LABORATORY COLONIZATION OF *MANSONIA* IN MALAYSIA: A PRELIMINARY REPORT

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Mosquitoes of the genus *Mansonia* are the vectors of filariasis due to *Brugia malayi* in South and Southeast Asia. Wharton (1962) found that besides *B. malayi,* various endemic *Mansonia* species were the natural hosts of *Brugia pahangi, Dirofilaria immitis, D. repens, D. magnilavatum* and three other unidentified filariae. Yen et al. (1982) recently described the infective larvae of a number of filaria species of animals of Malaysia using *Aedes togoi* (Theobald) as an experimental host. Further research on the identification of filaria species of the region within their natural *Mansonia* vectors, and on many aspects of the vector-parasite relationship is needed to better understand important aspects in the epidemiology of human filariasis. These studies await the availability of an adequate supply of suitable laboratory bred colony material.

Colonization of *Mansonia* species requires the maintenance of aquatic plants or substitutes which act as attachment hosts for the larvae and pupae in the culture medium. Jayewickreme and Niles (1952) reared *Mansonia uniformis* (Theobald) in small numbers in an infusion of dried powdered guinea pig dung in tap water using *Salvinia* and *Pistia* for larval attachment. Wharton (1957) reared larvae in Malaysia with the same infusion and had similar success using *Eichornia* for larval attachment. Laurence and Smith (1958) used a diluted infusion of guinea pig dung pellets suspended in tap water with a grass sod in the culture and yeast added to rear *Mansonia uniformis* and *Ma. africana* (Theobald). They used wet strength brown paper for larval attachment. Wharton (1962) made further improvements in his technique using rabbit dung infusion over a half-inch layer of fine sandy earth. Laurence, Page and Smith (1962) subsequently used a guinea pig diet pellet infusion in large scale colonization.

Mansonia uniformis has been maintained in culture for over 20 generations at the Institute for Medical Research, Malaysia (Cheong 1979) in a guinea pig dung infusion using *Eichornia* but it has been difficult to obtain adequate numbers of robust adults. Subsequently, *Eichornia* was replaced with a 'keaykolour' ruffia snow white paper (substance 250 GSM). Recently, in an attempt to provide a wider range of material suitable for studies on filariasis transmission our studies on *Mansonia* colonization were expanded to cover more species and to measure the yields of adults from cultures using different media and larval attachment hosts. Some preliminary results of the study are presented.

Egg masses are obtained from gravid females using the ovipot method described by Laurence and Smith (1958). They hatch in four days into dilute culture medium provided with plant or paper for larval attachment.

Two basal media, the original guinea pig dung infusion used in Malaysia (Wharton 1957) and an infusion of Bacto® liver powder in water and dried yeast powder (Samarawickrema 1968, Nayar 1973) are compared using either the paper or *Jussiaea repens,* an aquatic weed, for larval attachment.

A stock infusion of guinea pig dung is prepared by suspending oven dried ground dung at a concentration of 10 gm per liter of deionized water. One part of 48 hr old infusion is mixed with two parts of deionized water in the cultures. The liver yeast infusion is prepared by suspending a mixture of equal parts of Bacto liver powder and a local brand of ground dried yeast at a concentration of 3 gm per 3 liters of deionized water. A typical culture is set up with 250 newly hatched larvae in 3 liters of medium in a colorless transparent rectangular plastic container 30 cm × 16 cm and 20 cm high covered with a plastic lid and paper or plant for larval attachment. A small quantity of yeast powder is added to each culture once every three days when the paper is renewed. The experiments are being carried out in a humidified insectary at a temperature range of 24–26°C. Five species, *Ma. uniformis, Ma. indiana* (Edwards), *Ma. bonneae* (Edwards), *Ma. dives* (Schiner) and *Coquillettidia crassipes* (Van der Wulp) are being colonized for which data from the first three named are presented. This is probably the first time permanent colonies of *Ma. bonneae* and *Ma. indiana* have been established.

Table 1 shows the average percentages of pupation and percentages of emergence of adults in different types of culture together with the number of larvae pupating and the number of adults emerging. The highest pupation, 39% for *Ma. uniformis,* 38.7% for *Ma. bonneae,* and the highest adult emergence, 27.2% for *Ma. uniformis* and 25.9% for *Ma. bonneae,* have been obtained in cultures using liver and yeast infusions and paper for attachment. *Mansonia indiana* showed the least successful

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Table 1. Percentage pupation and emergence and number of pupae and adults from first instar larvae of *Mansonia* set up in different culture media with 250 larvae in each culture.

Type of culture	Species of *Mansonia*	No. of tests	No. of I stage larvae set up	Pupation			Emergence			
				Average percentage pupated	Range	Total number pupated	Average percentage emerged	Range	Total number ♂♂	Total number ♀♀
Guinea pig dung infusion	*uniformis*	24	6000	26.8	0–80.0	1608	18.7	0–55	561	563
+ yeast	*bonneae*	10	2500	17.5	0–35.2	438	12.2	0–26	162	144
+ paper	*indiana*	7	1750	29.1	18.8–46.8	510	11.4	0–27.6	110	90
Liver/ yeast + paper	*uniformis*	25	6250	39.0	1.2–80.0	2440	27.2	0–54	883	820
	bonneae	11	2750	38.7	22.4–61.6	1064	25.9	13.2–53.6	365	348
	indiana	3	750	27.2	10.4–40.4	204	16.3	6.8–25.6	62	60
Liver/ yeast + *J. repens*	*uniformis*	9	2250	36.3	7.6–74.4	816	27.6	6.0–59.2	280	341
	bonneae	6	1500	25.3	8.0–48.0	380	19.3	4.8–37.2	136	154
	indiana	8	2000	26.5	2.8–55.6	529	17.0	0–40.0	175	164

pupation and emergence among the three species.

The conditions provided in the insectary tend to prolong the life cycle with a consequent increase in the mortality of immature stages. Recently, parallel cultures have been set up in an outdoor insectary with the temperature ranging from 28–30°C. In a preliminary set of outdoor cultures of *Ma. uniformis* the average pupation was 49% and the average emergence 37%. Adequate numbers of robust adults are being obtained from both indoor and outdoor cultures and experimental transmission studies have commenced. Complete accounts of detail colonization experiments of the five species will be presented later.

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A BASIC PROGRAM FOR THE ANALYSIS OF ULV INSECTICIDE DROPLETS[1]

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In order to satisfy labeling requirements, improve application efficiency, and develop a legal historical record of applied droplet sizes, the New Jersey State Airspray Program regularly collects sprayed (airborne) insecticide droplets for microscopic measurement, and

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