Effects of Culture Age on the Shipment Survival of the Mosquito Parasite Romanomermis culicivorax

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The mermithid nematode *Romanomermis culicivorax* Ross and Smith has drawn considerable interest as a potential biological control agent for larval mosquitoes over the past 10 yr. Since the isolation and initial studies of this parasite at the Gulf Coast Mosquito Research Laboratory (GCMRL), Lake Charles, Louisiana, there has been much demand to supply researchers with cultures of this nematode. As an example, in the past 2 yr (1978–79), 86 shipments have been made from this laboratory to 35 locations, 24 of which were outside the United States. For shipment, cultures of *R. culicivorax* in sand were placed in plastic bags and then into styrofoam containers to protect them from temperature extremes. Nevertheless previous delivery reports usually indicated heavy losses in preparasite yields, often in excess of 80–90%. Direct observations of large numbers of cultures transported to El Salvador (3) and Africa revealed that the stress imposed upon the cultures during transportation resulted in an early egg hatch or the nematodes being killed by the shearing action of the culture sand.

Similar problems were encountered by Fairfax Biological Laboratories, Clinton Corners, New York, when they attempted to distribute *R. culicivorax,* and they discontinued their efforts to market the nematode. Since early egg hatch appeared to be a major cause of mortality in the infective-stage nematodes (preparasite), this study was initiated in cooperation with the Lee County Mosquito Control District (LCMCD), Ft. Myers, Florida, to determine if culture age at time of shipment effected nematode yields.

Cultures used in the study were obtained from normal stocks maintained at the GCMRL (2). As cultures matured to the desired age, half of each was placed into a plastic bag and shipped in styrofoam containers to the LCMCD. The half-culture

Received for publication 23 September 1980.

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that was retained was held undisturbed in the original culture tray. Half-cultures were shipped when they were 2, 4, 6, 8, 10, 12, and 14 wk old, and all age groups were replicated eight times.

Preparasite yields in the half-cultures were determined at both laboratories. Half-cultures were flooded with 800 ml of chlorine-free water for 16 h and the number of preparasites in suspension determined by counting the number of preparasites in three 1-ml samples from each of two dilutions (six counts) for each culture. The high and low counts were disregarded and the mean of the four remaining counts was used to determine the numbers of preparasites. Only actively swimming nematodes were counted. All tests were conducted at ambient temperatures (25–27 C).

The yields from half-cultures 10–14 wk old at the time of shipment were determined 1 wk after shipment; yields from younger half-cultures were determined when they were 10 wk old. Culture hatch was synchronized as much as possible between the two locations. After the preparasite counts were made, free moisture was removed and the half-cultures stored for an additional 3 wk and hatched a second time. If the total combined hatch from both locations for a given culture failed to yield one million or more preparasites, that culture was discarded and another replication shipped in its place.

The yield (both hatches) from shipped half-cultures of all age groups was reduced 29–87%, compared to the unshipped half-cultures (Table 1). However, the difference was not significant for half-cultures shipped when 2 wk old (actually three of the eight half-cultures shipped at this age yielded more preparasites than corresponding unshipped portions). Losses in total yield for shipped half-cultures increased linearly from 29 to 81% ($r = -0.99$) for the 2–8-wk-old cultures, remained constant at 79–81% for 8–12-wk-old cultures, and increased to about 87% for 14-wk-old cultures. Preparasite yield from only the first hatch followed a similar pattern.

The differences in yield from the second hatch between the shipped and unshipped half-cultures were not significant for five of the seven age groups, and the shipped half-cultures averaged higher yields than the unshipped half-cultures for the 6-wk-old cultures. Also, the yields declined with age from both shipped and unshipped half-cultures. The second hatch accounted for only 9–19% of the total yield for unshipped cultures.

These results confirm earlier observations that shipment of *R. culicivorax* cultures resulted in significant losses of yields of infective-stage nematodes. The correlation between increased loss of yield and increased age of the culture at the time of shipment strongly suggests that agitation during shipment resulted in the early hatch of nematode eggs. The high losses (81–90%) exhibited for the 8–14-wk-old half-cultures were sustained by mature eggs that hatched and were killed during shipping. Most preparasites obtained from these shipped

Table 1. Comparison of hatches of preparasites of *R. culicivorax* between shipped and unshipped cultures at seven ages.

	Number of hatched preparasites ($\times 10^3$)†								
	First hatch			Second hatch			Total hatch		
Age (wk)	Unshipped	Shipped	% loss	Unshipped	Shipped	% loss	Unshipped	Shipped	% loss
2	2,829	2,086 NS‡	27	680	413 NS	39	3,509	2,498 NS	29
4	2,765	1,634 *	39	566	495 NS	13	3,390	2,130 *	37
6	2,405	861 **	61	364	566 NS	36	2,769	1,425 *	49
8	2,588	340 **	87	388	222 **	43	2,976	563 **	81
10	2,447	319 **	87	474	240 *	49	2,796	559 **	80
12	1,968	366 **	81	262	98 NS	63	2,230	464 **	79
14	2,048	210 **	90	207	92 NS	56	2,256	302 **	87

†Means for eight replications.
‡NS = not significant; * significant at $P = 0.05$; ** significant at $P = 0.01$.

half-cultures were produced from eggs maturing subsequent to shipment. In 2-wk-old cultures, few if any eggs are mature (1), which suggests that the observed losses (29%) were a result of damage to either maturing and ovipositing adults or to immature eggs.

These data suggest that if *R. culicivorax* is to be successfully shipped, methods must be found to prevent disturbance of cultures being prepared for shipment and to pack cultures so as to prevent shock and shearing by the culture sand during shipment. Also a culture medium other than sand must be found to reduce shipping weight and damage to the nematodes. Until these are developed, shipment of very young cultures (2–4 wk) is recommended to increase the chances of successful delivery of *R. culicivorax* eggs.

LITERATURE CITED

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