SPATIAL DISTRIBUTION OF *AEDES TRISERIATUS* EGGS IN A SITE ENDEMIC FOR LA CROSSE ENCEPHALITIS VIRUS

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ABSTRACT. Egg laying behavior in *Aedes triseriatus* was studied in an oak woodlot endemic for La Crosse encephalitis virus in southwestern Wisconsin. Daily counts from oviposition traps allowed the calculation of spatial distribution parameters for eggs during 1988. Egg distribution along an ellipsoidal transect of 25 traps was clumped with some traps being more attractive to ovipositing female *Ae. triseriatus* than others. Females were estimated to deposit a mean of 31 ± 9.8 eggs per oviposition.

INTRODUCTION

Aedes triseriatus (Say) is a low density mosquito, common to woodlands in the upper midwestern United States. It breeds primarily in tree holes (phytotelmata), although other containers, including discarded tires, may be used as oviposition sites (Craig 1983). *Aedes hendersoni* (Cockerell), a sibling species of *Ae. triseriatus*, also oviposits in tree holes, but primarily uses arboreal tree holes, while *Ae. triseriatus* prefers basal oviposition sites (Scholl and DeFoliart 1977, Sinsko and Grimstad 1977).

Aedes triseriatus is the major vector of La Crosse encephalitis virus (LACV) in the midwestern United States (DeFoliart et al. 1986). LACV, a bunyavirus, produces an acute febrile pathogenesis which causes more childhood illness than any other arbovirus in the United States (Kappus et al. 1983). Since LACV can be transmitted transovarially in the mosquito (Watts et al. 1973), new breeding sites may become LACV foci. In Minnesota, Balfour (1976) related occurrences of human LACV infection to proximity of *Ae. triseriatus* breeding sites. For this reason the behavioral ecology of *Ae. triseriatus* oviposition is important in LACV epidemiology.

The selection and location of the oviposition site by a female mosquito determines the larval habitat of the species. The responses of *Ae. triseriatus* to various biological, chemical and physical factors have received some attention in laboratory studies, and these have been recently reviewed by Bentley and Day (1989). Relatively little is known, however, about the oviposition behavior of *Ae. triseriatus* in the field. Our purpose in this study was to determine the spatial distribution of *Ae. triseriatus* egg batches in the field, as well as to estimate egg batch size in a site endemic for LACV.

MATERIALS AND METHODS

The study site was an 8-hectare second growth white oak (*Quercus alba* (Linn.)) woodlot in Iowa County, WI. The woodlot is located on a hillside in a predominately agricultural area where the hillsides are too steep for cultivation. Twenty-one tree holes were found in the study area. LACV has been isolated repeatedly from larvae at the site (Litsitza et al. 1977; S. V. Landry, personal communication).

Loor and DeFoliart (1969) adapted the ovitrap monitoring method used in the *Ae. aegypti* (Linn.) eradication program for monitoring the presence of *Ae. triseriatus*. This method makes use of a 400-ml beverage can, painted black and filled with water, as an artificial oviposition site. A slat of balsa wood (Novak and Peloquin 1981) is clipped to the side of the container using a binder clip. Female *Ae. triseriatus* then oviposit on the balsa. Identical ovitraps may be arranged along transects to make spatial comparisons among these artificial oviposition sites.

Oviposition traps were placed on an elliptical transect in the study area with each trap located on the nearest tree no closer than 18 m from the previous trap. A hole was cut into the side of each can 25 cm from the top to allow attachment to the tree and as a drain to prevent overflow. Traps were hung from screw hooks at a height of 30 cm.

The traps were checked 3 times a week beginning June 6, 1988. After the first oviposition occurred the traps were checked daily until September 30. Balsa strips with eggs were removed and replaced with fresh strips. The eggs on the strips were then counted in the laboratory. Every 2 weeks all of the balsa strips were removed and replaced. Traps were filled weekly prior to the first oviposition and were filled to the drain hole daily after the first oviposition. The traps were rinsed and refilled on days when the balsa strips were replaced.

Several methods have been developed for determining the spatial distribution of individuals in the field. Two of the most common are based on the relationship between the sample mean and sample variance. Taylor (1961) noted that there is a linear relationship between the sample mean and variance. Regression analysis of this relationship can be used to determine a slope estimate which can serve as a dispersion index. Iwao (1968, 1970) uses a mean/variance relationship in the form of a crowding index (Lloyd 1967) that can be regressed against the mean to determine not only an index of dispersion (slope), but also an estimate of a "unit of contagion" (intercept). Both methods have been used extensively to determine dispersion indices for eggs, larvae and adults. Our data were analyzed using Minitab™ statistical software (Minitab Inc. 1986) and the methods of Taylor (1961) and Iwao (1968, 1970).

RESULTS AND DISCUSSION

The 1988 field season was shortened by drought. At the study site most tree holes were dry until late June. The first oviposition occurred June 20, but only a few traps were positive for eggs (oviposition events) in June (June 20, 29 and 30). Oviposition occurred almost daily from July 5 until early September. There were 69 days in which at least one trap contained eggs. Loor and DeFoliart (1970) reported that 94% of the eggs deposited at this site were *Ae. triseriatus* eggs. Eggs of *Ae. hendersoni* with several *Orthopodomyia signifera* (Coquillett) made up the remainder of the eggs. Landry and DeFoliart (1986) collected 622 adult *Ae. triseriatus/Ae. hendersoni* mosquitoes at the site and, on morphological examination, found all to be *Ae. triseriatus.*

The natural log of the variance of the daily count was regressed on the natural log of the mean for days that were positive for oviposition, yielding the following regression equation: $y = 1.196 + 1.467x$. Using a t-test based on the standard error estimate, the regression line had a slope significantly greater than one ($P < 0.001$).

Taylor (1961) stated that the slope of a mean/variance regression of field samples greater than one indicates a clumped distribution of individuals. The slope of 1.4 indicates a clumping of eggs among ovitraps. Some traps are more attractive to ovipositing female *Ae. triseriatus* and therefore have more eggs deposited in them. The intercept of the regression equation is thought to be a scaling factor and has no interpretive value.

Lloyd's (1967) mean crowding index was calculated for each of the 69 days in which eggs were deposited. These values were regressed against the mean egg value for the day. The regression equation is $y = 30.31 + 2.028x$; t-tests on both model coefficients showed the intercept to be significantly greater than zero ($P < 0.001$) and the slope to be significantly greater than one ($P < 0.001$).

Using the mean crowding index vs. the mean egg value regression (Iwao 1968, 1970) also results in an equation with a slope significantly greater than one, which also can be seen as evidence for a clumped distribution of eggs. Using this method, the intercept can be used as an estimator of clump size. In this case, an intercept of zero would indicate that eggs are clumped individually. An intercept of 30.3 suggests that individuals are clumped in groups of 31 ± 9.8 ($P = 0.05$). This model parameter can be seen as an estimate of egg batch size for a single *Ae. triseriatus* female for one oviposition.

This estimate of egg batch size falls between the estimates of Kitron et al. (1989). The confidence interval around the intercept estimator is smaller as a result of a more precise error estimate based on daily data rather than weekly counts. Kitron et al. (1989) reported egg batches of 29.3 ± 16.9 and 46.9 ± 25.3 based on weekly counts from ovitraps in 2 wooded areas situated in central Illinois.

In this study eggs were removed from traps daily allowing females to use a fresh oviposition strip without the influence of conspecific eggs. Egg pheromones have been reported in *Culex tarsalis* Coquillett and *Cx. pipiens* (Linn.), which increase oviposition on surfaces on which conspecifics have deposited eggs (Osgood 1971, Dadd and Kleinjan 1974). Surprisingly, Kitron et al. (1989) found that the presence of eggs on the oviposition strips resulted in a decrease in further oviposition.

Figure 1 shows the total number of eggs laid in each individual ovitrap over the course of the study. The mean number of eggs per trap over the study period was 630.8 ± 123.7. Figure 1 also shows the total number of days that each trap was positive for eggs (an oviposition event occurred). Traps had a mean of 14.6 ± 2.1 oviposition events. The pattern of oviposition was similar in both number of eggs deposited and number of times a trap was used during a season. These measurements are highly correlated. Several traps had very high numbers of eggs and others had very low numbers. These values reflect egg counts which are greater or less than a 95% mean confidence interval. We are unable to suggest a reason why particular traps were more attractive or unattractive, as tree diameter, species of tree on which the trap was attached, degree of shading and temperature did not seem to account for these differences in oviposition. Beier et al. (1982) were also unable to correlate *Ae. triseriatus* oviposition with habitat variables.

Although the distribution in egg laying among oviposition traps was contagious, few ovipostion traps were sufficient for detecting and monitoring populations of *Ae. triseriatus* in the field. We feel that 3 oviposition traps per hectare

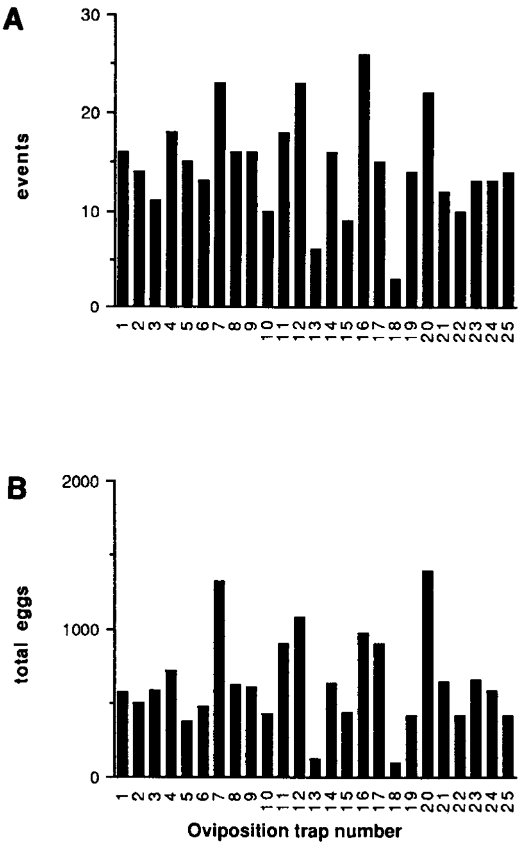

Fig. 1. Distribution of eggs in oviposition traps. A. Number of days each trap was positive for eggs (n = 366). B. Total number of eggs deposited in each trap (n = 15,998).

greatly reduces the probability of placing a trap in a site which is unattractive to ovipositing *Ae. triseriatus.* A reference distribution based on our field data shows the probability of placing a trap in one of these unattractive sites to be $P < 0.01$.

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