INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON THE VIABILITY AND HATCHING OF *AEDES AEGYPTI* (L.) EGGS

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A comprehensive book compiling research information accumulated on the yellow fever mosquito, *Aedes aegypti* (L.) was published recently by Christophers (1960). The chapter dealing with techniques and laboratory rearing describes various methods for the maintenance of laboratory colonies. In most of the papers dealing with rearing techniques, the problems of conditioning of the eggs and the hatching stimulus are discussed. Recently several such papers dealing with these problems in aedine eggs have been published (Judson 1963, Muspratt 1962, Mallack *et al.* 1964, Quraishi *et al.* 1963). Studies elucidating optimum temperature and relative humidity, however, are lacking.

During the course of maintaining a laboratory colony of *A. aegypti* in Riverside, difficulties were encountered in obtaining sufficient egg, larval, and pupal populations. The eggs of the colony during the conditioning period were kept at room temperature (80° ±2° F.) and a relative humidity of 50–80 percent. The magnitude of hatch in eggs treated in this manner was quite low. It was surmised that the ambient relative humidity (averaging about 65 percent) was suboptimum for conditioning of *A. aegypti* eggs. The current studies were initiated to investigate the role of various relative humidities and temperatures during the conditioning period of the eggs of *A. aegypti*.

Aedine eggs require hatching stimuli for proper hatching. Some of these stimuli have been reported to consist of reduction of ambient oxygen concentration (Gjullin *et al.* 1941). Several agents have been claimed to be effective in providing this stimulus (Borg and Horsfall 1953, Burgess

1959). In the maintenance of our *A. aegypti* colony tap water or tap water containing Fleischmann's yeast gave little hatch. The hatch was also low when tap water was deoxygenated by boiling. Moreover, this method was found to be inconvenient for continuous use. Horsfall (1956) used corn broth as a hatching stimulus for *A. vexans* (Meig.) eggs. Standardization of corn broth to get consistent results involves numerous inconveniences. Ascorbic acid has been used by some authors with good results (Borg and Horsfall 1953, Horsfall 1956, Judson 1960). The minimum optimum concentration of ascorbic acid for hatch of properly conditioned eggs in a certain period of time, however, has not been fully investigated. The efficacy of ascorbic acid solution as a hatching stimulus was, therefore, studied further.

MATERIALS AND METHODS. Various static relative humidity levels were obtained by using saturated salt solutions placed in desiccators (Wexler and Brombacher 1951, Rockland 1960). Two desiccators having top inside diameters of 158 mm. (depth to the plate being 95 mm.) and 100 mm. (depth to the plate being 65 mm.) were used for each saturated salt solution. Three incubating chambers were set up with constant temperatures of 80°, 90°, and 100° F. The variability in temperature level was ±2° F.

Strips of paper towels with eggs of an average age of 12 hours were removed from colony cages and cut into smaller strips, each holding a number of the eggs. Several such strips were placed in each of the desiccators maintained at the desired temperature. At intervals of 3, 4, and 5 days, egg strips were removed from each desiccator and submerged in 50 ml. of 0.025 percent solution of ascorbic acid in 4-oz. treated paper cup. This concentration of ascorbic acid was found to yield satisfactory hatch of conditioned eggs. Extent of hatch of eggs was assessed 24 hours after start of exposure in the ascorbic acid solution. Using a dissecting microscope, the hatched eggs with their opercula pushed off could be easily distinguished from the unhatched ones.

In order to study the efficacy of ascorbic acid solution as a hatching stimulus, eggs were conditioned for 5 days in 100 percent relative humidity at 80° F. Ascorbic acid solutions (wt./vol.) were made in tap water. Various dilutions were tested. The conditioned eggs were placed in the ascorbic acid solution for 24 hours and the extent of hatch assessed in the above manner.

RESULTS AND DISCUSSION. Effects of temperature and relative humidity during conditioning of *A. aegypti* eggs are shown in Table 1. The data indicate that there is a direct relationship between the level of relative humidity and the percent egg hatch. An inverse relationship, however, is evident between temperature and the percentage of hatch. Egg viability declined markedly at the lower relative humidities. At 80° F. and 90° F. at the suboptimum relative humidities of 67 and 84 percent level of egg hatch increased as the exposure period increased. This suggests that longer periods for conditioning are needed at these humidities. A period of three days was not sufficient for optimum conditioning at any of the optimum or suboptimum humidities.

Judson (1960) reported slightly reduced hatching response when eggs of *A. aegypti* were conditioned at 50 percent relative humidity for 2–8 weeks. In our studies, maximum hatch was obtained when the eggs were conditioned at 90 percent and 100 percent relative humidity with no significant difference in percent hatch between the two.

The highest temperature (100° F.) studied here was for all practical purposes lethal to the eggs of *A. aegypti* at all levels of relative humidities studied. Eggs at low relative humidities underwent considerable shrinkage, shriveling and disintegration due to desiccation. Effects of high temperature and low humidity were so severe that most of the eggs appeared ripped off longitudinally (Figure 1A). Normal eggs on the other hand maintained as controls at the high humidities

TABLE 1.—Effect of relative humidity and temperature on the viability of *Aedes aegypti* eggs exposed for various periods.

Relative humidity % and material used	Hatch of eggs (%), conditioned at various temperatures for various periods																	
	80° F.						90° F.						100° F.					
	3 days		4 days		5 days		3 days		4 days		5 days		3 days		4 days		5 days	
	No.	%H	No.	%H	No.	%H	No.	%H	No.	%H	No.	%H	No.	%H	No.	%H	No.	%H
100 (Dist. water)	148	79	269	95	160	95	36	50	52	69	37	78	120	4	176	0	84	0
90 (KNO_3)	101	78	149	94	127	95	50	52	68	66	58	79	194	20	177	0	109	0
84 ($Li_2SO_4H_2O$)	203	88	190	94	152	93	38	26	38	29	31	29	91	0	177	0	75	0
67 ($CuCl_2$)	247	28	154	62	170	82	42	10	53	0	57	28	110	0	173[a]	0	144[a]	0
32 ($MgCl_2$)	162	5	107	7	141	13	50	0	49	0	59	0	129	0	143	0	144	0
11 (LiCl)	136	4	227	0	174	0	57	0	53	0	60	0	83	0	193	0	133	0

[a] Some of the eggs conditioned at 80° F. and most of those conditioned at 90° F and 100° F. were shriveled due to excessive desiccation. Some eggs ripped off longitudinally.

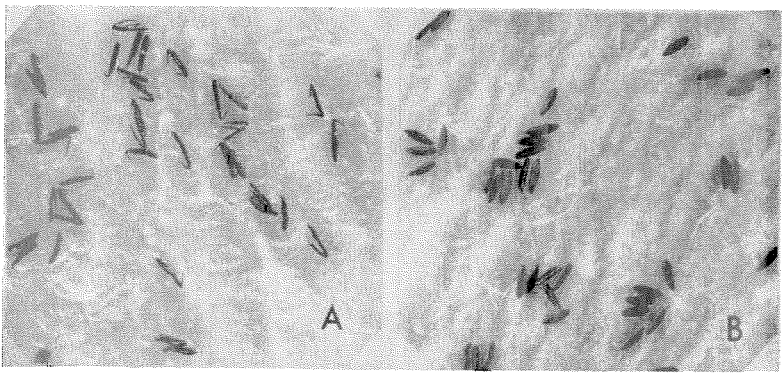


FIG. 1.—Effect of relative humidities on the eggs ot *Aedes aegypti* conditioned for 5 days. A—eggs conditioned at at 11% R.H. at 90° F.; B—eggs conditioned at 90% R.H. at 90° F.

were found to be viable and of normal appearance (Figure 1B).

Although the 100 percent relative humidity at 80° F. was found more suitable for conditioning the eggs, this relative humidity level was quite undesirable from the standpoint of accumulation of excessive moisture condensing on the sides of the chamber and the paper strips. Under these conditions many eggs would hatch on the strips and heavy loss of larvae would occur due to this earlier hatching. As the 90 percent relative humidity was found equally suitable at 80° F., this level has been successfully used in routine rearing of the species. There is no moisture condensation at this level of relative humidity and eggs conditioned at this humidity have high viability.

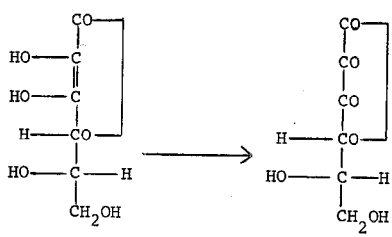
Results of different concentrations of ascorbic acid on the hatch of the conditioned eggs are shown in Table 2. Maximum hatch was observed in the 0.1 percent solution but this concentration was found to be toxic to the newly-hatched larvae, which died while in the solution for 24 hours. Concentrations lower than this were not toxic.

TABLE 2.—Effect of ascorbic acid on the hatch of *Aedes aegypti* eggs[a].

Conc. ascorbic acid (%)	No. submerged	% hatched
0.1	104	95
0.05	158	86
0.025	150	89
0.01	103	89
0.005	186	73
0.0001	147	57
0.0 (water)	138	52

[a] Eggs were conditioned for 5 days in 100% R.H. and 80° F.

Ascorbic acid is a powerful reducing substance (Smith 1954). The acid reacts readily with ambient oxygen to give the oxidized form dehydroascorbic acid:



Borg and Horsfall (1953) reported 100 percent hatch of properly conditioned *A. aegypti* eggs using 0.00006 M (0.0018 percent) ascorbic acid. Lower concentration like 0.00003 M (0.0009 percent), however, did not give any hatch within 24 hours. Judson (1960) reported 0–12 percent hatch in one hour using 0.005 M (0.11 percent) ascorbic acid, even though the eggs were conditioned for a fairly long time (2–8 weeks) in 90–100 percent relative humidity. A 0.1 percent solution was used

to obtain as much as 84 percent hatch of *A. vexans* eggs but no toxicity was reported (Horsfall 1956).

From the present findings it appears that the concentrations used by Borg and Horsfall (1953) were too low to obtain a high percentage of hatch in 24 hours. A 100 percent hatch of eggs using 0.00006 M ascorbic acid might be due to the use of water which was already low in its dissolved oxygen concentration. High concentrations as used by Horsfall (1956) and Judson (1960) demonstrated good hatch but these concentrations are not suitable for use in maintenance of a colony as these concentrations are detrimental to the newly-hatched larvae, particularly when the eggs are left in the solution overnight. The present study shows that almost 90 percent hatch can be obtained in 0.01 percent solution. This concentration is low enough so that larvae hatched do not suffer any loss due to mortality. There is no need to remove hatched larvae from this concentration for fear of mortality. After maximum hatch, which usually occurs within 24 hours, the larvae and ascorbic acid solution are transferred to rearing trays to which tap water and food were added. The 0.01 percent concentration of ascorbic acid is now routinely used in providing a hatching stimulus for the conditioned eggs of *A. aegypti*.

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