LABORATORY COLONIZATION OF *MANSONIA UNIFORMIS*, *MA. INDIANA* AND *MA. BONNEAE* IN MALAYSIA

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ABSTRACT. Methods are described for the laboratory colonization of *Mansonia uniformis, Ma. indiana* and *Ma. bonneae* in Malaysia. Gravid females oviposited in 500 ml beakers with a layer of water covered with small leaves of *Salvinia.* Newly hatched larvae were set up in a basal medium of guinea pig dung and water or liver powder, yeast powder and water. Larvae attached to aquatic plants or 'Keaykolour' ruffia snow white paper. The cultures with paper gave better yields than those with plants. Production of *Ma. uniformis* was higher than the other two species. Twelve generations of *Ma. uniformis* and 11 generations of *Ma. indiana* and *Ma. bonneae* were monitored in the laboratory.

INTRODUCTION

The study of filarial parasites in wild and domestic animals in Malaysia (Poynton and Hodgkin 1939, Edeson et al. 1955) and in Kenya (Buckley et al. 1958) which are morphologically similar to the human filariae *Brugia malayi,* and the necessity to identify these parasites provided an impetus to colonize their vectors, species of *Mansonia.* Jayawickreme and Niles (1952), following Bonne-Wepster and Brug (1939), described a technique for rearing *Mansonia uniformis* (Theobald) and *Ma. annulifera* (Theobald) in small numbers through six generations utilizing a larval medium of dried powdered guinea pig dung in water. Wharton (1957, 1962) used these methods to rear *Ma. uniformis* in Malaysia. A successful large scale colonization of *Mansonia africana* (Theobald) and *Ma. uniformis* from Africa and Malaysia was reported from England (Laurence and Smith 1958, Laurence et al. 1962).

Principal difficulties in the colonization of *Mansonia* have been the provision and maintenance of aquatic plants to which the larvae and pupae attach and the maintenance of an adequate food supply. Early workers used common aquatic plants such as *Pistia, Salvinia* and *Eichhornia* utilized by the immature stages in the field. Laurence and Smith (1958) and Laurence et al. (1962), following Wanson (1944), used crepe paper for larval attachment in infusions of guinea pig dung and animal diet. A piece of grass sod or turf was placed in the container with the newly hatched larvae. These methods were followed by Samarawickrema (1962, 1968) to colonize Sri Lankan strains of *Ma. uniformis* and *Ma. annulifera* in London. Later Samarawickrema (unpublished data) successfully colonized *Mansonia titillans* (Walker) in Florida, USA, using white offset board paper (Gestetner 223T), fish meal and liver yeast media.

Efforts of Wharton (1962) to colonize Malaysian *Mansonia* using guinea pig dung infusion and *Eichhornia* plants yielded small numbers which enabled him to study their susceptibility to human and animal filariae. Recently in Kuala Lumpur, efforts were intensified to standardize improved methods and production of *Ma. uniformis, Ma. indiana* (Edwards) and *Ma. bonneae* (Edwards). Preliminary results were reported by Cheong et al. (1984); details are recorded in this paper.

In the meantime Sucharit et al. (1981) in Thailand have reported on yields of adults obtained in their cultures of *Ma. uniformis, Ma. indiana, Ma. annulifera* and *Ma. bonneae.*

MATERIALS AND METHODS

MAINTENANCE OF ADULTS. Fifty to 100 mosquitoes were maintained in 250 ml unwaxed paper cups closed with netting arranged on a plastic tray. Humidity was maintained at 70–90% by placing wet cotton on cups and covering this with another tray in a humidified insectary at 24–26°C. Mating occurred readily in paper cups.

Adults fed on 10% sugar solution from cotton wool pads placed on the netting. Females were blood-fed either by exposing the mosquitoes to a human arm or a restrained white mouse.

Mansonia mosquitoes lay eggs on the under surface of leaves of aquatic plants. Following the method of Laurence and Smith (1958), bloodfed females were held in paper cups and when gravid were introduced into 500 ml beakers, closed with netting and containing water and young *Salvinia* plants. Most females oviposited immediately.

MAINTENANCE OF LARVAE. Leaves with egg masses were allowed to hatch into a dilute larval medium with a few young *Salvinia* plants in a humidified insectary at 24–26°C and photoperiod L:D 12:12. The eggs hatched mostly overnight between 1900 and 0600 hr. The eggs

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of *Ma. uniformis, Ma. indiana* and *Ma. bonneae* hatched in a mean period of 4, 6 and 5 days, respectively, after their deposition.

Larval cultures were set up in transparent plastic tanks 30 × 16 × 20 cm covered with plastic mesh. Media used were: (a) dried powdered guinea pig dung, 10 gm per liter of deionized water, allowed to stand for 48 hr and diluted 1:2 when used, (b) an infusion of equal parts of Bacto liver powder and a local brand of ground dried yeast, 1 gm per liter of deionized water.

The attachment sites for larvae were the aquatic plants *Eichhornia crassipes and Jussiaea repens,* the semi-aquatic weed *Alternanthera triandra* and 'Keaykolour' ruffia snow white paper (substance 250 GSM), manufactured by Wiggins Teape Paper Ltd., Birmingham, U.K. Selected plants were individually washed several times and the shoot and root systems cleaned of debris and visible organisms before placing them in the culture media. Rectangular pieces of paper 33 × 16 cm folded into two were used, one per culture.

A typical culture contained 250 newly hatched larvae in 3 liters of medium with paper or plant substratum. About 250 mg of dried yeast were added to each culture once in 3 days. Plants were replaced as they deteriorated in the cultures. Papers were replaced with new ones every third day. The pH of the medium in a sample of cultures in each generation was measured using an Orion research digital pH meter. It changed from 6.8 to 7.2. These agreed with the values reported by Laurence and Smith (1958) and Wharton (1962).

Initial cultures were maintained in an indoor humidified insectary at 24–26°C. Subsequently, most cultures were set up in an outdoor unhumidified insectary at 28–30°C.

MAINTENANCE OF PUPAE. In the indoor insectary, pupation of *Ma. uniformis, Ma. indiana* and *Ma. bonneae* occurred in (mean ± SD) 26±2.3, 33±4.6 and 35±7.5 days respectively in guinea pig dung cultures and in 28±3.2, 31±3.2 and 35±3.1 days in liver yeast cultures. In the outdoor insectary pupation in the three species occurred in 18±1.1, 24±1.6 and 28±3.4 days, respectively in guinea pig dung cultures and 19±1.7, 27±2.3 and 27±2.0 days, respectively in liver yeast cultures. When pupae appeared on the paper, they were gently detached with a soft paint-brush, counted and transferred to a beaker of water. Pupae harvested daily were placed inside a cage for adult emergence.

RESULTS

Tables 1–3 show the relative success of the cultures of the three species. The best overall results were obtained for *Ma. uniformis* on paper in guinea pig dung infusion at 28–30°C yielding 53.3% pupation and 37.6% adult emergence (Table 1). The next best was 37.3% pupation and 29.6% emergence in the liver yeast and paper cultures in the same insectary. In contrast, the yield of adults of *Ma. indiana* was very low. Paper and *Jussiaea repens* in dung infusion supported *Ma. indiana* best, yielding 37% mean pupation and 21% mean emergence in the outdoor insectary. *Mansonia bonneae* was most often cultured on paper. The mean emergence success of 18.1% in the indoor insectary was comparable to that of 22.3% in the outdoor insectary (Table 3).

Results from individual cultures of *Ma. uniformis* on paper showed 70.5–70.9% of the pupae emerging as adults and, on plants, 70.2–74.1%. The percentage of pupae emerging as adults of *Ma. indiana* was 47.0–57.2% on

Table 1. Percentage of pupation and emergence from first instar larvae of *Mansonia uniformis* set up in two culture media with 250 larvae in each culture.

Medium	Attachment host for larvae and pupae	No. of tests	Mean % pupation	(Range)	Mean % emergence	(Range)
INDOORS (24–26° C)						
Guinea Pig dung infusion	Paper	30	29.3	(0.0–80.0)	20.7	(0.0–55.2)
	Alternanthera	8	19.1	(4.4–33.2)	15.1	(3.2–26.4)
Liver yeast infusion	Paper	25	39.9	(1.2–77.2)	28.9	(0.4–54.0)
	Eichhornia	5	25.7	(9.6–37.2)	10.2	(6.4–20.0)
	Jussiaea repens	11	29.2	(0.0–74.4)	22.5	(0.0–59.2)
	Alternanthera	5	7.6	(0.0–18.0)	3.8	(0.0–10.4)
OUTDOORS (28–30° C)						
Guinea Pig dung infusion	Paper	25	53.3	(0.0–81.2)	37.6	(0.0–65.6)
	Eichhornia	6	27.3	(7.6–54.4)	20.3	(6.4–43.6)
	Alternanthera	6	11.5	(4.0–20.4)	8.3	(3.6–19.2)
Liver yeast infusion	Paper	27	37.3	(0.0–78.8)	29.6	(0.0–60.4)
	Eichhornia	9	24.3	(2.0–60.8)	18.0	(0.8–41.2)
	Alternanthera	5	*	*	14.7	(5.2–22.4)

* Pupae not counted.

Table 2. Percentage of pupation and emergence from first instar larvae of *Mansonia indiana* set up in two culture media with 250 larvae in each culture.

Medium	Attachment host for larvae and pupae	No. of tests	Mean % pupation	(Range)	Mean % emergence	(Range)
INDOORS (24–26° C)						
Guinea Pig dung infusion	Paper	26	18.7	(0.0–40.0)	8.8	(0.0–27.6)
Liver Yeast Infusion	Paper	27	21.9	(0.0–66.8)	9.6	(0.0–26.4)
	Jussiaea repens	8	23.2	(1.6–44.0)	12.1	(0.0–28.8)
OUTDOORS (28–30° C)						
Guinea Pig dung infusion	Paper	19	36.8	(10.0–67.2)	19.5	(2.0–34.8)
	Jussiaea repens	5	34.7	(27.2–46.4)	21.0	(17.2–24.4)
	Alternanthera	6	11.5	(2.4–20.4)	7.1	(1.6–14.8)
Liver yeast infusion	Paper	17	20.0	(4.4–46.4)	11.5	(1.2–28.0)
	Eichhornia	6	11.7	(2.0–19.2)	6.6	(1.2–11.2)
	Jussiaea repens	6	13.1	(2.0–26.4)	8.3	(0.0–19.6)

paper and 52–60% on plants. In *Ma. bonneae* the emergence was 69.8–74% on paper and 53.1–53.4% on plants. The comparable percentages of adult emergence from pupae in the paper and plant cultures of *Ma. uniformis* and *Ma. indiana* suggest that the gentle dislodging of the pupae attached to paper with a soft paintbrush did not increase their mortality. Larval counts 10–12 days after initiation of cultures showed less mortality in *Ma. uniformis* than in the other two species (Tables 1–3). In all species, mortality of the larvae in the guinea pig dung infusion cultures was more than in the liver-yeast cultures indoors at 24–26°C, while the reverse was true in the outdoor insectary at 28–30°C.

The numbers of adults produced during the colonization of 12 generations of *Ma. uniformis* and 11 generations each of *Ma. indiana* and *Ma. bonneae* are given in Table 4. These results show that even though the mean percentage yields were low, especially in *Ma. indiana* and *Ma. bonneae*, adequate numbers were produced continuously for experimental studies.

DISCUSSION

The cultures using paper gave better overall yields than those with plants in all but one comparison (Tables 1–3), confirming the observations of Laurence and Smith (1958). The papers were uniform, convenient to handle and the progress of larval development could be easily assessed by lifting the papers out of the cultures. In addition, there was less risk of introduction of contamination as in the case of plants.

The three plants tested gave varying results for the three species. Although the use of plants had the convenience of less frequent replacement of the attachment substrate as compared with paper, the debris of rotting plants, especially in the case of *Eichhornia*, probably interfered with larval development. The *Eichhornia* plants deteriorated and withered fast in the organically polluted medium under indoor conditions. It proved to be a poor host to *Ma. bonneae*. *Jussiaea repens* withstood the conditions of the culture medium better and its thick, hardy stem proved better for larval attachment. Predictably, *Alternanthera triandra* was the least effective.

The dung medium was used by Wharton (1957) as a finely ground powder. Laurence and Smith (1958) simplified the preparation by suspending dry dung pellets in tap water and diluting it when setting up the cultures. The guinea pig dung infusion has the disadvantage

Table 3. Percentage of pupation and emergence from first instar larvae of *Mansonia bonneae* set up in two culture media with 250 larvae in each culture.

Medium	Attachment host for larvae and pupae	No. of tests	Mean % pupation	(Range)	Mean % emergence	(range)
INDOORS (24–26° C)						
Guinea Pig dung infusion	Paper	22	21.1	(0.0–34.8)	14.7	(0.0–34.8)
Liver yeast infusion	Paper	51	29.3	(0.0–63.2)	18.1	(0.0–58.8)
	Jussiaea repens	6	34.1	(13.6–54.0)	19.1	(4.8–35.2)
OUTDOORS (28–30° C)						
Guinea Pig dung infusion	Paper	23	31.5	(4.4–50.0)	22.3	(4.4–47.2)
Liver yeast infusion	Paper	20	15.2	(0.0–46.0)	11.3	(0.0–31.2)
	Eichhornia	6	10.7	(0.0–24.8)	5.7	(0.0–13.6)

Table 4. Production of adults of *Mansonia* species in the laboratory through 12, 11 and 11 generations respectively.

	Ma. uniformis			*Ma. indiana*			*Ma. bonneae*		
	No. of adults emerged			No. of adults emerged			No. of adults emerged		
Generation	♂	♀	% emergence	♂	♀	% emergence	♂	♀	% emergence
F1	550	571	25.5	58	79	4.5	362	351	20.7
F2	439	332	26.1	117	124	10.7	226	236	13.2
F3	328	218	9.5	156	166	17.4	294	277	16.9
F4	430	325	14.4	56	42	9.8	117	113	18.4
F5	591	602	18.4	202	194	8.8	311	313	25.0
F6	673	586	27.4	67	81	8.2	438	343	14.9
F7	1888	1762	25.0	315	255	7.9	248	166	15.6
F8	482	462	19.5	500	435	13.8	826	736	20.0
F9	1233	1182	26.7	455	392	10.0	403	387	8.4
F10	1091	999	18.9	273	299	8.9	760	635	15.4
F11	1214	1001	16.4	327	242	13.4	330	250	6.9
F12	1558	1633	24.8						

of the unpleasant odor and the irritant effects on the skin. In London, this medium was abandoned in favor of an infusion of pellets of balanced animal diet in tap water (Laurence et al. 1962).

Larval media with liver powder and yeast have been widely used in mosquito cultures (Nayar 1967, Nayar and Sauerman 1973). Liver-yeast media have also been used to culture *Mansonia* (Samarawickrema 1968, Nayar et al. 1973). In our cultures liver yeast infusion was as effective as the guinea pig dung infusion.

Our results for *Ma. uniformis* are similar to those of Laurence and Smith (1958) and Laurence et al. (1962). Using a new infusion of liver powder and yeast powder, we have successfully colonized *Ma. uniformis, Ma. indiana* and *Ma. bonneae.* Colonization of four species, *Ma. uniformis, Ma. annulifera, Ma. indiana* and *Ma. bonneae* has been recently reported by Sucharit et al. (1981) in Thailand using guinea pig dung infusion and plants. No details have been reported on the numbers produced and the duration of the colonies. Our results using paper and liver yeast medium with better yields represent several improvements on the Thailand work. Our work aims to standardize liver-yeast medium and paper cultures in the colonization of *Mansonia.*

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