EFFECT OF FOOD AND TEMPERATURE ON *AEDES AEGYPTI* (L.) AND *AEDES TRISERIATUS* (SAY) LARVAL DEVELOPMENT[1]

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Detection of *Aedes aegypti* is based on larval searches and ovitrap examinations (Fay and Eliason, 1966). Water in the artificial containers acts as a hatching stimulus for the eggs. As subsequent temperature and food conditions would influence the occurrence of larvae and the activity of potentially ovipositing adults, a study of these factors would aid in determining when each detection method could be used to advantage.

Christophers (1960) reviewed studies on the duration of larval development of *Ae. aegypti*. The data were primarily concerned with constant temperatures, however, and not with the accelerating or inhibitory effect of varying temperatures or the amount of food available to the larvae. Studies on the larvae of *Ae. aegypti* have shown that the temperature limits for development to adults, although difficult to determine precisely, appear to be 57° F. and 96.8° F. (Bar Zeev, 1958).

The present study was undertaken to determine the effect of temperature in combination with varying amounts of food upon the larval development of *Ae. aegypti* and *Aedes triseriatus*. No attempt has been made to determine either the lethal or optimal developmental temperatures under the varying food supplies.

MATERIALS AND METHODS. The *Ae. aegypti* strain used in this study, colonized originally in Puerto Rico, has been maintained at this laboratory for 7 years. The *Ae. triseriatus* (Alabama strain) has been maintained here since 1965.

A properly conditioned egg strip of *Ae. aegypti* (Fay, 1964) or *Ae. triseriatus* (Hayes and Morlan, 1957) was placed in 95° F. water and the resulting first instar larvae, in groups of 25, were placed in 10-inch diameter enameled pans containing 1 liter of tap water of the temperature under consideration. The larval food was finely ground standard laboratory chow containing 23 percent crude protein, 4.5 percent crude fat, 6 percent crude fiber, and 9 percent ash. Full rations of food per larva, slightly modified from that of Morlan *et al.* (1963), were 0.15 mg. and 0.30 mg. per larva on days 0 and 1, respectively, and 0.60 mg. of food per larva thereafter. The larva raised on ½ and ¼ rations received proportionately less food per day.

Two controlled-atmosphere chambers were used for each experiment, one at a constant temperature, the other at a 20 degree fluctuating temperature on a 24-hour cycle. The temperatures used were 90° F. versus 80°–100° F. (Condition A), 80° F. versus 70°–90° F. (B), 70° F. versus 60°–80° F. (C), and 60° F. versus 50°–70° F. (D). All experiments were conducted at 80 percent relative humidity.

Six enameled pans each containing 25 larvae were shelved in each chamber in the following positions:

Top left-bottom right=¼ food
Top center-bottom center=½ food
Top right-bottom left=full food

The resulting pupae were held at a temperature of 78° F. and 60 percent relative humidity. The adults emerging into gallon ice cream cartons covered with 40 mesh nylon netting were fed 10 percent honey water and given three successive blood meals (rabbit) on days 3, 4 and 5 of adult life. The day of initial oviposition was recorded.

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RESULTS AND DISCUSSION. Standard Food Conditions (*Ae. aegypti*). At temperatures of both 90° F. and 80°–100° F. (A), the first pupae appeared on day 5 and 90 percent pupation occurred on days 9 and 7, respectively (Table 1). At 80° F. and 70°–90° F. (B), initial pupation took place on day 6 and 90 percent pupation occurred on day 8. The 10 degree drop in test temperatures had the net effect of a 1-day lag in pupation. In both experiments the fluctuating temperature had approximately the same effect as the constant temperature.

Another 10 degree drop in temperature to 70° F. and 60°–80° F. (C) resulted in an initial pupation time of 10 days and 9 days, respectively, with 90 percent pupation on day 13 at 70° F. constant and on day 12 for 60°–80° F. During each 24-hour period the chamber temperature changes at a uniform rate from 70°–60° F. and back to 70° F. in 12 hours and then from 70°–80° F. and back to 70° F. in the succeeding 12 hours. A slight acceleration of development apparently occurs during the 70°–80° F. period without a corresponding inhibitory effect during the 60°–70° F. period under the fluctuating temperature conditions.

At 60° F. and 50°–70° F. (D) initial pupation took place on days 33 and 21, respectively. Ninety percent pupation was not reached at either of these experimental temperatures due to high larval mortality. Results at these temperatures showed a great deal of variation but the percentage pupation was always greater at the 50°–70° F. conditions. Exposures of 12 hours at 50°–60° F. are offset by the acceleration of development which takes place in the 12 hours spent in temperatures from 60°–70° F.

The temperatures (D) at which this last experiment was conducted approach the threshold temperature for *Ae. aegypti*. The larvae are not active, do not feed well resulting in accumulation of food, fouling of the water, and high larval mortality not evidenced at the other temperatures.

One-Half and One-Fourth Food Conditions (*Ae. aegypti*). When compared with full food ration (Table 1), there is an average 1-day lag in initial pupation at ½ food and an average 2-day lag at ¼ food levels for all temperatures except (D), 60° F. and 50°–70° F. where results were less consistent. As with full ration at 60° and 50°–70° F., the initial pupation at 50°–70° F. at both ½ and ¼ food was faster than at 60° F. constant. It should be noted that those larvae which did not pupate died.

The time periods to 90 percent pupation emphasize the effect of the availability

TABLE 1.—Rate of development of *Ae. aegypti* with various food and temperature conditions. Values represent mean of two replicates.

	Temp. conditions (° F.)	Initial pupation (days)			90% pupation (days)			Initial oviposition (days)		
		Full food	½ food	¼ food	Full food	½ food	¼ food	Full food	½ food	¼ food
A	90	5	6	7	9	9	12	12	13	14
	80–100	5	6	8	7	11	15	12	13	15
B	80	6	7	8	8	10	13	13	14	16
	70–90	6	7	8	8	9	(66%)*	13	13	16
C	70	10	10	11	13	15	16	16	18	20
	60–80	9	11	11	12	15	(88%)	16	19	20
D	60	33	28	36	(46%)	(32%)	(17%)	45	36	51
	50–70	21	21	23	(61%)	(62%)	(52%)	30	30	32

* Ninety percent pupation not obtained, percent pupation indicated in parentheses.

of food on the rate of development more than do the intervals for initial pupation.

Ae. triseriatus. Similar larval development tests were made with *Ae. triseriatus,* since this species has comparable ecological habitats to *Ae. aegypti* and its eggs are commonly found in ovitraps.

Larval development of *Ae. triseriatus* (Table 2) when compared with *Ae. aegypti* (Table 1) for the temperature conditions (A), (B) and (C) showed larval development of the former to be 1–4 days slower and the time to initial oviposition to be 7–10 days slower. These factors, combined with 3 days for conditioning of the eggs of *Ae. aegypti* and 6 days for *Ae. triseriatus,* give a mean generation time for temperatures (A), (B) and (C) of 21.1 days for *Ae. aegypti* as compared to 29.6 days per generation for *Ae. triseriatus.*

In considering condition (D) for *Ae. triseriatus,* no pupation occurred in the 90-day test period at 60° F. at ¼ food. At the 50°–70° F. condition of (D) only a few larvae pupated (Table 2) at any food level and in contrast to *Ae. aegypti* the remaining larvae entered diapause in the fourth instar with relatively little mortality. These data agree with the results obtained by Wright (1966) who, working with the same strain of *Ae. triseriatus,* found that a low temperature of 65° F. would maintain larval diapause. Whether these test conditions pertain in field populations of these two species is another question, but it is evident that at temperatures of 70° F. or higher *Ae. aegypti* has a reproductive advantage over *Ae. triseriatus.* At the 60° F. or 50°–70° F. conditions, although *Ae. aegypti* completes development to the pupal stage more successfully than *Ae. triseriatus,* the larval mortality is considerable. The fourth instar larvae of *Ae. triseriatus* are able to withstand these temperature conditions for extended periods of time.

After the 90-day test period one-half of the diapausing *Ae. triseriatus* larvae in each of the ¼, ½ and full food regimes at 60° F. and 50°–70° F. were removed and held at 80° F. and 80 percent relative humidity. Under these conditions, there was a subsequent 7 percent larval and 30 percent pupal mortality of the larvae reared for 90 days at 60° F. and a 2 percent larval and 12 percent pupal mortality of those reared at 50°–70° F. Pupation commenced on day 96, resulting in normal adults, and oviposition was recorded in samples from all food and temperature combinations. All fourth instar larvae

TABLE 2.—Rate of development of *Ae. triseriatus* with various food and temperature conditions. Values represent mean of two replicates.

	Temp. conditions (° F.)	Initial pupation (days)			90% pupation (days)			Initial oviposition (days)		
		Full food	½ food	¼ food	Full food	½ food	¼ food	Full food	½ food	¼ food
A	90	6	7	8	9	11	17	19	23	22
	80–100	9	9	11	11	16	19	20	22	23
B	80	9	10	10	12	15	19	21	20	26
	70–90	10	10	11	13	16	20	21	23	25
C	70	13	13	14	16	17	20	26	27	27
	60–80	12	12	12	15	17	21	26	27	27
D	60	30	30	*	(4%)‡	(2%)	(0%)	†	†	†
	50–70	24	27	34	(52%)	(12%)	(2%)	40	44	†

* No pupation in 90-day test period.
‡Ninety percent pupation not obtained, percent pupation indicated in parentheses.
† No oviposition.

kept at 60° F. or 50°–70° F. receiving ¼, ½, or full rations remained in diapause during this period.

It is hoped the data from this study showing the minimum time periods from egg to egg under different conditions of temperature and food may be correlated with the rainfall and temperature data in local areas, to estimate the number of generations in any given year, as well as to guide the operational surveillance techniques. It must be borne in mind, however, that since *Ae. aegypti* eggs in the field will be stimulated to hatch by varying amounts of successive rains, there may be overlapping of generations.

SUMMARY. Using 90°, 80°, 70°, and 60° F. constant temperatures and regular temperature cycles of ±10° F., from these values combined with full, ½, and ¼ food rations, the effects on larval development of *Ae. aegypti* and *Ae. triseriatus* were studied in the laboratory using initial and 90 percent pupation and initial oviposition as evaluation criteria. At 90° F. or 80°–100° F., initial pupation of *Ae. aegypti* occurred at 5, 6, and 7–8 days and oviposition occurred at 12, 13, and 14–15 days at full, ½, and ¼ rations, respectively. The above values were extended by 1 day at 80° F. or 70°–90° F. and by 3–6 days at 70° F. or 60°–80° F. At 60° F. or 50°–70° F., deleterious effects of the test conditions were evident. In general, values for *Ae. triseriatus* showed the same pattern but the time periods were prolonged and the species was better adapted to the colder test conditions.

References

BAR ZEEV, M. 1958. The effect of temperature on the growth rate and survival of the immature stages of *Aedes aegypti* (L.). Bull. Ent. Res. 49:157–163.

CHRISTOPHERS, S. R. 1960. *Aedes aegypti,* the yellow fever mosquito, its life history, bionomics and structure. Cambridge Univ. Press, XII, 739 pp.

FAY, R. W. 1964. The biology and bionomics of *Aedes aegypti* in the laboratory. Mosq. News 24(3):300–308.

FAY, R. W., and ELIASON, D. A. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti.* Mosq. News 26(4):531–535.

HAYES, R. O., and MORLAN, H. B. 1957. Notes on *Aedes triseriatus* egg incubation and colonization. Mosq. News 17(1):33–36.

MORLAN, H. B., HAYES, R. O., and SCHOOF, H. F. 1963. Methods for mass rearing of *Aedes aegypti* (L.). Pub. Hlth. Rept. 78(8):711–719.

WRIGHT, J. E. 1966. Diapause induction, maintenance, and termination studies on larvae of *Aedes triseriatus* (Say) (Diptera:Culicidae). Ph.D. thesis. The Ohio State University, 81 pp.

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