Table 1. Effect of exposure of field collected aedine eggs to hot water on rate of recovery in 0.3% solution of hydrogen peroxide.

Pre-separation treatment	Eggs Treated[a] No.	Percentage Eggs Recovered		
		Minimum	Maximum	Mean & S.E.
Not heated	1000	89	98	94.0 ± 2.9
Heated w/o drying	1000	94	100	98.6 ± 1.8
Heated and dried	1000	94	100	97.0 ± 1.6

[a] Number of replicates 10 for each treatment.

wax and placed in water to cleanse them of hydrogen peroxide prior to examination.

RESULTS AND DISCUSSION. Yields of eggs are uniformly high following separation from extraneous organic debris by the method described. Table I shows the effect of pre-separation treatments on the percentage of eggs recovered. Eggs not heated and assumed to be viable are not recovered as readily, or with equal regularity, as heat treated eggs. The percentage of recovery for heat treated eggs flattened by air drying is comparable to that for eggs heat treated but not dried.

Heating the eggs and debris prior to separation not only increases the likelihood of the eggs remaining submerged after addition of the solution, but also prevents eggs from hatching while under examination.

Several features of hydrogen peroxide separation make it adaptable to egg surveys for both abatement operations and investigative purposes. No appreciable difference has been observed in the percentage of recovery among eggs of aedine species commonly found in soil samples collected in Central Illinois (*Aedes vexans, Ae. trivittatus, Ae. sticticus, Ae. dupreei, Psorophora horrida, Ps. confinnis, Ps. ciliata*). Since nonviable eggs are also recovered using hydrogen peroxide separation, their relative frequency in a sample can be determined. Eggs intended for rearing may be separated using hydrogen peroxide if the recovery rate shown for eggs lacking pre-separation heat treatment is acceptable. Materials used in the procedure are relatively inexpensive and readily available. Above all, the method provides satisfactory terminal egg separation without the time consuming manipulation of the sample as required by optical separation.

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FURTHER OBSERVATIONS ON THE HOST RANGE OF THE MOSQUITO FUNGUS *CULICINOMYCES*

A. W. SWEENEY

Malaria Research Unit, RAAMC, Ingleburn
NSW 2174, Australia

Mosquito pathogenic fungi of the recently described genus *Culicinomyces* Couch, Romney and Rao have been discovered independently in Australia (Sweeney et al. 1973) and in the United States of America (Couch et al. 1974). A study of the Australian fungus has shown that it is pathogenic to larvae of Culicidae, Chironomidae, and Ceratopogonidae but not to some other aquatic insect larvae, the mosquito fish *Gambusia,* and freshwater shrimps (Sweeney 1975a). As these observations suggested that the susceptible hosts of this fungus may be restricted to certain dipterous families, an additional series of infection experiments was performed with aquatic larvae from other families of the Diptera.

Aquatic larvae of the families Tipulidae, Simuliidae, Stratiomyiidae, and Syrphidae were collected from fresh water habitats near Sydney and were transported to the laboratory

for infection studies. The tipulid, stratiomyiid, and syrphid larvae were placed in plastic trays 18 × 12 × 5 cm containing water from their respective collection sites. Simuliid larvae of the genus *Austrosimulium* were collected from a swiftflowing creek near Ingleburn. They were difficult to maintain in the laboratory though preliminary observations showed that their survival was best in strongly aerated water. Those used for these experiments were placed into ten liter plastic buckets containing 2 liters of water from the creek in which they were collected and were aerated vigorously with an air pump.

The test larvae were exposed to inocula prepared either *in vitro* (from agar culture) or *in vivo* (from dead infected larvae) as described previously by Sweeney 1975b. Some other larvae of each family were left untreated as a control. Living uninfected larvae of *Culex quinquefasciatus* Say (=*fatigans*) were added to each of the test trays and buckets as an indicator; their subsequent infection and death confirmed the infectivity of the inocula. Dead specimens were examined for the presence of the fungus and each experiment was not concluded until after the death of all the indicator *Culex* larvae.

The results showed no appreciable test mortality with larvae of the families Tipulidae, Stratiomyiidae, and Syrphidae (see Table 1). Most test and control simuliid larvae died within 2 days though a small proportion survived as long as 4 days. Two of the latter showed signs of internal mycelia and subsequently developed an external sporulating layer with the characteristic morphology of *Culicinomyces*.

Nematocera. Within the Nematocera, only members of the Division Culicimorpha were susceptible (families Culicidae, Chironomidae, Ceratopogonidae, and Simuliidae), whereas species of the Division Psychodomorpha (family Psychodidae) and Tipulimorpha (family Tipulidae) were not susceptible. Within the Suborder Brachycera, larvae of the families Stratiomyiidae and Syrphidae did not appear to be affected by the fungus. Although large numbers of tipulid and stratiomyiid larvae were not available for testing and many other insects remain to be tested for susceptibility, these observations support the view that *Culicinomyces* may be restricted to aquatic larvae of the Culicimorpha and thus it may have a potential for use against midges, biting midges and black flies as well as mosquitoes. It would appear to be particularly worthy of consideration for development as a candidate biological control agent as its host range may be wide enough to include many insect species of medical and veterinary importance but not so wide as to adversely affect many other harmless or beneficial insects.

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Table 1. Mortality of larvae exposed to *in vitro* and *in vivo* inocula of *Culicinomyces*.

Test Larvae	*in vitro* Inoculum		*in vivo* Inoculum		% control Mort.	% *Cx. quinquefasciatus* Mort.
	No. tested	% Mort.	No. tested	% Mort.		
Tipulidae	9	11	9	0	16.6	100
Simuliidae	143	100*	144	100	100	100
Stratiomyiidae	20	0	20	0	0	100
Syrphidae	78	0	49	0	0	100

* Two of these specimens developed *Culicinomyces* sporulation on the exterior cuticle of thorax and abdomen.

The only susceptible hosts of *Culicinomyces* within the range of animals tested in these experiments and in those conducted previously (Sweeney 1975a) were in the suborder

Sweeney, A. W. 1975b. The mode of infection of the insect pathogenic fungus *Culicinomyces* in larvae of the mosquito *Culex fatigans*. Aust J. Zool. 23:49–57.
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AEDES ATROPALPUS IN ABANDONED TIRES IN JEFFERSON COUNTY, KENTUCKY[1]

CHARLES V. COVELL JR. AND ALAN J. BROWNELL

Department of Biology, University of Louisville, Louisville, Ky. 40208

Answering a complaint as an employee of the Louisville—Jefferson County Department of Public Health Mosquito Control Project, the junior author collected 31 mosquito larvae from abandoned tires behind a tire store in downtown Louisville, Kentucky, on 27 July, 1978. The senior author identified all the larvae as *Aedes atropalpus* (Coquillett). More larvae were taken on 11 and 18 August and reared and identification of the adults substantiated the original determination. During the 18 August visit the tires were treated with a suspension of chlorpyrifos (Dursban®) in water. No other mosquito species was found cohabiting the tires during these 3 visits.

No more larvae were found in these tires until 11 September, when 11 *atropalpus* larvae were recovered along with several *Culex pipiens* Linnaeus and *Cx. restuans* Theobald. At another site about a block away, other tires were found to harbor 16 *Ae. atropalpus,* along with *Ae. triseriatus* (Say), *Cx pipiens,* and *Cx. restuans* (collections on 7 and 18 September). No collections were made after 18 September, and the Health Department had by that time initiated legal measures to force the owners to remove the old tires.

Although *Ae. atropalpus* had not been reported from Kentucky by Covell (1968), a single specimen was reported from Cumberland Falls, Whitley Co. (in southeastern Kentucky) by Zavortink (1972), collected by N. E. Good on 12 August, 1948. Our collections of this species represent the first record for Jefferson County, and apparently for the lower Ohio Valley area, representing a larval population extending from 27 July to 18 September, 1978. More important, here is solid evidence that *Ae. atropalpus* will breed in old tires. Breeding sites for this species are usually characteristic of those cited by Zavortink (1972): "holes in rock and concrete, and in rock-filled pools."

Larval and adult specimens referred to here are deposited in the collection of the University of Lousiville. This addition brings to 42 the number of mosquito species recorded from Jefferson County, Kentucky. The authors thank Maj. Edward S. Saugstad, U. S. Army, for his input.

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[1] Contribution No. 193 (New Series) of the Department of Biology, University of Louisville.

A METHOD FOR DEMONSTRATING MOSQUITO EGG HATCH TO LARGE AUDIENCES USING A 35 mm SLIDE PROJECTOR[1,2]

A. A. DIEDWARDO

Mosquito Research and Control, P. O. Box 231, New Brunswick, NJ 08903

Demonstration of mosquito egg hatch and other phases of mosquito biology usually requires providing microscopes for each individual or for small groups of individuals. This does not pose a serious problem in the laboratory-classroom; however it can be time-consuming if not impossible when large audiences are involved, particularly away from the classroom.

The method herein described involves construction of a small glass cell which can be

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