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Movement of Five Nematode Species through Sand Subjected to Natural Temperature Gradient Fluctuations[1]

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Abstract: Temperature gradient fluctuations that occur naturally as a result of heating and cooling of the soil surface were reproduced within 15-cm-d, 15-cm-long acrylic tubes filled with moist sand. Sunny and rainy periods during the late summer in eastern Texas were simulated. Five ecologically different nematode species were adapted to fluctuating temperatures for 20–36 hours at a simulated depth of 12.5 cm before being injected simultaneously into the centers of tubes at that depth. When heat waves were propagated horizontally to eliminate gravitational effects, the movement of *Ditylenchus phyllobius, Steinernema glaseri,* and *Heterorhabditis bacteriophora* relative to the thermal surface was rapid and largely random. However, *Rotylenchulus reniformis* moved away from and *Meloidogyne incognita* moved toward the thermal surface. When heat waves were propagated upward or downward, responses to temperature were the same as when propagated horizontally, irrespective of gravity. The initial direction of movement 1.5 hours after introduction to 20-cm-long tubes at five depths at five intervals within a 24-hour cycle indicated that *M. incognita* moved away from and *R. reniformis* moved toward the temperature to which last exposed. Differences in movement of the five species tested relative to gravity appeared related to body length, with the smallest nematodes moving downward and the largest moving upward.

Key words: behavior, *Ditylenchus phyllobius, Heterorhabditis bacteriophora, Meloidogyne incognita,* nematode, *Rotylenchulus reniformis, Steinernema glaseri,* temperature, thermotaxis.

Knowledge regarding behavioral responses of nematodes to temperature has accumulated slowly over several decades, and the topic probably has been reviewed more than it has been studied. In short, many nematodes migrate along temperature gradients on the surface of transparent gels (1–3,5,11,14,19,20,23). In some cases, the behavioral mechanism is taxis (4), and in others kinesis has been implicated (6). When the direction of movement at different temperatures is compared, a complex response pattern usually is observed that indicates net movement toward heat at some temperatures and away from heat at others. Most species exhibit a preferred and an avoided temperature, both of which can be shifted by thermal adaptation.

Are these responses artifacts? In sand, *Ditylenchus dipsaci* and *Rotylenchulus reniformis* exhibit responses generally similar to those on gels (3,16), but on Baermann funnels, temperature gradients appear to affect movement of *R. reniformis* differently than on gels (15). Nematode responses to temperature in sand and soil, as on gels, have been obtained only by subjecting nematodes to unnaturally abrupt changes in temperature. Effects of the natural, gradual fluctuations in vertical gradients that occur as the soil surface heats and cools during the day have not been examined. Computer simulations of the effects of these fluctuations predict that nematodes could move deep into the soil or to the soil surface, depending on relationships between the ambient, avoided, and adaptation temperatures, the stimulus threshold, rate of movement, rate of adaptation, and limits of adaptation (7,8). Thus, ecologically different species could move in quite different directions. This prediction can be tested mechanistically by comparing response thresholds, rates of adaptation, etc. A more direct approach is to expose ecologically diverse nematodes simultaneously to natural patterns of change and monitor their behavior. The objective of this study was to compare the

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movement of vermiform developmental stages of two sedentary root parasites, one leaf and stem parasite, and two entomogenous species when placed in moist sand subjected to controlled temperature gradient fluctuations, representative of those measured during the summer in eastern Texas.

Materials and Methods

Nematode populations: Heterorhabditis bacteriophora strain NC1 and *Steinernema glaseri* strain NC (provided by H. D. Kaya and T. M. Burlando, University of California, Davis) were propagated separately on *Galleria mellonella* at 24 ± 2 C. Infective juveniles were collected on White traps (24), transferred to deionized water, and used immediately for behavior experiments or stored in loosely capped culture bottles at 10 C for no more than 30 days. Nematode suspensions stored at 10 C were transferred to 17 C for 24 hours and then to ambient laboratory temperature (ca. 23 C) for another 24 hours before use.

Ditylenchus phyllobius (origin, College Station, TX) was obtained from foliar galls of *Solanum elaeagnifolium* that had been dried and frozen the year before. Fourth-stage infective juveniles were extracted from galls (18) and maintained at ambient laboratory temperature for 24 hours before use.

Rotylenchulus reniformis (originally from Baton Rouge, LA) was propagated on tomato (*Lycopersicon esculentum*) in a greenhouse (20–34 C). Mixed, vermiform developmental stages (approximately equal numbers of adult males, preparasitic females, and juveniles) were obtained from soil by direct Baermann funnel extraction (15) and used the same day. *Meloidogyne incognita* race 3 (originally from eastern Texas) was propagated on tomato in the same greenhouse as *R. reniformis*. Eggs were extracted from roots by the sodium hypochlorite method (12) and placed on hatching trays at ambient laboratory temperature. Second-stage juveniles were collected twice during a 3-day period immediately preceding each experiment and used immediately or stored at 17 C and transferred to ambient laboratory temperature 24 hours before use.

Over 95% of the nematodes of each species were motile when used experimentally.

Temperature control and analysis of nematode movement: Acrylic tubes were made 15.0 cm long with 15.0-cm inside diameter or 20.0 cm long with 3.8-cm inside diameter. A 2-mm-d hole, to be used for injecting nematodes, was drilled into the wall of each 15-cm tube halfway along its length. Five 1-mm-d holes were drilled into each 20-cm tube at distances 2, 6, 10, 14, and 18 cm from one end. Thus, holes in the 15-cm tubes were one tube radius from the ends, and holes in the 20-cm tubes were centered on five 4-cm sections such that each hole was one tube radius from the ends of the section on which it was centered. The holes were sealed with vinyl tape. Tubes were packed with moist brick sand by taping a short extension tube to each end and fastening the bottom extension to a tissue-covered perforated plate to which vacuum could be applied. Dry sand was poured into the tube and saturated with water by gravity flow. Excess water was removed under vacuum for 2 minutes (65-cm water column for the 15-cm tubes and 80-cm water column for the 20-cm tubes), to achieve an average moisture content of 12–16% by weight (ca. 0.1 bar water potential).

After packing tubes, extensions were removed, excess material was sliced cleanly off each end, and a cover was taped tightly over each end. Narrow tubes were covered with aluminum foil, and wide tubes were covered with 3-mm thick, circular acrylic plates. Into each nematode injection hole was inserted a syringe needle just long enough to reach the center of the tube. Needles were connected to capillary tubing through which nematodes were later introduced.

Temperature gradient fluctuations were achieved by positioning the tubes lengthwise between two rectangular heat transfer coils (automobile transmission coolers) in an insulated box filled with moist sand

(Fig. 1). Two boxes were used; in experiments with the 15-cm tubes, two tubes were buried in each box. Also buried in each box parallel to the tubes into which nematodes were injected was an extra 4-cm-d, 20-cm-long tube of moist sand with seven thermocouples inserted equidistantly along its length. During experiments, temperatures were measured with a data logger every 30 minutes. The temperature of water pumped through the transmission coolers was controlled and altered through time with the aid of multiple-endpoint ramp-and-soak profile controllers (LFE Instruments, Chesterland, OH). Controllers were programmed to mimic selected patterns of temperature change measured previously in cotton fields near College Station, Texas, during various months of the year. One heat transfer coil in each box, denoted the thermal surface, mimicked temperatures 5 cm deep, and the other mimicked temperatures 25 cm deep. To remove bias due to moisture gradients generated during tube packing, tubes in all experiments were prepared and buried in pairs with the ends that were upward during tube preparation oriented oppositely relative to the thermal surface coil.

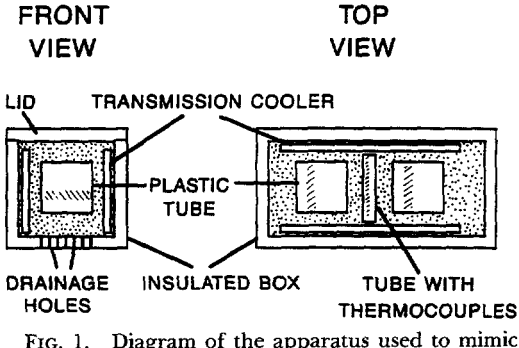

FIG. 1. Diagram of the apparatus used to mimic natural patterns of temperature change in tubes filled with moist sand. All components illustrated within the insulated box were buried in moist sand that was tightly packed around the components by flooding the box with water and allowing excess water to drain from the bottom by gravity flow. Not illustrated are fine screens covering the drainage holes, thermocouples, and water lines leading from the transmission coolers to water baths controlled by multiple-endpoint ramp-and-soak profile controllers.

Nematodes were preadapted for 20–36 hours, depending on the experiment, by burying them within a loosely capped or aerated vial at the same distance from the thermal surface coil as the hole through which they were injected. Aeration was achieved by pumping hydrated air through capillary tubes inserted into holes drilled in vial caps. In experiments with 20-cm tubes, five vials were used, one for each injection hole. Temperatures within the vials were measured in preliminary experiments to verify that they closely followed sand temperatures. Nematodes were injected into each 15-cm tube in 1.0 ml of water and into each 20-cm tube in 250 µl water per injection hole, at a rate of 500–2,000 individuals of each species per injection.

After exposure to experimental temperatures, final distributions of nematodes within the 15-cm-d tubes were determined by extruding the contents of the tube vertically with the aid of a specially made plunger and positioner. As it was extruded, the sand was cut into seven equally thick disks (denoted A–G), and each disk was cut into nine 42-cm^3 sections (denoted 1–9) using specially made cutting tools (Fig. 2). Final distributions of nematodes within the 3.8-cm-d funnels were determined by slicing the sand as it was ex-

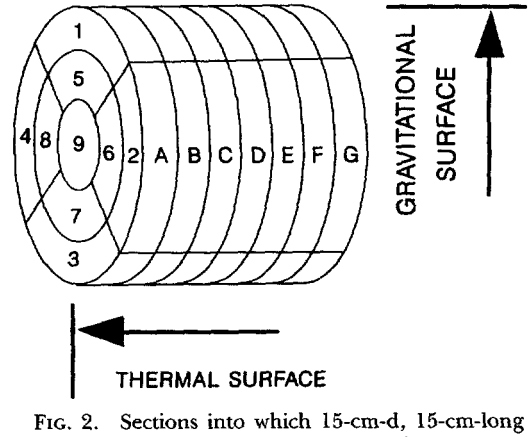

FIG. 2. Sections into which 15-cm-d, 15-cm-long cylinders of sand were cut after exposing them to temperature gradient fluctuations propagated from the thermal surface. Nematodes were introduced into section D-9.

truded from each tube into five 4-cm-long cylinders, and slicing each cylinder in turn into five equally thick disks (0.8 cm thick, 9 cm^3 volume). Just before extrusion, the nematode injection holes were marked by inserting a 36-mm piece of aluminum wire into each hole to ensure that every fifth disk was centered on an injection point. For both tube sizes, the sand that was at the top of the tube during packing was always extruded first. Nematodes were extracted from each section or disk by Baermann funnel or by flotation, depending on the aim of the experiment. Flotation extraction was achieved by vigorously agitating each section of sand in 300 ml water and decanting the water into 400-ml funnels within which the nematodes were allowed to settle overnight. All nematodes collected from funnels were counted.

Experiments: Following several exploratory trials, six experiments were conducted. In the first, movement of all five species was compared in four horizontally oriented 15-cm-d tubes subjected to temperatures representative of three consecutive, sunny summer days (Fig. 3). Temperature gradients were generated horizontally, parallel to the tube axis, so that the effects of temperature on movement could be examined independently of the effects of gravity. Nematodes were injected and tubes were sectioned when the thermal surface coil was at its maximum daily temperature. Tubes were analyzed by Baermann funnel extraction 24 and 48 hours after nematode injection; two tubes were included for each sampling interval.

The second experiment was identical except that nematodes were extracted by flotation. Flotation extraction of the 42-cm^3 sections yielded silty suspensions of nematodes that were difficult to count but eliminated possible bias due to the dependency of Baermann funnel extraction on nematode motility. The third experiment was identical to the first except that the temperature pattern simulated was a rainy period immediately following the sunny period simulated in the first experiment (Fig.

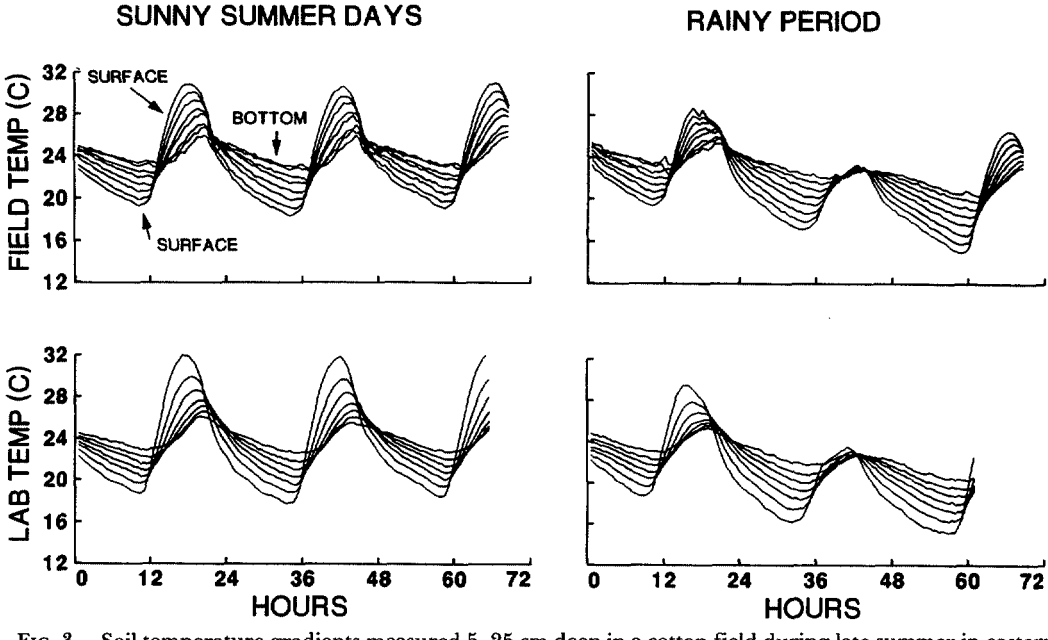

FIG. 3. Soil temperature gradients measured 5–25 cm deep in a cotton field during late summer in eastern Texas and laboratory simulations of those gradients. Thermocouples were positioned at 2.5-cm intervals in the field and at 3.3-cm intervals in the laboratory. Because the uppermost thermocouple in the field malfunctioned during these measurements, the corresponding field temperatures are not given.

3). These first three experiments employed sand passed through a 450 μm-pore sieve.

The fourth and fifth experiments were the same as the first experiment except that the sand was 150–250 μm in diameter. Also, the two tubes in each box were oriented vertically rather than horizontally, with the thermal surface coil on top in one box and on bottom in the other. Nematode distributions in the four tubes in each experiment were analyzed after 24 hours. The fourth and fifth experiments differed from each other only in the omission in the fourth of *R. reniformis,* which was unavailable.

A sixth experiment was designed to compare the initial direction of movement by the root parasites just before sunrise, during midmorning, during midafternoon, at sunset, and during the middle of the night. The narrow, 20-cm-long tubes (five injection holes per tube) were packed with sand <250 μm d, oriented horizontally, and submitted to the same temperature patterns as in the first experiment. *Rotylenchulus reniformis* and *M. incognita* were injected into all holes in four tubes at each of five intervals (denoted I–V) during the 24-hour cycle (20 tubes altogether). Two tubes were analyzed 1.5 hours after each injection and the other two, 3 hours after injection. Nematodes were extracted by flotation.

Results

In the first two experiments, in which 15-cm-d tubes and temperature gradients were oriented horizontally, the most obvious result obtained for the leaf and stem parasite, *D. phyllobius,* was rapid dispersal throughout the length of the tubes. Although movement relative to the thermal surface was inconsistent, appreciably greater movement toward the gravitational surface than downward occurred in seven of the eight tubes examined (Figs. 4, 5). In contrast, the root parasites exhibited a pronounced response to temperature, with *M. incognita* moving toward and *R. reniformis* moving away from the thermal surface in every tube. Both species moved away from the gravitational surface, with greater downward movement by *R. reniformis* than by *M. incognita.* Because Baermann funnel and flotation extraction yielded similar dispersal patterns for these two species (Figs. 4, 5), the method of extraction from soil did not seem an important factor. Movement of the two entomogenous species appeared to be rapid and largely random, although the data suggested a tendency for both species to move away from the thermal surface and for *S. glaseri* to move toward the gravitational surface. Movement of *H. bacteriophora* relative to gravity appeared erratic.

In the rainy period simulation (third experiment), *D. phyllobius* appeared to move toward the thermal surface in three tubes and toward the gravitational surface in all four tubes (Fig. 6). The directions of movement of the two root parasites, as in the sunny day simulations, differed markedly from each other, with *M. incognita* again moving toward and *R. reniformis* moving away from the thermal surface. Both species moved away from the gravitational surface as before, with greater downward movement by *R. reniformis* than by *M. incognita.* At 24 hours, the distributions of the entomogenous nematodes were generally inconsistent and difficult to interpret. After 48 hours, however, nematode distributions indicated differential movement, with *S. glaseri* moving toward and *H. bacteriophora* moving away from both surfaces.

In the fourth and fifth experiments, when the 15-cm tubes were oriented vertically and subjected to the sunny period simulation, strong responses to temperature were again observed for the two root parasites, with *M. incognita* moving toward the thermal surface and *R. reniformis* away from it irrespective of gravity (Fig. 7). *Ditylenchus phyllobius* tended to move away from the thermal surface in all tubes. Movement of the insect pathogens again

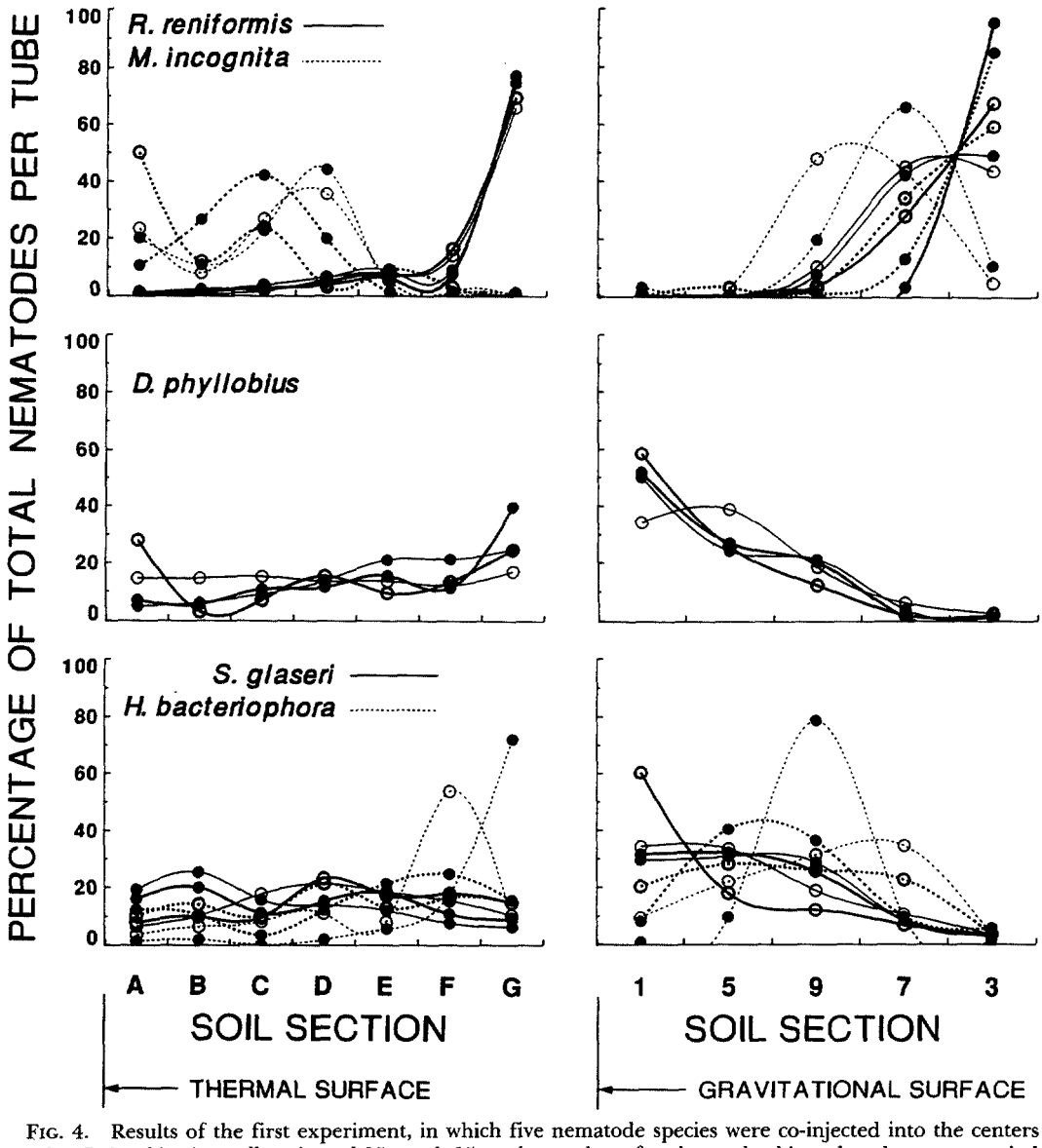

FIG. 4. Results of the first experiment, in which five nematode species were co-injected into the centers (section D-9) of horizontally oriented 15-cm-d, 15-cm-long tubes of moist sand subjected to the sunny period simulation. Nematodes were extracted by Baermann funnel 24 hours (thin lines) and 48 hours (thick lines) after injection. Open circles indicate tubes with the end that was upward during packing oriented toward the thermal surface. Solid circles indicate tubes oriented in the opposite direction. Soil was sectioned and labeled as indicated in Figure 2. Curves were generated by spline algorithm.

was largely random, with a possible tendency of both species to move toward the gravitational surface. The average number of *S. glaseri* in sections 1–4 (the outer cylinder) was similar to that in other soil sections, which indicated uniform radial dispersion from the injection point. In contrast, *M. incognita* and *R. reniformis* were recovered predominantly from sections 5–9.

When the initial directions of movement by *M. incognita* and *R. reniformis* were directly compared in horizontally oriented 20-cm tubes at five depths relative to the thermal surface at five intervals during one 24-hour cycle of the sunny day simu-

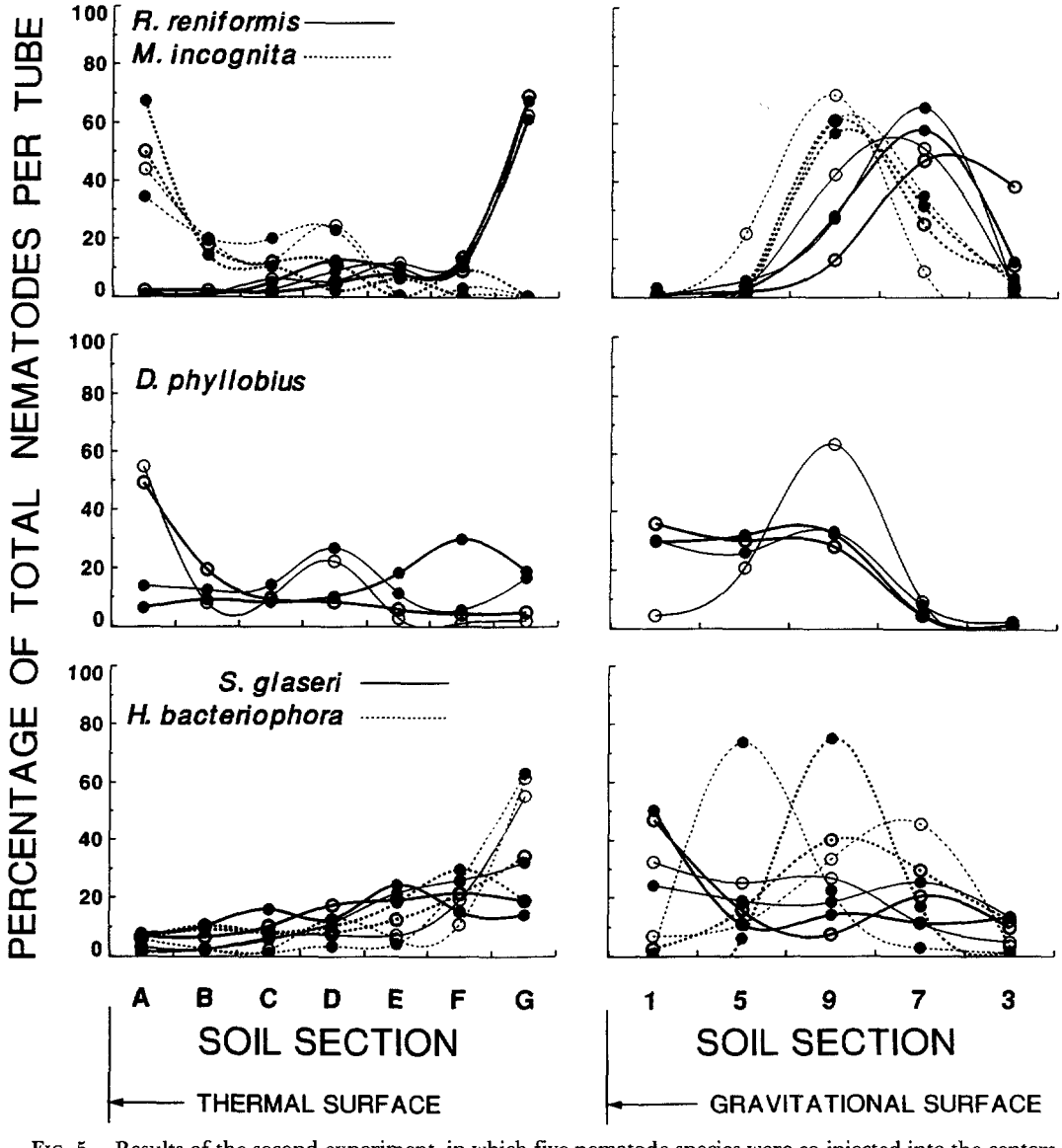

FIG. 5. Results of the second experiment, in which five nematode species were co-injected into the centers (section D-9) of horizontally oriented 15-cm-d, 15-cm-long tubes of moist sand subjected to the sunny period simulation. Nematodes were extracted by direct flotation 24 hours (thin lines) and 48 hours (thick lines) after injection. Open circles indicate tubes with the end that was upward during packing oriented toward the thermal surface. Solid circles indicate tubes oriented in the opposite direction. Soil was sectioned and labeled as indicated in Figure 2. Curves were generated by spline algorithm.

lation, distributions about injection points after 3 hours in most cases overlapped and were virtually impossible to interpret (data not presented). Considerably less dispersal had occurred at 1.5 hours, however, and distribution patterns confirmed previous results. Differential movement could be interpreted for 20 of the 25 depths and time intervals examined (Fig. 8). In all cases, the thermal distribution pattern of *R. reniformis* was shifted downward relative to the distribution of *M. incognita* about the injection point. Greatest dispersal and differential movement occurred at the times and depths when temperatures were highest or gradients strongest, and was consistent re-

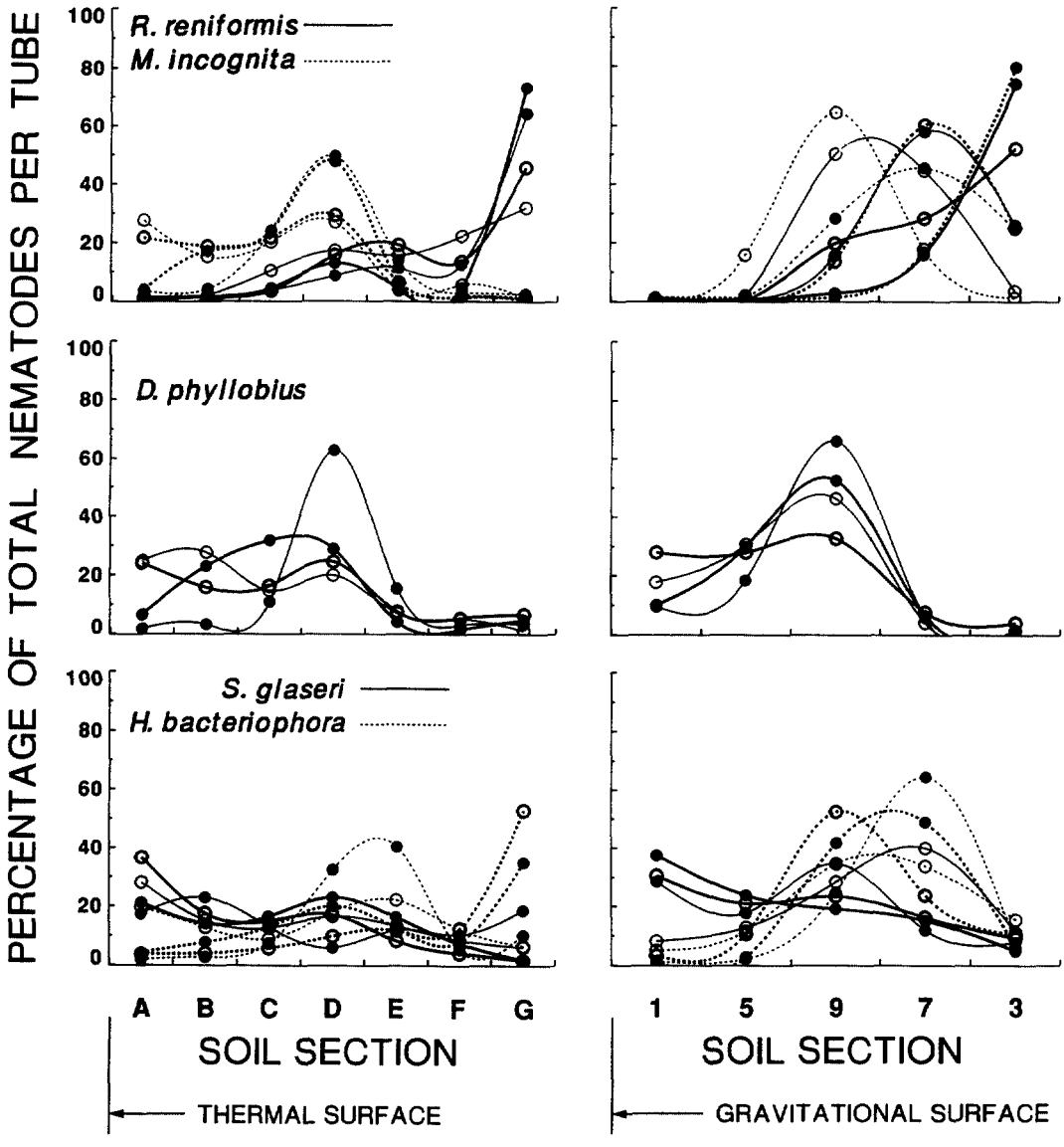

FIG. 6. Results of the third experiment, in which five nematode species were co-injected into the centers (section D-9) of horizontally oriented 15-cm-d, 15-cm-long tubes of moist sand subjected to the rainy period simulation. Nematodes were extracted by Baermann funnel 24 hours (thin lines) and 48 hours (thick lines) after injection. Open circles indicate tubes with the end that was upward during packing oriented toward the thermal surface. Solid circles indicate tubes oriented in the opposite direction. Soil was sectioned and labeled as indicated in Figure 2. Curves were generated by spline algorithm.

gardless of the gradient direction. For example, appreciable differential movement during Interval II (midmorning) occurred only near the surface, whereas during the warmest part of the day (Interval III) comparable differential movement occurred throughout the length of the tubes. During the late afternoon (Interval IV), when the gradient had become inverted near the surface due to surface cooling, differential movement was apparent at all but the greatest depth, where the gradient was still flat. During the night (Interval V), dispersal about the injection points was appreciably less than during the afternoon, but the gradient throughout the tubes was

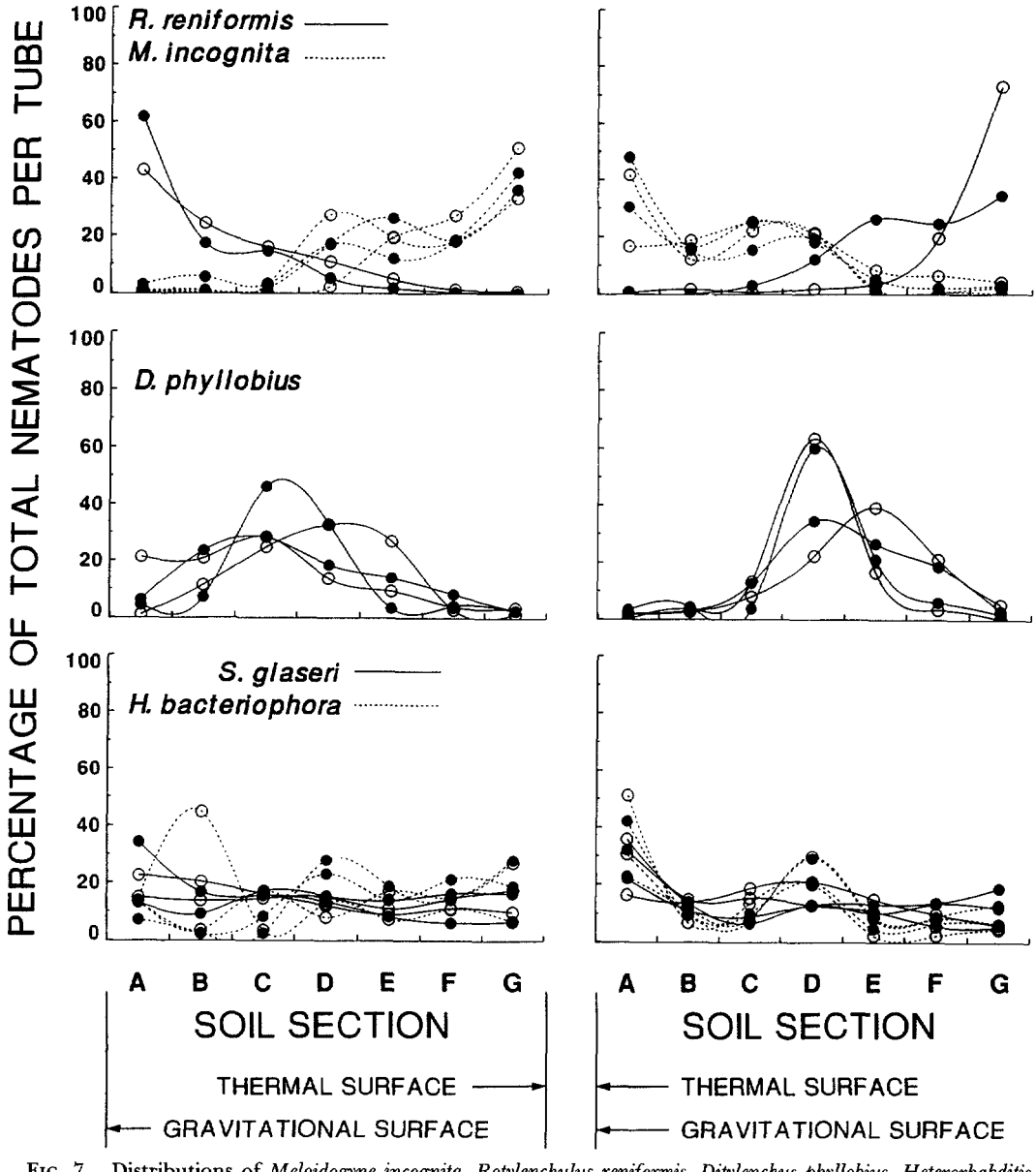

FIG. 7. Distributions of *Meloidogyne incognita, Rotylenchulus reniformis, Ditylenchus phyllobius, Heterorhabditis bacteriophora,* and *Steinernema glaseri* 24 hours after introduction to vertically oriented 15-cm-d, 15-cm-long tubes of moist sand subjected to the sunny, summer day simulation. In tubes on the left the thermal surface heat transfer coil was buried below the tubes, and in tubes on the right it was placed on top. Open circles indicate tubes with the end that was upward during packing oriented upward with respect to gravity. Solid circles indicate tubes oriented in the opposite direction. Soil was sectioned and labeled as indicated in Figure 2. Curves were generated by spline algorithm.

greater than during the early morning and late afternoon, and noticeable differential movement consistent with that during the day occurred at all thermal depths despite the low temperatures and gradient inversion.

DISCUSSION

This research was designed to investigate the effects of temperature gradients on the direction of movement of nematodes in a thermally natural particulate

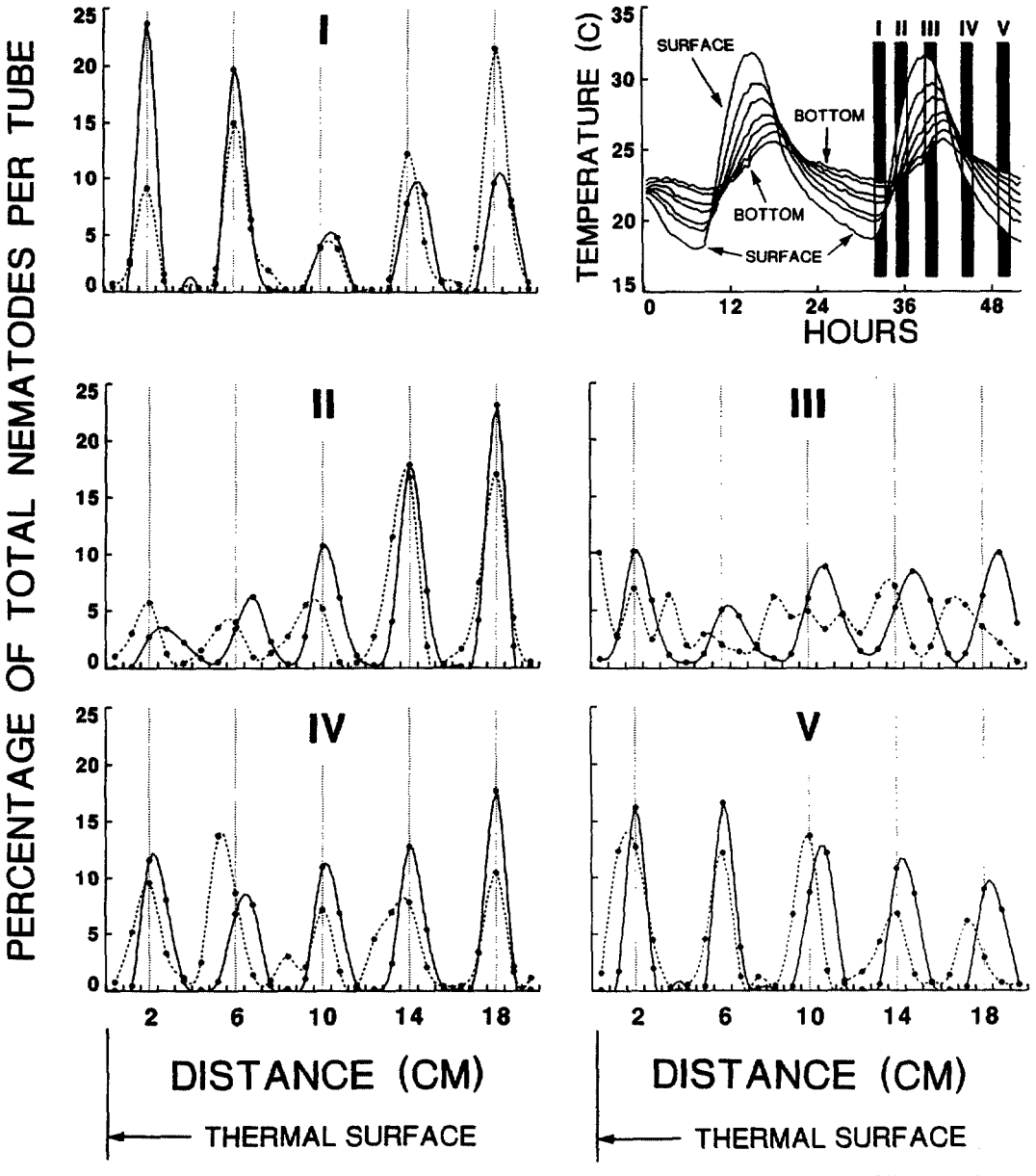

FIG. 8. Distributions of *Meloidogyne incognita* (dashed lines) and *Rotylenchulus reniformis* (solid lines) 1.5 hours after simultaneous injection into horizontally oriented 3.8-cm-d, 20-cm-long tubes of moist sand subjected to the sunny, summer day simulation. Roman numerals indicate the intervals during which movement was examined, and vertical dotted lines indicate the points along the tube where nematodes were introduced. Each datum is the mean of two replications. Curves were generated by spline algorithm.

matrix and compare the direction of movement with that previously observed under thermally unnatural conditions. In six experiments in six different months of the year, *M. incognita* moved toward and *R. reniformis* moved away from the thermal surface, irrespective of gravity. Interestingly, this marked difference was not predictable from previous responses measured on gels. The vermiform stages of *M. incognita* and *R. reniformis* are similar in size when compared with the other species tested, have similarly low rates of spontaneous motility in water, and have exhibited

generally similar responses to temperature under static conditions (5,15).

A much slower rate of thermal adaptation by *M. incognita* than by *R. reniformis* could place *M. incognita* near its avoided or dispersion temperature during parts of the day when temperatures are optimum for movement, while *R. reniformis* would adapt quickly enough to remain near its thermal preferendum. As a result, when the soil is warming *R. reniformis* would move away from heat (downward, most of the time) and when the soil is cooling it would move toward heat (again downward, most of the time). *Meloidogyne incognita* would move the opposite direction. This interpretation is consistent with results of computer simulations considering all patterns of change during the day (7,8).

Other explanations are conceivable. When nematodes are given freedom of movement in three dimensions, responses to temperature could differ substantially from those observed on gels. Alternatively, both species may adapt rapidly and respond over a common range of temperature by kinesis with a resultant tendency to move downward, but also move rapidly toward heat by thermotaxis as occurs in hookworms, when the environment experienced by the moving nematode is sufficiently hot and is gradually warming (4). If the threshold temperature for the taxis were several degrees lower for *M. incognita* than for *R. reniformis,* then gradients during the hottest period of the day could induce *M. incognita* to move rapidly upward with no effect on the kinesis-mediated downward movement of *R. reniformis*. Results obtained in the sixth experiment, in which initial directions of movement during five intervals of the day were compared, indicate this effect probably did not occur, because similarly differential movement appeared to occur during the night and day. More data are needed, though, for these and other species.

The most obvious effects of gravity were a consistent tendency for *D. phyllobius* and *S. glaseri* to move upward and for *M. incognita* and *R. reniformis* to move downward. Vermiform stages of the root parasites, *M. incognita* and *R. reniformis,* are similar in size and about 350–400 μm long. Infective juveniles of *H. bacteriophora, D. phyllobius,* and *S. glaseri* are approximately 600, 650, and 1,100 μm long, respectively. Perhaps body length relative to soil particle size influences vertical movement. Previous studies on *Globodera rostochiensis* (22) and *R. reniformis* (16) in soil and sand with coarse particle or soil crumb sizes revealed downward movement, which was reversed when in a finer textured matrix. Downward movement by *R. reniformis* and *G. rostochiensis* was associated with head-down descent in water and was suggested to result from differences in the specific gravities of the two ends of the body. Upward movement is more difficult to explain and suggests geotaxis. Pronounced upward movement by *S. glaseri* in sandy soil 1 m from the point where introduced has been observed at a constant temperature of 26 C (21), but in another study (at 23 C) a slightly greater tendency to move downward than upward occurred in four soils greatly differing in texture (10). Effects of gravity on nematode movement in soil deserve further study.

Interpretations regarding the ecological significance of dispersal patterns in relation to temperature gradients and gravity should be made with an awareness that these are only two of many factors that might influence movement in natural soil profiles. Nematodes probably respond to various stimuli interactively or sequentially in a species-specific manner that increases the chances of completing the life cycle. The most obvious characteristic of movement of the insect pathogens, *S. glaseri* and *H. bacteriophora,* from a point source was rapid dispersal throughout the tubes. Much larger tubes, as have been used in previous dispersal studies (10,21), may be required to detect responses of these rapidly moving nematodes to vertical temperature gradients. Perhaps, however, the responses of these species to natural patterns of temperature change are unimportant compared with the effects of gradients of

carbon dioxide and substances released by the insect host (9,13).

Upward movement of *D. phyllobius* during the rainy day simulation is consistent with its known movement from soil onto plant stem surfaces during rainy periods (17). However, fewer than 30% of the applied juveniles reached the section at the end of the tube after 48 hours, compared with 60–85% for *R. reniformis* in most tubes, even though *D. phyllobius* is a larger and much faster moving nematode in water. In vitro, *D. phyllobius* is strongly attracted to an unidentified compound that apparently is specific to the *Solanum* plants it parasitizes (18). Perhaps gradients of this compound around stems in the upper few centimeters of soil induce movement to the soil surface. Movement by *D. phyllobius* toward low temperatures (ca. 17 C) in vitro is apparently unaffected by thermal adaptation (14) and could play an important role in movement on wet stems chilled by evaporation, yet be unimportant in soil.

In conclusion, this study indicates that both temperature and gravity can differentially influence vertical dispersal of ecologically different nematodes in thermally natural particulate matrices. However, much more information is needed regarding interactive and sequential responses to these and other factors affecting movement before we clearly understand the role of nematode behavior in host finding and stress avoidance in the soil.

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