OCCURRENCE AND OVITRAP SITE PREFERENCE OF TREE HOLE MOSQUITOES: *AEDES TRISERIATUS* AND *AEDES HENDERSONI* IN EASTERN KENTUCKY[1]

ELLEN M. BALLARD,[3] JEROME H. WALLER[2] AND F. W. KNAPP[3]

ABSTRACT. This ovitrap study examined the effects of altitude above sea level, tree species, and tree trunk diameter on the distribution of eggs of the 2 tree hole mosquitoes, *Aedes hendersoni* and *Ae. triseriatus*. Only tree species and trunk diameter affected the distribution significantly. *Aedes hendersoni* eggs were found more frequently associated with trees of border and sunny habitat, while *Ae. triseriatus* eggs were more frequently found in association with trees of mesic habitat. Oviposition of *Ae. hendersoni* occurred more often at trees with smaller diameter at breast height than did *Ae. triseriatus*. These differences in ovipositing frequency appear to be related to the microhabitat associated with different sample sites.

INTRODUCTION

Aedes hendersoni Cockerell and *Aedes triseriatus* (Say) are 2 closely related species of tree hole mosquitoes whose most notable difference lies in their varying ability to serve as competent vectors for the LaCrosse encephalitis virus. *Aedes triseriatus* is the principal vector for the virus in the Midwest. *Aedes hendersoni* is not able to transmit the virus (Thompson et al. 1972, Watts et al. 1975). In recent years, several states in the Midwest have added *Ae. triseriatus* to the list of mosquitoes that are monitored for arborvirus activity. Failure of *Ae. triseriatus* adults to respond to light and carbon dioxide traps has led to the use of ovitraps as the principal method of monitoring population levels of this mosquito (Loor and Defoliart 1969). One disadvantage of this method is that the eggs of *Ae. triseriatus* are indistinguishable from those of *Ae. hendersoni*. In order to identify the species captured, additional time and money must be spent to hatch eggs and rear larvae to the fourth instar (Breland 1960). The purpose of the present research was to investigate various environmental factors that might influence the distribution of eggs.

MATERIALS AND METHODS

All field data were collected in Madison County, Kentucky in woodlands belonging to Berea College. Ovitraps similar to those used by Novak and Peloquin (1981) were employed in the study. Wooden tongue depressor blades which (15 cm length) had been scratched vertically with a saber saw blade were used as oviposition sticks. All oviposition sticks were collected at approximately 2 week intervals, placed individually in plastic bags and stored at 24°C with a 16 h light/8 h dark photoperiod. To insure proper embryonic development, all oviposition sticks were kept for at least 2 weeks before hatching was attempted. Each stick was examined under a dissection microscope to locate, count and record all eggs present.

Larval counts. Egg sticks were moistened with tap water 24 h prior to exposure to the hatching medium (0.1 g Fleishman's dry yeast/liter tap water). Egg sticks were kept in hatching medium, and emerged larvae were fed daily with 0.1 ml of liver-powder/liter H_2O.

Larvae were reared in 250-ml Erlenmeyer flasks at 20°C and identified to species using the characteristics given by Breland (1960). Larval color and gill size were readily seen at 25× magnification using a dissecting microscope.

Sample sites. This sample site selected was within the foothills of the Cumberland Mountain range, in Madison County, Kentucky. Sampling designs consisted of line transects and area plots. Four transects with ovitraps positioned every 30 m were set up on 4 different hillsides. These transects followed intermittent streams in the lower elevations. As the transect continued into the upper elevations, the streams tapered off and the vegetation and surroundings were more arid. In these arid elevations the transect followed an upward course of steadily increasing altitude.

All traps were attached to the east sides of the trees and were positioned every 30 m throughout the length of the transect. The trees used within each transect were selected to reflect the tree species distribution within the area. The altitude at each ovitrap was determined with an altimeter which was calibrated to a benchmark located on the Berea College campus. The diameter of each tree in the study was measured at the height of attachment of the ovitrap, i.e., approximately 1 m from ground level. This value ap-

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[2] Department of Biology, Pikeville College, Pikeville, KY 41501.

[3] Department of Entomology, University of Kentucky, Lexington, KY 40546-0091.

proximated the BDH (diameter at breast height) measurement used in forestry sampling methodology.

Statistical analysis. Two-way analyses of variance were conducted on the data of Table 1 (relative numbers of eggs of the 2 species) and Table 2 (proportions of tree species and proportions of eggs of the 2 mosquito species). The Pearsonian correlation coefficient was used to test the association between altitude above sea level and the numbers of eggs of the 2 mosquito species (Snedecor and Cochran 1969).

In the analysis of the effect of DBH the relationship between the diameter of trees upon which the ovitraps were hung and the number of eggs found was estimated by use of the Pearson product-moment correlation coefficient (Snedecor and Cochran 1969), and by a *t*-test of the significance of the difference in mean DBH between trees bearing ovitraps that had no eggs of *Ae. hendersoni* with those that did. The trees used in these analyses were those of the first 3 lines of Table 2, namely *Quercus alba, Q. rubra, Acer saccharum* and *A. rubrum.*

RESULTS

Results from the 4 line transects are summarized in Table 1. A total of 91,306 eggs were counted for the 103 ovitraps located on these transects. The eggs of *Ae. triseriatus* were found in greater proportions in all 4 transects. All traps contained eggs of *Ae. triseriatus*, while only 39% were positive for *Ae. hendersoni*. All traps that were positive for *Ae. hendersoni* also contained eggs of *Ae. triseriatus*.

Table 1. Distribution of eggs of *Aedes hendersoni* and *Ae. triseriatus* collected from line transects near Berea, Kentucky in 1982.

Transect	No. of traps	Mean number of eggs collected/trap/sample: *Ae. hendersoni*	*Ae. triseriatus*
1	29	6.0 ± 7.5*	126.8 ± 99.1
2	37	1.0 ± 3.0	86.5 ± 96.7
3	22	5.1 ± 29.5	79.0 ± 43.2
4	15	10.6 ± 14.3	58.4 ± 50.0

* no. eggs/trap/sample ± SD

A two-way analysis of variance (mosquito species by transect) showed no significant transect effect ($F = 0.82$, d.f. = 3,3 and $p = 0.439$). The difference in numbers of the 2 species that is apparent from inspection of the table yielded a significant species effect ($F = 29.31$, d.f. = 1,3 and $p = 0.012$).

Altitude effect. The effect of altitude on the distribution of *Ae. hendersoni* vs. *Ae. triseriatus* was studied using the results obtained from the four transects. The correlation between altitude in meters above sea level and the total number of eggs found in the ovitrap was not significantly different from zero for either species. The value of the Pearson r for *Ae. hendersoni* was −0.06, and for *Ae. triseriatus* was −0.09. A value of −0.23 is required for significance at the 0.05 level. Thus there was no association between altitude and frequence of egg-laying in the ovitraps for either species.

Tree species effect. Inspection of the data in Table 2, where tree species that bore ovitraps with more than 3,000 eggs during the sampling season are arranged in descending order of total eggs, suggests that numbers of eggs collected are associated with tree species upon which the ovitraps were hung. Specifically, it appears that there is an over-representation of the eggs of *Ae. hendersoni* in ovitraps hung on *Acer saccharum* and *Acer rubrum* and an over-representation of the eggs of *Ae. triseriatus* in ovitraps hung on *Quercus alba*. The null hypothesis of no difference in proportions was not supported: a two-way analysis of variance of the transformed proportions (arcsin p) in the 3 × 8 table showed a statistically significant difference among the 8 rows (species of trees). The value of F for the 8 rows was 6.28 (d.f. 7,14 and $p = 0.004$). The F value for the 3 sets of proportions was 0.13 (d.f. 2,14 and $p = 0.12$). Therefore the observed dif-

Table 2. Comparison of proportions of ovitraps on tree species with proportions of eggs of *Aedes hendersoni* and *Ae. triseriatus*. Data are in descending order of total eggs found.

Tree species	Ovitrap proportion (number)	*Ae. hendersoni* Proportion (number)	*Ae. triseriatus* Proportion (number)	Total proportion (number)
Acer saccharum	0.203 (14)	0.222 (680)	0.212 (12,146)	0.213 (12,826)
Quercus alba	0.145 (10)	0.146 (448)	0.211 (12,085)	0.208 (12,533)
Acer rubrum	0.159 (11)	0.322 (987)	0.157 (9,001)	0.166 (9,988)
Carya spp.	0.159 (11)	0.049 (149)	0.159 (9,109)	0.153 (9,258)
Platanus occidentalis	0.087 (6)	0.079 (24)	0.086 (4,927)	0.086 (5,168)
Tilia heterophylla	0.087 (6)	0.037 (113)	0.064 (3,688)	0.063 (3,811)
Nyssa sylvatica	0.058 (4)	0.045 (139)	0.060 (3,415)	0.059 (3,554)
Totals	1.000 (69)	1.000 (3,064)	1.000 (57,263)	1.000 (60,327)

ferences would bear investigation in subsequent studies.

Tree size. Among trees of the genera with the highest number of eggs collected (*Quercus* and *Acer*), there appeared to be a difference in the relationship of the measured diameter to presence of eggs of the 2 mosquito species. For the 35 trees of the 3 species shown in the first 3 lines of Table 2 the Pearsonian correlation between these variables was opposite in sign for the 2 species, although in neither case was it statistically significantly different from zero. The correlation between diameter and number of eggs of *Ae. hendersoni* was −0.16; for diameter and number of eggs of *Ae. triseriatus* it was +0.21. A value of 0.33 is required for significance at the 0.05 level for the 34 degrees of freedom of these cases.

DISCUSSION

Sinsko and Grimstad (1977) found evidence for vertical stratification of the 2 tree hole mosquitoes within a level woodlot in Indiana. Substituting the variation in altitude above sea level for the vertical displacement of that study, the present study found no statistically significant difference in occurrence of eggs of the 2 species among 3 broad altitude ranges. The association of altitude with numbers of eggs of *Ae. hendersoni* is positive: It is negative for *Ae. triseriatus.* A plausible explanation is that in both studies the operative variables lie in the light levels afforded the mosquitoes. Ovitraps placed higher above ground in the Indiana study and at higher elevations in this study were both in relatively more open and sunny sites, which seem to be favored by *Ae. hendersoni.* The trees where the largest numbers of *Ae. hendersoni* eggs were found are either colonizers of open or cleared areas (e.g., *Acer rubrum*), trees found most often on the border of a forest (e.g., *Fraxinus americana*), or trees of high, dry sunny habitat (e.g., *Quercus prinus* and *Q. veluntia*).

In summary, the data of this study are consistent with the findings of vertical stratification of the 2 mosquito species *Ae. hendersoni* and *Ae. triseriatus.* They lead to the suggestion that the variables of (1) height above ground, (2) varying frequency by altitude, and (3) tree species at which eggs are found in ovitraps are indicators of preference of the 2 mosquito species for ovitraps in different degrees of light and shade as sites for oviposition.

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