OBSERVATIONS ON THE MASS PRODUCTION OF *ROMANOMERMIS CULICIVORAX*, A NEMATODE PARASITE OF MOSQUITOES[1]

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ABSTRACT. In 115 mass-rearing cycles of *Romanomermis culicivorax* Ross and Smith, ratios of nematodes to mosquito larvae of 12, 13, 14, and 15:1, produced mean levels of parasitism of 75, 80, 85, and 88%, respectively. Levels of parasitism between 51 and 60% produced an average 6.3 g of postparasitic nematodes compared with 10.9 g at levels of parasitism between 91 and 100%. Nematode cultures averaged 1912×10^3 preparasites if they were flooded for the first time when they were between 11 and 19 weeks old; hatch declined with age when the cultures were more than 19 weeks old at the time of the first flooding. Total yields were highest (5316×10^3 preparasites) when cultures were flooded for the first time when 8–10 weeks old and then at 3–4 week intervals thereafter.

A number of mermithid nematodes have been reported as potential control agents of their respective hosts. However, to date, only *Romanomermis culicivorax* (= *Reesimermis nielseni* auct. partim.) Ross and Smith, a parasite of larval mosquitoes, has been produced in sufficient quantities to permit substantial field testing. The basic procedure for the mass-rearing of this parasite was developed in 1972 (Petersen 1973, Petersen and Willis 1972). Since then *R. culicivorax* has been reared in a number of laboratories in several countries in quantities sufficient to permit lab-

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oratory and limited field studies. As a result, there are several laboratories and one commercial company interested in establishing large-scale rearing facilities. Reported here are the results of measurements and observations made of 115 generations of mass-reared *R. culicivorax* to better determine the most efficient methods for culturing this parasite.

The basic procedures used in rearing *R. culicivorax* were previously described by Petersen and Willis (1972). Only the parasite-host ratios at the time of exposure and the frequency and intervals between floodings of cultures have been modified from the original procedures.

RESULTS AND DISCUSSION

The 115 mass-rearing cycles averaged 11 (4–25) rearing trays/cycle and ca. 20,000 exposed hosts (*Culex quinquefasciatus* Say)/tray (Petersen and Willis 1972). However, mortality was high during some cycles, and some measurements were not recorded during the 2.5 years of observation, so much of the information reported was derived from only 100 of the cycles.

The preparasite-host exposure ratios were increased from 12 to 13 to 14:1 over the study period. Also, 3 populations were exposed at ratios of 15:1. High levels of parasitism (> 95%) were achieved at all 4 ratios (Table 1). A majority of the exposures at the 12:1 ratio and later at the 13:1 ration failed to produce the desired levels of parasitism (85–90%). As a result the ratio was increased to 14:1 during the latter part of the study. This need to increase the ratios to maintain adequate levels of parasitism indicated that host resistance may have developed.

The mean production of postparasites per mosquito rearing tray was lower at a ratio of 14:1 than at a ratio of 13:1 because several of the cycles at 14:1 produced unusually low yields. The reason for this wide divergence for a given dosage was not determined at the time. However, it has since been learned that the standard treatment (sodium thiosulphate, $Na_2S_2O_3$) of water used in the rearing system to remove free chlorine (CL_2) was not always effective because the city added ammonia (NH_3) to the water supply. The resultant chloramine complex complicated the removal of chlorine and was undoubtedly responsible for much of the variation. Well water is now used exclusively to avoid this problem.

Yield of postparasites averaged 10.9 (5.0–16.9) g/rearing tray and increased proportionately as the level of parasitism increased (Table 2). However, on occasion, some of the higher levels of parasitism (85–100%) gave less yield, perhaps because of premature host mortality caused by multiple parasitism. Also, some mosquito populations were less healthy because of unknown contaminants in the rearing system or because of poor quality food or improper feeding. Any of these factors can result in premature host mortality or in the production of excess male nematodes, both of which reduce nematode weight yields (Petersen 1973).

Table 1. Parasitism by *Romanomermis culicivorax* of *Culex quinquefasciatus* at 4 parasite to host ratios under mass-rearing conditions.

Exposure ratio [a]	No. rearing cycles	Mean % parasitism (range)	Mean nematode yield in g (range) [b]
12:1	29	75 (36–98)	8.8 (2.9–16.9)
13:1	28	80 (55–98)	9.9 (4.4–15.4)
14:1	40	85 (69–98)	9.6 (3.2–13.5)
15:1	3	88 (82–97)	10.7 (10.2–11.0)

[a] Preparasite-host ratio.
[b] Mean yield per rearing tray (20,000 hosts).

Table 2. Yields of postparasitic *Romanomermis culicivorax* obtained from various levels of parasitism of *Culex quinquefasciatus* under mass rearing conditions.

% parasitism	No. rearing cycles	Mean yield in g (range) [a]
31–40	2	3.1 (2.9–3.2)
41–50	1	3.5 —
51–60	5	6.3 (3.6–8.1)
61–70	15	8.1 (4.4–10.8)
71–80	22	9.0 (3.8–13.8
81–90	20	9.9 (5.3–13.3)
91–100	35	10.9 (5.0–16.9)

[a] Mean yield per rearing tray (20,000 hosts).

The maximum yield per tray (16.9 g) resulted from a parasite-host ratio of 12:1 and a 94.8% parasitism of the host population.

Complete histories were kept for only 321 cultures hatched as needed for routine laboratory tests and colony maintenance. Therefore, the flooding patterns that were developed were based on the large number of observations rather than a designed study.

Cultures flooded for the 1st time produced an average 1421 (0–7485) x 10^3 preparasitic nematodes. Those cultures flooded for the 1st time when 11–19 wk old (n = 161) averaged 1912 (0–7485) x 10^3 preparasites. Those flooded for the 1st time after 19 wk produced progressively fewer preparasites (decline in mean hatch of 117 x 10^3/week) until there was little or no hatch after 36 wk at ambient temperatures (26–28°C) (Fig. 1). The maximum single hatch of 7485 x 10^3 preparasites was obtained from a culture that was 12 wk old at the 1st flooding. Though significant hatches occasionally occurred in some cultures at all ages up to 32 weeks, they became less frequent with age. When the total hatches were figured on the basis of age of culture at the time of the 1st flooding, a negative regression (210 x 10^3) preparasites/week was observed after the cultures were 8 wk old (no data are available for younger cultures) (Fig. 1). Also, the average total yield was highest (5316 x 10^3) from cultures flooded between wk 8 and 10 (n = 5). Though these data suggest that greater yields can be obtained by flooding very young cultures (< 10 week), it becomes impractical to flood cultures younger than 8 wk because of the very low hatches. If preparasites of *R. culicivorax* are to be used in mosquito control, the maximum numbers must be obtained from a given flooding. Therefore, cultures 11–13 weeks old at the time of the 1st flooding will generally give the highest single hatch (ca. 2100 x 10^3); also, they will then produce that many again in 2 or 3 subsequent floodings. In contrast, cultures flooded 1st between the 17th and 19th week give about the same yield from the 1st flooding but averaged only half again as many preparasites in subsequent floodings.

The interval between floodings of a culture also had an effect on the total yield. Generally, if cultures were flooded within 2 wk after a previous flooding, the yields of preparasites were small, and efficiency was lost. Cultures flooded at 3-wk intervals produced mean yields of 800 and 725 x 10^3 preparasites after the 2nd and 3rd floodings, respectively; cultures flooded at 4-wk intervals produced mean hatches of 1325 and 590 x 10^3 preparasites; and cultures flooded at 5-wk intervals averaged 810 and 560 x 10^3 preparasites. However, the floodings at 4-wk intervals, though they produced a higher mean hatch after the 2nd floodings compared with other flooding intervals, did not produce as many after the 3rd and 4th floodings as did cultures flooded at 3-wk intervals. Also, floodings at intervals of 5 or more wk usually meant that the culture was past the period of prime productivity by the 3rd flooding.

There was much variation in nematode production from culture to culture within a series and from series to series. Some cultures were mature at 8–9 weeks but others still contained a large proportion of postparasites after 18 weeks. Total hatches ranged from 0 to 13.7 x 10^6 preparasites; ca. 25% produced hatches in excess of 3 x 10^6 preparasites and ca. 10% produced hatches in excess of 5 x 10^6 preparasites.

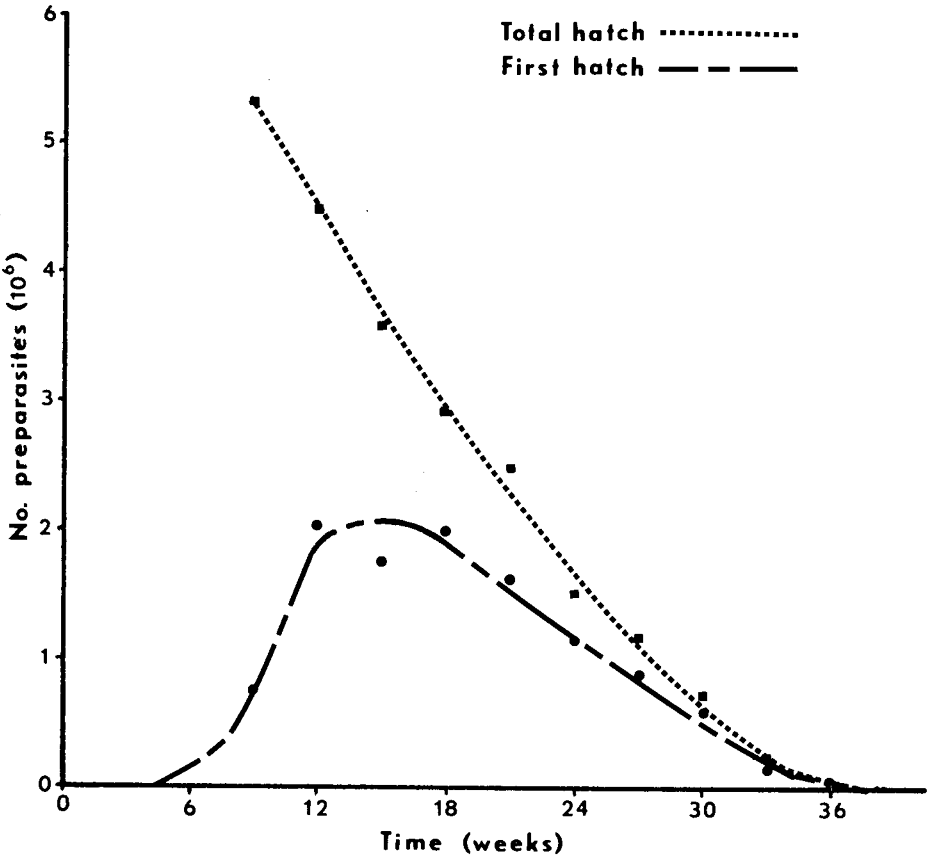

Figure 1. Mean hatches of *R. culicivorax* resulting when cultures were flooded for the first time at the given ages (weeks) and mean total hatches for the same cultures.

The mean hatch for the 321 cultures was 2.47 x 10^6 preparasites.

Although *R. culicivorax* has been studied extensively, little is known about the environmental factors that affect egg development, maturation, and hatching. Also, unexplained mortality greatly reduced the efficiency of some cultures. The causes of this mortality, and methods for controlling it remain to be determined. Culture substrates other than sand are needed, temperature and its effect on storage of cultures must be understood, and density factors within cultures must be resolved before the true production potential of this parasite can be achieved and quality control can be assured.

Literature Cited

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