

SURVIVAL OF *Aedes triseriatus* PUPAE IN THE ABSENCE OF FREE WATER¹

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ABSTRACT. Although pupae are able to survive in the absence of free, unabsorbed water, the timing of disappearance of free water relative to age post-ecdysis is an important factor. Pupae are more vulnerable to the

disappearance of free water within 1 hr following ecdysis than subsequently. Most pupae exposed to conditions devoid of free water die during ecdysis.

It is well-established that immature stages of mosquitoes can survive to adult emergence under conditions devoid of free, unabsorbed water, e.g. in muck, moist soil, etc. (Al-Azawi & Chew 1959; Bick 1948; Bick & Penn 1947; Gunstream & Chew 1957; Headlee 1945; Husbands 1953; Markos & O'Bergh 1958; Nielsen & Nielsen 1953; Schoof, Schell & Ashton 1945; Thurman & Mortenson 1950; Woodard & Fukuda 1971).

The purpose of this study was to determine survival of *Aedes triseriatus* (Say) pupae in the absence of free water as a

function of age post-larval-pupal ecdysis. We particularly wanted to establish whether or not it would be feasible to hold pupae on moist filter paper for experimental purposes.

Ae. triseriatus is found throughout the eastern U.S. and most of the Great Plains, is the most abundant tree hole breeder in North America and will oviposit in almost any artificial aquatic habitat (Jenkins & Carpenter 1946). Tree holes form in various ways including rot cavities where branches have been removed, rotted-out tree stumps and the trunks of adjacent trees growing together. Since tree holes and similar habitats are likely to be temporary water containers, *Ae. triseriatus* is

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probably naturally subjected to conditions where free water has disappeared.

MATERIALS AND METHODS

Eggs (Alabama strain) were hatched in 100 ml of previously boiled tap water and 1st instar larvae were transferred to plastic rearing containers (75 × 63 × 25 cm) with screen floors held about one-third of the way from the bottom. Twenty-four round plastic cups (5 cm diam.; 3.5 cm high) were placed on the screen floor and each cup was half-filled (30.0 ml) with tap water. The box was filled with water to the level of the menisci in the cups. This minimized temperature variation when a rearing container was removed from the incubator. Day old larvae were added, 20–25/cup, along with several pinches of dried beef blood. Food was added as necessary.

To obtain pupae of known ages cups were checked at designated intervals and new pupae removed and held for various lengths of time.

Conditions with no free, unabsorbed water were produced by placing two circles (5.0 cm diam.) of moist filter paper (Whatman #1) in a plastic cup (described above), saturating the paper with water and pouring off unabsorbed water. Pupae were placed in the cup and a lid secured to confine all adults and to prevent drying. All experiments were carried out in an incubator at 27±1°C.

To compare survival on moist filter paper with free water conditions, newly emerged pupae (0–1 hr post-ecdysis) were divided into 3 groups (3 replicates per group): (1) those which would be put on moist paper immediately, (2) those which would be put in free water immediately and (3) those which would be held in water for 24 hr and then be put on moist paper. The number of adults emerging and the number which died before or at some point during pupal-adult ecdysis were recorded.

To evaluate survival as a function of age post-ecdysis, pupae of known ages (0, 5, 15, 30 and 45 min and 1, 2, 3 and 4 hr

post-ecdysis) were held in free water for varying periods of time and then placed on moist paper. The accuracy of age post-ecdysis was to the minute for the 0, 5 and 15 min groups, within 1 to 2 min for the 0, 5 and 15 min groups and 2 to 3 min for the 45 min and 1, 2, 3 and 4 hr groups. Twenty mosquitoes were used in each age group. Again, the number of adults successfully emerging and the number which died before or at some point during pupal-adult ecdysis were recorded.

Before statistical analysis, percent data were transformed to increase the likelihood of approximation to a normal distribution (arcsine transformation—Sokal and Rohlf 1969).

RESULTS

There was no significant difference in mean mortality between pupae placed in free water immediately after ecdysis (Fig. 1, W) and those held in free water for 24 hr and then placed on moist paper (H) ($t = 0.95$, d.f. = 4, $P > 0.05$). Mean mortality in pupae placed on moist paper immediately following ecdysis (P) was significantly greater than in the other 2 groups (P-W: $t = 3.81$, d.f. = 4; P-H: $t = 4.61$, d.f. = 4; $P \leq 0.05$).

Mortality before and during ecdysis is compared in Fig. 2. Regardless of treatment, the proportion of total mortality before ecdysis (1) versus during ecdysis (2) was the same ($F = 0.19$, d.f. = 2 & 6, $P > 0.05$). Thus the results from all 3 treatments could be combined (G). More pupae died during ecdysis (G2) than before ecdysis (G1) ($t = 3.68$, d.f. 8, $p \leq 0.05$).

As holding time in free water increased, percent mortality decreased rapidly (Fig. 3). After 1 hr. mortality averaged 20% (range = 10–25%).

The data in Fig. 3 were divided into 0–1 hr and 2–4 hr groups (Fig. 4). Individuals which died during ecdysis were classified relative to the degree of ecdysis as follows: (1) emerged, but some legs torn off or bent; (2) mostly emerged, but

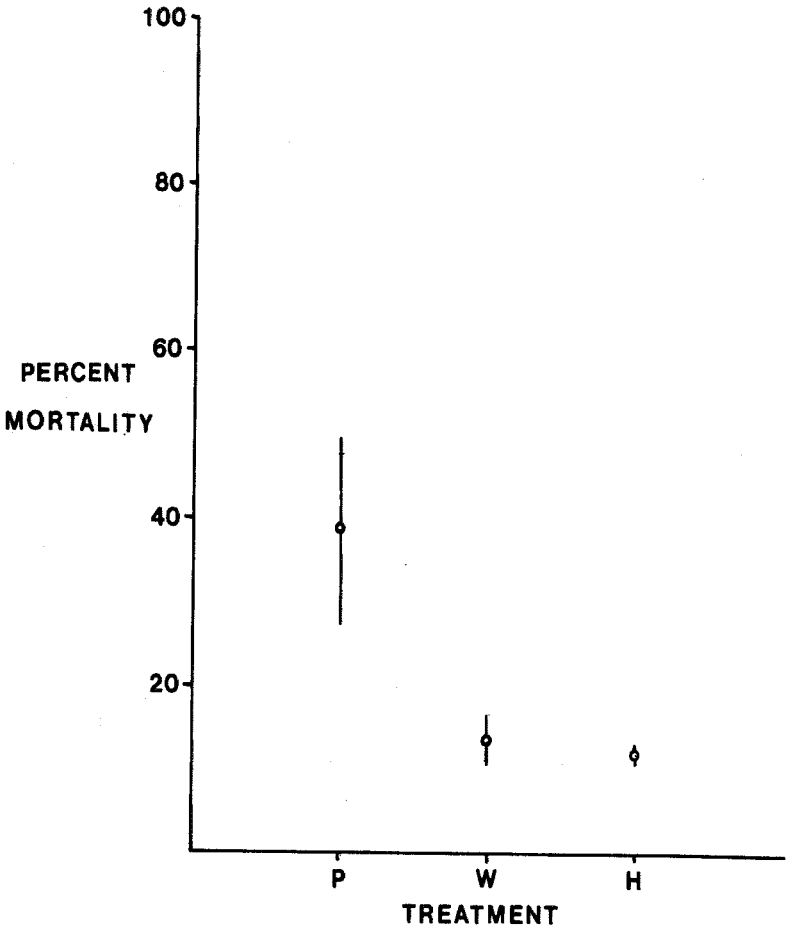


Fig. 1. Mortality: moist paper vs. free water vs. free water/moist paper. P, on moist paper only (number/replicate = 104, 76, 62); W, in free water only only (number/replicate = 108, 59, 64); H, in free water for first 24 hr, then on moist paper (number/replicate = 102, 60, 63); circle = mean; vertical line = standard error.

legs, some or all, still within the pupal exuvium; (3) most of the thorax out of the pupal exuvium; (4) abdomen out of the pupal exuvium, but thorax still enclosed; (5) thorax barely beginning to protrude through dorsal split. Although there was higher mortality in the 0-1 hr group than the 2-4 hr group, a larger proportion of the 2-4 hr group died during ecdysis (83.3 vs. 53.3%). Also among those indi-

viduals which died during emergence, all of the 2-4 hr individuals emerged, but lost legs in the process or emerged except for one or more legs being caught in the pupal exuvium. Individuals in the 0-1 hr group experienced the full range from the thorax just beginning to protrude to complete emergence.

In both 0-1 hr and 2-4 hr groups, a greater proportion of pupae died during

ecdysis than before ecdysis (53.3 vs. 46.5% in the 0-1 hr group; 83.3 vs. 16.7% in the 2-4 hr group). These results are consistent with those shown in Fig. 2.

DISCUSSION

Pupae are more vulnerable to the absence of free water prior to 1 hr following ecdysis than after 1 hr. When pupae are placed on moist paper 24 hr or more following ecdysis mortality is not different from that of pupae which remain in water for the entire pupal-pharate adult period. More pupae die during ecdysis than before ecdysis regardless of treatment. Among pupae which die during ecdysis,

those older than 1 hr, when placed on moist paper, tend to have progressed farther toward complete emergence than those placed on moist paper younger than 1 hr. Difficulty in ecdysis is probably related to excessive loss of moisture.

Our results on survival in the absence of free water are consistent with those of other workers and demonstrate that although pupae are capable of surviving under these conditions, the time of the loss of free water is an important factor.

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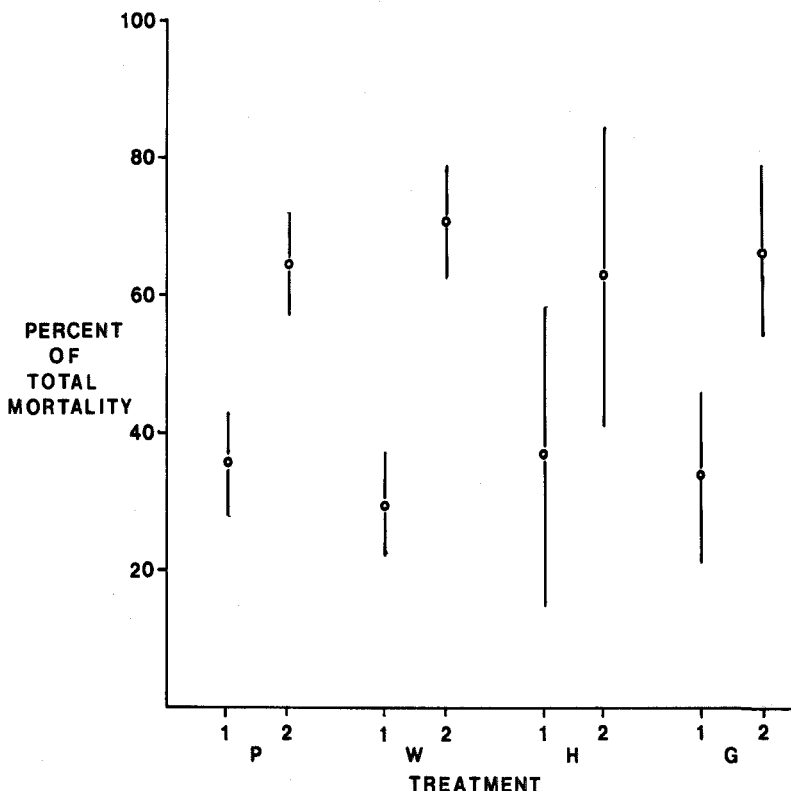


Fig. 2. Percent of total mortality before (1) and during ecdysis (2). P, W, H, circles and vertical lines same as Fig. 1; G = all data combined.

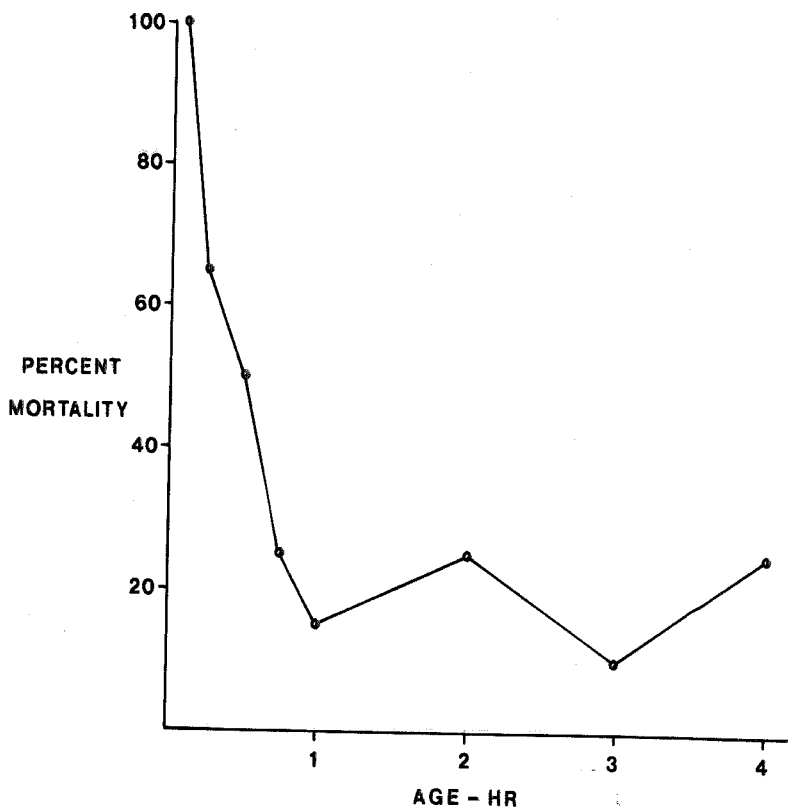


Fig. 3. Mortality and post-ecdysial age when placed on moist paper (20/ each age).

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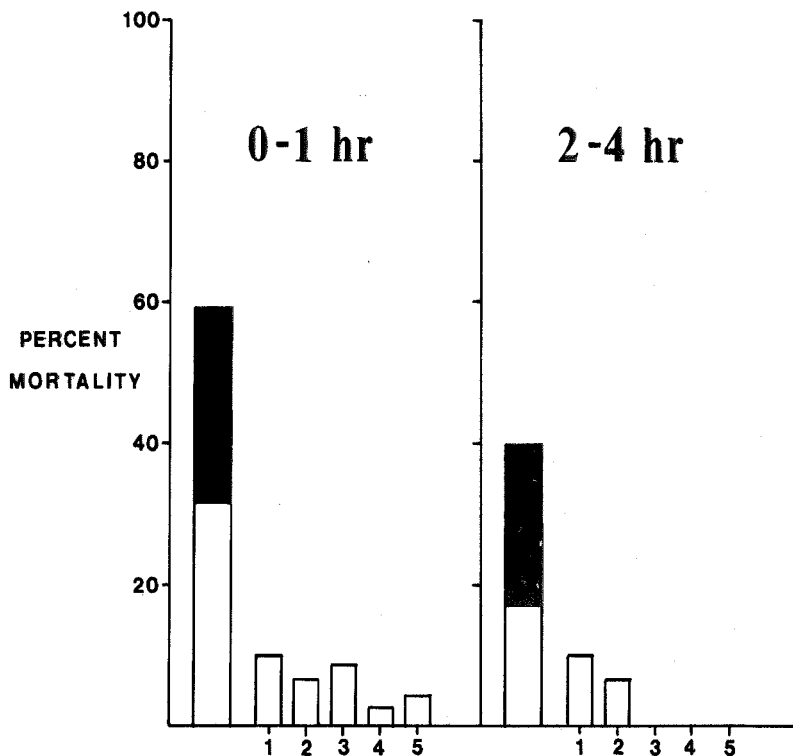


Fig. 4. Mortality, post-ecdysial age when placed on moist paper and extent of ecdysis. Based on data from Fig. 3 divided into 0-1 hr and 2-4 hr groups. Black = died before ecdysis; white = died during ecdysis; 1-5 explained in text.

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