A FIELD EVALUATION OF TWO SUGGESTED *AEDES TRISERIATUS* OVIPOSITION ATTRACTANTS

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ABSTRACT. Two reported oviposition attractants for *Aedes triseriatus*, fish oil emulsion and water of high optical density, were evaluated in ovitraps in the field. Solutions of fish oil emulsion at 1% repelled ovipositing mosquitoes, whereas water containing vegetable dye increased oviposition up to 4-fold over control traps. Laboratory bioassays with fish oil emulsion at both 1 and 5% confirmed the field results.

Oviposition site selection is an important part of the behavioral ecology of a mosquito species as it determines the larval habitat (Jenkins 1946). Oviposition site selection by the eastern tree hole mosquito, *Aedes triseriatus* (Say), is of particular interest because it is the major vector of La Crosse (LAC) encephalitis virus in the upper Midwest (DeFoliart et al. 1986). *Aedes triseriatus* is a sylvan species breeding primarily in tree holes (rot holes containing water, leaf detritus and stem flow), but also in other small water containers including scrap tires (Craig 1983).

As *Ae. triseriatus* responds poorly to light traps (Craig 1983) (the most common surveillance tool used by public health agencies and abatement districts), Loor and DeFoliart (1969) adapted the ovitrap method from the *Ae. aegypti* (Linn.) eradication program to monitor *Ae. triseriatus*. Although primarily a relative population measure (Berry et al. 1980), ovitrapping is commonly used to monitor populations of *Ae. triseriatus*. The traps can be made and a large number placed in the field at relatively low cost.

The response of ovipositing *Ae. triseriatus* to various biological, physical and chemical factors has been recently reviewed by Bentley and Day (1989). Although there have been few field studies, fish oil emulsion plant food made from by-products of the commercial fishing industry has been reported to attract *Ae. triseriatus* to ovitraps in the field (Holck et al. 1988). Oviposition water of high optical density has been shown in several laboratory studies to attract ovipositing *Ae. triseriatus* (Wilton 1968; Beehler et al., unpublished data). We wanted to determine the effectiveness of these 2 factors in increasing trap sensitivity when compared with a distilled water control. If these 2 factors are attractive in the field, they could easily be incorporated into an ovitrapping program to increase the competitiveness of ovitraps with naturally occurring oviposition sites.

Thirty oviposition traps were placed along a 120 m transect through a second growth white oak (*Quercus alba* (Linn.)) woodlot in Iowa County, WI, on July 27, 1988. The traps were arranged in groups of 3 on each of 10 trees, approximately 30 cm above the ground and at least 15 cm apart on the tree. The traps were attached to trees using screw hooks inserted into holes 2.5 cm from the top of the trap. These holes also served to prevent overflow. The next group of traps was placed on the first oak large enough to hold a group, but not less than 9 m from the previous group.

Within each group, the center trap served as a control and was filled with tap water. The trap on the left was filled with water to which 3 drops of odorless green vegetable dye and 3 drops of red dye had been added. The trap on the right was filled with a 1% solution of fish oil emulsion (Fish Oil Emulsion Plant Food, Green Light Co., San Antonio, TX).

The 10 trap groups were checked once weekly for 6 weeks. Egg sheets (2.5 × 5 cm strips of balsa wood) were removed, taken back to the laboratory and the eggs counted. The control trap and the trap with dyed water were emptied, washed with tap water, and reversed in position on the tree each week. They were then refilled with the appropriate treatment. The trap containing fish oil emulsion solution was refilled only every other week and left in the same position. The first week, traps on trees 1–3 and 9–10 were filled with emulsion solution. When these traps were checked, they were washed and left empty on the tree. The second week traps on trees 4–8 were treated with emulsion solution. This alternating process was repeated throughout the study period in an effort to quantify any olfactory cues at a trap group.

All eggs taken in the field study were assumed to be *Ae. triseriatus*. Loor and DeFoliart (1970), working at this same site, found that 94% of eggs laid in oviposition traps were *Ae. triseriatus* whereas only 6% were the sibling species, *Aedes hendersoni* (Cockerell). Also, morphological examination by Landry and DeFoliart (1986) showed that all of 622 *Ae. triseriatus/Ae. hendersoni* adults trapped at this site were *Ae. triseriatus*.

For laboratory evaluation of the attractancy of fish oil emulsion to ovipositing female mosquitoes, 7 to 10-day-old F_2 *Ae. triseriatus* were used in cage oviposition bioassays. Original egg stock came from eggs collected from ovitraps in Dane County, WI. Two to 4-day-old mosquitoes were placed in a 1 m^3 cage and were given 3 days in which to mate. During this time they were provided with a 5% sucrose solution. All cages were kept in an insectary under standard conditions with a photoperiod of 14L:10D including 1 h of evening twilight. Temperature was maintained at 24±2°C and humidity was kept at approximately 95%. After 3 days, females were allowed to feed on an anesthetized mouse (University of Wisconsin-Madison animal welfare assurance #A1457) and randomly chosen blood-engorged females were placed into cages (1 m^3). For the 1% solution, 30 females were used in each cage and for the 5% solution, 25 females were used.

One day later, 2 oviposition containers (plastic dental cups 6.5 cm diam) were placed in each cage. Inserted into each container was a 2.5 × 7.6 cm piece of balsa wood held in place by a #20 binder clip. Placement of one fish oil emulsion treatment and one distilled water control were randomly assigned within each cage, 63 cm apart, with a sucrose source between them. The blood-engorged females were left in the cage for 1 week. Cages were maintained at the environmental regime described above. Three replicates each of a 1% solution and a 5% solution were tested against controls. Each replicate was a 1 m^3 cage. The balsa strips were then removed and the number of eggs counted. A 2-way analysis of variance was performed on the data to determine the effect of both the fish oil emulsion and the effect of emulsion concentration.

The 1988 field season was shortened by drought, and there were few adult *Ae. triseriatus* ovipositing until mid-July. In this truncated field season, data could be collected for only 6 weeks.

In each week of the field experiment, the number of eggs deposited in traps containing dyed oviposition water exceeded those in control traps (Fig. 1). Eggs were not laid in any of the traps containing fish oil emulsion solution. Data were log transformed to prevent the variance from increasing as mean egg number increased. Regression analysis showed that treating water with vegetable dye increased the number of eggs deposited in ovitraps ($P < 0.001$). The total number of eggs laid each week did not vary significantly during the study period ($P = 0.27$). Color of the oviposition water accounted for 76% of the variation in the data. These data provide field confirmation of the laboratory findings of Wilton (1968) that water of high optical density is attractive to ovipositing *Ae. triseriatus*.

Although we used the same concentration of fish oil emulsion solution (1%) as used by Holck et al. (1988), in our tests the emulsion was not attractive to ovipositing mosquitoes in the field. Within a few days, the solutions became cloudy and the oviposition substrate (balsa strips) became covered with a bacterial film. Holck et al. (1988) apparently did not experience this problem.

In the laboratory tests with fish oil emulsion, again, the emulsion solution was not attractive to ovipositing mosquitoes. As in the field studies, the emulsion became covered with a bacterial scum after several days. This material often covered the balsa oviposition substrate making its surface inaccessible. Lower concentrations of emulsion in solution may have reduced bacterial growth. Analysis of variance showed that both the higher and lower concentrations had a strong negative effect on oviposition. Control water received a significantly higher ($P < 0.001$) number of eggs in all cases (Table 1).

In conclusion, fish oil emulsion solution did not attract ovipositing *Ae. triseriatus* females either in the field or in the laboratory; instead,

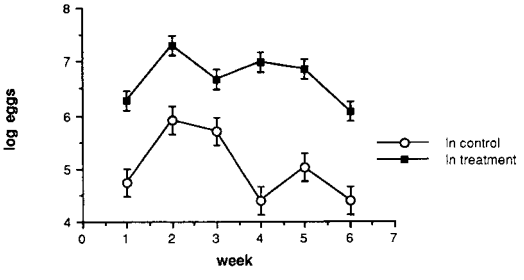

Fig. 1. Total number of eggs laid in paired oviposition traps in the field. Treatment traps contained dyed oviposition water whereas control traps contained tap water. No eggs were laid in traps containing 1% fish oil emulsion during the study period.

Table 1. Laboratory oviposition bioassays with fish oil emulsion solution and a distilled water control.

Concentration of fish oil (%)	No. of eggs deposited*	
	Treatment	Control
5[1]	0	906
5[1]	0	1,054
5[1]	0	1,285
1[2]	1	277
1[2]	5	200
1[2]	7	166

[1] 25 females/cage.
[2] 30 females/cage.
* All treatments significant at $P < 0.001$.

analysis of variance showed the emulsion to act as a repellent. Adding vegetable dye to water to increase its optical density significantly increased the number of eggs deposited in traps in the field. This simple procedure for increasing the sensitivity of ovitraps might warrant the attention of mosquito abatement districts, public health agencies and others charged with monitoring the presence of *Ae. triseriatus* in the field.

The authors wish to thank Joyce Gibbons and Winston Nunes for their assistance during the course of this study. This research was supported in part by the Research Division, College of Agricultural and Life Sciences, University of Wisconsin, and by NIH grant #5-RO1-AI-07453.

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