

IMPROVED OVIPOSITION MEDIUM FOR *MANSONIA* COLONIZATION¹

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ABSTRACT. In an attempt to develop more suitable oviposition media for *Mansonia* colonization, 6 aquatic plants and styrofoam² were offered as substrates to determine oviposition preferences by placing them in the same ovipot. Female *Mansonia* laid eggs on each, i.e.; *Pistia stratiotes*, *Salvinia cucullata*, *Lemna minor*, *Alternanthera philoxeroides*, *Jussiaea repens*, *Eichornia crassipes* and styrofoam. The oviposition percentages were 50.2, 23.6, 22.2, 1.3, 0.9,

0.5 and 1.3, respectively. Since the experiment indicated that the mosquitoes would oviposit in the absence of living aquatic plants a new ovipot with sliced styrofoam floating on the surface of tap water in a small plastic jar was prepared. Since our results were similar to those obtained using Wharton's ovipot, this technique is now routinely used in our laboratory for the oviposition of *Mansonia uniformis*, *Ma.indiana* and *Ma.annulifera*.

INTRODUCTION

Because of difficulties in rearing *Mansonia* mosquitoes, important vectors of Brugian filariasis, various procedures to improve their colonization have been tried in our laboratory where colonies of *Mansonia uniformis*, *Ma.indiana* and *Ma.annulifera* have been established. Some well known aquatic plants such as *Pistia stratiotes* were used for oviposition by Wharton (1957) but the drawbacks are: 1) the plants are not always available, 2) they die before the experiment ends and 3) they can be infested with insects or worms. Laurence et al. (1962) used impregnated paper discs instead of aquatic plants but that type of paper is expensive and difficult to obtain. This paper describes an easy, practical and successful medium for *Mansonia* oviposition.

MATERIALS AND METHODS

Three species of *Mansonia*, *uniformis* (Theobald), *indiana* Edwards and *annulifera* (Theobald) collected from Bangluek canton, Chumphon province,

south Thailand were used. They have been established and maintained in our laboratory for 30, 26 and 26 generations, respectively by a modification of Wharton (1957) using *Jussiaea repens* as host plant for the larvae.

The aquatic plants used in the experiment (*Pistia stratiotes*, *Salvinia cucullata*, *Lemna minor*, *Jussiaea repens*, *Alternanthera philoxeroides* and *Eichornia crassipes*) were collected locally around Bangkok.

The sliced styrofoam (1-2 mm thick, cut into pieces 1 × 1 cm) used for oviposition was the common expanded styrofoam used for packing electronic equipment.

Our ovipot was modified from that described by Wharton (1957) and differs as described below. The sliced styrofoam pieces were placed on the surface of the tap water contained in a plastic jar, 9.5 cm diam × 10 cm covered with a netting lid. Only a small amount of water was used and the foam pieces were so arranged that little exposed water surface was left. Gravid *Mansonia* mosquitoes were placed into the jar for oviposition.

To study the oviposition preference on host plants and styrofoam, the 6 species of aquatic plants and styrofoam were placed in Wharton's ovipot of 20 cm diam and 30 cm height. The surface area of each oviposition substrate was approx-

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² Styrofoam[®], expanded polystyrene.

imately the same. Twenty gravid females of each species that had been held in small paper cups for 2-3 days after feeding on human blood were placed into the ovipot. Oviposition results were subsequently recorded until no egg-clusters were deposited.

The relative efficacy of Wharton's ovipot and the new ovipot were compared by using the numbers and hatching rates of egg-clusters and mortality rates of adults. All experiments were carried out in the insectary with 28-30°C and 80-90% relative humidity with 4 replicates.

RESULTS

Egg-clusters were deposited on *P.stratiotes*, *S.cucullata*, *L.minor*, *A.philoxeroides*, *J.repens*, *E.crassipes* and styrofoam. While most egg-clusters were found on *P.stratiotes*, some were found on *S.cucullata* and *L.minor* and few on the rest of the plants and styrofoam. All *Mansonia* species had similar patterns of host plants preference (Table 1). The presence of egg-clusters on foam led to further experiments using sliced styrofoam for oviposition.

In a direct comparison, gravid female mosquitoes of *Ma.uniformis*, *Ma.indiana* and *Ma.annulifera* were allowed to lay eggs in Wharton's ovipot containing only *P.stratiotes* and in the new ovipot containing sliced styrofoam. Collectively, for the 3 species, 95.8% of the females deposited in the new ovipot and 95.0% in Wharton's ovipot (Table 2). All egg-clusters from foam substrates showed hatching and 91% of all eggs hatched. In Wharton's ovipot, 100% of the egg-clusters showed hatching and 93% of the eggs hatched. Some females died on the surface of the water and accounted for mortality rates of 2.1% in our ovipot and 2.9% in Wharton's ovipot (Table 2).

DISCUSSION

The results suggest that the gravid females of these 3 *Mansonia* species have

Table 1. Oviposition preference of *Mansonia* mosquitoes on various kinds of host plant and foam (20 mosquitoes/test for 4 replicates).

Species of <i>Mansonia</i>	Percentages and total numbers of egg-clusters ()						Foam	Total
	<i>P. stratiotes</i>	<i>S. cucullata</i>	<i>L. minor</i>	<i>A. philoxeroides</i>	<i>J. repens</i>	<i>E. crassipes</i>		
<i>uniformis</i>	45.4(35)	26.0(20)	23.4(18)	1.3(1)	1.3(1)	0 (0)	2.6(2)	100.0(77)
<i>indiana</i>	50.7(38)	17.3(13)	26.7(20)	2.7(2)	0 (0)	1.3(1)	1.3(1)	100.0(75)
<i>annulifera</i>	54.8(40)	27.4(20)	16.4(12)	0 (0)	1.4(1)	0 (0)	0 (0)	100.0(73)
Total	50.2(113)	23.6(53)	22.2(50)	1.3(3)	0.9(2)	0.5(1)	1.3(3)	100.0(225)

Table 2. Comparison between Wharton's ovipot and the new ovipot on egg-laying and mortality rate of *Mansonia* mosquitoes (20 mosquitoes/test for 4 replicates).

Species of <i>Mansonia</i>	Wharton's ovipot (<i>P. stratiotes</i> as medium)		New ovipot (styrofoam as medium)	
	% and no. of egg-clusters ()	% and no. of dead mosquitoes ()	% and no. of egg-clusters ()	% and no. of dead mosquitoes ()
<i>uniformis</i>	95.0(76)	2.5(2)	100 (80)	0(0)
<i>indiana</i>	97.5(78)	2.5(2)	95.0(76)	2.5(2)
<i>annulifera</i>	92.5(74)	3.6(3)	92.5(74)	3.6(3)
Total	95.0(228)	2.9(7)	95.8(230)	2.1(5)

particular oviposition preferences on aquatic plants. When given a choice, they select *P. stratiotes* (Table 1).

The observation that a few egg-clusters were laid under the rough surfaces of styrofoam suggested that egg-laying could be induced in the absence of living plants. Thus, a new ovipot using sliced styrofoam was developed. In direct comparisons between Wharton's ovipot and the new ovipot, the gravid females readily laid eggs in the latter as well as in the former. The similarity of egg-hatching between both ovipots clearly demonstrated that the new ovipot could be used. The advantages of the new ovipot are that egg-clusters are more clearly observed, it is inexpensive and reusable. The sliced foam is always available and easily arranged in the ovipot so that little water surface is available, preventing the mosquitoes from drowning. The new ovipot is now routinely used in our laboratory.

Styrofoam was also successful for larvae and pupae of *Mansonia* and *Coquillettidia* (Breeland et al. 1981). Ten percent of adults emerged when the rough surfaced styrofoam was used and it was about the same rate as in our observations, using *M. uniformis*. Further research on using styrofoam for larval and pupal development is needed.

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