The Effects of Temperature on the Infectivity of Romanomermis culicivorax

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Abstract: The survival time (ST) of the preparasitic larvae of *Romanomermis culicivorax* was determined by measuring motility at 1, 6, 12, 18, 21, 27, 30, and 37 C; the ST_{50} at each of these temperatures was 2.3, 2.2, 2.0, 2.0, 1.7, 1.6, 0.9, and 0.7 days, respectively. About one-third of the preparasites infected first-instar larvae of *Culex pipiens* within 24 h at 27 C. The preparasites were infective at 12 to 33 C with the optimum infectivity at 21-33 C. Lower temperatures decreased the percent infectivity but increased the time that the nematodes remained infective. The time required for host infection increased as the preparasitic larvae aged at 15, 21, and 27 C. *Key Words:* motility, *Culex pipiens*.

Romanomermis culicivorax Ross and Smith, 1976 (18) has promise as a biological control agent for mosquitoes (13, 22). It can be mass reared readily in the laboratory (17); it has been used successfully in several field experiments; and it has a broad host range—at least 52 species of mosquitoes (13). A major environmental factor affecting its activity as a biological control agent is temperature. Petersen and Willis (16) reported that *R. culicivorax* was active when water temperatures were above 18 C. Low water temperatures (daily minimums of 6-8 C and maximums of 13-22 C) generally prevent infection of *Culex pipiens fatigans* larvae by *R. culicivorax* (11). However, survival of preparasitic stages of *R. culicivorax* was prolonged by low temperatures, but the preparasites were more infective at higher temperatures (9).

If *R. culicivorax* is to be used with maximum efficiency in the management of mosquito populations, the effects of physical environmental factors on its infectivity require intensive study. This study was designed to determine the effects of temperature, age of preparasitic nematodes, and exposure time to the host on the infectivity of *R. culicivorax* for *Culex pipiens*.

MATERIALS AND METHODS

Romanomermis culicivorax was obtained from Dr. J. J. Petersen, USDA, and was propagated in *Culex pipiens* according to the procedures developed by Petersen and Willis (17). This nematode was known previously as *Reesimermis nielseni* but has been redescribed as *Romanomermis culicivorax* sp. n (18). The Italian strain of autogenous *Culex pipiens* was obtained from Dr. A. R. Barr at UCLA and used for the production of *R. culicivorax*. The female mosquitoes of this strain produce one egg raft (70 eggs)/individual without a blood meal (21) and this ability facilitates the production of the mosquito host.

Preparasitic larvae were obtained and unhatched eggs were removed by methods similar to those described previously (14, 17). Preparasitic nematodes for each replicate were pipetted into plastic petri plates (36x15 mm) and the total volume of water in each dish was adjusted to 2.5 ml. The dishes were covered with plastic lids with 0.2-cm holes for air exchange and placed in the dark at the appropriate temperatures.

The infectivity of preparasitic larvae was tested with the first-instar larvae of *C. pipiens*. The mosquito larvae were captured with a large-bore Pasteur pipette and counted as they were released from the pipette onto a 4-cm square of silk bolting cloth. The mosquito larvae were transferred, by immersing the bolting cloth, to 250-ml polystyrene food containers which contained 125 ml dechlorinated water. Unless indicated otherwise, dechlorinated tap water was used in all experiments. Twenty mosquito larvae were placed in each container and the natural mortality was less than 20% in the control containers. The mosquito larvae were fed ca 75 mg of finely ground rabbit chow (Purina), 3 parts, and brewer's yeast (ICN Pharmaceuticals), 1 part. Preparasitic larvae were rinsed from petri dishes into the containers with mosquitoes. The containers were covered with polyethylene lids (0.2-cm hole for aeration) and placed in the dark at the experimental temperatures for 24 h. After

Received for publication 22 October 1976.
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24 h, the infection process was terminated by pouring the contents of each container on a screen with 180-μm openings which retained mosquito larvae but allowed the nematodes to pass through (15). The mosquito larvae were returned to the containers with 125 ml water, fed, and maintained for 10 days at 27 C. After this interval, the number of postparasitic nematodes and the number of adult mosquitoes in each container were counted.

The recovery of postparasitic nematodes, based on the number of preparasites added initially, was used as the criterion of successful infection since such nematodes represented viable and competent members of the preparasitic nematode population. This estimate of infectivity did not account for nematodes that may have killed hosts soon after infection or nematodes that died before emergence from the host. Possibly, it provided a lower estimate of infectivity than one made on the basis of infections determined by dissecting host larvae soon after penetration. However, this approach was advantageous because it gave an estimate of nematodes that were capable of completing development in the host and perpetuating the survival of the species.

Data from all experiments were analyzed by analysis of variance and Duncan's multiple range test (20).

RESULTS

Infection competency of preparasites: The ability of *R. culicivorax* to infect autogenous *C. pipiens* was studied at 27 C. Five infection ratios of preparasites to mosquitoes were used with five replications for each ratio (Table 1). The average recovery of postparasites was 30.5% for all infection ratios. Dead mosquitoes were not dissected to recover nematodes that were incapable of emerging from host cadavers. The number of mosquitoes that completed development decreased with increasing infection ratio. Mosquito mortality at the lower infection ratios was higher than postparasite recovery, an indication that natural mortality from unknown causes in this experiment was elevated.

Effects of temperature on survival of preparasites: The effect of temperature on the survival of preparasitic nematodes was determined in a factorial experiment. Motility or lack of motility was used as the criterion to distinguish between living and dead nematodes. Also, nonmotile nematodes were crenated and more transparent than motile nematodes. The factors studied were temperature and age of preparasitic nematodes. Five replications of 30 six-hour-old, preparasitic nematodes were taken from two different sand cultures of *R. culicivorax* and placed at the temperatures tested. At 24-hour intervals, the temperature of the dishes was allowed to equilibrate at room temperature for 0.5 h and the number of motile preparasites in each replication was counted. Survival times were derived from a graphical plot of the combined data from sand cultures 1 and 2. Motility of preparasitic nematodes decreased more rapidly at higher temperatures. Total survival time was shortest, 2 days, at 37 C and longest, 6 days, at 1 to 12 C. The times at which 50% (survival time, ST_{50}) of the preparasites remained motile at 1, 6, 12, 18, 21, 27, 30, and 37 C were 2.3, 2.2, 2.0, 2.0, 1.7, 1.6, 0.9, and 0.7 days, respectively. The times at which 10% of the preparasites remained motile were 5.1, 5.2, 5.7, 4.5, 4.4, 2.0, 2.8, and 1.6 days, respectively.

Relationship of motility of preparasites to infectivity: The relationship of motility to infectivity of preparasitic nematodes was studied at six temperatures (Fig. 1). A preliminary experiment showed that infec-

TABLE 1. Recovery of the postparasitic larvae of *Romanomermis culicivorax* from *Culex pipiens* at various infection ratios[y].

Infection ratio	Number of postparasites recovered	Post-parasite recovery (%)	Mosquitoes emerged (%)
1:1	37	37.0 a[z]	25.0 a[z]
2:1	52	26.0 ab	19.0 ab
3:1	93	31.0 ab	14.0 b
4:1	134	33.5 ab	0.0 c
5:1	126	25.2 b	4.0 c

[y]Infection obtained during 24-h period at 27 C. Five replications were used per infection ratio. Twenty *C. pipiens* were used per replication with the appropriate number of preparasites in 125 ml water.

[z]Results of Duncan's multiple range test. Means which have letters under the same subgroup are not significantly different ($P < 0.05$).

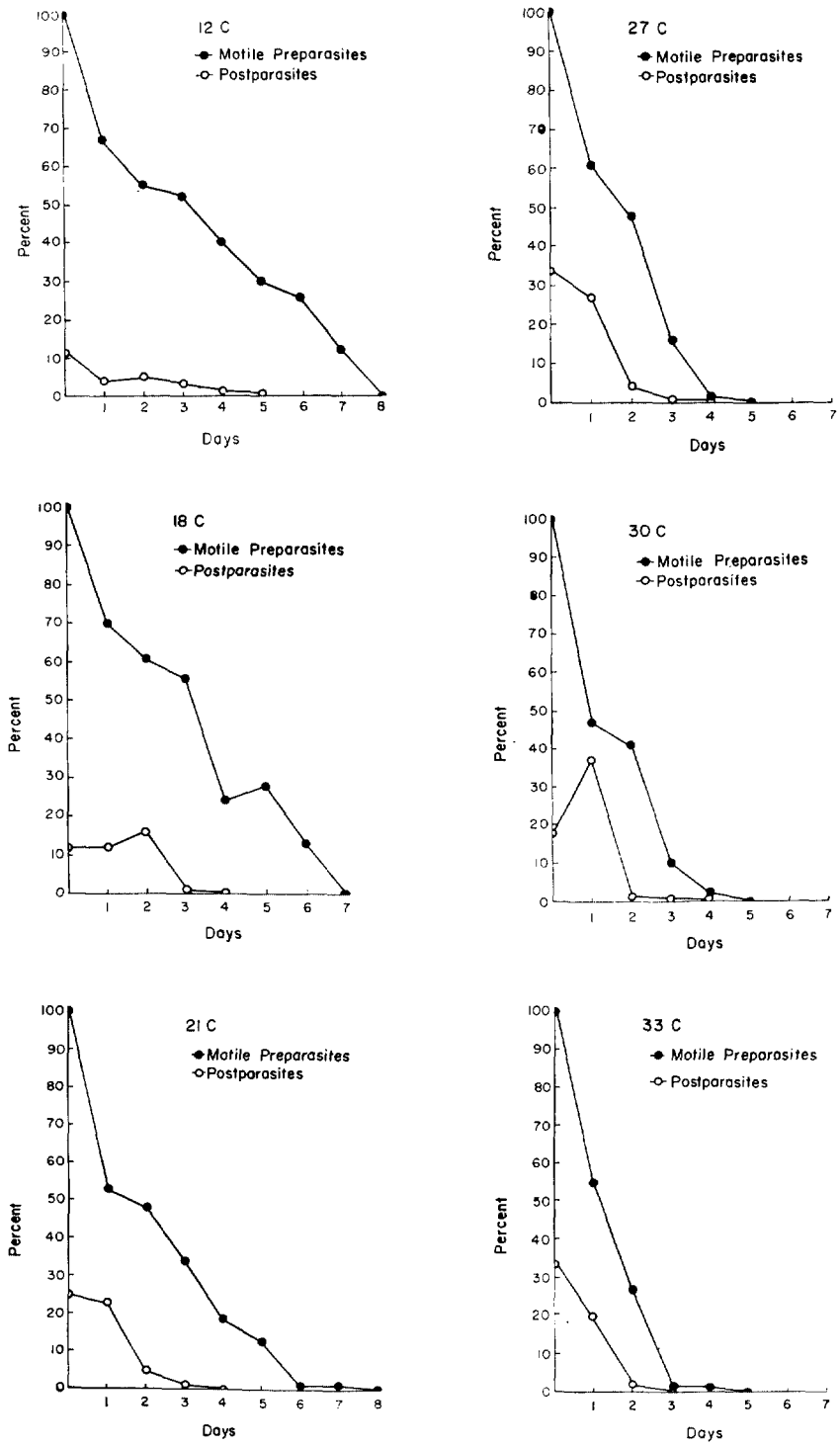

FIG. 1. Relationship of motility to infective ability of preparasitic *Romanomermis culicivorax* at 12, 18, 21, 27, 30, and 33 C. Infectivity during a 24-h period was measured by recovery of postparasitic nematodes. Each point is the mean of 5 replications, with 20 preparasites and 20 first instar *Culex pipiens*/replication.

tion occurred rarely at 6 C and that the host mosquitoes were killed at temperatures above 35 C. Five replicates of 20 preparasitic nematodes each were used for 24 h intervals at each temperature. After daily counts, the preparasites were transferred into containers with mosquitoes previously equilibrated at the experimental temperature, and the infectivity occurring during the succeeding 24-hour-period was determined.

In general, the infection competency of preparasites was lower than those remaining motile (Fig. 1). The preparasites initially infective at 12, 18, 21, 27, 30, and 33 C were 11, 12, 25, 24, 18, and 34%, respectively. With the exception of 18% at 30 C, the infectivity of preparasites on days 0 and 1 was lower at 12 and 18 C than the four higher temperatures tested. There were no differences in the infectivity of preparasites on days 2 and 3. Although the infectivity of preparasites was lower at the lower temperatures, nematodes retained their infective ability longer at lower (12, 18 C) than at higher temperatures.

Temperature greatly influenced the time (ST_{50}) required for 50% of the preparasites to become nonmotile and the time (I_{50}) for 50% of those initially infective to lose their infectivity (Table 2). Motility decreased more slowly than did infectivity at the lower temperatures tested (12, 18 C), whereas at the higher temperatures (21, 27, 33 C), preparasites lost their infectivity at a rate similar to that for loss of motility.

Effects of age of preparasites and length of exposure on infectivity: The effects of preparasite age and length of exposure time to *C. pipiens* on infectivity of *R. culicivorax* were studied in a factorial experiment. Tests were conducted at three temperatures (Fig. 2) with three age groups of nematodes (newly hatched = 0, 1, and 2 days old), and with five exposure times to mosquitoes. Five replications of 60 preparasites and 20 mosquitoes were used for each treatment. At the intervals designated, the preparasites were transferred into containers with mosquitoes and treated as described for the previous experiments.

Infection levels obtained at 15 C were highly variable and thus no differences in infectivity were found. At 21 C, the early phases of infection were variable but the maximum levels of infection achieved by 0- and 1-day-old preparasites was higher than that of 2-day-old preparasites. At 27 C, 0-day-old preparasites were more infective than 1- and 2-day-old preparasites after 4 h of exposure, and 1-day-old preparasites were more infective than 2-day-old preparasites after 6 h of exposure to mosquitoes.

Newly hatched preparasites were more infective at 21 and 27 C than at 15 C. Infections by 1-day-old preparasites initially took place faster at 27 C than at 21 and 15 C, but at longer exposure times there were no differences between 27 and 21 C. Comparison of the 2-day-old preparasites showed the lowest infectivity at 27 C and the highest at 21 C. The low level of infection at 15 C was due probably both to inhibition by low temperature and to aging, whereas the low level of infection at 27 C was due to aging alone.

TABLE 2. Effects of temperature on the survival and infectivity of preparasitic *Romanomermis culicivorax*[a].

Temperature C	Motility ST_{50} (days)	Infectivity I_{50} (days)
12	3.2	2.0
18	3.2	2.7
21	1.6	1.6
27	1.8	1.4
30	1.0	1.4
33	1.2	1.2

[a]The percent motility or infectivity was plotted versus time and the time (days) for survival (ST) or infectivity (I) of 50% of the larvae was determined by interpolation.

DISCUSSION

Petersen (12), in an earlier study with *C. pipiens quinquefasciatus,* obtained 30-50% of the expected maximum yield of postparasites from preparasites used in his infections. These results are similar to the recovery level obtained in our experiments. A reduction in postparasite recovery occurred at the higher infection ratios in these experiments, possibly because the mosquitoes were not able to handle the high parasite burden and died before the nematodes developed the capacity for survival in the external milieu. Although the average infectivity of *R. culicivorax* in *C.*

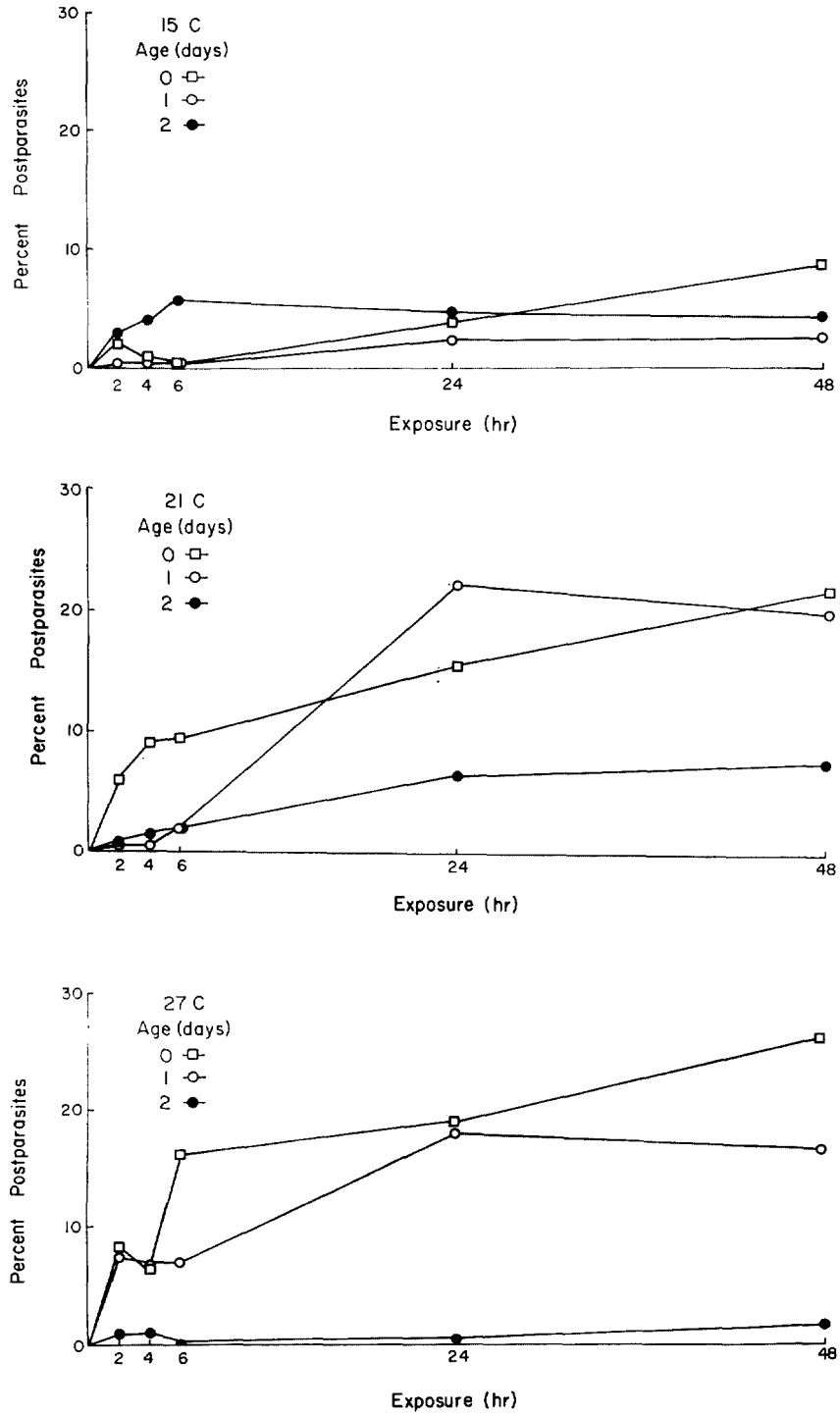

FIG. 2. Recovery of postparasitic *Romanomermis culicivorax* as affected by age of preparasites and length of exposure of preparasites to *Culex pipiens* at 15, 21, and 27 C. Each point is the mean of 5 replications. Each replication consisted of 60 preparasites and 20 mosquitoes in 125 ml tap water.

pipiens may seem low, similar levels of infectivity have been reported for both animal and plant parasitic nematodes (7, 19). In our subsequent experiments, the recovery of postparasitic nematodes from 3:1 infection ratios was used as an estimate of the infectivity of preparasites because such an estimate would be a measure of those nematodes capable of penetrating hosts, completing the parasitic phase of development, and, thereby perpetuating the survival of the species.

Motility of preparasitic nematodes decreased more rapidly at the higher temperatures tested than at the lower temperatures. The optimum for survival lies from 1 to 21 C for the larvae. It was not realized in an earlier report (4) in which preliminary survival times were reported that the larval suspensions were contaminated with viable eggs. This fact resulted in the reporting of longer survival times than were found in our work. Kurihara (9) has also reported similar prolonged survival times for the preparasites of *R. culicivorax,* and it seems likely that his tests were also contaminated with late-hatching eggs. However, our results suggest that incorporation of eggs in material released in field experiments would be a feasible procedure for extending the infectivity of the inoculum.

Initially, infective ability was higher at the higher temperatures tested (21-33 C) than at the lower temperatures (12, 18 C). Infective ability fell off faster at the higher than at the lower temperatures. Similarly, Petersen (14), in a study in which temperatures varied from 24-27 C, found that the percent of mosquitoes infected by *R. culicivorax* declined very rapidly after 24 h and that little infectivity remained after 72 h. The optimum range for infection by *R. culicivorax* found in our study is from 21 to 33 C. This range is not unusual when it is compared to the range for other nematodes (1, 5, 7, 10, 19).

Petersen and Willis (15) found that exposure time greatly influences the extent of parasitism in populations of mosquitoes by *R. culicivorax*. We encountered much variation in the rate of infection at the three temperatures used. This variation decreased as temperature increased. Low temperature had the effect of slowing down the infection process but increased the length of time over which the nematodes remained infective. Similar results have been found with other nematodes (19).

Information is available on the effects of temperature on three other genera of insect-parasitic nematodes. The optimum temperature for the development of all life cycle stages of *Hexamermis brevis* was 10-20 C with 30 C being lethal to the nematode (1). Branch et al. (3) reported that infective stage *Heterotylenchus* sp. were killed quickly by temperatures lower than −4 C and higher than 58 C. The temperature threshold for infectivity by this nematode was 17-18 C. Females were infective for at least 36 h and could survive up to 4 days in dung at 27 C. Jackson (8) reported that 19 C was the optimum temperature for growth of *Neoaplectana,* whereas Veremchuk (23) reported that development of a *Neoaplectana* sp. obtained from an elaterid beetle was best at 21 to 24 C. Jackson (8) reported that activity and morphological development of *Neoaplectana* ceased below 5 C and that dauer larvae were unable to withstand temperatures above 34 C.

Various species of mosquitoes are able to survive exposure to freezing temperatures, whereas other species can survive temperatures as high as 47 C (2, 6). However, the development of larvae to adults usually does not occur at these extremes of temperature (2) and the favorable temperature range for development of mosquito larvae probably lies between 10 and 27 C. It appears that *R. culicivorax* can be an effective means of controlling a large number of mosquito species between 21 and 33 C, but other means of control would be necessary outside this temperature range.

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