NOTES ON *AEDES TRISERIATUS* EGG INCUBATION AND COLONIZATION

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Aedes triseriatus (Say) has been found to be a natural or laboratory vector of bird malaria (Huff, 1932), dog heartworm (Phillips, 1939), and eastern equine encephalitis (Davis, 1940). The development by Repass (1952) of a laboratory technique for colonizing this species has greatly enhanced its usefulness in laboratory research. However, several instances in which low percentage egg hatches jeopardized the existence of established laboratory colonies have been brought to the attention of the authors. Baker (1935) reported a case in which the hatching of *A. triseriatus* was apparently influenced by the amount of daylight available and he believed the photosensitive ova would not hatch unless exposed to sufficient light. Love and Whelchel (1955) obtained no indication of this effect and concluded that the reduction of the photoperiod in the fall influenced only the time required for the completion of larval development. The present study of the requirements for egg development, hatching, and conditioning was undertaken to determine the means of insuring consistently satisfactory *A. triseriatus* egg hatches.

METHODS. The *A. triseriatus* colony was established with larvae collected from artificial containers in Bonaventure Cemetery, Savannah, Georgia, and was maintained in a cage (22 x 22 x 22 inches) at $75 \pm 5°$ F. and 75 ± 10 percent relative humidity. The cage was screened on three sides and the top with 20-mesh plastic screen. The back and the floor were 0.5 inch plyboard. A screened tunnel (6.5 x 7 x 22 inches) extended from the front to the back of the cage about 8 inches above the floor on the left side. A platform hinged to the back of the cage, and 2 inches above the floor of the tunnel, extended through the tunnel to the front of the cage. An opening, 7 x 9 inches, was provided in the lower right front corner of the cage for the attachment of a cloth sleeve.

A blood meal was offered to the adults for 3 hours each day by placing a rabbit in a 21 x 6 x 1.5 inch metal tray on the tunnel platform which was then wedged upward to force the clipped back of the rabbit against the 20-mesh screen of the tunnel. The adult female mosquitoes readily fed through the screen. A wood block, 6 x 5.5 x 1.5 inches, placed in the tray beneath the rabbit's abdomen forced the rabbit's back against the screen. The outer end of the tray, 3.5 inches high, served as a barrier preventing the rabbit's escape. This type of cage eliminated the loss of mosquitoes usually encountered while placing an animal into, and removing it from, a stock cage. Seedless raisins, a pad soaked with a one-molar sucrose solution, and water were provided fresh each week as mosquito nourishment.

From September through mid-January, an automatically-operated light system furnished a 45-minute evening crepuscular period followed by 10 hours of darkness.

The use of saturated paper toweling as an oviposition site, instead of chips or blocks of wood as devised by Repass (1952), facilitated egg handling and storage. Eggs were laid upon a strip of moist paper toweling 3 inches wide, lining an uncovered 600-ml. beaker containing about 200 ml. of water. The water and the toweling were placed in the beaker approximately 24 hours before being placed in the colony cage.

To determine the time needed for completion of embryonic development, eggs

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of known ages were tested at 79 ± 3° F. Beginning on the third day with a given egg batch and continuing through the eighth day, replicate lots of about 100 eggs were removed on successive days from the oviposition beakers and immersed in tap water. After submergence in tap water for 24 hours, the percentage of egg hatch was determined. All unhatched eggs were dissected, and the degree of embryonic development recorded.

Tests were conducted to compare the percentage of hatch obtained among eggs immersed in tap water and in other media. In preliminary trials, the following media were tested: 50 milligrams of ground dog chow per liter of water, and 100 milligrams, each, of ground dog chow and of yeast per liter of water. Subsequent tests compared the percentage of hatch obtained in four mixtures of dog chow and yeast in water. The proportions of dog chow to yeast were 50:50, 100:50, 50:100, and 100:100 milligrams per liter of water. The eggs from a single batch were divided into four approximately equal groups and were submerged in one of the four media for either 18 or 22 hours. In any given test the eggs in each of the four groups were submerged for the same time period. All unhatched eggs were dissected to determine the percent viable.

Dissection of the eggs was accomplished under a dissecting microscope using *minuten Nadeln* attached to applicator sticks. To prevent the eggs from rolling during the dissection, they were placed in drops of clean water on the adhesive surface of drafting tape attached, adhesive surface up, along the length of a 1 x 3 inch microscope slide.

Each dissected egg containing a larva showing complete embryonic development and movement was considered viable. Well developed embryos in which the cephalic egg breaker was darkly pigmented constituted the criterion for complete embryonic development.

Eggs from given batches were matured under different relative humidities at 79 ± 3° F. to determine which would give the more satisfactory hatches. Hatches obtained in paired tests utilizing approximately equal numbers of eggs from a given egg batch were first compared with eggs incubated at relative humidities of 70 and 80 percent and later with eggs incubated at relative humidities of 80 and 90 percent.

The 70, 80, and 90 percent relative humidities, maintained with saturated salt solutions of sodium nitrate, potassium bromide, and barium chloride, respectively, varied ± 2 percent. Eggs were subjected to a saturated atmosphere by placement on moist paper toweling in covered oviposition beakers. The effect of the direct contact of the toweling with water may be important, but its significance was not tested.

On each successive day following oviposition, the saturated toweling containing a batch of eggs laid in a single day was removed from the oviposition beaker. Two strips of toweling, each containing about 100 eggs, were cut from the egg batch. Each of the strips was hung on a rack in a chamber maintaining one of the desired relative humidities. The remainder of the egg batch was returned to the saturated atmosphere in the oviposition beaker. Thus a series was obtained in which 6-day-old and 8-day-old eggs had been incubated at saturation, at either 70 or 80 percent relative humidity, and at all of the possible daily combinations of 70 percent relative humidity and saturation, or 80 percent relative humidity and saturation. Following storage at each of the test conditions, the percentage hatch of 6-day-old and of 8-day-old eggs was determined by immersing them for 22 hours in equal quantities of a standard hatching medium. The percentage of hatch was based upon egg and larval counts made at the end of the immersion period.

At weekly intervals, samples of eggs were taken from a given batch to determine the effect of aging on the percentage of hatch. Ten egg batches, each laid

in successive weeks, were used. Two batches contained only enough eggs for testing four consecutive weeks, and no samples from any batch were tested beyond the eleventh week. Although attempts were made to use 100 eggs in each weekly sample, the variation in egg density and the number of eggs per batch resulted in samples ranging from 32 to 340 eggs with a mean of 106 eggs for the 63 samples tested. All of the eggs were incubated for 6 days in the oviposition beaker before being stored at 80 percent relative humidity. The temperature for incubation, storage, and testing was $75 \pm 5°$ F. In each test, the eggs were submerged for 4 hours in equal quantities of a standard hatching medium.

RESULTS. Mating in the colony was observed to occur during periods of semi-darkness. The males grasped the females during flight, the pair fell to the floor of the cage, and mating was completed in about 5 seconds.

Oviposition occurred principally at night. Egg batches ranged in size from 159 to 1,120 eggs.

At $79 \pm 3°$ F. a minimum of 4 days was found necessary for complete development of the *A. triseriatus* embryo. Six days was the minimum time in which complete development by all the eggs within a batch resulted in a 100-percent hatch. Based on the number of viable eggs, averages of 0, 43, 48, 74, 75, and 95 percent hatched when 3 through 8 days old, respectively; based on the total number of eggs, averages of 0, 19, 18, 56, 16, and 78 percent hatched.

Preliminary trials showed that 1-day-old mixtures of dog chow and brewers' yeast in water stimulated hatching more than fresh or 1-day-old media of tap water, dog chow in water, yeast in water, or fresh mixtures of the dog chow and yeast in water. On the basis of 12 tests of four different dog chow-yeast combinations, the mixture containing 0.2 gram each of ground dog chow and of powdered brewers' yeast per liter of water yielded the largest mean percentage hatch of viable eggs and of total eggs. A 1-day-old formulation of this mixture was adopted as the standard hatching medium.

No hatches greater than 30 percent were obtained in 38 replicate tests obtained from either 6- or 8-day-old eggs incubated continuously at 70, 80, or 90 percent relative humidity. These poor hatches emphasized the need for an incubation period in a saturated atmosphere. Six-day-old eggs incubated continuously in a saturated atmosphere averaged 78 percent hatch, the best average recorded for that age group; the 8-day-old eggs continuously held in a saturated atmosphere averaged 75 percent hatch, but this was not the highest average hatch among this age group.

Six- and 8-day-old eggs incubated for a portion of their prehatching period at 80 percent relative humidity consistently yielded larger mean percent hatches than eggs incubated for similar periods of time at either 70 or 90 percent relative humidity. Nineteen replicate paired tests compared hatches between 6-day-old eggs incubated for known portions of their prehatch period at either 70 or 80 percent relative humidity and at either 80 or 90 percent relative humidity. An average 69 percent hatch of eggs, conditioned 5 days in the saturated atmosphere of the oviposition beaker and 1 day in the 80 percent relative humidity chamber, was the best result obtained in the series. In a similar series with 8-day-old eggs, the best mean hatch of 90 percent was obtained from eggs incubated 6 days in the saturated atmosphere of the oviposition beaker and 2 days in the 80 percent relative humidity chamber. Eight-day-old eggs yielded 100 percent hatches in two cases. One of these was with eggs incubated 7 days in the oviposition beaker and 1 day in the 80 percent relative humidity chamber and the other was incubated under the preceding conditions for 5 and 3 days, respectively.

Egg hatch decreased as egg age increased beyond 2 weeks. An average of 75 percent of the eggs 1 and 2 weeks old

hatched. Slightly over 50 percent of all the eggs 3 through 8 weeks old hatched but only 33 percent of all the eggs 9 through 11 weeks old hatched. However, in one egg batch, hatches averaging 92 percent per trial occurred through the eighth week. The first trial in which no hatch was obtained occurred during one of the two trials with 11-week-old eggs.

The larvae were fed Purina dog chow which had been ground and sifted through a 40-mesh screen. Ten to 14 days usually were required for development from the egg to the adult stage. Crowding, overfeeding, underfeeding, or reduction of temperature increased the time required for larval development.

SUMMARY. A self-sustaining laboratory colony of *A. triseriatus* was readily established and maintained from field-collected larvae. Oviposition upon moist paper toweling showed the use of wooden blocks for oviposition sites to be unnecessary. A technique utilizing the adhesive surface of drafting tape to prevent eggs from rolling during dissection is described.

A minimum of 4 days at approximately 80° F. was found necessary for the development of the embryo, and 6 days was the minimum time in which complete development by all the eggs in a batch provided a 100 percent hatch.

The percentage of egg hatch was increased by hatching eggs in day-old media consisting of 0.2 gram each of ground dog chow and of powdered brewers' yeast per liter of water.

The need for a period of incubation in a saturated atmosphere (over water on a moist substrate) was indicated by the low hatches obtained in paired tests of 6- and 8-day-old eggs incubated continuously at about 80° F. and 70, 80, or 90 percent relative humidity.

Six- and 8-day-old eggs matured for a portion of their prehatch period at 80 percent relative humidity consistently produced larger hatches than those which had been incubated a similar portion of their prehatch period at either 70 or 90 percent.

A 78 percent hatch of 6-day-old eggs incubated continuously in a saturated atmosphere was the best average recorded for that age group. The best mean hatch of 8-day-old eggs, 90 percent, was obtained from eggs incubated the first 6 days in a saturated atmosphere and the last 2 days in 80 percent humidity.

A reduction in the percentage of eggs hatching was found to occur when egg age exceeded 2 weeks.

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