ENVIRONMENTAL INFLUENCES ON THE VIABILITY OF OVERWINTERING *AEDES AEGYPTI* (L.) EGGS[1]

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INTRODUCTION. In the northern part of its range in the United States the yellow fever mosquito, *Aedes aegypti,* overwinters in the egg stage, yet there is little information on its ability to survive this hazardous period. In laboratory studies Meola (1964), using eggs conditioned for 4 days, obtained hatches of over 50 percent for eggs stored at 50° F. or 80° F. in saturated humidity for 5 months, and hatches above 90 percent for eggs held at 60° F. or 70° F. for similar periods. In evaluating the resistance of the various stages of this mosquito to an extreme of 0.5° C., Bar-Zeev (1957) lists the egg stage as the most resistant with young pupae, larvae, old pupae and adults showing progressively less resistance.

In field tests in Oklahoma, Rozeboom (1939) exposed eggs partially protected in an open bottle from December to April, and only a single egg hatched, whereas in a more fully protected bottle within a screened shed, an unspecified but significant number hatched. During this test period there were up to 9 successive days when the ambient temperature remained below 32° F. In Houston, Texas, Hatchett (1946) allowed *Ae. aegypti* eggs to incubate 72 hours and then placed them dry for 22–109 days under fully exposed conditions for a resultant 17.7 percent total hatch. During this period there were only 5 hours of freezing weather and the minimum temperature never fell below 30° F.

The present paper describes tests made in Savannah, Georgia, and in Meridian, Mississippi, to obtain more complete information on the resistance of *Ae. aegypti* eggs to various factors involved in overwinter exposures. Both conditioned and unconditioned eggs were tested on different substrates for various exposure periods with the subsequent percent hatch being used as the evaluation criterion.

TEST A. MATERIAL AND METHODS. Unconditioned, approximately 24-hour-old eggs of *Ae. aegypti* (wild Meridian strain) deposited on aluminum paddles (Wilton *et al.,* 1968) were clipped inside styrofoam cups and water was added to the egg line at the 2-inch level. The cups were then placed outside and exposed to environmental conditions; all water subsequently supplied to the cups was from natural precipitation. Air temperatures and larval counts were recorded daily to determine the percent hatch as well as the time when hatching ceased in the field. At the same time, naturally deposited eggs in various types of containers in field situations were also under observation.

RESULTS AND DISCUSSION. Observations on 200 naturally flooded unconditioned eggs placed in the field daily from November 1–25, 1968, at Meridian indicate the last seasonal hatch occurred on December 4, 1968, whereas the last hatch of natural field eggs was recorded on November 6, 1968.

From November 15–December 5, 1968, the period of late season hatch, maximum air temperatures averaged 62° F. with a minimum average of 30.4° F. for a 5-day period November 18–22, 1968. A hatch

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TABLE 1.—*Ae. aegypti* egg hatches as influenced by temperatures during the fall season transitional period at Meridian, Miss., 1968

Date	Temperature °F. Max.	Avg.	Min.	Avg.	Paddles showing hatch	Larvae Recorded	Precip. (inches)
Nov. 15	71		49		0	0	0.07
16	77		53		0	0	T
17	72		58		0	0	1.00
18	66)		35)		6	18	0
19	51)		29)		5	49*	0
20	57)	62	28)	30.4	5	17*	0
21	63)		27)		2	2*	0
22	73)		33)		4	5*	0
23	71		41		0	0	0
24	61)		38)		8	30	0.35
25	66)		33)		1	2*	0
26	71)	69	31)	39.6	11	44	0
27	76)		55)		14	42	0
28	72)		41)		26	193	0.32
29	59		35		0	0	T
30	56		45		0	0	0
Dec. 1	63		50		0	0	0.77
2	60		47		10	27*	0
3	60		39		0	0	0.60
4	54		31		2	17*	0
5	64		31		0	0	0

* First-instar larvae dead when counted.

of 91 larvae from 22 paddles showed only 18 to be alive when counted (Table 1). In contrast, average maximum air temperatures of 69° F. with an average minimum of 39.6° F. for the 5-day period, November 24–28, 1968, resulted in a hatch of 311 larvae from 60 paddles, and only two larvae were not alive when counted. On November 17 and 24 rainfalls of 1.00 and 0.35 inch, respectively, flooded all eggs and triggered the two hatch periods mentioned above.

Some eggs did hatch at approximately 62° F., which is above the 55° F. which Christophers (1960) indicates as the minimal hatching temperature. Low temperatures averaging 30.4° F. did, however, kill the newly emerged larvae within a few hours. An increase of 7–9° F. of both maximum and minimum temperatures was sufficient to increase hatch and to sustain living larvae for at least 24 hours as shown during November 24–28 (Table 1).

Of a total of 5,000 eggs placed outside and exposed under these test conditions, 8.9 percent did hatch during late season, and observations of naturally deposited eggs in field containers indicated similar size hatches. Such hatches with subsequent death of the larvae would, in effect, reduce potential spring populations.

TEST B. MATERIAL AND METHODS. At Savannah, Georgia, 48 aluminum paddles, each containing 24-hour-old unconditioned *Ae. aegypti* eggs, were obtained from an insectary colony. Each paddle was clipped inside an individual 1-pint mason jar. Twenty-four jars were held in the laboratory at 80° F. and 65 percent relative humidity, and the remainder were placed outside in a roofed cage protected from rainfall. The temperature was monitored inside the cage. Twelve of the jars in the cage were designated "WET TEST" and 12 "DRY TEST." Twelve jars held in the laboratory were designated WET CONTROL and 12 DRY CONTROL. On week 0–1 (October 18–25, 1968) and for 11 consecutive weeks one jar in the cage (WET TEST) and one jar in the laboratory (WET CONTROL) were flooded with tap water. After being flooded 1 week, larval counts were recorded and all water was removed. The WET TEST jar and one DRY TEST jar were then brought into the laboratory and along with the WET CONTROL jar and one DRY CONTROL jar were flooded with enriched hatching medium for 24 hours and the hatch was recorded. Eggs were allowed to dry for 24 hours and were reflooded to detect any delayed hatch.

RESULTS AND DISCUSSION. The results of the exposure of unconditioned eggs on aluminum paddles at Savannah, Georgia, for up to 12 weeks indicate that WET TEST yielded an overall average hatch of 21.8 percent, whereas the DRY TEST gave only 13.3 percent (Table 2). Only during week 3–4 did DRY TEST hatching exceed that of the WET TEST. Both DRY and WET CONTROL groups gave consistently higher hatches, averaging 72 and 76 percent, respectively. A significant trend toward reduced hatches is evident after 3–4 weeks of exposure under both WET and DRY TEST conditions. The WET TEST eggs held outside appear to have benefitted by the eggs being beneath the water either through the insulating effect or the prevention of desiccation, or through both.

Temperatures during the 12 weeks shown in Table 2 declined with averages in the low 40°'s during weeks 2–4, with extremes in the low 20°'s F. With the exception of the hatches of 25.8 percent and 15.1 percent by outside WET TEST eggs on weeks 4–5 and 5–6, respectively, there were no appreciable hatches after the above-mentioned temperatures occurred.

The cumulative effects of temperatures of 32° F. or lower on these test eggs produced a decrease in the size of hatches in both WET and DRY TEST eggs. Again the WET eggs, in general, revealed the largest hatches, with a marked decline after 3–4 weeks in both WET and DRY eggs.

TEST C. MATERIAL AND METHODS. Eggs of colonized *Ae. aegypti* were deposited directly in #2½ tin cans, in square 4 x 4 x 4½ inch plastic containers and in ⅓ sections of auto tires, all containing 1½ inches of water. Eggs were collected for 24–72 hours in various containers and conditioned at room temperature for an additional 72 hours. A group of these containers with eggs, consisting of two of each type were prepared each week and placed outside for 6 consecutive weeks from December 12, 1968, through January 16, 1969. After each of 12 exposure periods ranging from 800–3064 hours, one set of containers (one tire section, one tin

TABLE 2.—Resultant hatches after field exposure of unconditioned *Ae. aegypti* eggs at Savannah, Ga., 1968–69.

Week	Average Temperature ° F. Max.	Min.	Week	Cumulative hr. at less than 32° F.	Percent Hatch Wet test	Wet control	Dry test	Dry control
0–1	65	37	56.0	13	68.7	97.2	44.9	95.2
1–2	62	37	49.5	28	39.7	63.1	35.1	72.3
2–3	50	30	40.0	68	51.3	86.5	9.1	71.1
3–4	57	30	43.5	103	39.1	68.2	64.9	69.2
4–5	63	39	56.0	113	25.8	83.0	0.0	10.5
5–6	60	39	49.5	118	15.1	24.5	0.6	72.8
6–7	70	46	58.0	127	7.4	41.0	2.2	100.0
7–8	55	39	47.0	*	3.9	90.2	0.8	65.4
8–9	49	32	40.5	*	0.6	100.0	0.3	100.0
9–10	56	33	44.5	*	6.1	61.4	0.3	33.3
10–11	58	31	44.5	*	2.5	97.6	1.0	75.9
11–12	61	37	49.0	*	1.1	87.6	0.3	73.4
Average values					21.8	76.6	13.3	72.4

* Not continued, since egg hatches were less than 7%.

TABLE 3.—*Ae. aegypti* egg hatches under increasing periods of exposure to varying temperature range combinations. Meridian, Miss., 1968–69.

	Total hours				
Exposure	20° F. or less	21°–31° F.	32–55° F.	Above 55° F.	Percent hatch
2688–3064	6	199–275	1528–1631	955–1152	0.0–0.7
800–1200	6	147–200	463–716	131–328	4.4–9.1
1225–2400	0	56–101	712–1237	325–1062	12.3–53.4

can and one plastic container) was brought into the lab and flooded with tap water. Larval counts were made after 24 hours; the container was allowed to dry for 24 hours, was again reflooded, and final counts were made at 72 hours. Each container was cut into sections, and the entire surface examined under a binocular dissecting microscope to determine the number of eggs present, and the percentage hatch for the set under consideration.

While in the field, the above containers, placed directly on the ground, were sheltered by a roof of 4 x 8-foot plywood on 4-foot supports. Since no precipitation could fall into these containers, the water level was artificially maintained at 1½ inches.

RESULTS AND DISCUSSION. The results of the temperatures and exposure periods on percent hatch in groups of containers as previously described are presented in Table 3. With exposure periods from 1225 to 2400 hours and with less than 101 hours exposure at 21–31° F. and no exposure below 20° F., hatches from 12.3 to 53.4 percent resulted. With shorter total exposure periods from 800–1200 hours with 6 hours exposure at 20° F. or less and 147 to 200 hours at 21–31° F., the hatch was reduced to a range of 4.4–9.1 percent. With longer exposure periods from 2688–3064 hours with 6 hours exposure at 20° F. or less and from 199–275 hours at 21–31° F., the hatch was further reduced to a range of 0.0–0.7 percent.

Low temperatures have a decided lethal effect on conditioned eggs with increasing exposure age.

TEST D. MATERIAL AND METHODS. Investigations were also undertaken to determine the effect of 20° F. on *Ae. aegypti* eggs for periods of 2, 3, 4 or 8 consecutive hours' exposure under varied water levels. At the 2-inch level, on each of 24 aluminum paddles, approximately 100 eggs, ascertained through microscopic examination, were collected for 24 hours and conditioned for 72 hours. Another group of 24 paddles were used in the unconditioned state. One paddle was then clipped into each of 48 styrofoam cups.

To three cups holding conditioned eggs, sufficient water was added to establish the water level of 1½, 2 and 2¾ inches. Thus, eggs on the aluminum paddles were fully exposed with water levels at 1½ inches, were at the water-air interface at 2 inches and were flooded at 2¾ inches. Four series of such test cups were prepared. Like series were prepared from unconditioned test eggs, and all eight series were placed immediately into the freezing chamber of a frostless refrigerator at 20° F. Similarly, four control series of conditioned eggs and four of unconditioned eggs were prepared and held at insectary conditions, 80° F. and 80 percent relative humidity.

After exposure periods of 2, 3, 4 or 8 hours, respectively, series of conditioned and unconditioned test eggs were removed from the 20° F. chamber and held with the conditioned and unconditioned control eggs at 80° F. and 80 percent relative humidity for hatch evaluations. After all ice melted, all cups bearing conditioned test eggs and a series of conditioned control eggs were flooded to the top. The series of cups bearing unconditioned test and control eggs were held for an additional 72 hours to allow conditioning and were then flooded. The percent hatch was determined after 24 and 48 hours of flooding. All eggs were then allowed to dry for 24 hours; they were again reflooded for 48 hours, and total hatches were determined.

RESULTS AND DISCUSSION. The effects on eggs of short-term exposures (2–8 hours) at 20° F. (±2°) and of icing conditions with a consideration of water levels in regard to flooded and nonflooded eggs are presented in Table 4. It should be recognized, however, that under normal circumstances viable eggs found in the field under winter temperature regimes are conditioned. The unconditioned eggs were included in this study for comparative purposes.

Findings indicate that conditioned test and control eggs both yielded larger hatches than unconditioned test and control eggs. Generally, however, there were no clear cut distinctions in the hatch rates between eggs exposed to 20° F. for 2 up to 8 hours or between the hatch rates of the refrigerated eggs from the control eggs.

Significant hatches occurred under all test and control conditions regardless of the water levels. With eggs continuously flooded (2¾-inch water levels), hatching also occurred without a prerequisite drying period. Although ice formed on all cups under test conditions, it did not appear to have inhibited viability to any degree, even though at the 2-inch water level most eggs were wholly or partially embedded. External examination of the eggs, microscopically, revealed only a very few which may have been crushed or otherwise damaged by the freezing forces. Obviously, under these test conditions short term exposures were not detrimental to *Ae. aegypti* eggs.

SUMMARY. *Ae. aegypti* (L.) eggs were exposed to field and laboratory test conditions at Meridian, Mississippi, and Savannah, Georgia, to determine their resistance to fall and winter climatic conditions, as measured by their subsequent hatchability. Approximately 8.9 percent of the conditioned eggs hatched during these periods, and the larvae eventually died, thus reducing potential spring populations. With increased exposure, the percent hatch was reduced, but the degree

TABLE 4.—*Ae. aegypti* egg hatches after exposure at 20° F. or 80° F. for periods ranging from 2–8 hours. Meridian, Miss., 1969.

		Conditioned Eggs				Unconditioned Eggs			
		Percent Hatch				Percent Hatch			
Test temp. °F.	Hours of exposure	1½" water[a]	2" water[b]	2¾" water[c]	Average hatch	1½" water	2" water	2¾" water	Average hatch
20	2	52	68	72	60	30	19	19	22
	3	85	73	69	76	20	43	44	34
	4	100	75	67	87	25	15	17	19
	8	84	83	76	81	34	25	93	43
80	2	73	45	84	63	..[d]	..	..	..
	3	..	50	..	..	..	39	..	..
	4	60	46	98	72	10	41	27	33
	8	55	54	70	59	8	32	53	44

[a] Eggs not flooded.
[b] Eggs at air-water interface.
[c] Eggs flooded.
[d] No data.

of reduction was less for eggs which were flooded. Cumulative effects of temperatures at 32° F. or lower also had detrimental effects on hatch of conditioned eggs, whereas cumulative long term exposures at 20° F. were critical. However, short term exposures at 20° F. of both conditioned and unconditioned eggs under laboratory test conditions were not detrimental to egg viability, nor did icing conditions, similar to those encountered in the field, have a significant effect on egg viability.

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DISTRIBUTIONAL RECORDS OF MOSQUITOES ON THE SOUTHERN HIGH PLAINS WITH A CHECKLIST OF SPECIES FROM NEW MEXICO AND TEXAS

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One of the earliest works concerning the mosquito fauna of New Mexico was that of Theobald (1903) in which he described a new species. This was followed by the monograph of Dyar (1922) entitled "The Mosquitoes of the United States," which listed eight species for New Mexico.

An interest in the problem of malaria resulted in surveys of the anophelines of New Mexico and a list of the known mosquitoes of the state. (Barber, *et al.*, 1929; Barber and Forbrich, 1933, and Barber, 1939).

Ferguson and McNeel (1954) published a list of thirty mosquitoes known from New Mexico. With the omission of *Culex apicalis* Adams and *Aedes campestris* Dyar and Knab, these are the records given by Carpenter and LaCasse (1955). Possibly *A. campestris* was omitted because it was listed as a queried species in earlier works (Barber, 1939; Ferguson and McNeel, 1954).

Hill, Smittle and Philips (1958) recorded thirty-three species of mosquitoes from New Mexico but actually added five new records to the Ferguson and McNeel list as they omitted *Orthopodomyia signifera* (Coquillett) and the queried *A. campestris*.

Recent reports by Miller (1962) and Miller, *et al.*, (1964) listed *Orthopodomyia alba* Baker from the Rio Grande Valley, *Aedes thelcter* Dyar from the Pecos Valley and *A. pullatus* (Coq.) from Colfax County, New Mexico, as well as additional distributions of other species previously reported. Miller (1965) keyed the known larvae without giving localities.

Zavortink (1968) included a list of known records of the genus *Orthopodomyia* while Neilsen, *et al.* (1968) included known distribution of those species occurring in tree holes in New Mexico.

The nomenclature used in this paper is after Stone (1965).

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