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A SUBSTRATE MODIFICATION FOR THE OVIPOSITION TRAP USED FOR DETECTING THE PRESENCE OF *AEDES TRISERIATUS*[1]

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The artificial oviposition trap developed by Loor and DeFoliart (1969) provided a means to determine the presence or absence of *Aedes triseriatus* (Say), the vector of LaCrosse encephalitis virus. Prior to their report, the only reliable method available to investigators was by sampling adult female populations by landing-biting rates with human volunteers or estimating the larval population by tree-hole sampling.

The oviposition trap of Loor and DeFoliart (1969) consists of a 12-ounce aluminum can painted black, lined inside with a strip of black muslin cloth, which provided the substrate for ovipositing females. The can was filled with a mixture of organic debris and water in order to wet the cloth, which provided an attractive moisture gradient and resembled tree-hole water in consistency. To assess the number of eggs laid per black cloth liner, the cloth has to be submerged in water and viewed microscopically, or the cloth has to be bleached white. Both procedures are cumbersome, with the former process entailing difficulties in accurate counting and the latter process injuring the eggs.

The purpose of this paper is to report about an oviposition substrate modification such that; (1) the presence or absence of eggs can be determined visually in the field, and (2) eggs can be counted accurately without prior treatment of the substrate.

Preliminary laboratory trials with black oviposition cans using strips of several light wood veneers, pressed board and balsa wood were tested against black muslin cloth liners. All of these trials were done in 1 m^3 colony cages using 25 gravid *Ae. triseriatus* per cage. The infusions used in conjunction and in various combinations with these substrates were dried materials of oak leaf, maple leaf, cottonwood leaf, beech leaf and grass. The results revealed that the balsa wood strips used with oak leaf infusion was the most attractive combination to ovipositing females and that the eggs present could be seen and counted with ease on the light colored background of the balsa wood.

Field trials to test the comparative effectiveness between oviposition substrates of balsa wood and black muslin cloth were carried out at Potato Creek State Park, Indiana during August, 1978. A transect having 4 stations with 3 oviposition cans per station was established. Each of the 4 stations had an oak tree (*Quercus* spp.) to which the oviposition cans were fastened, with the bottom of the oviposition cans resting on the ground. All of the oviposition cans contained approximately 250 ml of oak leaf infusion. The 3 substrates used at each station were: (1) a balsa wood strip, (2) a black muslin cloth liner, and (3) a balsa wood strip plus a black muslin cloth liner. The oviposition cans were serviced every 2 days at which time the infusions and substrates were changed. The substrates were rotated to different cans at this time to inhibit position biases. The substrates were returned to the laboratory to determine the number of eggs present.

During the course of the field experiment, 1754 eggs of *Ae. triseriatus* were collected. The data in Table 1 show that a strong preference for the balsa strips was exhibited by gravid females. Of the eggs collected, 87% were on the balsa strips with the remaining 13% on the black cloth liners. Periodic challenges of the balsa wood strip, modification with black muslin cloth lined cans during the summer of 1979

[1] This study was supported in part from NIH research grant AI-02753, and NIH Training Grant 5-T32-AI 07030-02.

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Table 1. Substrate preference of gravid *Aedes triseriatus* using artificial oviposition traps charged with oak leaf infusion with balsa wood strips and black cloth liners, at Potato Creek State Park, Indiana, August, 1978.

Station	Percent eggs deposited		
	Balsa strip	Black cloth	Balsa & cloth (b/c)
1	0	5	9/5
2	6	1	0/0
3	18	1	1/0
4	47	0	6/1
Total	71	7	16/6

at 4 other wood lots in Northwest Indiana, substantiated the results of these experiments. In all of these challenges, less than 10% of all oviposition occurred on black cloth liners.

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FILTER PAPER TEST FOR RAPID DETERMINATION OF PHENOTYPES WITH HIGH ESTERASE ACTIVITY IN ORGANOPHOSPHATE RESISTANT MOSQUITOES

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Organophosphate (OP) resistance is associated with high esterase activity in several species of mosquitoes (Yasutomi 1970, 1971, Pasteur 1977, Georghiou and Pasteur 1978). In *Culex quinquefasciatus* Say from California and in *Cx. pipiens* L. from France, the high esterase activity is due to a dominant allele (*A*) of a specific gene and cannot be separated from OP resistance; therefore, all phenotypes with the highly active esterase (*AA* homozygotes or *AO* heterozygotes) are resistant (*RR* or *RS*, respectively) while those without it (*OO* homozygotes) are OP-susceptible (*SS*) (Georghiou et al. 1980, Pasteur 1977). Thus, in any experiment, the determination of the proportion of individuals that do not have high esterase activity is analogous to the determination of mortalities at insecticide dosages that kill susceptible insects but do not affect resistant individuals (Pasteur and Georghiou 1980).

Until recently, esterase phenotypes were determined by starch electrophoresis of homogenates of individual mosquitoes. We describe here a filter paper test that accomplishes almost the same tasks, but avoids time-consuming manipulations and expensive apparatus. The same principle has been employed in Japan in studies of OP resistance in the leafhoppers *Nephotettix cincticeps* and *Laodelphax striatellus* (Ozaki 1969).

DESCRIPTION OF THE TECHNIQUE

EQUIPMENT. Strip of Whatman No. 2 filter paper, 12 × 15 cm; one plexiglass plate, 20 × 20 × 0.5 cm; one hemolysis test tube of ≃ 1 cm diam.; one "Pipetman" or "Eppendorf" pipette for measuring 15 μl volumes (or if not available, a Pasteur pipette, the tip of which has been pulled over flame).

STOCK SOLUTIONS. Phosphate buffer (pH 6.5) composed of 4.8 g Na_2HPO_4 and 9.2 g KH_2PO_4 per liter of water; substrate solution composed of 1% α-naphthyl acetate (Sigma No. 6750) in acetone; fixing solution composed of 10% acetic acid in water.

WORKING SOLUTIONS. *A.* 100 ml of phosphate buffer and 10 ml of substrate solution; *B.* 300 mg of Fast Garnett GBC salt (Sigma No. F0875) in 100 ml of water; *C.* fixing solution (as described above). These solutions should be prepared just before use.

PREPARATION OF THE MOSQUITOES. Adults or young 4th-instar larvae may be used. Anesthetized adults or larvae that have been carefully dried on tissue paper are placed at freezing temperature for a minimum of 20–30 min before use. Insects can be stored for 1 or 2 weeks at −20°C and many months at −50°C without deterioration of esterase activity.

PROCEDURE. Single mosquitoes are deposited in a 15 μl drop of distilled water on the plexiglass plate and then thoroughly crushed with the bottom of the hemolysis tube. After each homogenization, the bottom of the test tube is firmly blotted on the Whatman filter paper, which has been placed on several layers of tissue paper. When 10 to 20 mosquito

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