LABORATORY STUDIES ON THE SUITABILITY OF MUD AS AN OVIPOSITION SUBSTRATE FOR *ANOPHELES ALBIMANUS* WIEDEMANN [1]

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Introduction

The climate of El Salvador, like that of other Central American countries, is characterized by an extended dry season that usually lasts from November to May. During this period, favorable habitats for both larval and adult mosquitoes are minimal. Floodwater mosquitoes, inhabiting the same areas as anophelines during the rainy season, survive the dry season adversity in the same manner as floodwater mosquitoes survive a winter in North America, i.e., by dormant eggs in the soil. The question arises as to how anophelines survive this period in numbers sufficient to account for rather dramatic population increases associated with the advent of the rainy season.

Stone and Reynolds (1939) reported that in Panama sudden increases in anopheline larvae and adults occurred 7 to 10 days after the onset of the rainy season. In early June 1968, a large population of anopheline and floodwater aedine larvae was observed coexisting in a coastal pasture flooded in the initial rainy season inundation. Other unconfirmed reports of similar observations have been received. In light of these occurrences, and notwithstanding the possibility of such survival mechanisms as aestivation and the fact that some anopheline breeding continues throughout the dry season, it was concluded that further studies on possible dry season survival mechanisms associated with the egg stage of Central American anophelines are warranted.

The general question of anopheline resistance to desiccation is discussed by Howard, Dyar, and Knab (1912) who refer to reports from several sources of both egg and larval survival after varying degrees of desiccation. They state, however, that exact observations are needed. Such observations are still lacking, although there are scattered records in the literature which lend impetus to further studies. Stone and Reynolds (1939) recovered *Anopheles albimanus, A. punctimacula,* and *A. tarsimaculatus* (sic) larvae by flooding slightly moist earth from a seepage area that they estimated had not received rain for at least a month prior to the collection of the samples. These authors believed that the larvae came from eggs present in the soil. Buxton and Breland (1952) reported the recovery of *Anopheles,* presumed to be *pseudopunctipennis,* from flooded dry material in Texas. Holstein (1954) treated the subject with reference to *A. gambiae* in Africa. Although this author played down the likelihood of *A. gambiae* being assured dry season survival by means of eggs resistant to desiccation, he did allow a conclusion from his own data that, in regions of light rainfall, *gambiae* breeding places could become dry without ill effect if the interval between two consecutive falls of rain was in the neighborhood of 14 days. This was based on his findings of *gambiae* egg resistance to desiccation up to 16 days under certain conditions.

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This study is concerned with the laboratory aspects of the problem, i.e., the relative suitability of mud as an oviposition substrate and the fate of eggs that are deposited on mud.

MATERIALS AND METHODS

To determine the suitability of mud as an oviposition substrate, a series of tests was conducted in each of which two gravid females of *A. albimanus* from an insectary colony were introduced into lantern globes placed over field mud from a source known to support *albimanus* breeding (Pántano Ticuiziapa, near La Libertad, El Salvador). The mud was saturated with local, bottled drinking water as is used in the insectary colonies. (Because of a surface film, the local tap water is unsuitable for colony work.) Care was taken that no free water was present. The females were provided with a sugar-water pad on the screened top of the globe, and the open bottom of the globe fit snugly inside the rim of the petri dish. This procedure was followed in obtaining eggs for various experiments and was also used with materials other than mud for comparing oviposition on different substrates.

Upon obtaining eggs on mud substrate, the petri dishes were labeled and served as stock egg supplies for various experiments to determine the fate of eggs under different drying conditions. Some mud surfaces were allowed to air dry gradually, others were allowed to dry more slowly in covered petri dishes, and others were kept moist by adding bottled water as necessary.

For studies on egg survival and hatching rates of eggs held under different storage conditions before inundation, a portion of mud harboring eggs was scooped from the stock dishes with a small spatula and transferred to watch glasses for flooding and hatching observations. All experiments involving mosquitoes and eggs in the lantern globe setups were conducted at room temperature (77°–84° F.).

For experiments requiring large numbers of eggs of a known age or for experiments concerning colony mosquito reaction to mud or other substrates, petri dishes containing the substrate(s) were introduced into colony cages, and the eggs obtained were handled in the same manner as those from globes.

Any departures from the methods described above, as well as methods used in adapting the use of mud to operational colony work, are described elsewhere.

RESULTS

SUITABILITY OF MUD AS AN OVIPOSITION SUBSTRATE. The initial experiment in which two gravid females of colony *albimanus* were introduced into each of four lantern globes over petri dishes containing mud and into four setups each containing other substrates showed that the species readily deposited its eggs on mud and in greater numbers than on the other media provided. The data presented in Table 1 show that equal numbers (8)

TABLE 1.—Number of eggs deposited by *A. albimanus* females on different substrates in petri dishes. July 31, 1968.

Dish No.	No. of Females	Substrate* Mud	Water over Mud	Water over Sand	Saturated Sand	Water
1	2	82	54	0	0	0
2	2	86	0	28	0	75
3	2	57	65	0	0	0
4	2	120	0	0	0	68
TOTAL	8	345	119	28	0	143

* Beach sand, field mud, bottled drinking water.

of females deposited an overnight total of 345 eggs on mud, 143 on water, 119 on water over mud, 28 on water over sand, and none on water-saturated sand. Only in the case of the mud did mosquitoes oviposit on all four of the replicates. The mud was from a field habitat known to support *albimanus* breeding, the sand was filtered beach sand, and the water was locally bottled, nonchlorinated drinking water as is normally used in oviposition bowls in the insectary colonies.

MUD VERSUS WATER AS AN OVIPOSITION SUBSTRATE. Although eggs were readily deposited on mud, a sizable number were also deposited on water. To determine which would be used by choice, a total of 14 dishes of mud and 14 of water were put in seven colony cages, 2 mud dishes and 2 water dishes in each cage.

Care was taken to eliminate position-bias of the dishes by alternating positions within the various cages. After one night's oviposition (August 19, 1968), a total of 3,351 *albimanus* eggs were recovered from the 14 mud dishes and only 175 from the corresponding water dishes. An additional observation showed a total of 30 dead adults on the water surfaces as compared with only 14 on the mud surfaces.

The results of the experiment are shown in Table 2. These data, supported by many subsequent observations, indicate that not only do our colony *albimanus* accept mud as an oviposition medium but that they actually favor mud over the bottled water being used in insectary operations.

There is evidence that field water is more acceptable to *A. albimanus* as an oviposition medium than bottled water, but mud is even better. On August 22, 1968, various substrates were introduced into two colony cages, including field mud, field water (both from Pántano Ticuiziapa, near La Libertad, El Salvador), and bottled water. Two replicates of each yielded the following total egg counts after one night's oviposition: mud —3,819; field water—1,931; bottled water —176. Additional data involving other substrates showed 652 eggs from sponge soaked in field water as compared with 105 from sponge soaked in bottled water, and 797 from filter paper over saturated mud versus 104 from filter paper over saturated sand (bottled water in both cases). These data and subsequent observations show a decided preference by *albimanus* for mud-associated media.

HABITAT MUD VERSUS NONHABITAT MUD. To determine if *albimanus* shows a preference for field mud from a known anopheline habitat over mud from a non-mosquito habitat, petri dishes were prepared using field mud from Pántano Ticuiziapa (known habitat) and mud from the garden of the Central America Malaria Research Station (a non-anopheline habitat). Two such dishes, one with mud from each source, were placed in each of three colony cages on the night of August 20, 1968. One night's oviposition yielded a total of 2,025 eggs from the six dishes containing field mud and 1,943 eggs from the six counterpart dishes containing garden mud. The results shown

TABLE 2.—Number of eggs deposited by *A. albimanus* when offered mud and water in insectary colony cages, August 19, 1968. Two petri dishes of mud and two of water in each of seven colony cages.

Colony Cage No.	Petri Dish Containing Mud	Petri Dish Containing Water
R–1	435 (2)*	10 (5)
	360 (1)	46 (9)
R–2	280 (2)	0 (0)
	633 (3)	53 (1)
R–3	212 (0)	25 (6)
	340 (1)	17 (2)
R–4	520 (2)	13 (2)
	350 (3)	11 (2)
S–1	27 (0)	0 (0)
	62 (0)	0 (0)
S–2	0 (0)	0 (1)
	0 (0)	0 (0)
S–3	132 (0)	0 (2)
	0 (0)	0 (0)
Total	3,351 (14)	175 (30)

* Numbers in parentheses indicate dead mosquitoes on the surface of the oviposition dish.

TABLE 3.—Number of *A. albimanus* eggs deposited on field mud (from a known habitat) and garden mud (from a non-anopheline habitat), August 20, 1968. Two petri dishes of each substrate in each of three colony cages.

Cage No.	Field Mud	Garden Mud
R–2	250	280
	148	135
R–3	137	88
	310	380
R–4	180	310
	1,000	750
TOTAL	2,025	1,943

in Table 3, and subsequent observations on the use of both types of mud in insectary operations, indicate no real preference by *albimanus* for the habitat mud. An advantage in actual use, however, is that habitat mud has a better texture for handling eggs than garden mud has.

FATE OF EGGS DEPOSITED ON MUD. Having discovered that *albimanus* would readily deposit eggs on a mud surface under laboratory conditions, it remained to determine the fate of such eggs under varying conditions.

1. Eggs flooded immediately (morning following oviposition) tended to hatch as though they had been deposited on water, i.e., hatching began 2 days after flooding and was somewhat erratic, with most hatching occurring over a period of several hours but with some stragglers continuing to hatch for up to 2 days after hatching began, or 4 days after flooding.
2. Eggs on mud which was allowed to air dry gradually for at least 48 hours after deposition hatched spontaneously within minutes after being inundated. This was true even after the mud had reached a powdery-dry state, but no hatching was observed in such groups after being held for more than 5 days in a gradually drying state (Table 4: S–7, S–8, S–11, R–2, R–9, R–10). However, the holding period among the gradually air-dried groups was extended to as much as 11 days when the soil was remoistened prior to reaching a very dry condition (Table 4: S–1 and R–5).
3. Eggs held on mud maintained in a moist condition exposed to air remained hatchable in one group for up to 15 days (Table 4: S–2). However, in other lots, the time possible to hold the eggs under such conditions and still get hatching was less (Table 4: S–8—8 days, S–10—9 days). Subsequent efforts to hatch eggs held longer than 15 days on mud were unsuccessful.
4. Eggs held on mud in covered petri dishes without further handling showed hatching periods ranging up to 12 days, with hatching occurring spontaneously in minutes or even seconds after being inundated at any time after 48 hours (Table 5).

In all of the pre-inundation hatching experiments, data were obtained by inundating separate portions of egg-containing mud at fixed intervals after oviposition. This was accomplished by simply removing a portion of the mud containing eggs, submerging the contents in a watch glass, and observing hatching under a stereoscopic microscope.

A limited number of experiments were done with wild-caught *albimanus,* and no differences from colony mosquitoes were noted in either their deposition of eggs on mud or the fate of the eggs after deposition.

OPTIMUM FLOODING TIME. Table 6 shows hatching patterns of eggs held on mud in covered petri dishes without further handling and flooded at 24-hour intervals. Twelve lots of eggs recovered from four petri dishes in each of three colony cages on August 21, 1968, served as the stock egg supply. A portion (at least 20) of the eggs from each dish was flooded 24 hours after deposition and other portions at 24-hour intervals thereafter until there was no further hatching or until hatching percentages were negligible. The data (Table 6) show both the rate and completeness of hatch. Eggs held on the oviposition mud for less than 48 hours hatched as though they had been deposited on water, e.g., eggs inundated after being held on mud for 24 hours began hatching 24 hours after inundation, 50 percent had hatched by 33 hours, 90 percent had hatched by 48 hours; and it was 54 hours after inundation before hatching was complete—308 of 325 eggs (94.8%) hatched.

By contrast, eggs held for 48 hours on the oviposition mud before inundation began hatching 1.3 minutes after inundation, 50 percent had hatched within 5.5 minutes, 90 percent within 21.4 minutes, and ultimate hatching had occurred by 78.7 minutes—253 of 260 (97.3%) of the eggs hatched.

A pre-inundation time of 72 hours was similar to the 48-hour pattern. However, holding the eggs for 96 or 120 hours before inundation produced optimum results in terms of immediate hatching and completeness of hatch. For example, eggs held on mud for 96 hours began to hatch in seconds after inundation, 50 percent

TABLE 4.—Hatching records of *A. albimanus* egg batches resulting from inundation of a portion of the eggs on mud at fixed intervals after deposition, August 1968.

Dish No.	No. of Eggs Deposited	Date of Oviposition	Condition of Mud (Exposed to Air)	Date of First Hatching	Date of Last Hatching	No. Days Remained Hatchable
S–1	170	Aug. 4	Aug. 6, hard, cracked; Aug. 8, re-moistened	Aug. 6	Aug. 15	11
S–2	102	Aug. 3	Flooded immediately (20) *	Aug. 5	Aug. 6	3
			Air dried gradually (44)	Aug. 6	Aug. 6	3
			Moisture maintained (38)	Aug. 7	Aug. 18	15
S–3	122	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S–4	155	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S–5	350	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S–6	86	Aug. 4	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S–7	28	Aug. 3	Aug. 6, hard, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	5
S–8	150	Aug. 3	Flooded immediately (110)	Aug. 5	Aug. 6	3
			Air dried gradually (25)	Aug. 5	Aug. 8	5
			Moisture maintained (15)	Aug. 9	Aug. 11	8
S–9	105	Aug. 4	Aug. 6, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	4
S–10	114	Aug. 3	Flooded immediately (20)	Aug. 5	Aug. 5	2
			Air dried gradually (64)	Aug. 5	Aug. 6	3
			Moisture maintained (30)	Aug. 5	Aug. 12	9
S–11	73	Aug. 3	Aug. 6, dry, cracked mud; Aug. 8, crusty dry mud	Aug. 6	Aug. 8	5
R–1	205	Aug. 3	Aug. 6, powdery dry	Aug. 5	Aug. 7	4
R–2	226	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 8	5
R–3	136	Aug. 4	Aug. 6, cracked, dry	No hatch	......	..
R–4	25	Aug. 4	Aug. 6, cracked, dry; Aug. 8, crusty	Aug. 6	Aug. 8	4
R–5	165	Aug. 4	Aug. 6, cracked, dry; Aug. 8, re-moistened	Aug. 6	Aug. 14	10
R–6	200	Aug. 4	Aug. 6, cracked, dry	Aug. 6	Aug. 8	4
R–7	140	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 6	3
R–8	32	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 6	3
R–9	130	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 8	5
R–10	83	Aug. 3	Aug. 6, cracked, dry	Aug. 6	Aug. 8	5
R–11	65	Aug. 4	Aug. 6, powdery dry	Aug. 6	Aug. 8	4
R–12	52	Aug. 4	Aug. 6, cracked, dry	Aug. 6	Aug. 8	4

* Numbers in parentheses refer to sub-lots of eggs subjected to distinct treatments.

TABLE 5.—Hatching records of *A. albimanus* egg batches inundated at fixed intervals after being held on oviposition mud in covered petri dishes.

Dish No.	No. of Eggs	Date of Oviposition	Date of		No. Days Remained Hatchable
			First Hatching *	Last Hatching	
1	108	Aug. 18	Aug. 20	Aug. 28	10
2	92	Aug. 17	Aug. 20	Aug. 28	11
3	63	Aug. 17	Aug. 20	Aug. 28	11
4	78	Aug. 18	Aug. 20	Aug. 28	10
5	91	Aug. 16	Aug. 20	Aug. 28	12
6	34	Aug. 16	Aug. 20	Aug. 23	7
7	127	Aug. 17	Aug. 20	Aug. 28	11
8	65	Aug. 18	Aug. 20	Aug. 28	10
9	None	....	....	....	..
10	138	Aug. 16	Aug. 20	Aug. 28	12
11	206	Aug. 17	Aug. 20	Aug. 28	11
12	84	Aug. 18	Aug. 20	Aug. 28	10
13	45	Aug. 16	Aug. 20	Aug. 28	12
14	150	Aug. 16	Aug. 20	Aug. 28	12
15	None	....	....	....	..
16	95	Aug. 17	Aug. 20	Aug. 28	11
17	142	Aug. 18	Aug. 20	Aug. 28	10

* Hatching occurred in seconds after inundation during first several days and increased to a matter of several minutes toward the final hatching date. Note that no hatching was attempted earlier than 48 hours after oviposition.

had hatched within 2.3 minutes, 90 percent had hatched within 4.6 minutes, and ultimate hatching had occurred by 5.0 minutes. Moreover, 265 of 266 eggs (99.0%) so flooded successfully hatched. The 120-hour eggs showed a slightly slower hatching ultimate (7.1 minutes) and slightly lower completeness of hatch (96.0%).

In eggs held on mud longer than 120 hours (5 days), the hatching became more erratic and required more time, with 53.3 percent hatching in the 144-hour group and 32.8 percent hatching in the 168-hour group; after 216 hours (9 days) on mud only 1.7 percent (4 of 240) of the inundated eggs hatched.

These data and subsequent observations demonstrate that the optimum time for flooding *albimanus* eggs deposited on mud under laboratory conditions is 96 to 120 hours (4 to 5 days) after deposition.

INSECTARY APPLICATION OF RESULTS. Since *A. albimanus*, under laboratory conditions, more readily deposited eggs on mud than on water and the eggs hatched almost instantly and simultaneously when conditioned for at least 2 days on slowly drying mud in covered petri dishes, we have used mud for part of our insectary colony operations. For several months mud alone has been used as an oviposition medium in half of our colony cages. The results have been a saving of shelf space, more uniform larval growth, a cleaner operation, less manipulation, and, above all, increased mosquito production. To date, no problems have been encountered by the use of mud in place of water. Insectary attendants greatly prefer the mud.

TESTS WITH OTHER SPECIES. In limited tests with wild-caught *A. pseudopunctipennis* from a local source, there was a high mortality among females and when eggs were obtained they showed no signs of adaptability to a mud substrate. In dishes allowed to air-dry, the eggs collapsed and, in those where moisture was maintained, the eggs hatched after 2 days even in the absence of free water. This, of course, was fatal to the hatching larvae.

SUMMARY AND CONCLUSIONS. Females of *Anopheles albimanus* from both colony

Table 6.—Average hatching time in minutes and hatching percentages of 12 lots of *Anopheles albimanus* eggs held on mud in covered petri dishes for varying periods of time prior to inundation.

Hatching Pattern	Pre-Inundation Holding Time 24 Hr.	48 Hr.	72 Hr.	96 Hr.	120 Hr.	144 Hr.	168 Hr.	168+ Hr.
Initial hatch	*	1.3 Min.	<1.0 Min.	<1.0 Min.	<1.0 Min.	2.0 Min.	2.8 Min.	**
50% hatch		5.5 "	5.7 "	2.3 "	2.8 "	7.6 "	7.6 "	
90% hatch		21.4 "	31.5 "	4.6 "	6.7 "	15.0 "	16.1 "	
Ultimate hatch		78.7 "	104.0 "	5.0 "	7.1 "	26.6 "	18.6 "	
No. eggs flooded	325	260	279	266	254	240	240	
No. eggs hatched	308	253	258	265	244	128	79	
Percent hatch	94.8	97.3	92.5	99.0	96.0	53.3	32.8	

* Eggs held on mud for less than 48 hours behaved as though they were deposited on water, e.g., eggs inundated after being held for 24 hours on mud showed the following hatching pattern after being inundated: initial hatch—24 hours; 50% hatch—33 hours; 90% hatch—48 hours; complete hatch— 54 hours.

** Even after 120 hours on mud (5 days) the hatching pattern became erratic, and a smaller percentage hatched. Hatching was progressively reduced from 32.8% at 168 hours to only 1.7% (4 of 240) after 216 hours (9 days).

and wild sources readily deposited their eggs on mud substrates under laboratory conditions. The source of the mud (anopheline habitat or nonhabitat) did not appear to be important. When given a choice, the species preferred mud to either bottled or field-collected water. The length of time that eggs deposited on mud survived and remained hatchable depended upon the rate of drying of the mud substrate. Eggs were commonly held on mud in covered petri dishes for 10 to 12 days after oviposition and remained hatchable. They remained hatchable, even on powder-dry substrate, for up to 5 days after deposition when the medium was allowed to air-dry gradually. Some eggs from one batch remained on moistened mud for 15 days and hatched upon inundation.

Hatching occurred almost immediately after inundation when eggs on mud were successfully held for at least 48 hours under any conditions of drying. The optimum time for flooding was found to be 96 to 120 hours after deposition. Eggs so handled began hatching in seconds, and hatching was complete in from 5 to 7 minutes with 96 percent to 99 percent of all eggs hatching.

These studies indicate that *A. albimanus* eggs exhibit a considerable degree of resistance to desiccation. This characteristic has already been manipulated to improve the efficiency of operating the insectary colony of this species. Further, we hope that these results will lend impetus to additional investigations into the possible involvement of egg resistance to desiccation in studies on the dry season survival of *A. albimanus* in Central America. Limited tests with *A. pseudopunctipennis* indicated no adaptation to depositing eggs on mud or egg resistance to desiccation.

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