Mass Production of the Mosquito Parasite Romanomermis culicivorax: Effect of Density

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Abstract: When numbers of *Romanomermis culicivorax* Ross and Smith were varied in containers with a constant surface area and depth of sand, densities of 12–24 nematodes per cm^2 yielded significantly more preparasites than higher densities. When container surface area and numbers of nematodes were constant and sand volume and depth were varied, yields did not differ significantly. When numbers of nematodes and sand volume were constant and surface area and sand depths were varied, yields were significantly higher for a density of 24 nematodes per cm^2. Yields of preparasites were tripled by simply setting up three cultures, each containing 5 g of nematodes, instead of a single culture containing 15 g.

The increasing interest in biological control of mosquitoes with the nematode parasite *Romanomermis culicivorax* Ross and Smith has led to an increasing demand for commercial production of this agent. Such production has been slow in developing, however, because of many inherent problems (potential market, shelf-life, patent protection, production costs, and shipping). Interest in commercial production expressed by the Fairfax Biological Laboratory, Clinton Corners, New York, and by Nutrilite Products, Buena Park, California, has stimulated investigations at the Gulf Coast Mosquito Research Laboratory to define more critically the important factors associated with mass production of the nematode. The study reported here was made to determine the effects of densities of postparasite and adult nematodes in sand cultures on production of the preparasite (infective) stage of *R. culicivorax*. Previous general practice has been to place 15 g of postparasites into a culture tray (22 × 33 × 4 cm) containing about 1000 cc of sand (ca. 33 postparasites/cc of sand) (2). Under these conditions, a culture flooded for the first time during its prime (11–19 weeks) produced an average 1.4×10^6 preparasites; the average total hatch was 4×10^6 preparasites over the life of the culture (1).

MATERIALS AND METHODS

The postparasites used in producing the test cultures were reared in *Culex pipiens quinquefasciatus* Say by using procedures described earlier (2); those used to establish the test cultures for any one trial were all produced from a single rearing cycle. Within 5 days of emergence, postparasites from the various emergence trays were pooled, washed, and weighed out to provide the desired numbers. Numbers of postparasite and adult nematodes used in setting up the cultures were estimated by assuming 2200 postparasites per gram (weight determined by volumetric displacement of water) on the basis of counts made of five 1-g populations. In test 1, different numbers of postparasites were set up in plastic shoe boxes (15 × 30 × 9 cm) each containing 1,000 cc of washed commercial blasting sand and 600 ml of well water. In two trials, postparasites were separated into four populations each of 2.5, 5, 7.5, 10, and 15 g (20 populations). In the third trial the 15-g populations were not included (16 populations). In test 2, each of the three trials included 16 cultures placed in aluminum cake pans (18.5 × 18.5 × 5 cm) lined with plastic. Four each contained 250, 500, 750, and 1,000 cc of sand, 500 ml well water, and 5 g of postparasites of *R. culicivorax*. In test 3, each of the three trials included cultures containing 5 g of postparasites, four of which were placed in each of four types of containers: plastic containers (11 × 13 × 7.5 cm), small plastic-lined aluminum cake pans (18.5 × 18.5 × 5 cm), plastic shoe boxes (15 × 30 × 9 cm), and large plastic-lined aluminum cake pans (22 × 33 × 5 cm). All contained 750 cc of sand and 500 ml of well water (16 cultures/trial).

After cultures were set up, they were covered and stored for 1 week before the excess water was poured off, carrying with it any dead or dying nematodes left on the surface of the sand. After an additional 2 weeks, all remaining free moisture was re-

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moved by using absorbent paper towels, and the cultures were stored an additional 5 weeks. Thus, they were 8 weeks old when they were flooded with 1,000 ml of well water to produce the first hatch of preparasites. The number of hatched preparasites was determined 16 h later by volumetric dilution and by direct counting with a dissecting microscope. Then the free water was removed again, and the cultures were stored an additional 3 weeks before the second and succeeding floodings were made. When the cultures had been flooded four times (8, 11, 14, and 17 weeks old), they were discarded. All tests were conducted at ambient temperature (25–27°C).

RESULTS

In test 1, the container size, sand volume, and depth were constant and the number of postparasites was varied, resulting in 12.2, 24.4, 36.7, 48.9, and 73.3 postparasites per cm^2 of bottom surface area and 5.5, 11.0, 16.5, 22.0, and 33.0 postparasites per cc of volume for cultures containing 2.5, 5, 7.5, 10, and 15 g of postparasites, respectively. The lowest number (2.5 g of nemas/container) produced the highest average yield of preparasites (1.15×10^6/g of postparasites) (Table 1). Moreover, production of preparasites decreased linearly as the density of postparasites increased [$y = 1.36 - 0.07x$, where y = average total culture yield of preparasites ($\times 10^6$ preparasites per culture) and x = postparasite density (grams postparasites added per culture)]. Thus, with 15 g of postparasites per culture only 0.33×10^6 preparasites were produced per gram of postparasites. Though the actual numbers of preparasites produced was highest for the 10-g amount of postparasites (7.17×10^6), there was an actual loss of 37%; the loss was 71% for the 15-g amount.

As the populations of postparasites were increased, the range in the hatch of preparasites at a given concentration increased noticeably. Thus, total hatches ranged from 1.65 to 3.77, 1.69 to 8.79, 0.59 to 10.44, 0.90 to 13.31, and 0.050 to 14.40×10^6 for the cultures at 2.5, 5, 7.5, 10, and 15 g, respectively. There seemed to be a tendency for the cultures with the larger populations of postparasites to mature more slowly, though the trend was not statistically significant.

In test 2, constant numbers of postparasites (5 g) were placed in cultures of constant size but with four sand volumes (250, 500, 750, and 1,000 cc) and corresponding sand depths (0.75, 1.50, 2.25, and 3.00 cm). Preparasite yields did not differ significantly with sand depth and volume.

In test 3, constant numbers of postparasites (5 g) were placed in constant sand volumes in four sizes of containers, achieving surface areas of 143, 342, 450, and 726 cm^2 and sand depths of 0.95, 1.50, 2.25, and 5.40 cm. The yield of preparasites (1.19×10^6/g of postparasites) was significantly greater from containers with a surface area of 450 cm^2 than the other three sizes, and yield was significantly higher from containers with a surface area of 726 cm^2 than from the two smaller containers. The difference in production between the two smallest containers was not statistically significant (Table 3).

DISCUSSION

The results show that densities of post-

TABLE 1. Numbers of preparasites ($\times 10^3$) produced from cultures established with 5 concentrations of postparasites and constant container size, sand volume, and depth.

	Production from indicated number of postparasites (g)[a]									
	2.5		5		7.5		10		15	
Trial	Total[b] pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post
1	3,493	1,397	6,916	1,383	6,213	828	8,575	857	—	—
2	2,464	986	3,434	687	4,787	638	2,900	290	1,351	90
4	2,595	1,038	5,991	1,198	6,869	916	10,028	1,003	8,491	566
x̄	2,851	1,140 a[c]	5,447	1,089 ab	5,956	794 abc	7,168	717 bcd	4,922	328 d

[a]Values are means of four replications.
[b]pre = preparasites; post = postparasites.
[c]Values for means followed by the same letters not significantly different.

TABLE 2. Number of preparasites (× 10^3) produced from cultures established with a constant number of postparasites (5 g), a constant container size and surface area, but four sand volumes and depths.

Trial	Preparasites produced in cultures with indicated volumes (cc) of sand[a]							
	250		500		750		1000	
	Total[b] pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post
1	3,543	709	4,782	956	4,011	802	4,045	809
2	4,533	907	2,975	595	2,092	418	1,557	311
3	2,235	447	4,384	877	2,727	545	2,961	592
$\bar{x}$[c]	3,437	687	4,038	808	2,943	589	2,854	571

[a]Values are means of four replications.
[b]pre = preparasites; post = postparasites.
[c]Values for means not significantly different.

parasite and/or adult nematodes have a significant effect on yields of preparasites (Table 1). Since only volume and surface-area densities were varied in test 1 and since there were no significant differences in production in test 2 when bottom-surface-area densities were held constant and depth and volume densities were varied, bottom surface density is apparently the most important factor in determining the optimum number of postparasites in a culture. Also, production of preparasites differed when bottom-surface-area densities and depth were varied but volume densities remained constant (Test 3). Then, since depth did not have a significant effect in test 2, bottom surface area again appeared to be the most significant factor. However, when the sand is too shallow (<1.0 cm), drying of the culture may become a problem, and when the depth is too great (>2.0 cm), excess moisture at the bottom of the container can result in premature hatching of the preparasites. Also, greater depth would create weight, which would be undesirable for shipping and handling.

The cause of the reduced yield of preparasites (preparasites/g postparasites) in cultures with higher densities of postparasites is not known, but the greater range in hatch at the higher densities indicates that the potential for higher yields is there (14.4 × 10^6 preparasites from one 15-g culture). The failure to achieve the potential, whatever the cause, presently prevents economical use of cultures at the higher densities.

The data indicate that yields of preparasites can be increased some threefold by reducing the density of postparasites in the cultures. Earlier 15-g cultures flooded for the first time at 8–10 weeks produced an average total hatch of ca. 4–5 × 10^6 preparasites (Petersen 1978); in this study, 4.92 × 10^6 were produced (Table 1); however, 5-g cultures produced a mean total of 5.45 × 10^6 preparasites. Therefore, 15 g of postparasites will be utilized most efficiently in producing subsequent generations of pre-

TABLE 3. Number of preparasites (× 10^3) produced from cultures established with constant numbers of postparasites (5 g) and sand volumes but four container sizes, sand depths, and surface areas.

Trial	Preparasites produced in cultures with indicated surface areas (cm^2)[a]							
	143		342		450		726	
	Total[b] pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post	Total pre	Pre/g post
1	2,504	501	2,853	571	5,279	1,056	5,355	1,071
2	2,065	413	3,008	602	6,903	1,381	4,850	970
3	2,051	410	2,232	446	5,601	1,120	3,298	660
$\bar{x}$	2,207	441 a[c]	2,698	540 a	5,928	1,186 b	4,501	900 c

[a]Values are means of four replications.
[b]pre = preparasites; post = postparasites.
[c]Values for means followed by the same letters not significantly different.

parasites if they are placed in three 5-g cultures rather than one 15-g culture. Also, when sand is the culture medium, the density at the bottom surface should be 15–25 postparasites/cm² (0.7–1.1 g wet nemas/100 cm²). Sand depth should be 1–2 cm for best results.

LITERATURE CITED

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