

# The Great Lakes Entomologist

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Volume 25  
Number 4 - Winter 1992 *Number 4 - Winter*  
1992

Article 4

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October 1992

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### Recommended Citation

Beehler, Jeffrey; Lohr, Sharon; and DeFoliart, Gene 1992. "Factors Influencing Oviposition in *Aedes Triseriatus* (Diptera: Culicidae)," *The Great Lakes Entomologist*, vol 25 (4)  
Available at: <https://scholar.valpo.edu/tgle/vol25/iss4/4>

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FACTORS INFLUENCING OVIPOSITION IN *AEDES TRISERIATUS*  
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## ABSTRACT

Five factors associated with natural oviposition sites were tested for their attractancy to ovipositing *Aedes triseriatus*, including dyed oviposition water, presence of decaying organic matter, a dark oviposition container, water in which conspecific larvae have been reared to the 4th instar and the presence of eggs on the balsa wood oviposition substrate. A replicated fractional factorial design was used to examine the effects of the above factors on oviposition behavior in laboratory experiments. Regression analysis showed dyed oviposition water and eggs on the oviposition substrate to be statistically significant attractants for ovipositing *A. triseriatus* females. The attraction to dyed oviposition water indicated that dyed water in oviposition traps may greatly increase their competitiveness with naturally occurring oviposition sites.

*Aedes triseriatus* (Say) is a common woodland mosquito in the upper midwestern United States. It breeds primarily in treeholes, although other containers such as discarded tires may also be used as oviposition sites. This species is the vector of La Crosse encephalitis virus (DeFoliart et al. 1986) and thus, there is much interest in monitoring its presence. Unfortunately, *A. triseriatus* responds poorly to light traps (Craig 1983), and since it breeds in containers, it is not subject to sampling by dipper. To detect its presence, Lohr and DeFoliart (1969) adapted the ovitrap previously used for monitoring *Aedes aegypti* (Linn.) during the Pan American Health Organization *A. aegypti* eradication program. The trap consists of a water-filled, black-painted, 400 ml beverage can containing a balsa paddle (Novak and Peloquin 1981) which serves as an artificial oviposition site.

A number of physical and biological factors have been suggested as oviposition attractants for *Aedes triseriatus*. Physical factors which influence *A. triseriatus* oviposition include the orientation of the opening of the oviposition container, texture and coloring of the container walls, and the optical density of the oviposition water (Wilton 1968). Novak and Peloquin (1981) used dark oviposition containers when determining if egg counts differed in relation to the oviposition substrate provided in the ovitrap. They found balsa substrates to be best suited for use.

A biological factor reported as an oviposition attractant for *A. triseriatus* is the presence of decaying organic matter in the oviposition water (Wilton

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1968). Decaying organic matter has often been reported as an oviposition attractant with other *Aedes* and *Culex* species (e.g. Kramer and Mulla 1979, Hazard et al. 1967). Hazard et al. (1967) also found that the volatiles produced from the bacteria associated with organic infusions induced oviposition.

Egg pheromones have been reported as oviposition attractants in *Culex tarsalis* Coquillett and *Culex pipiens* (Linn.) (Osgood 1971, Dadd and Klienjan 1974). These volatiles induce oviposition when eggs of conspecifics are present. Another volatile attractant is the larval factor (LF), which is produced by 4th instar conspecifics and is present in larval holding water. Bentley et al. (1976) showed that LF was present in the holding water of 4th instar *A. triseriatus*. The LF was also shown to be present in the volatile fraction of holding water. By treating water with kaolin, McDaniel et al. (1979) showed that LF was not associated with waste products or larval gut bacteria. LF attractive to conspecifics has also been reported in *A. aegypti*, *A. atropalpus* (Coquillett), *A. togoi* (Theobald), *A. communis* (DeGeer), *Culex quinquefasciatus* Say and *Culiseta incidens* (Thomson) (Soman and Rueben 1970, Bentley et al. 1976, Maire 1984, Maire and Langis 1985, Trimble and Wellington 1980, Wilmot et al. 1987).

The objective of this study was to test 5 reported oviposition attractants in the laboratory to determine if there are simple ways of increasing ovitrap efficiency. The 5 factors: (1) dyed oviposition water, (2) presence of decaying organic matter, (3) presence of a dark oviposition container, (4) presence of eggs on the oviposition substrate, and (5) presence of conspecific LF were compared using a replicated fractional factorial design.

## MATERIALS AND METHODS

A replicated  $2^{5-2}$  (Resolution III) fractional factorial design was used to analyze the effects of factors that may influence the oviposition behavior of *A. triseriatus*. A standard design matrix and generators were used in the experimental design (Box et al. 1978). Each of the factors examined could be coded as present (+) or absent (-). A third replicate using a "fold-over" design (Box et al. 1978) was used to clarify the effect of the presence of decaying organic matter from interactions with other factors. Six experimental blocks were used. Each block represented a group of 4 oviposition cups within a single replication placed in an individual cage.

Fractional methods have 3 main advantages. First, they allow the comparison of a number of factors in a small number of experimental trials. Second, fractional designs allow the estimation of two-factor interactions. For example, if the combination of LF and decaying organic matter are important factors in inducing oviposition when present in tandem this interaction can be quantitatively considered. Third, fractional designs allow the use of small experimental blocks.

The first factor, dyed oviposition water, was produced by using 3 drops of red odorless vegetable dye and 3 drops of odorless green dye in 150 ml of distilled water. Water containing organic matter was produced for this study by placing 3 g of shredded, dried white oak (*Quercus alba*) leaves in 950 ml of distilled water 5 days before the beginning of the selection trial. This infusion was stored at 26° C until the trial. A darkened oviposition container was made by encircling the normally gray oviposition container with black construction paper. Balsa wood strips, 2.5 x 7.6 cm, with 40-100 *A. triseriatus* eggs/strip were kept at 3° C until the start of the experiments. Balsa wood strips without eggs were soaked for 24 hours and then stored at 3° C with the strips positive for eggs. The LF, water in which *A. triseriatus* larvae had been reared

at least to the 4th instar, was provided by the water in which the experimental mosquitoes were reared. Larvae were reared in pans with a density of approximately 15 larvae per 150 ml of distilled water. Ground Tetramin™ tropical fish food was supplied *ad libitum* as a larval food source. When pupation began, pupae were removed and the water was stored at 3° C until the start of the assay. Particulate matter was removed by passing the water through filter paper (Whatman #1, Qualitative) prior to the start of the experiments.

Approximately 100 one-to two-day old female *A. triseriatus* were placed in a cage (1 m<sup>3</sup>) with approximately 150 males. The mosquitoes were kept at 26 ± 2° C in an insectary with a photoperiod of 14L:10D including a 1 hr evening twilight period. Humidity was maintained at approximately 90%. Mosquitoes were allowed 5 days in which to swarm and mate during which they were provided a 5% sucrose solution. After 5 days, females were fed on an anesthetized mouse (University of Wisconsin-Madison animal welfare assurance #A1457). The following day 30 blood-engorged females were placed in each of 2 separate cages (1 m<sup>3</sup>).

Plastic dental cups (6.5 cm diam) were used as oviposition containers. Inserted into each container was a 2.5 x 7.6 cm piece of balsa wood held in place by a #20 binder clip. Cups were placed near the cage corners and treatments were assigned randomly within each cage.

Each container held 150 ml of water. If the design matrix called for LF in the water, then 75 ml of LF water was placed in the cup along with 75 ml of distilled water (-for organic matter) or 75 ml of oak infusion water (+ for organic matter). Distilled water (75 ml) was added to a container negative for LF and either distilled or oak infusion was added to bring the volume up to 150 ml per cup. After the cups were filled, vegetable dyes were added to cups positive for dyed oviposition water.

Blood-engorged females were left in the cages for 6 days, with access to a 5% sucrose solution. Cages were maintained at the environmental regime described above. The balsa strips were then removed and the number of eggs counted. The number of eggs on the strips positive for eggs at the start of the bioassay were subtracted to determine the number of eggs deposited during the study period. Data were analyzed using multiple regression. Since the measured response was an egg count, the square root of the eggs laid was used as the dependent variable in the regression. The square root transformation was used because it stabilizes the variance in count data. For this data, the square root transformation was also close to the Box-Cox estimate (Box and Cox 1964) of an appropriate transformation.

## RESULTS AND DISCUSSION

A total of 2,367 eggs were laid in the first replicate, 1,958 in the second and 3,529 in the third. Clearly the strongest attractant for ovipositing *A. triseriatus* in this study was dyed oviposition water. Of 7,854 eggs laid, 6,959 (89%) were deposited in containers containing dyed water (Table 1).

Regression analysis was performed to clarify the effects of the different factors on oviposition behavior. The regression coefficients for the final model, along with their standard errors and *P*-values can be found in Table 2. The *R*<sup>2</sup> for this regression was 0.92. This analysis confirmed that dyed oviposition water had the greatest effect on oviposition (*P* < 0.00001). Beehler and DeFoliart (1990) showed in a field study that oviposition traps which contained dyed oviposition water collected up to 4 times as many *A. triseriatus* eggs as traps containing water without dye. The presence of eggs on the oviposition substrate also increased oviposition (*P* < 0.001). Sixty-nine percent

Table 1. — The effect of 5 factors on the oviposition behavior of *A. triseriatus*.

Factor <sup>1</sup>	Percent of Total Eggs Deposited
Dyed water	89%
Decaying oak leaves	31%
Darkened container	47%
Eggs on oviposition substrate	69%
Larval factor	41%

<sup>1</sup>Factors in fractional factorial designs are not presented singly against other factors, but are presented in combination. Therefore percentages will not total 100%.

of the eggs laid were in oviposition cups which had eggs on the oviposition substrate. This increase in oviposition could be caused by an egg pheromone or some other factor associated with *A. triseriatus* eggs which is consistent with the findings of Osgood (1971) and Dadd and Klienjan (1974) in *Culex*. This result contradicts that of Kitron et al. (1989) who, in field studies found that the presence of eggs on the oviposition substrate decreased the oviposition of *A. triseriatus*.

The presence of decaying organic matter ( $P < 0.001$ ) and LF ( $P < 0.05$ ) also affected oviposition. Both factors have negative regression coefficients. The negative effects of organic matter and LF do not necessarily imply that they deter oviposition compared to distilled water. These two results merely reflect the great attractiveness of dyed oviposition water. Given a choice between dyed water with no organic matter and water with decaying organic matter and no dye, mosquitoes prefer the former. When comparisons were made within experimental blocks, water with decaying organic matter did prove attractive compared to distilled water. Within each block (an individual cage) there is not true statistical independence between treatments, and dyed oviposition water is such a strong oviposition attractant that fair comparisons for water with decaying organic matter and LF cannot be made using this design as these factors are not both present (+) and absent (-) within a block with the other factors held constant. In this study attractiveness of LF to ovipositing *A. triseriatus* would be confounded with the effect of bacteria and their metabolites present in the larval rearing pans. McDaniel et al. (1976) showed that there was a strong interaction between container color and the presence of LF. A dark oviposition container had no effect on oviposition in this analysis.

Table 2. — Multiple regression coefficients ( $\sqrt{x}$  transformed data), standard errors and significant *P*-values for factors assayed for *A. triseriatus* oviposition attraction.

Factor	Coefficient + Std. Error
Constant	15.2+0.7****
Dyed oviposition water	8.5+0.8****
Decaying oak leaves	-2.5+0.7**
Dark oviposition container	-0.04+0.7
Conspecific eggs on substrate	3.0+0.7***
Larval factor	-1.6+0.7*
Interaction between dye and organic matter	-3.6+0.7***
Interaction between dye and eggs on substrate	-2.2+0.7**

\* $P < 0.05$

\*\* $P < 0.01$

\*\*\* $P < 0.001$

\*\*\*\* $P < 0.0001$

Again, the extreme attractiveness of the dyed oviposition water within each block make comparisons of this interaction with a distilled water control difficult.

In most previous studies the hypothesized attractants were compared singly to a distilled water control. This study is unique in that possible attractants were compared directly with each other. Thus, these data give an indication of which oviposition attractants will perform well when competing with other naturally occurring factors, rather than distilled water controls. The extremely significant attraction to dyed oviposition water indicates that is a very important factor in selection of oviposition sites by *A. triseriatus*, especially in conjunction with the presence of eggs on the oviposition substrate.

Wilton (1968) examined six environmental variables using an overlapping fractional factorial design. He concluded that a horizontal opening, rough-textured and dark colored walls, and a dark background were important oviposition attractants for *A. triseriatus*. He also suggested that water of high optical density and decaying organic matter were strong oviposition attractants. All containers used in our studies had horizontal openings. The balsawood oviposition substrate was light in color and had a consistent texture between treatments. The container walls were uniform in color making comparisons with the containers of Wilton impossible. The attractiveness of dyed oviposition water to ovipositing females is consistent with the results of Wilton (1968).

Our results indicated that dyeing the oviposition water dark when sampling for *A. triseriatus* could greatly improve trap competitiveness with naturally occurring oviposition sites. These results also suggest the presence of an *A. triseriatus* egg factor which is attractive to ovipositing females.

#### ACKNOWLEDGMENTS

We thank Joyce Gibbons for her technical assistance during the course of this study. Dr. Kinley Lantz, Department of Applied Statistics, University of Minnesota kindly provided assistance in data analysis.

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