Proceedings of the Indiana Academy of Science
(1988) Volume 98 p. 215-220.

OVIPOSITION PREFERENCE AND LARVAL DEVELOPMENT OF THE MOSQUITO SPECIES *AEDES TRISERIATUS* (SAY) IN ACIDIC WATER

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ABSTRACT: Effects of acidic water on oviposition preference, survival, and larval development of the mosquito species *Aedes triseriatus* (Say) were studied in the laboratory. Oviposition preference was assessed by allowing gravid females to deposit eggs on oviposition sites containing rainwater adjusted to pH 2, 3, 4, 5, 6, and 7. Results show that pH 2 and 3 were unfavorable for oviposition. However, mosquitoes laid eggs in water ranging from pH 4 through 7. A significant difference between selection of oviposition sites existed, when pH 2 and 3 were compared with pH levels 4, 5, 6, and 7 ($p <$ 0.01). Larval development was assessed by allowing 50 first instar larvae to develop in separate glass containers at five levels (pH 3-7) and a control. Mortality was greatest at pH 3. At pH 4 and below, larval development was adversely affected.

INTRODUCTION

The increasing acidity of rain caused by the conversion of air-borne nitric and sulfur oxides to nitric and sulfuric acids has been of concern in North America for nearly two decades. Regions, which are susceptible to acidification, are those which have a low buffering capacity. In such areas, the threat of acidification has become especially apparent in freshwater ecosystems. Although the deleterious effect of acid precipitation on aquatic animals has yet to be fully evaluated, evidence shows that increases in hydrogen ion concentration are responsible for the decrease of several amphibian (Freda, 1986; Neville, 1979; Pierce, 1985) and invertebrate (France and Stokes, 1987; Sehgal and Pillai, 1970) populations.

There is a paucity of information on the effect of acid precipitation on the development of aquatic insects, particularly mosquitoes. Most studies on the effect of pH on mosquito biology are simply field observations reporting the occurrence and distribution of particular mosquito species in acidic habitats (Peterson and

Chapman, 1969; Sehgal and Pillai, 1970) or laboratory studies in which the toxicity of different acids is compared (Ingram, 1954; Kurihara, 1959; MacGregor, 1929; McGaughy, 1968; Pillai and Madhukar, 1969).

Aedes triseriatus (Say), sometimes called the eastern tree hole mosquito, is the primary vector of LaCrosse Encephalitis virus in the north-central United States. Its natural larval habitat is the water that collects in tree holes, tree crotches, or shaded tree stump holes. It also makes use of water that collects in automobile tires and to a lesser degree in tin cans and other artificial containers. Adult females are similar in appearance to another, less common tree hole mosquito, *Aedes hendersoni* (Cockerell) but can be separated with some difficulty using differences in color (Truman and Craig, 1968) and shape of tarsal claws (Harmston, 1969). Larvae and males are very difficult to identify from each other.

Both *Ae. triseriatus* and *Ae. hendersoni* can be distinguished easily from the recently introduced Asian tiger mosquito, *Aedes albopictus* (Skuse). The latter has a distinct white stripe extending along the middle of the back from the anterior margin of the scutum nearly to the scutellum. The remainder of the thorax is dark. The legs of the Asian tiger mosquito are brightly marked with bands of white scales, absent in *Ae. triseriatus* and *Ae. hendersoni.*

Unlike previous studies, our investitagtion was designed to 1) determine the effects of pH on the oviposition response and to 2) continuously monitor the effects of various concentrations of sulfuric acid on larval survival and development. Since sulfuric acid comprises 70% of the acid rain in the eastern United States, adults and larval *Aedes triseriatus* were exposed to various concentrations of this acid.

MATERIALS AND METHODS

Colony establishment and maintenance: *Aedes triseriatus* larvae were collected from discarded tires in Muncie, Indiana and reared in the laboratory. Larvae were fed finely ground Tetra Min fish food daily. Pupae were transferred to 60 X 60 X 60 cm cages in which the adults emerged. These adults were provided with a 10% sucrose solution, which was removed 24 hours before bloodfeeding. Blood meals were provided by an anesthetized guinea pig. Eggs were collected on black cloth and were held for 14 days under conditions described below to permit adequate time for embryonation. They were then placed in a hatching medium composed of a 1:1000 solution of nutrient broth and distilled water. Experimental work was restricted to the 3rd generation raised in captivity.

Mosquitoes were maintained at $23 \pm 2°C$, $75\% \pm 3\%$ RH, and a photoperiod of 15 hours of daylight per 24 hours. Fluorescent lamps controlled by a timing device provided a 14.25 h intensive light period. A rheostat attached to a clock decreased incandescent light intensity between photophase and scotophase.

This study was divided into two phases. In the first phase, oviposition site selection at pH levels 2, 3, 4, 5, 6, and 7 was determined. The second phase was a study of larval development time as well as pupation and emergence success at pH levels 3, 4, 5, 6, 7, and control.

Oviposition preference. Sixty 0.47-liter cylindrical paper containers were used to hold individual female *Ae. triseriatus* mosquitoes. The center portion of the lid was removed and replaced with mosquito netting. Six equally spaced holes,

TABLE 1. Percent mortality of mosquito larvae exposed to pH 3, 4, 5, 6, 7, and control (N = 50 at each pH level).

pH level	Instars				Pupae	Total %
	1	2	3	4		
3	36	64	0	0	0	100
4	12	28	4	0	0	44
5	10	10	0	0	2	22
6	0	10	0	2	2	14
7	2	6	0	0	6	14
Control	0	6	0	0	4	10

1.8 cm in diameter, were punched in the bottom of each container. Each hole accommodated a glass vial (7 cm he. x 1.8 cm I.D.), which served as the water reservoir. Each vial contained water previously adjusted to one of six pH levels (2-7) and a presoaked balsa wood strip which served as an oviposition site. The acidity of the water was increased with sulfuric acid or decreased with calcium oxide.

Sixty gravid females were then transferred to the 0.47 liter containers, one female per container allowing each gravid female a choice of oviposition sites at six different pH levels. These cages were rotated daily to prevent mosquitoes from establishing location preference. A fresh cotton ball soaked in 10% sucrose solution was placed on top of each cage daily.

After oviposition, the mosquitoes were removed from the cages and each balsa-wood strip examined for the presence of eggs. If one or more eggs were observed on the strip, the oviposition response was recorded as positive. Data were analyzed to determine statistical significance of oviposition preference for specific pH levels using the Cochran Q-test (Siegel, 1956).

Larval development. Three hundred newly hatched, third generation, 1st instar larvae were placed in 120-ml glass jars, one larva per jar. Each jar contained rainwater previously adjusted to one of five pH levels: 3, 4, 5, 6, or 7. Fifty individuals were exposed to each pH treatment level and a control treatment. Hydrogen ion concentration in each jar was measured daily with a pH meter. When a change in pH was observed, the solution was readjusted to the original treatment level. The exception was the control treatment consisting of ambient rainwater with an initial pH of 5.6 After larval food was added, the pH increased to 7.5 and remained at that level for the remainder of the study.

Mortality, molting, pupation, and emergence of adults were recorded daily. A two-way analysis of variance was used to determine differences in mortality of groups at different pH levels and to assess differences in larval development time at various pH levels.

RESULTS AND DISCUSSIONS

Mosquitoes did not oviposite on balsa wood in water with pH of 2. Only one cluster of eggs was found at pH 3. However, clusters of 11, 16, 12, and 13 were found at pH 4, 5, 6, and 7, respectively. Similar results were obtained by Pillai

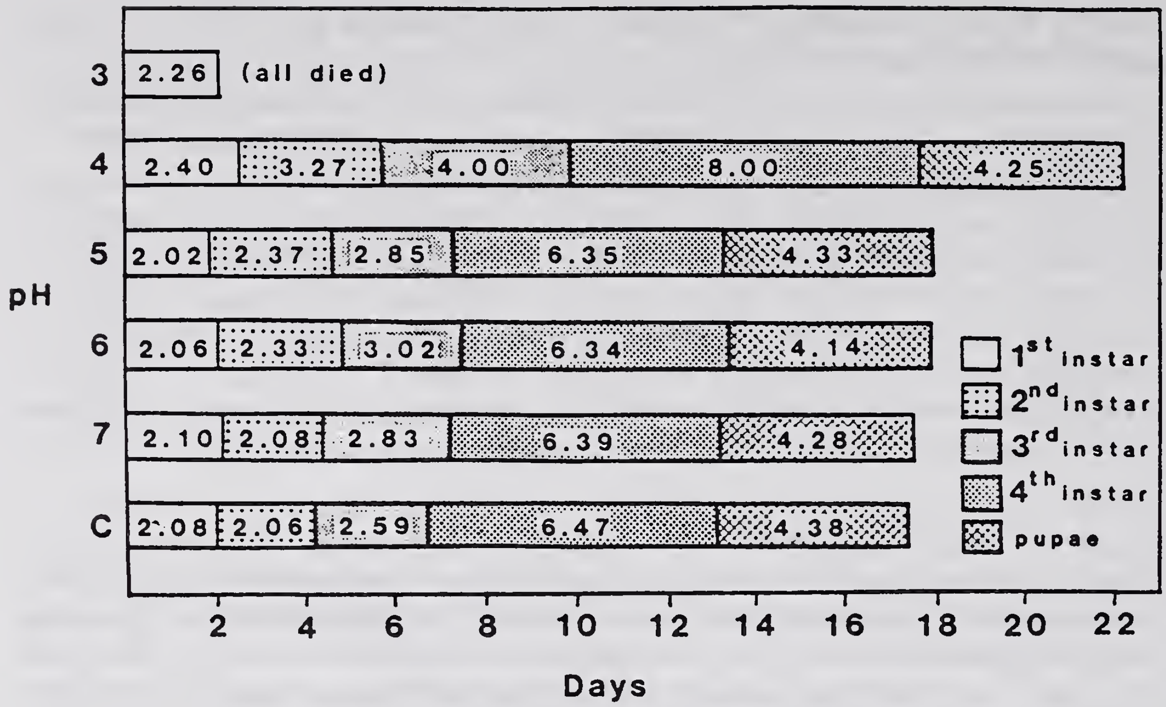

FIGURE 1. Average number of days required by *Aedes triseriatus* to complete instars 1, 2, 3, 4, and pupal stage at pH levels 3, 4, 5, 6, 7, and control.

and Madhulear (1969), who observed the lowest number of *Ae. aegypti* eggs at pH 2. Although no significant differences between responses at pH 4, 5, 6, and 7 were found, these levels were significantly higher than those at pH 2 and 3 ($p < 0.01$). The failure of mosquitoes to respond to oviposition sites at pH 2 and 3 suggests that highly acidic substrates are avoided by gravid *Ae. triseriatus* females.

When *Ae. triseriatus* were reared in media at pH 3, 4, 5, 6, and 7, mortality increased with increases in hydrogen ion concentration (Table 1). No larvae survived past the second instar in water at pH 3. Differences in mortality at pH 4, 5, 6, 7, and control were not statistically significant. However, the Cochran Q-test indicated a significant difference in mortality between groups raised in water at pH 4 and 5 ($p < 0.05$). The difference in mortality between larval groups reared at pH 3 and 4 was highly significant ($p < 0.001$). These data indicate a marked detrimental effect on larvae in water between pH 4 and pH 5. Similar results were obtained by Ingram (1954) and Tomkiewicz and Dunson (1977), who studied various aquatic insects. In this study, many of the deaths of mosquito larvae occurred during ecdysis.

Clements (1963) suggested that the fluid which digests the inner layers of the old cuticle may be altered by a high hydrogen ion concentration, inhibiting ecdysis and subsequently resulting in death. Mosquitoes in the early stages of development appeared most affected by highly acidic conditions. At lower pH levels (3, 4, and 5), most mortalities were observed at the first and second instar stages. Apparently, the body resistance to hydrogen ions increased with age. Craig and Clark (1938) found the buffer value of the larval blood in *Pieris rapae (L.)* and *Heliothis obsoleta (F.)* to be greatest in the last instar. Furthermore, Hoskins (1932) suggests that mosquito larvae are more affected by acidity then pupae, because the lining of the larval digestive tract is more permeable than the outer covering of the pupae.

A summary of the time required by *Ae. triseriatus* from hatching to emergence of adults is presented if Figure 1. Times required for completion of instars 1, 2, and 3 were significantly longer at pH 4 than at higher pH levels ($p < 0.05$). Fourth instar larvae also required a longer period for development at pH 4, but this difference was not statistically significant. Similarly, no significant differences between stages at other pH's or between duration of pupal stages were found.

Observations from this study indicate the *Ae. triseriatus* is adversely affected by excessive hydrogen ion concentration derived from sulfuric acid, a major constituent of acid rain. The data suggest that this species may be restricted in growth and development, if the pH of ambient breeding water falls below pH 4. Similarly, a decrease in pH may limit the distribution of this species by rendering potential breeding sites unsuitable for oviposition.

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