture techniques and their application to original isolation, growth, and assay of poliomyelitis and orphan viruses. Annal. New York Academy of Sciences, 61:754–772.

4. CHAMBERLAIN, R. W., SIKES, R. K., NELSON, D. B., and SUDIA, W. D. 1954. Studies on the North American arthropod-borne encephalitides. IV. Quantitative determinations of virus-vector relationships. Amer. Jour. Hyg., 60, 278.

5. BARBUTUS, PAUL P. 1956. Personal communication.

6. CHAMBERLAIN, R. W., RUBIN, H., KISSLING, R. E., and EIDSON, M. E. 1951. Recovery of virus of eastern equine encephalomyelitis from a mosquito, *Culiseta melanura* (Coquillett). Proc. Soc. Exper. Biol. and Med., 77, 396.

7. HOLDEN, P., MILLER, B. J., and JOBBINS, D. M. 1953. Isolations of eastern equine encephalomyelitis virus from mosquitoes (*Culiseta melanura*) collected in New Jersey, Proc. Exper. Biol. and Med. 87, 457.

8. HAYES, RICHARD O. 1957. Personal communication.

9. BARR, A. RALPH. 1956. Personal communication.

COLONIZATION OF *AEDES TAENIORHYNCHUS*

A. NELSON DAVIS
Entomology Research Division, Agr. Res. Serv., U.S.D.A.

Salt-marsh mosquitoes, *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.), have developed resistance to DDT, BHC, and dieldrin in several coastal areas of Florida where these insecticides have been used extensively. Laboratory investigations of more effective materials and methods of control for these species have been carried out in the past with field-collected larvae and pupae. These investigations have been hampered by the irregular occurrence of broods, even during the normal breeding season, and by difficulty in getting sufficient numbers in suitable condition for testing. For these reasons the laboratory studies could not be carried on continuously in keeping with the importance of the problem.

To meet the demands for specimens, insectary rearing of *taeniorhynchus* was begun at the Orlando, Fla., laboratory in April, 1957. The colony was started with field-collected eggs, larvae, and pupae from salt marshes in Brevard and Volusia Coun-

ties, Fla. These marshes produced mosquitoes that were difficult to control with chlorinated hydrocarbon insecticides. Since no successful colonization of this species had been reported, various techniques were tried in an effort to find the most suitable conditions for maintaining the adults and for oviposition, as well as methods for hatching the eggs and rearing the larvae. Satisfactory procedures have been developed and a colony established which will provide an adequate supply of *taeniorhynchus* larvae and adults for insecticide studies.

The adults are kept in a cage 30 inches square by 25 inches high, with sides of 18-mesh screen and top and bottom of ¼-inch plywood. A front opening, 12 by 15 inches, is closed by a cloth sleeve 30 inches long. Temperature is maintained at about 84° F. and relative humidity at 70 percent. The cage is located in the north side of the room in front of a window through which natural light is available. Undiluted honey and fresh water are provided at all times. A shaved rabbit is placed in the cage daily for 2 to 3 hours. The racks used to hold the rabbits were patterned from a holder designed by Laug (1944). Eggs are collected on damp sphagnum moss,[1] contained in a white enameled pudding pan 9 inches in diameter and 3 inches deep.

For the parent generation approximately 6,000 pupae were placed in the breeding cage for emergence. Three days after the first emergence a shaved rabbit was placed in the cage for the initial blood meal. The sphagnum moss was introduced 3 days later and allowed to remain for 25 days, after which time most of the adults were dead. The moisture content was checked (by touch) daily and water added as needed. When the moss was examined for eggs, great numbers were observed at all levels.

Approximately 20 percent of the moss was transferred to a clean pudding pan and inundated with 2 liters of tap water. Most of the eggs were washed from the moss and settled to the bottom of the pan. The remaining eggs were separated by gently agitating by hand. Hatching began within a few minutes after inundation and was apparently complete within a few hours. About 10,000 larvae were produced. Twenty-four hours after inundation the larvae were distributed in 20 pudding pans, each containing 2 liters of infusion water. This water was obtained by inundating a salt-marsh sod 8 inches in diameter by 1 inch thick with 2 liters of tap water and allowing it to stand for 24 hours. The larvae were fed powdered dog food twice daily. Pupation commenced in 6 days and was completed in 12 days; most of the larvae pupated in 10 days. About 9,150 pupae were placed in the breeding cage for emergence of adults.

From 50 to 75 percent of the insects produced each generation have been utilized in testing, and the colony has been maintained with 10,000 to 20,000 adults. Now in the F^5 generation, the insects are of normal size and vigor. There has been no indication of a reduced fecundity in successive generations.

[1] J. S. Haeger suggested the use of sphagnum moss as an oviposition medium.

Reference Cited

LAUG, E. P. 1944. A multiple rabbit holder. Jour. Lab. and Clin. Med. 29:308–311.