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Accelerated Movement of Nematodes from Soil in Baermann Funnels with Temperature Gradients

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Abstract: Baermann funnels were modified to eliminate or reverse the small temperature gradient (1–2 C/cm) across the soil layer that normally results from water evaporation. Effects of modifications on extraction efficiency were examined at various ambient temperatures and after overnight adaptation of three nematode species at 20 and 30 C. Extraction of *Meloidogyne incognita* from sandy loam, *Tylenchulus semipenetrans* from sandy clay loam, and *Rotylenchulus reniformis* from silt was greatly accelerated simply by covering funnels to prevent evaporation. In most cases, covering increased the nematodes extracted by 10–100 times after 5.5–48 hours. Faster and more efficient extraction of *R. reniformis* occurred over a wide range of ambient temperature (18–29 C). Effects of ambient temperature and temperature gradient direction on Baermann funnel extraction of *R. reniformis* were partly inconsistent with the behavior of *R. reniformis* in agar. Nematodes in agar moved toward cold at some ambient temperatures and toward heat at other temperatures. They always appeared to move toward cold on Baermann funnels. Differences were not attributable to blockage of gas exchange by covers. In agar and in funnels, the patterns of response to ambient temperature were shifted in the direction of the storage temperature.

Key words: Baermann funnel, behavior, *Meloidogyne incognita*, nematode extraction, *Rotylenchulus reniformis*, thermal adaptation, thermotaxis, *Tylenchulus semipenetrans.*

Two fundamentally different approaches are used for extraction of invertebrates from soil: mechanical separation by sieving, sedimentation, or flotation; and dynamic or behavioral separation that depends on animal motility (15,16,27). Each approach has disadvantages that are well-appreciated by nematologists and entomologists. Mechanical separations usually require more labor, time, and apparatus, whereas behavioral separations do not recover nonmotile animals and are greatly affected by the behavioral and physiological condition of those that move. Inadequate motility limits the effectiveness of simple, economic Baermann methods for extracting root-parasitic nematodes from soil (1,2,4,7,9,14,18,20,27). Many behavioral methods for isolating arthropods are quite effective; they differ from standard Baermann methods for extracting nematodes in that organisms are induced to orient toward or away from a stimulus such as light, heat, or gases (27).

Application of a stimulus to extract nematodes has been explored. Overgaard (19) achieved extraction efficiencies greater than 90% by heating water and soil within Baermann funnels, from above with incandescent light bulbs, to expel nematodes presumptively via negative thermotaxis. Heating soil, however, was not predicated on knowledge of nematode behavior. Oostenbrink (18) proposed that Overgaard's extraction efficiency resulted from the small sample sizes used (1–4 cm^3) rather than from heating. Most nematodes that have been reported to respond to temperature gradients moved toward heat, often suicidally (3,6,12,13), not away from it. Until recently, only four ecologically diverse species of nematodes were known to be negatively thermotactic: *Ditylenchus dipsaci* (Kuhn) Filipjev (5), *Globodera rostochiensis* (Wollenweber) Behrens (24), *Terranova decipiens* Krabbe (a cod and seal parasite) (25), and *Caenorhabditis elegans* (Maupas) Dougherty (11). All were examined in artificial gels rather than soil, except for *D. dipsaci* which was examined in agar (13) and in sand (5). For each species, positive and negative thermotaxes could be induced at appropriate ambient temperature or after appropriate thermal adaptation. When responses were compared at various

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ambient temperatures, nematodes appeared to move toward a preferred temperature, exhibiting thermal preferendum (13,24). Within the last 2 years, four additional plant-parasitic nematode species—*Meloidogyne incognita* (Kofoid and White) Chitwood (21), *Ditylenchus phyllobius* (Thorne) Filijev, *Tylenchulus semipenetrans* Cobb, and *Rotylenchulus reniformis* Linford and Oliveira (23)—have been found to aggregate directionally on artificial gels in response to small temperature gradients (0.001–1.0 C/cm). Each moves toward and away from heat, depending on ambient and adaptation temperatures. There is some question whether the behavioral mechanism is taxis or klinokinesis. Computer tracking studies on *M. incognita* J2 indicated both mechanisms were operative (8).

The behavioral sensitivity to temperature gradients reported for various nematode species suggests that temperature gradients may influence Baermann funnel extraction. The objective of our study was to determine the effects of gradient direction, thermal adaptation, and ambient temperature on the rates and efficiency of Baermann funnel extraction of *M. incognita, T. semipenetrans,* and *R. reniformis* from three soils. These nematodes and *G. rostochiensis* are the root parasites for which the greatest information is available regarding behavioral responses to temperature gradients in artificial gels. Responses to gradients in soil and agar were directly compared for *R. reniformis.*

Materials and Methods

Three soils were used, each previously infested with one of the nematode species to be examined: sandy loam with *M. incognita* and silt with *R. reniformis,* both propagated on tomato in a greenhouse, and sandy clay loam with *T. semipenetrans* from a citrus orchard. The soil in every experiment was collected on the previous day, thoroughly mixed, and stored overnight at a controlled temperature. Each soil was moist and workable but moisture percentages were not measured. In all experiments, the Baermann apparatus was a 15-cm-d polyethylene funnel, a latex drain tube with clamp, and a cylindrical ring cut from polyvinyl chloride pipe (4.5 cm high × 10 cm d). Funnels were filled with distilled water supplemented with 3.4 mM $CaCl_2$ to reduce clay fraction dispersion (W. H. Thames, pers. comm.). A uniformly thin layer of soil (100 g, 1.3 cm thick) was supported in the ring by two layers of two-ply facial tissue (Scotties, Scott Paper Co., Philadelphia, PA) stretched across its bottom and secured with a rubber band. Excess tissue was trimmed with scissors. The ring was gently lowered into the water until it was supported by the wall of the funnel and the upper surface of the soil layer was covered by water 2 cm deep.

This standard funnel was modified as required with various accessories to alter water evaporation, gas exchange, and temperature gradients through the soil layer (Fig. 1). Funnels were always exposed to a gentle air current created by an electric fan to increase the uniformity of ambient air temperature. Temperatures were recorded manually with precision thermistors (Yellow Springs Instruments, Yellow Springs, OH) interfaced to digital panel meters readable to 0.01 C, or automatically with thermocouples read by two 9-channel electronic data loggers (Polycorder, Omnidata, Logan, UT). Where stated, temperatures at strategic points within funnels were continuously monitored and visualized in real time through a CRT display of the thermal history of each thermocouple updated at 30-second to 5-minute intervals by a computer interfaced to one of the data loggers. Four experiments were done; the first three were repeated at least once for each species indicated.

Covering tops of funnels or submersing bottoms in water: Soil containing a given nematode species was divided into halves that were stored at 20 and 30 C. Three replicates of three kinds of funnels (Fig. 1, diagrams 1–3) were set up for soil from each storage temperature and placed at ca. 26 C ambient. Evaporation of water from open funnels chilled the funnel contents, causing the center of the soil layer to be 3–4

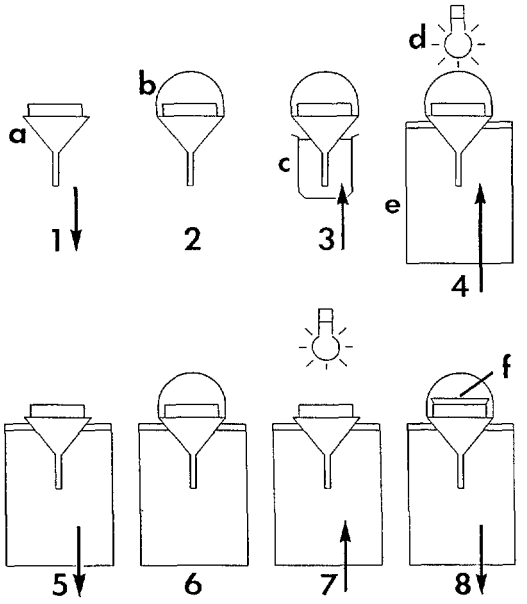

FIG. 1. Baermann funnel modifications (diagrams 1–8) and their components: a) standard open funnel; b) inverted bowl as cover; c) beaker of water; d) light bulb; e) water bath at controlled temperature; f) aluminum dish of ice placed over soil retaining ring. Arrows indicate direction in which temperature increases.

C cooler than otherwise and the top of the soil layer to be 1–2 C cooler than the bottom of the soil layer. A loose cover almost eliminated the gradient (0.0–0.1 C), and a loose cover plus immersion of the funnel bottom in a 1,000-ml beaker of water, chilled by evaporation, generated a 0.4–0.6 C decrease from top to bottom. These temperature differences were measured several times in every funnel the first time the experiment was conducted and were verified for replicate experiments in representative funnels. Usually, 5–10 ml water was drawn from each funnel after 2.5, 5.5, 24, and 48 hours, and nematodes were counted. In one of the experiments with *M. incognita,* seven replicates instead of three were run; they were partitioned among funnel racks in three rooms with ambient temperatures of 24, 27, and 30 C. This permitted examination of the effect of the decrease in mean soil temperature due to evaporation of water from open funnels. The effect of overnight refrigeration of soil containing *R. reniformis* also was examined on four replicate funnels with loose covers. These were set up to compare soil from storage at 5 and 30 C; they were drained for counting at the intervals described.

Movement of Rotylenchulus reniformis in agar and from soil in Baermann funnels: To obtain physiologically similar populations of nematodes in soil and in agar, the incubations, extractions, and experiments were coordinated as follows. On day 1, greenhouse soil (30 C) containing *R. reniformis* was thoroughly mixed and divided into two parts which were held overnight at 25 and 30 C. On day 2, the soil stored at 25 C was put at 20 C and the soil stored at 30 C was subdivided. One part was split among 12 covered funnels at 25 C which were drained after 10 hours to provide a nematode suspension which was then divided and stored at 20 and 30 C for use on day 3. Also on day 2, the second part of the 30-C soil was divided among two sets of 12 funnels. One set was covered and heated from above (Fig. 1, diagram 4) and one set was open and cooled from above by evaporation (Fig. 1, diagram 5); water baths maintained soil layers on the funnels at 12 temperatures (17–33 C). Thus, positive and negative temperature gradients of 1–2 C/cm down through the soil layer were maintained over a wide range of median soil temperature. The temperature of the top and the bottom of the soil layer in each funnel was measured hourly. After 5.5 hours, funnels were drained (40–50 ml) and nematodes were counted. On day 3, soil stored overnight at 20 C was subjected to the same procedure and the nematode suspensions stored overnight at 20 and 30 C were used to test for behavioral responses to gradients in agar. Nematodes were suspended randomly in 0.3 ml of 0.75% water agar within thin-bottomed channels (4 × 40 mm). A two-dimensional temperature gradient plate, described in detail by Robinson (23), was used to simultaneously expose two replicates of channels to 1 C/cm along their lengths at 12 channel midpoint temperatures (15–32 C). Gradient linearity was confirmed in representative channels with

tissue implantation thermistors (YSI), and plate temperatures were continuously monitored by eight thermocouples interfaced to a computer as described. Suspensions stored at 20 and 30 C were examined concurrently. After 5.5 hours, the distribution of nematodes within each channel was determined and expressed as the percentage of nematodes on the warmer half. The experiment was repeated with a new collection of soil containing *R. reniformis.*

Baermann funnels modified to generate a series of gradients with and without covers: Soil containing *R. reniformis* was stored at 30 C, then placed in three replicates of four kinds of funnels (Fig. 1, diagrams 5–8). There were standard open funnels and three covered funnels. A light bulb made soil within each of three additional open funnels 2 C warmer on top rather than cooler on top and an aluminum dish of ice over the funnel ring made soil in each of three additional covered funnels 2–3 C cooler on top than on bottom. Water bath temperatures were adjusted to maintain temperatures of the centers of soil layers at 25 ± 1 C in all cases. Thus, a series of temperature gradients was established at a fixed median soil temperature with alternating funnel covering status: 2–3 C cooler on top, covered; 1 C cooler on top, open; nearly isothermal, covered; and 2 C warmer on top, open. Temperatures at the upper and lower soil surfaces were monitored continuously in one replicate of each kind of funnel by a computer interfaced to thermocouples, as described. The corresponding temperatures in other replicates were monitored manually at 30-minute intervals. After 5.5 hours, funnels were drained and nematodes were counted.

Saturating soil overnight or deleting $CaCl_2$ supplement: In the foregoing experiments, the moist, aerated soil in which nematodes were stored overnight became saturated immediately when placed on funnels, thus subjecting nematodes to an abrupt change in conditions. For the fourth test, soil with *R. reniformis* was divided in two parts. One part was saturated with water. After storing both parts overnight at 30 C, three replicates of each part were run on open and covered funnels. After 5.5 hours, funnels were drained and nematodes were counted. In a similar fashion, the effect of deleting the $CaCl_2$ supplement from the funnel water was tested. Funnels of the $CaCl_2$ experiment were sampled after 5.5 and 24 hours.

Results

Covering tops of funnels or submersing bottoms in water: At 26 C ambient air temperature, loosely covering Baermann funnels always increased the number of *M. incognita, T. semipenetrans,* and *R. reniformis* obtained after 2.5, 5.5, and 24 hours, usually by one to two orders of magnitude (Fig. 2). Submersing the bottoms of covered funnels in water to slightly cool them did not increase extraction. After 48 hours, open funnels yielded quantities of *R. reniformis* comparable to those from covered funnels. Open funnels never yielded quantities of *M. incognita* or *T. semipenetrans* comparable to those from covered funnels, and more nematodes were obtained from covered funnels than by sugar flotation, where this comparison was made. For all species extracted at 26 C, similar numbers of nematodes were obtained from soil stored at 20 and 30 C. Soil stored at 5 C, however, yielded half as many *R. reniformis* as soil stored at 30 C (Fig. 3). Effects of ambient air temperature differences between 24 and 30 C were negligible compared with the effect of covering funnels for *M. incognita* extraction (Fig. 4).

Movement of Rotylenchulus reniformis in agar and from soil in Baermann funnels: After 5.5 hours on temperature gradients in agar, *R. reniformis* had moved toward heat and away from it, depending on ambient and storage temperatures (Fig. 5). Generally, nematodes moved toward a temperature near the storage temperature. Nematodes stored at 20 C moved toward 23 C and those stored at 30 C moved toward 32 C. The only exception to this result was the accumulation of nematodes stored at 30 C on the coolest ends of channels with midpoints below 21 C. Concurrent extraction of the same soil

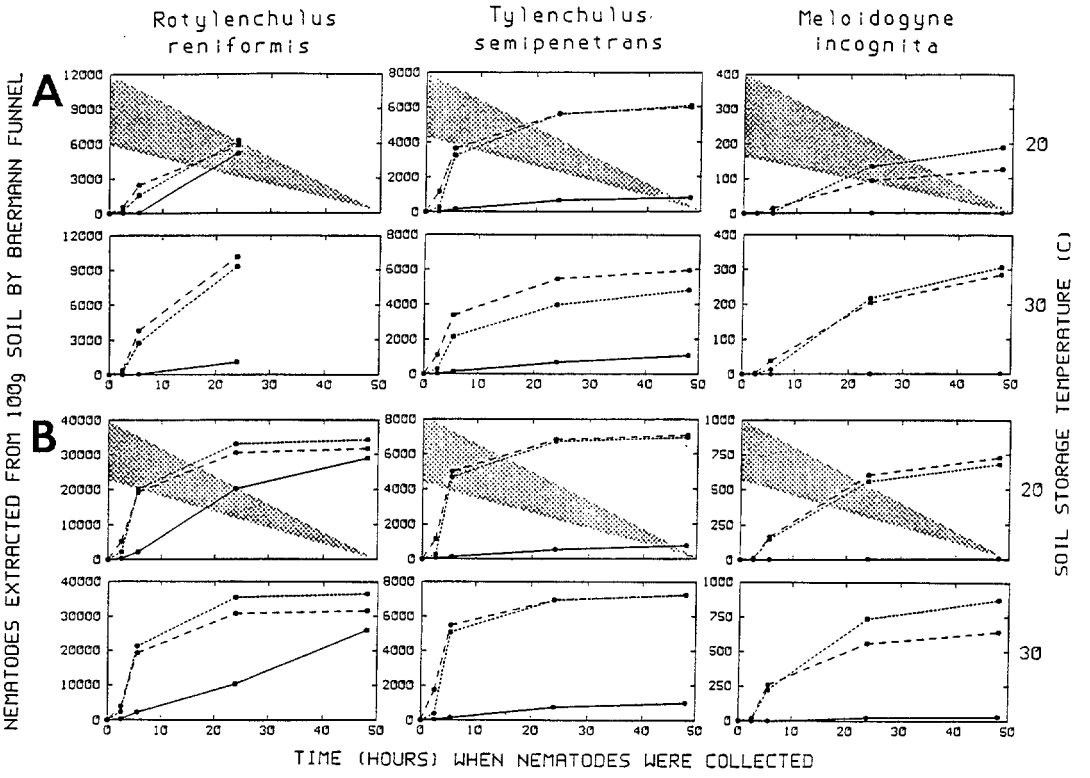

FIG. 2. Nematodes extracted from three soils in Baermann funnels that were open (solid line), covered (dotted line), or covered and submersed in a beaker of water (dashed line) after overnight storage of soil at 20 or 30 C. A) First run. B) Repeat. Each circle is the mean of three replicates. Nematode counts were exponentially distributed and therefore were analyzed after ln (X + 1) transformation. Crosshatched areas indicate separately for each experiment the back-transformed 95% LSD, which varies with mean nematode count as indicated when expressed on an arithmetic scale. There is no intended relationship between the LSD and the ordinate axis.

population by Baermann funnels with positive and negative temperature gradients at 12 ambient temperatures yielded data partly inconsistent with movement in agar. In all cases, far more nematodes moved downward from soil that was warmest on top (Fig. 5); i.e., nematodes appeared to move away from heat over certain ranges

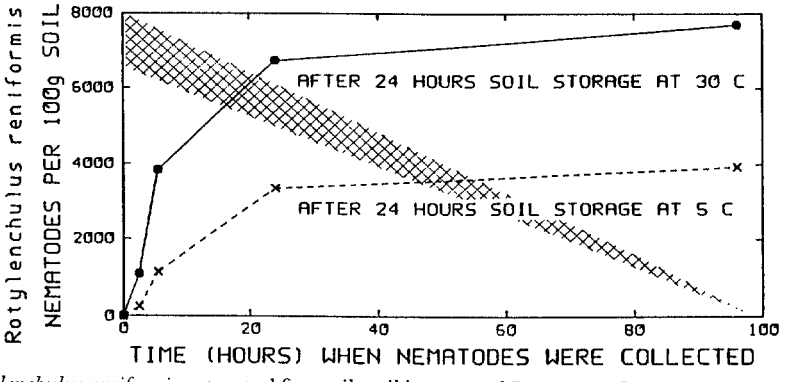

FIG. 3. *Rotylenchulus reniformis* extracted from silt soil in covered Baermann funnels after overnight storage of soil at 5 or 30 C. Each circle is the mean of three replicates. Nematode counts were exponentially distributed and therefore were analyzed after ln (X + 1) transformation. Crosshatched area indicates the back-transformed 95% LSD, which varies with mean nematode count as indicated when expressed on an arithmetic scale. There is no intended relationship between the LSD and the ordinate axis.

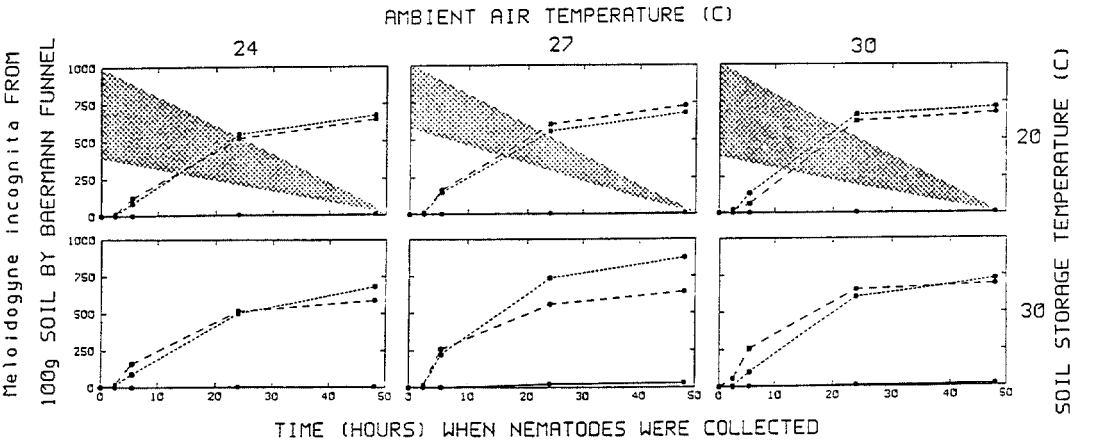

FIG. 4. *Meloidogyne incognita* extracted from sandy loam in Baermann funnels that were open (solid line), covered (dotted line), or covered and immersed in a beaker of water (dashed line) as influenced by ambient and adaptation temperatures. Each circle is the mean of three replicates. Nematode counts were exponentially distributed and therefore were analyzed after ln (X + 1) transformation. Crosshatched areas indicate separately for each ambient temperature the back-transformed 95% LSD, which varies with mean nematode count as indicated when expressed on an arithmetic scale. There is no intended relationship between the LSD and the ordinate axis.

of ambient temperature in which nematodes in agar moved toward heat. Increases in ambient temperature from 18 to 29 C greatly increased the number of nematodes that moved down from soil in covered funnels heated from above. The optimum range of ambient temperature for extraction of *R. reniformis* was lower for soil

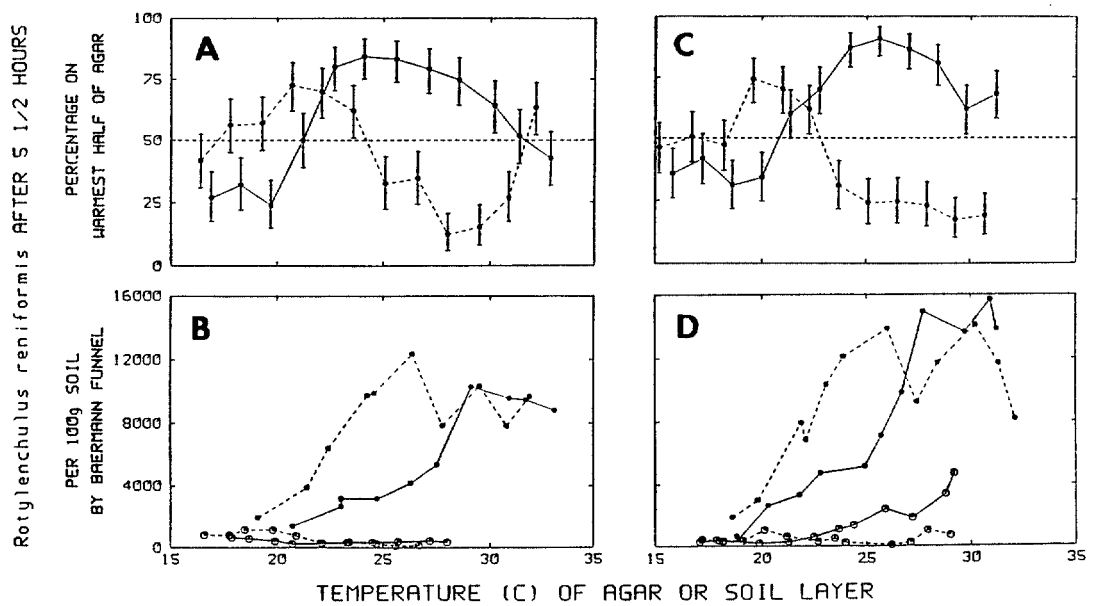

FIG. 5. Comparison after 5.5 hours between the effect of ambient temperature on the movement of *R. reniformis* on temperature gradients (1 C/cm) in agar (A) and their extraction from silt soil in Baermann funnels (B). The funnels (B) were open and cooled from above by evaporation (open circles) or covered and heated from above (closed circles). Before the experiment, nematode suspensions (A) and soil (B) were stored overnight at 20 C (dashed lines) or 30 C (solid lines). Brackets (A) indicate confidence limits for two replicates after arcsine transformation. Each symbol in B represents the nematodes from a single funnel because only one funnel was run for each temperature indicated. C, D) Repeat of A, B.

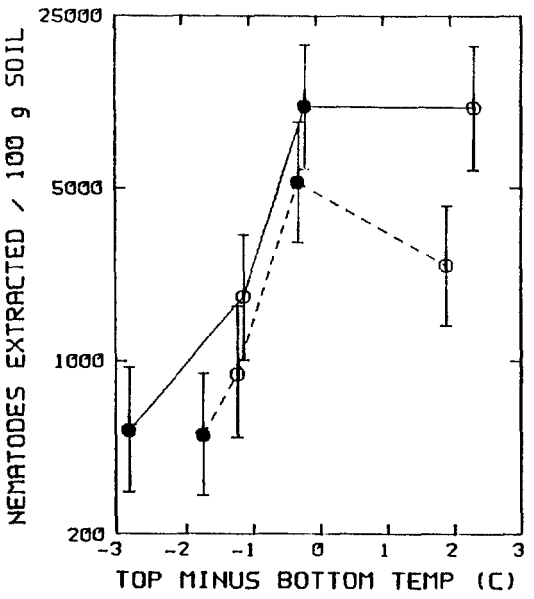

FIG. 6. *Rotylenchulus reniformis* extracted after 5.5 hours from silt soil in Baermann funnels modified to generate a series of temperature gradients down through the soil layer with covers (closed circles) and without covers (open circles). Water baths maintained centers of soil layers at 25 ± 1 C. Solid and dashed lines indicate replicate experiments. Temperature differences are averages derived from measurements taken at upper and lower surfaces of soil layers 10 times during the experiment. Nematode counts were analyzed after ln (X + 1) transformation. Brackets indicate confidence limits at the 95% level.

stored at 20 C than for soil stored at 30 C. Between 19 and 26 C, a 1–5 C higher temperature was required to extract the same number of nematodes from soil stored at 30 C as from soil stored at 20 C.

Baermann funnels modified to generate a series of gradients with and without covers: Accelerated movement of *R. reniformis* from soil continued to be related to manipulation of temperature gradients rather than covering status (Fig. 6). Maximum extraction occurred in funnels that were warmer on top or nearly isothermal.

Saturating soil overnight or deleting $CaCl_2$ supplement: Storing soil saturated with water overnight reduced the number of *R. reniformis* extracted after 5.5 hours by 28% (Table 1). This effect was small, however, compared with the effect of covering, which increased nematode extraction by threefold. Deletion of the $CaCl_2$ supplement from funnel water decreased overall extraction of *R. reniformis* somewhat at 5.5 hours but did not alter the effect of covering funnels.

DISCUSSION

Our results indicate that a behaviorally mediated phenomenon related to subtle temperature differences can greatly influence the movement of nematodes from soil in Baermann funnels. Accelerated extraction of three species occurred whenever it was ensured that the upper surface of the soil was not appreciably cooler than the bottom. This condition could be achieved by covering the tops of funnels or by heating open funnels from above with a light bulb to prevent or offset chilling caused by evaporation. Thus, the phenomenon does not appear directly related to evaporation, to blockage of gas exchange by the loose covers, or to stirring caused by air currents. The magnitude of the gradient required to produce the effect (ca. 1 C/cm) was small compared with the ranges of ambient temperature over which it was observed: 24–30 C for *M. incognita* and 19–30 C for *R. reniformis*. Therefore, it seems unlikely that nematodes failed to move because they were immobilized by cold. Instead, directional movement resulting from a behavioral mechanism such as klinokinesis or orthotaxis is indicated.

Overnight storage of soil at 20 and 30 C did not measurably alter the apparent direction of nematode response to gradients in funnels maintained at 26 C. Storage temperature did appear to shift the optimum temperature range for extraction of *R. reniformis*. Therefore, the data suggest that, in this case, soil storage altered the thermal optimum for motility, but not thermotactic preferences, i.e., not the direction nematodes would move on a temperature gradient. Thermal adaptation has been reported to alter thermal optima for isothermal dispersion of *R. reniformis* and *T. semipenetrans* in agar (23), for Baermann funnel extraction of *M. incognita* (17), and for motility of *D. dipsaci* in water (5).

It would be instructive to know why evidence for thermotactic adaptation was not

TABLE 1. Effects of overnight saturation of soil and deletion of $CaCl_2$ supplement from funnel water on extraction of *Rotylenchulus reniformis* from 100 g silt soil after 5.5 hours in covered or open Baermann funnels.

	Nematodes extracted			
	Overnight saturation		$CaCl_2$ supplement	
	With	Without	With	Without
		Covered		
Limits†	(2,650–6,460)	(3,460–8,440)	(11,325–13,690)	(8,020–9,700)
Mean	4,140	5,410	12,450	8,820
		Open		
Limits	(820–2,010)	(1,280–3,110)	(6,170–7,460)	(2,940–3,550)
Mean	1,280	1,990	6,780	3,230

† Back-transformed 95% confidence limits for three replicates based on residual error of one-way classified analysis of ln (X + 1) transformed data.

detected in Baermann funnels. Partial or complete thermotactic adaptation occurs within 24 hours in *C. elegans* (11), *D. dipsaci, T. semipenetrans, R. reniformis* (23), and *M. incognita* (21). Both times that we extracted *R. reniformis* from soil at 12 funnel temperatures, parallel experiments in agar on nematodes from storage in water at 20 and 30 C indicated thermotactic adaptation had occurred. Funnel results did not. Perhaps adaptation takes longer in soil than in water under our conditions. Also, it should be considered that the physical, geometric, and chemical conditions nematodes were exposed to in agar were quite different from those in soil. In particular, an additional nematode attractant, such as carbon dioxide (10,22) could be operative in soil. Further research is needed.

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