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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homogenates have been deposited, the filter paper is immersed in solution *A* for 90 sec. It is then blotted between two tissue papers and transferred to solution *B* for 60 sec. Finally, it is dipped in solution *C* and then allowed to dry for recording of results.

RESULTS AND DISCUSSION

Mosquitoes that give faintly stained spots have an *OO* phenotype for esterase and are OP-susceptible (*SS*); those that give a strongly stained spot have an *AO* or *AA* phenotype for esterase and are OP-resistant (*RS* or *RR*) (Fig. 1).

The filter paper test has been used in our laboratory to study the linkage relationships between the gene that codes the highly active esterase (*EST-B*) in *Cx. quinquefasciatus* and various morphological genes, e.g. *y* (yellow larva), *pl* (plum eye) (Pasteur et al., (unpublished), and OP and permethrin resistance genes (Halliday et al., (unpublished). The technique proved extremely reliable and allowed us to survey much larger samples than would have been possible using electrophoresis techniques. Thus, the filter paper test has made the *Est-B* an excellent marker gene for future formal genetic studies without the need for special equipment to analyze it.

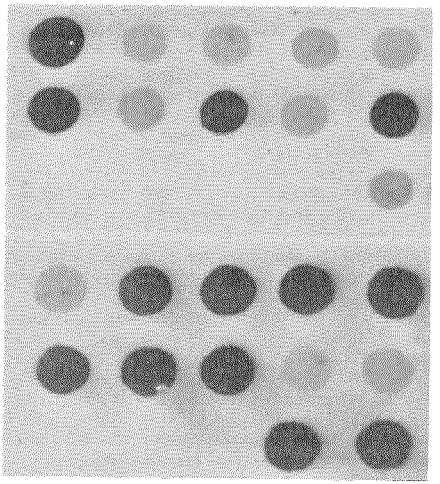

Figure 1. Filter paper showing the differences in esterase staining intensities among the offspring of a *SR* × *SS* cross. Dark spots correspond to individuals possessing the highly active esterase B, i.e., *SR* OP-resistant, light spots to individuals without esterase B, i.e., *SS* susceptible.

However, we believe that the filter paper test could provide more rewarding applications in natural population surveys for the detection of OP resistance and the estimation of its frequency. Up to now, natural populations of only 2 species, *Cx. quinquefasciatus* from California and *Cx. pipiens* from France, have been investigated thoroughly enough to show that they segregate for a single OP-resistant gene and a single gene of high esterase activity (Pasteur 1977, Pasteur and Georghiou 1980, Pasteur et al. 1980).

The applicability of the filter paper test to cases in which more than one gene are known to code highly active esterases remains to be examined.

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BITING FLIES COLLECTED FROM RECUMBENT BLUETONGUE-INFECTED SHEEP IN IDAHO

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A severe outbreak of bluetongue (BT) disease occurred in a flock of sheep near Bruneau, Owyhee Co., Idaho, August, 1973: 25% of 650 ewes and 12% of their lambs died. The probable vector was the biting midge or gnat, *Culicoides variipennis* (Coquillett), as reviewed by Jones and Foster (1978).

The presence of recumbent BT-infected sheep allowed the recovery of biting flies attacking sheep by mouth aspirator. The number of flies collected in 3 morning collections at about sunrise on August 24 and 27 and on September 12, 1973 are as follows:

CERATOPOGONIDAE	
Culicoides	
variipennis (Coquillett)	27
owyheensis Jones and Wirth	7
Leptoconops	
americanus Carter	4
SIMULIIDAE	
Simulium	
vittatum Zetterstedt	89
CULICIDAE	
Anopheles	
freeborni Aitken	13
Aedes	
dorsalis (Meigen)	6
nigromaculis (Ludlow)	4

Two additional species of *Leptoconops* were recovered on August 26, 1973, in an evening collection just before dusk: 1 *L. knowltoni* Clastrier and Wirth, and 15 *L. reesi* Clastrier and Wirth.

Bluetongue virus was recovered from parous females of *C. variipennis* collected during the outbreak (Barber and Jochim 1975), but not from any other species of biting fly.

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A SECURITY MODIFICATION FOR THE "AMERICAN MODEL" MOSQUITO LIGHT TRAP

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The San Mateo County Mosquito Abatement District employs 15 modified "American Model" light traps (Mulhern 1953) in its adult mosquito surveillance program (Fig. 1). The District, composed largely of suburban type communities with middle income families, has experienced increased problems of light trap security in the past few years. On occasion, traps placed in some areas have been the target of vandals. More importantly, to this District, was the potential hazard to unauthorized personnel who may tamper with the exposed cyanide kill jars used in the traps. These con-