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Preferred Temperature of *Meloidogyne incognita*[1]

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Abstract: In laboratory thermal gradients, newly hatched infective juveniles of the plant-parasitic root-knot nematode *Meloidogyne incognita* migrated toward a preferred temperature that was several degrees above the temperature to which they were acclimated. After shifting egg masses to a new temperature, the preferred temperature was reset in less than a day. Possible functions of this type of thermotaxis are discussed, including the use of thermal gradients around plant roots to locate hosts and to maintain a relatively straight path while ranging in the absence of other cues (a collimating stimulus).

Key words: acclimation, collimating, eccritic, *Meloidogyne incognita*, root-knot nematode, temperature, thermotaxis.

Thermal gradients produce oriented locomotion in a variety of small organisms (18). The responses of *Paramecium* (12,15,21), pseudoplasmodia of the slime mold *Dictyostelium* (1,6,13,19), and nematodes (2,3,6–9,11,20) have been relatively well characterized. A common observation is that the organisms move toward a "preferred" temperature. In many cases, the preferred temperature has been equated to the temperature to which the animals have been previously exposed. Such behavior is easily explained as choosing a temperature to which the organism is physiologically adapted. In other cases, e.g., the slime mold, the organisms move away from an "avoided" temperature. The adaptive value of an avoided temperature is not as clear, but computer modeling studies suggest that, in the dynamic thermal environment of soil, it would cause the organism to move toward the surface (6).

Juveniles of the root-knot nematode *Meloidogyne incognita* respond to temperature changes of 0.001 C (7) and migrate in thermal gradients as shallow as 0.001 C/cm (17). This high degree of sensitivity suggests the response plays an important but unknown role in the life of the nematode. In order to investigate the significance of this sensitivity to temperature, we determined the direction of migration in a thermal gradient under a variety of conditions.

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MATERIALS AND METHODS

Meloidogyne incognita (Kofoid & White) Chitwood were grown on tomato plants (10). Egg masses were picked from the roots and incubated on screens under a shallow layer of water at 18, 23, 28, or 33 C. Second-stage juveniles (J2) were used within 1 day of hatching.

Thermal gradients were established by connecting an insulated aluminum channel (133 cm long, 3 cm wide, 2 cm high, 1.5 mm thick) between a heat source and sink. The J2 migrated on agar in small plastic trays (6 cm long, 2 cm wide, 2 cm high) placed end-to-end in the channel. The thermal source and sink were a hotplate and a thermoelectric cooler. The channel was insulated with 1-cm-thick plastic foam. The thermal gradient was about 0.1 C/cm, which is much above threshold (17) but below typical values in the top 30 cm of soil (5). Thus, each tray included a temperature range of less than 1 C.

Approximately 150–200 J2 were pipetted onto the center of each plastic tray containing 3 or 4 ml of 2% agar. The trays were covered and placed in the channel which was covered with a strip of insulation. The J2 were allowed to move on the agar surface for 2 hours before their distribution was quantified.

Nematodes were counted in each of 12 equal sections of agar along the length of each plastic tray. Numbers were weighted to reflect the distance of movement from the center. The weighted numbers of J2 moving down a gradient were subtracted

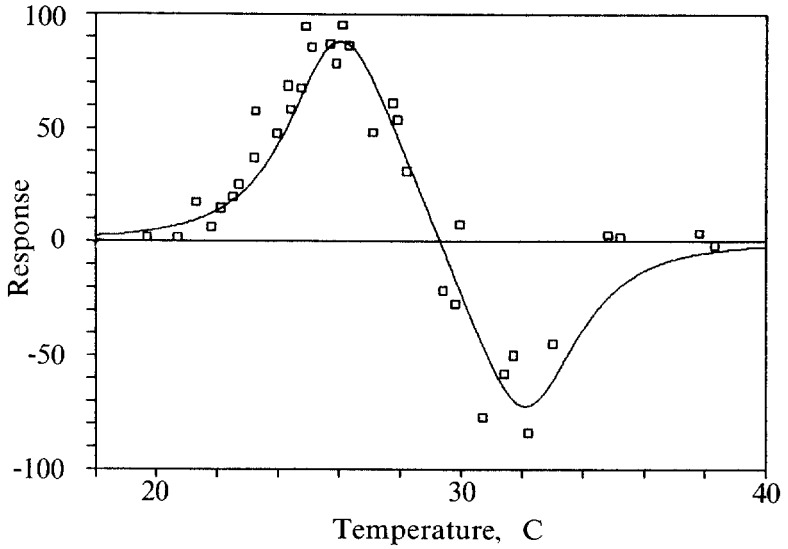

FIG. 1. Profile of responses of second-stage juveniles of *Meloidogyne incognita* hatched from egg masses held at 28 C. Temperature is that at the middle of the plastic tray containing agar and nematodes. Curve is the equation $R = 33(29.3 - T)/(1 + |(29.0 - T)/4|^5)$.

from those moving up the gradient, then divided by the sum of all weighted numbers and multiplied by 100, to yield the response (R):

$$R = 100 \times \frac{\begin{array}{l}[6(N_1 - N_{12}) + 5(N_2 - N_{11}) \\ + 4(N_3 - N_{10}) + 3(N_4 - N_9) \\ + 2(N_5 - N_8) + (N_6 - N_7)]\end{array}}{\begin{array}{l}[6(N_1 + N_{12}) + 5(N_2 + N_{11}) \\ + 4(N_3 + N_{10}) + 3(N_4 + N_9) \\ + 2(N_5 + N_8) + (N_6 + N_7)]\end{array}}$$

where N_1 is the number of J2 found in the section closest to the heat source and N_{12} the number found in the section closest to the heat sink. This response index has maximum and minimum values of 100 and −100, indicating the migration of all J2 to the warmest and coolest sections of a tray, respectively. If J2 are distributed symmetrically around the center starting position, the response index is zero.

In order to measure the rate of temperature acclimation of the response, J2 from a pooled group of plants were split into four samples which were incubated at the four different temperatures. Since the plants were subjected to diurnal temperature fluctuations, the previous acclimation temperature was not well defined but was the same for all the samples in this experiment. Thus, any difference in response between the groups is indicative of thermal acclimation.

RESULTS

The direction of migration of J2 depended on their temperature (Fig. 1). At extremely low or high temperatures migration did not occur and the response was zero. At temperatures just above the lower limit of migration, the J2 migrated toward warmer temperatures, as indicated by the positive response. Conversely, at temperatures just below the upper limit, the J2 migrated toward cooler temperatures, as indicated by the negative response.

The response curve was shifted to different temperatures when J2 acclimated to different temperatures were used (Fig. 2). The peak of movement toward warmer temperatures corresponded roughly to the acclimation temperature, and the preferred temperature was usually several degrees above the acclimation temperature. This shift of the response curve was fully developed by the first day after the temperature difference was established, except for the anomalously behaving J2 incubated

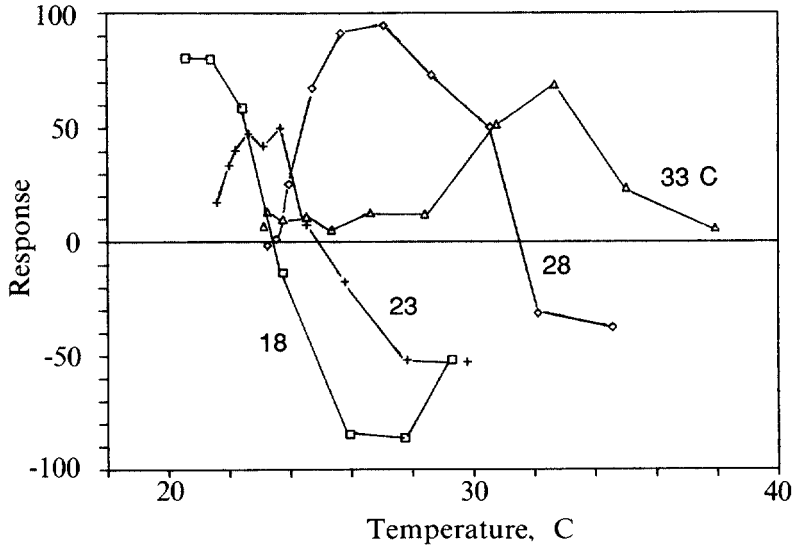

FIG. 2. Response curves of second-stage juveniles of *Meloidogyne incognita* acclimated to different temperatures. Egg masses were picked and held for 4 days at the four different temperatures.

at 33 C (Fig. 3). If the peaks of the response curves are plotted, the acclimation process has a similar time course (not shown).

To define the relationship between acclimation temperature and the response curve more clearly, preferred temperatures (for acclimation times from 1 to 7 days) were compared to the corresponding acclimation temperatures (Fig. 4). Data for 33 C acclimation were excluded because a true preferred temperature does not exist in this case. In all other cases the preferred temperature was above the acclimation temperature. At the low acclimation tem-

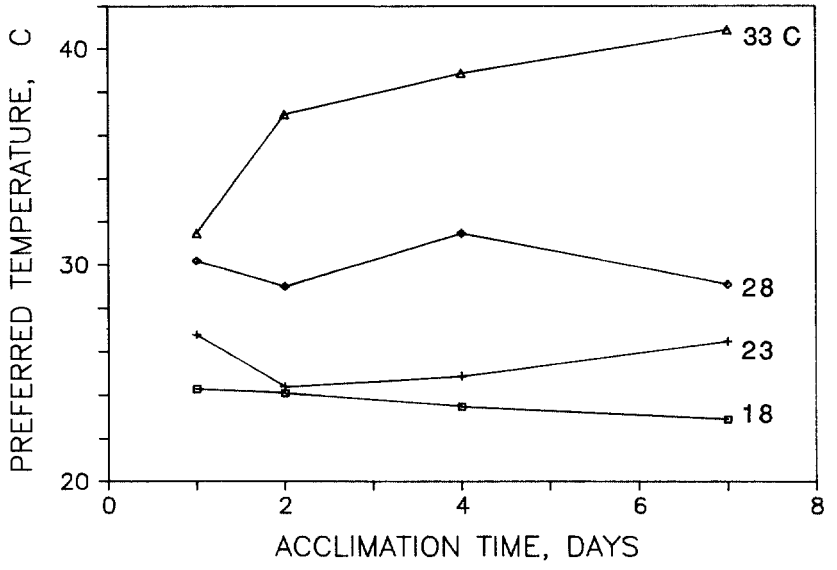

FIG. 3. Time dependence of preferred temperature after shift in acclimation temperature. Egg masses of *Meloidogyne incognita* were picked and held for the indicated times at the four different temperatures. Since a true preferred temperature was not present for second-stage juveniles incubated at 33 C, an apparent value was estimated by extrapolation.

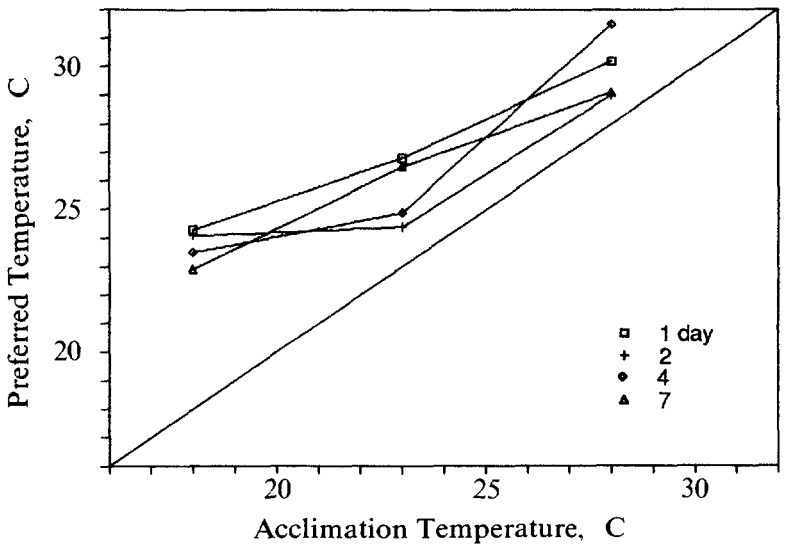

FIG. 4. Preferred temperature as a function of acclimation temperature. Data are from egg masses of *Meloidogyne incognita* held at the indicated acclimation temperature for 1, 2, 4, or 7 days. Straight line is the locus of points for which the preferred temperature equals the acclimation temperature.

perature the offset was about 6 C, while higher temperatures had an offset of about 2 C. (The estimated offset for 33 C was larger.)

DISCUSSION

If the temperature at which the J2 start is not too extreme, they move away from both extremes toward an intermediate temperature which is referred to as their preferred temperature (Fig. 1). This temperature is identified as the temperature where the curve of the responses crosses zero with a negative slope. It represents the temperature at which the J2 tend to accumulate. The steep slope of the curve defines the preferred temperature precisely; any region of indifference to temperature must be less than 1 C wide.

The J2 incubated at 33 C appear not to have a true preferred temperature; they simply move up the gradient until disabled by the high temperatures. As a result, the response curve simply goes to zero rather than going negative (Fig. 2). What has been plotted in Figure 3 for J2 acclimated to 33 C is the point of zero response from an extrapolation of the negative slope. This lack of avoidance of high temperature is probably a pathological consequence of exposure to high temperature.

These results are the first demonstration that the preferred temperature of an organism is offset from the acclimation temperature. In other organisms, the preferred temperature is apparently set at the acclimation temperature (2,3,11), the organisms move away from an avoided temperature (1,13,19), or the organisms simply move up the gradient no matter how the temperature relates to their acclimation temperature (14). It should be pointed out that our experiments have a higher temperature resolution than most of those previously performed. Thus, a similar offset may exist in some other organisms but not have been observed.

The fact that acclimation occurs and that the preferred temperature is higher than the acclimation temperature means that these nematodes tend to move toward ever higher temperatures. In a stationary gradient, they would move to their preferred temperature. As they acclimated to that temperature, they would again move upward to the new preferred temperature and so on. Thus, they would slowly move up the gradient at a speed limited by their rate of acclimation.

Plant-parasitic nematodes might locate plant roots by the higher temperatures caused by greater metabolic activity around

roots than in soil generally (9). Recent calculations indicate, however, that the thermal gradients due to metabolic heating of roots are generally much smaller than those due to other sources in soil and are comparable to the long-term gradient associated with the cooling of the earth (5).

Another possible function of this response is simply that it serves the nematode as a means of maintaining a straight course. All organisms have limits on how accurately they can move along a straight line without reference to an external stimulus. In the absence of any cues as to the location of a target, the best strategy for an organism may be to move along a straight line in order to sample as large an environment as possible in a given time. It is not efficient to go around in circles, which is what invariably happens to an animal that has no directional references. Stimuli that serve such a purpose have been termed "collimating stimuli" (16).

The thermal gradient in soil may be the best collimating stimulus available to nematodes. In the absence of cues indicating the direction of a potential host, following the thermal gradient may be a good strategy even if the gradient does not originate from a root. Consistent with this hypothesis, we have observed that when a chemical gradient conflicts with a thermal gradient, the nematodes respond predominantly to the chemical gradient (unpubl.). Rode (20) observed that the distance covered by nematodes was greater in the presence of a thermal gradient than in its absence. A simple random walk calculation suggests *M. incognita* J2 would travel more than 100 times further in a day if its movements were oriented than if it moved at random (4,16).

Another consideration is that, in the normal environment of these organisms (soil near the surface), thermal gradients are not stationary. Since soil temperature undergoes daily fluctuations and the nematodes have this complicated behavior, it is not clear how thermotaxis would cause the nematodes to move. Recent computer modeling of this behavior suggests that the behavior will cause most individuals to move toward a particular soil depth (Dusenbery, unpubl.).

Literature Cited

1. Bonner, J. T., W. W. Clarke, Jr., C. L. Neely, Jr., and M. K. Slifkin. 1950. The orientation to light and the extremely sensitive orientation to temperature gradients in the slime mold *Dictyostelium discoideum.* Journal of Cellular and Comparative Physiology 36:149–158.

2. Burman, M., and A. E. Pye. 1980. *Neoaplectana carpocapsae*: Movements of nematode populations on a thermal gradient. Experimental Parasitology 49: 258–265.

3. Croll, N. A. 1967. Acclimatization in the eccritic thermal response of *Ditylenchus dipsaci.* Nematologica 13:385–389.

4. Dusenbery, D. B. 1987. Theoretical range over which bacteria and nematodes locate plant roots using carbon dioxide. Journal of Chemical Ecology 13:1617–1624.

5. Dusenbery, D. B. 1988. Limits of thermal sensation. Journal of Theoretical Biology 131:263–271.

6. Dusenbery, D. B. 1988. Avoided temperature leads to the surface: Computer modeling of slime mold and nematode thermotaxis. Behavioral Ecology and Sociobiology 22:219–223.

7. Dusenbery, D. B. 1988. Behavioral responses of *Meloidogyne incognita* to small temperature changes. Journal of Nematology 20:351–355.

8. Dusenbery, D. B., and J. Barr. 1980. Thermal limits and chemotaxis in mutants of the nematode *Caenorhabditis elegans* defective in thermotaxis. Journal of Comparative Physiology 137:353–356.

9. El-Sherif, M., and W. F. Mai. 1969. Thermotactic response of some plant parasitic nematodes. Journal of Nematology 1:43–48.

10. Goode, M., and D. B. Dusenbery. 1985. Behavior of tethered *Meloidogyne incognita.* Journal of Nematology 17:460–464.

11. Hedgecock, E. M., and R. L. Russell. 1975. Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans.* Proceedings of the National Academy of Science, USA 72:4061–4065.

12. Hennessey, T., and D. L. Nelson. 1979. Thermosensory behaviour in *Paramecium tetraurelia*: A quantitative assay and some factors that influence thermal avoidance. Journal of General Microbiology 112:337–347.

13. Hong, C. B., D. R. Fontana, and K. L. Poff. 1983. *Thermotaxis* of *Dictyostelium discoideum* amoebae and its possible role in pseudoplasmodial thermotaxis. Proceedings of the National Academy of Science, USA 80:5646–5649.

14. McCue, J. F., and R. E. Thorson. 1964. Behavior of parasitic stages of helminths in a thermal gradient. Journal of Parasitology 50:67–71.

15. Nakaoka, Y., and F. Oosawa. 1977. Temperature-sensitive behavior of *Paramecium caudatum.* Journal of Protozoology 24:575–580.

16. Pline, M. J., and D. B. Dusenbery. 1987. Responses of the plant-parasitic nematode *Meloidogyne incognita* to carbon dioxide determined by video camera–computer tracking. Journal of Chemical Ecology 13:873–888.

17. Pline, M., J. A. Diez, and D. B. Dusenbery. 1988. Extremely sensitive thermotaxis of the nematode *Meloidogyne incognita.* Journal of Nematology 20: 605–608.

18. Poff, K. L., D. R. Fontana, and B. D. Whitaker. 1984. Temperature sensing in microorganisms. Pp. 137–162 *in* G. Colombetti and F. Lenci, eds. Membranes and sensory transduction. New York: Plenum Press.

19. Poff, K. L., and M. Skokut. 1977. Thermotaxis by pseudoplasmodia of *Dictyostelium discoideum.* Proceedings of the National Academy of Science, USA 74:2007–2010.

20. Rode, H. 1970. Zur Orientierungsweise von Larven des Kartoffelnematoden in Temperaturgefallen. Nematologica 16:258–266.

21. Tawada, K., and F. Oosawa. 1972. Responses of *Paramecium* to temperature change. Journal of Protozoology 19:57–63.