Thermal Tolerance of Romanomermis culicivorax, a Nematode Parasite of Mosquitoes[1]

R. LEVY and T. W. MILLER, JR.[2]

Laboratory tests were conducted to determine the effects of high temperatures on the viability and infectivity of the preparasitic (infective) stage of the mermithid nematode *Romanomermis culicivorax* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.). In separate tests, cultures containing *R. culicivorax* eggs were flooded for 1.5 h (Test 1), 6 h (Test 2) and 15 h (Test 3) with 1,000 ml of dechlorinated water [water purified by a reverse osmosis (RO) system]; the water containing hatched preparasites was decanted into flasks (2, 3) and 5 and 10 ml of water (about 1,000 and 720 preparasites, Tests 1 and 2, respectively) were added to 400-ml beakers containing 50 ml of RO water that was preheated in water bath. In Test 1, 1- to 6-h-old preparasites were placed at 37.7, 40.5, and 43.3 C; for Test 2, 1- to 8-h-old preparasites were placed at 43.3 C. In Test 3, 10 ml of RO water containing about 2,800 1- to 19-h-old preparasites were introduced into beakers containing RO water at room temperature (24 C). These beakers were placed into the water bath, and the temperature was slowly raised to 40.5 C over a 7-h period. Each treatment was replicated 4 times.

In Tests 1 and 2, preparasites were held at a given temperature for 60 min and then allowed to cool for about 45 min before the percent mortality was determined. Mortality of preparasites was determined according to procedures established by Levy and Miller (1). Control preparasites were tested in water held at 24 C (ambient).

To investigate the infectivity of the heat-treated preparasites, the volume of water in all the beakers was increased to 150 ml and 25 second-instar, laboratory–reared *Culex pipiens quinquefasciatus* Say larvae were added to each. Larvae were fed ground rabbit chow daily and reared at 26 C (ambient) throughout the remainder of the experiment.

The percent host infectivity was determined 5 days post-treatment (all surviving *C.p. quinquefasciatus* were examined). Postparasite viability was also recorded for each test group as an indicator of the biocontrol potential of the heat-treated preparasites.

Data were analyzed by simple regression analyses and the "t" test.

Received for publication 31 January 1977.

[1] In cooperation with the Gulf Coast Mosquito Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Lake Charles, Louisiana 70601.

[2] Lee County Mosquito Control District, Post Office Box 2237, Fort Myers, Florida 33902. We sincerely thank L. J. Murphy, Jr., Research Technician, Lee County Mosquito Control District, Fort Myers, Florida, for his technical assistance.

Data from thermal tolerance Test 1 indicated that the average percent mortality of *R. culicivorax* preparasites held in water preheated to 37.7, 40.5, and 43.3 C for 60 min was 11.7 (10.5-12.5)%, 36.5 (30.3-47.6)%, and 93.2 (90.6-97.4)%, respectively. Regression analyses resulted in the model: percent preparasite mortality = $-544.04 + 14.59$ C ($r = 0.976$; $P \leq 0.01$). On the basis of this equation, we can estimate that water temperatures required to induce 50% and 90% preparasite mortality in 1 h would be 40.7 and 43.5 C, respectively. Preparasites surviving heat-treatment produced host parasitism of 100% at 37.7, 39.4% at 40.5, and 0% at 43.3 C at an initial parasite to host ratio of 40:1 (Test 1). Parasitism in control was 100%.

Test 2 was conducted at 40.5 C since Test 1 indicated that extreme responses in preparasite mortality and host parasitism occurred at the upper and lower limits of the estimated thermal death range of 37-44 C. These results occurred from the 60 min temperature exposure. Preparasites not killed in the 40.5 C water produced 66.3 (52.6-76.2)% host parasitism.

In addition, postparasites obtained from mosquito larvae infected with heat-treated preparasites in Tests 1 and 2 (37.7-40.5 C) appeared to exhibit no unusual behavioral characteristics when they were compared with postparasites from controls (24 C).

Preparasites (Test 3) in water raised from 24-40.5 C over a 6- to 7-h period resulted in 42.7 (40.0-61.9)% mortality. This occurrence indicated that similar preparasite mortality would result from either a direct introduction into 40.5 C water (Tests 1 and 2) or from introduction into water gradually increased to 40.5 (Test 3).

Less than 1% preparasite mortality was observed in controls for Tests 1, 2, and 3.

Observations indicated that preparasite survival and infectivity were inversely related to temperature. It is interesting to note that preparasite mortality in Test 2 was greater than in Test 1 at 40.5 C; however, a greater degree of host parasitism was achieved in Test 2 even though the initial preparasite to host ratio was greater in Test 1 than in Test 2 (i.e. 40:1 and 29:1, respectively). These results were presumed to be related to inherent differences between nematode populations (the age and number of floodings between the two cultures used in the experiments). Test 2 was conducted with a nonflooded nematode culture while Test 1 was conducted with a nematode culture that had been subjected to several previous floodings and was about 6 months old.

These data suggest that *R. culicivorax* has little value in habitats with temperatures in excess of about 40 C. However, temperatures of this magnitude are not typically encountered in most mosquito-breeding areas of southwest Florida.

LITERATURE CITED

1. LEVY, R., and T. W. MILLER, JR. 1977. Susceptibility of the mosquito nematode Romanomermis culicivorax (Mermithidae) to pesticides and growth regulators. Environ. Entomol. (In Press).
2. LEVY, R., L. J. MURPHY, JR., and T. W. MILLER, JR. 1976. Effects of a simulated aerial spray system on a mermithid parasite of mosquitoes. Mosq. News 36:498-501.
3. PETERSEN, J. J., and O. R. WILLIS. 1972. Procedures for mass rearing of a mermithid parasite of mosquitoes. Mosq. News 32:226-230.