Techniques in Nematode Ecology

The concept for this Ecology Manual was developed by the SON Ecology Committee, which envisioned it as a continually updated resource for soil ecologists and nematologists interested in nematode and soil ecology. The idea was ahead of its time and the project languished due to problems associated with traditional publication and distribution costs. Online publication seems to be the ideal way to make these contributions available to a wide audience while allowing updates and additional contributions over time. We thank the authors for their patience while technology caught up with their contributions. Editors who worked with authors to develop this manual were: T. Wheeler, T. Forge, and E. Caswell-Chen. Comments about this manual or ideas about future contributions may be directed to the Society of Nematologists Ecology Committee. The current committee chair for 2000 is: J. A. LaMondia(lamondia@caes.state.ct.us).

Table of Contents

Techniques for individual or small numbers of nematodes:

Techniques for nematode populations or communities:

Techniques for Studying Nematode Movement and Behavior on Physical and Chemical Gradients

A. F. Robinson

Nematologist, USDA ARS,
Southern Crops Research Laboratory,
2765 F & B Road,
College Station, TX 77845.
Tel: 409-260-9234
Fax: 409-260-9333
forestr@acs.tamu.edu

A comprehensive description of methods for studying nematode behavior is not possible in this brief chapter. My goal is to provide some published examples that will help the reader to select and develop techniques. This review is organized by methods rather than biology. For example, techniques for testing volatiles, whether for pheromone activity or for root attractant activity, are discussed together, while techniques that could be used to examine the roles of light, temperature, gravity, and moisture in the vertical movement of a foliar parasite are discussed separately. Like most research on nematode movement, this review focuses on motile stages outside host tissue; only a few references to methods for observing movement within plant tissue are included. Motility assays conducted as part of studies on toxicology or survival are generally not included, nor are studies on hatching stimulants. For additional information the reader is referred to previous reviews (16,31,45,119,143,183,184,185,186,194,196).

GENERAL CONSIDERATIONS

Optical constraints: Most nematodes are microscopic, translucent and colorless, and are seen best with transmitted light. Nematodes on opaque surfaces usually are difficult to observe even at high magnification. The natural surface or matrix on or through which investigators usually wish to understand nematode movement, however, is opaque (e.g. leaf surfaces, soil, plant tissue, animal tissue) and destructive sampling at timed intervals often is required to monitor movement under natural conditions. Many investigators, therefore, have used transparent gels, recognizing that conditions on gels, on other moist surfaces, and within soil will differ. Differences would be expected, for example, in surface tension, surface tortuosity, solute and gas diffusion, and water potential (24). By using gels it has been possible to study responses of individual nematodes to gain insight regarding specific behavioral mechanisms, such as tropotaxis and klinokinesis (31,53,63,71). Recently, the thin translucent roots of *Arabidopsis thaliana* have been used to elucidate feeding and migrations by nematodes in roots (161,174,182,196)

Space and time constraints: The size of most nematodes presents challenges in the design of devices small enough to control and measure ecologically meaningful gradients. Where movement of root parasites through soil is of interest, their typically slow movement relative to rates of change in temperature, gas tensions, and moisture (24) can make the simulation of natural fluctuations in gradients useful. Studies examining temperature gradient fluctuations, for example, indicated that differences in the rate of thermal adaptation by a nematode can reverse the net direction it moves vertically in response to soil surface heating and cooling (44,127). Another consideration is the geometry of both the stimulus source and gradient, which can alter the pattern of aggregation or dispersal (45,164,165).

TECHNIQUES FOR MONITORING RESPONSES

Hundreds of research articles and abstracts describe responses of nematodes to known stimuli, or to chemically unknown stimuli from plants, insects, and other nematodes. Many different kinds of containers, matrices and extraction techniques have been used to monitor movement by large numbers of nematodes. Responses of individual nematodes have been examined for the most part on transparent gels or on gels coated with sand grains to induce nictating (68,78,170,177). Paths taken by individual nematodes have been recorded and analyzed by photographing nematode tracks on agar (3,134,138,139), analyzing videotapes (21,113,164), and employing digital image analysis to track as many as 200 nematodes simultaneously (41,42,43,95,112,171). A powerful technique that so far has been exploited little is to tether individual nematodes by sucking the tail (or head) into a finely drawn capillary. Tethering permitted measurement of responses by *Caenorhabditis elegans* (40) and *Meloidogyne incognita* (61) to rapid changes in solutes by recording body movement with an array of miniature photodetectors connected to a multichannel stripchart recorder. More recently, tethering permitted insertion of electrodes into microscopic nematodes to obtain recordings of action potentials in response to changes in concentrations of external solutes (83,188,189). This technique has considerable potential to accelerate the identification of pheromones and other specific attractants. Video monitoring of events during root invasion has been employed in several studies and has revealed that highly specific behaviors are involved in successful parasitism (161,174). Repeated direct observations of events under the microscope without specialized equipment, however, still works well to characterize root invasion and mating (176).

CONTROL OF PHYSICAL STIMULUS GRADIENTS

Touch: Most vermiform nematodes move when touched or alter their patterns of spontaneous movement upon encountering objects or physical discontinuities in an otherwise homogenous matrix. The typical response is to stop forward movement, move back a short distance, and then proceed in a new direction (31). In only one case, apparently, have probes been employed that can touch individual cephalic papillae or other sensors (83); techniques have not been developed to test for neurosensory involvement in the synchronous movements that touching individuals of various species commonly exhibit on moist surfaces and during so-called swarming or clumping events (31,78,195). Placement of nematodes on a wet wick with one end immersed in a well of water was used to test

Aphelenchoides besseyi for orientation to water current flow (rheotaxis) in experiments investigating upward movement on rice stems (1). Rheotaxis was implicated in upward movement by *Meloidogyne chitwoodi* (110) but not by *Globodera rostochiensis* (155) in soil.

Gravity: Many studies have sought to identify factors mediating vertical movements in soil and on aerial plant surfaces by nematodes that infect vertebrates (17,70,73,94,106,121), insects (60,102,142,158), and plants (1,82,110,115). Compelling evidence of the ability to sense gravity has been provided only for the insect parasite, *Mermis nigrescens* (55). Statocysts are unknown in nematodes. However, nematodes might be able to orient to gravity directly by proprioception or indirectly by sensing related gradients in soil moisture, gases or temperature. *Turbatrix aceti* (108,109), *G. rostochiensis* (156), and *Rotylenchulus reniformis* (131) orient vertically in water, likely due to differences between anterior and posterior body densities. The extent of downward orientation by *R. reniformis* was measured by suspending nematodes in the narrow channel of a sealed, hookworm counting slide and videotaping the slide in a vertical orientation through a microscope laid sideways (131). Passive vertical orientation was proposed to explain downward movement by *G. rostochiensis* in coarsely aggregated soil (156) and by *R. reniformis* in coarse sand (131).

Vertical movement in soil most commonly has been studied in open-ended containers (usually circular but sometimes square in cross section) that can be stacked to facilitate layered vertical sampling at the end of the experiment. Buckley (17), though, examined vertical movement of strongylid larvae on 2.5-cm 7.5-cm microscope slides that were covered with moist sand and enclosed within culture tubes. Composite containers as large as 15 cm in diameter and 2 m long often have been employed in various laboratory and field experiments (110,197). Usually, polyvinyl chloride pipe (PVC), ceramic sewer pipe tiles, or acrylic tubing has been used. Acrylic tubing is expensive but transparent, permitting direct observation of tube contents. When using acrylic tubes 20 cm or less in length, I usually have found it adequate to extrude sand or soil vertically with a clamp, plunger, and spacers, and slice it into 8-mm (or thicker) disks during extrusion. If the sand or soil is near field capacity, subsections are easily and accurately cut from the disks with home-made tools similar in design to cookie dough cutters.

Electric and magnetic fields: Responses to magnetic fields were tested by Sukul et al. (146) and Bessho et al. (169) but such experiments are rare. Responses to electric fields have been examined more often (28,137,147,151). Sand saturated with various salt solutions usually has been employed. Viglierchio and Yu (151) noted that attempts to use agar resulted in excessive surface drying and movement of water to the electrodes. Their apparatus, which employed quartz sand as a nematode and salt migration bed, was modified from paper electrophoresis equipment (151). The sand was layered onto a circulating water bath constructed from glass plates to dissipate heat.

Light: Photoreceptors and orientation to light have been described only in certain mermithid and marine species, although at least one rhabditid seems to have a dermal light sense (19). Behavioral arenas have ranged from moistened black or white felt within an empty, 30-cm 60-cm fish aquarium for studying the 100-mm-long adult females of *M. nigrescens* (20,22), to 0.75% water agar within 2-cm X 2-cm 10-year pin boxes for the 2-mm-long infective juveniles of *Agamermis catadecaudata* (129). Responses to radiant heat can be eliminated by passing light through water or infrared filters (19,105). Narrow bandpass filters, neutral density filters, and a radiometer with a known, preferably flat, spectral response can be used to control wavelength, measure threshold intensities and obtain action spectra (103) as a basis for formulating hypotheses regarding photosensitive pigments. The half-maximal response threshold for *M. nigrescens* was comparable to photon fluence 30 minutes before dawn (22), emphasizing the need to eliminate peripheral illumination during phototaxis experiments. Studies on transverse phototaxis in *A. catadecaudata*, on the other hand, indicated that irradiance equal to direct sunlight was required to elicit a maximal response (129); therefore, weak illuminators may inadequately simulate light levels naturally encountered by species that normally occur on foliage or the soil surface during the day.

Temperature: Temperature gradients around heat sources and sinks have been generated in agar and water with various simple devices, including hot glass rods (84), tungsten heating wire (32,48,88), light emitting diodes (23), living insects taped to the outside of a dish (23), containers of germinating seeds (48), small beakers of frozen acetic acid (72), and light from a fiber optic (130). The ability of *M. incognita* juveniles to detect a 0.001 C change in ambient temperature was discovered using computer-video tracking, two telethermometers, a 100-W spotlight, and a household dimmer switch (43). Responses of *Ditylenchus dipsaci* to linear gradients were studied by Wallace (157) and Croll (30) with a 15-cm-long, 6-mm-d polyethylene tube that was filled with sand, wrapped in steel wool, and inserted into a glass culture tube. One end of the culture tube was placed in a thermos of ice and the other in a tin can containing more steel wool and an incandescent light bulb.

The discovery of behavioral adaptation to temperature in nematodes (30,134) led to studies requiring maintenance of linear gradients within many containers simultaneously at different median temperatures. Several kinds of temperature gradient plates were devised, large enough to accommodate a number of petri dishes or rectangular containers of agar. Most gradient plates consisted of metal slabs up to 1 m long fastened to metal pillars immersed in hot or cold liquids (18,72,111). Rode described a more complex gradient plate onto which agar was poured directly (133,135). Another study utilized a system of narrow, 4-cm-long containers with thin bottoms made from acetate film, with each chamber clamped to its own, miniature gradient plate made from an aluminum turnbuckle (125). When 96 of these plates were fastened to two 8-m lengths of copper pipe, and hot and cold water was pumped through the two pipes in opposite directions at a balanced flow rate controlled by a dual-piston metering pump, the countercurrent heat exchange principle maintained the same gradient steepness in all channels, but at median temperatures uniformly spaced between 5 and 36 C. A subsequent study utilized countercurrent heat exchange to maintain gradients in a similar fashion inside 4-cm-d acrylic tubes containing sand; details were provided regarding construction of the apparatus (131). Studies with vertebrate parasites have required large containers of liquid matrix. In their work on juveniles of the dog heartworm (*Dirofilaria immitis*), Mok et al. (100) used a 43-cm-long glass trough of liquid medium supported on one end by a hot plate. Ronald (136) used an 88-cm tube containing dilute sea water in his work on the seal parasite, *Terranova decipiens*. Baffles were used in both of these studies to eliminate convection currents. Ronald's tube was wrapped in heating tape on one end, and packed in dry ice on the other.

One of the earliest intentional exposures of nematodes to vertical temperature gradients in soil was Overgaard-Nielsen's use of a light bulb within a wooden box to heat Baermann funnels from above (104). A reexamination of temperature gradient effects during Baermann funnel extraction (130) also employed light bulbs as heat sources but combined them with the immersion of funnel bottoms

in warm or cool water baths and the chilling of funnels from above; chilling was achieved by placing an aluminum dish containing cold water and a copper chill coil on the soil retaining ring. Natural diurnal fluctuations in vertical temperature gradients within soil were subsequently achieved in large acrylic tubes (15 cm d, 15 cm long) by sealing tube ends with circular plates and burying the tubes in moist sand within insulated boxes (127). Automobile transmission coolers were buried on either side or on top and bottom of the sand mass in each box and water was pumped through coolers at variable temperatures using ramp-and-soak controllers programmed for 96 1-hour ramps, with ramp endpoints identical to hourly temperatures previously measured at corresponding depths in a natural soil profile. Heat wave propagation across the tubes closely mimicked the sinusoidal, downward heat waves of the natural soil. An alternative, cheaper way to mimic natural temperature fluctuations at the soil surface is described by Beerwinkle and March (11).

Soil moisture and water potential: The reader is referred to Kramer's text on plant water relations (90) and to Wallace's classical studies on the effects of soil water potential on nematode movement (152,153,154). Soil moisture gradients, of course, are more difficult to maintain than are uniform levels and rigorous techniques are lacking. An obvious but crude method is to pack layers of soil possessing gravimetrically adjusted, increasing or decreasing moisture contents into a container (118). Another way is to saturate soil within an open-ended container on a sintered glass funnel, or suitable substitute, and either equilibrate the soil with a water column or else extract excess water with a partial vacuum, so as to leave more moisture at the bottom than at the top (49,131,156). Much of the gradient generated remains for a number of hours, even when the container is removed and inverted, or oriented horizontally. An apparently unused technique which would seem to provide more accurate control as well as a means to alter the gradient during the course of an experiment is to sandwich soil between two sheets of dialysis membrane, and continuously expose each sheet to a different concentration of polyethylene glycol in water. Related devices are described and discussed by DeGuiran (35) and Zur (162). If nematode responses to moisture gradients are detected, it is essential to know the matric potentials achieved because it is matric potential rather than moisture content that most directly affects physiological water regulation and the suitability of water films for nematode movement (152). Matric potentials can be estimated by measuring water content, if the moisture characteristic of the soil has been determined (96). Additional characteristics of the soil, such as textural class, particle size distribution of the sand fraction, volumetric porosity, and bulk density (24,90,96) can also profoundly influence the suitability of conditions for nematode movement, and should be determined and reported if possible.

CONTROL OF CHEMICAL GRADIENTS

Diversity of bioassays used: In more than 100 studies where nematode responses to chemical stimuli have been examined, at least 50 different kinds of behavioral assays have been employed. Responses have been examined to many known compounds and to unknown compounds from insect hosts (67,78), insect vectors (54,98,99), plants (2,13,91,97,107,119,159,160), bacteria (69), fungi (144), and other nematodes (16,173,178,188). Although the sources of the stimuli of interest in most cases are ecologically obvious, there are few obvious patterns relating nematode ecology to the kinds of containers, migration matrices and behavioral arena geometries that have been used.

Most studies have used 0.5 to 3% agar as a migration matrix, but others have used natural sand (5,33,102,118,128,142), manufactured silica sand (93,120), soil (9,27,130,163,190), balanced salt solutions (6,56,124,181), or the lining of the mouse duodenum (168). Influence of matrix heterogeneity on movement of *C. elegans* was analyzed on agar covered with a monolayer of sand (164). The optimum agar concentration depends on the strength and the optimum sand particle size depends on the length of the nematode studied (154,156). Test substances as well as nematodes have been introduced to migration matrices in virtually every way imaginable. Nematodes, nematode secretions (64) or test solutions have been applied to the matrix on a piece of filter paper (6,29,34,54,75,89,126,132,166), within agar disks (4,29,52,54,66,89,124), in cotton pads (54,148,149), with a fine nylon brush (179), or within water droplets that were allowed to dissipate (4,12,79,80,81,101,114,145,190,191). Alternatively, nematodes have been mixed uniformly with sand (5,7,33) or with agar before solidification (8,14,132,150), or portions of agar have been removed and replaced with agar containing nematodes or test compounds (15,148,175). In track analysis studies, nematodes are applied to the matrix surface with a drawn capillary or a small pick, such as a dental file, bamboo splinter, or flattened platinum wire (29,47,66,74,123).

In many assays, other living organisms have been included but restrained from moving about the arena. Examples include: miniature sand baskets containing mycelium and culture broth of *Arthrobotrys* spp. (nematode trapping fungi) (5,7); agar-filled drinking straw sections containing *Escherichia coli* and males or females of the bacteriophagous nematode, *Panagrellus redivivus* (36); nutrient agar cores with or without *E. coli* placed directly on the arena agar surface (196); sealed dialysis tubing containing males or females of *Pellioditis pellio* (50,51,77); pipet tips containing *Galleria mellonella* caterpillars restrained by cotton plugs or steel wool (9,57,140,141); a female of *Heterodera schachtii* stuck to the agar-coated undersurface of a coverslip that was elevated and supported by glass beads 1 mm above the surface of an agar plate (62), to evaluate involvement of volatiles in sex attraction; passing air through filter holders containing *Monochamus alternatus* pupae to collect volatiles attractive to the pinewood nematode (99); suspending infected beetles in a wire basket above water containing test compounds, to study host exit behavior of the pinewood nematode (192); embedding and incubating male or female *Radopholus similis* within agar discs to collect pheromones (74); and use of partitioned sections within petri dishes (69) or plastic tubes (65), respectively, to expose *C. elegans* or *Panagrolaimus rigidus* to volatiles from bacteria or oppositely sexed nematodes.

Entomopathogenic nematode assays: No less than five distinct behaviorally based assays have been developed to characterize dispersal behavior and monitor population densities of entomopathogenic nematodes in soil (172,180,187).

Arena geometry: Usually, petri dishes or other, shallow circular containers have served as behavioral arenas, but in many cases the arena geometry has instead been cylindrical (118,128), tubular (120), rectangular (33,76,77,112), linear (25,26), tri-lobed (113,122), quadra-lobed (4), cross-shaped (89), T-shaped (193), U-shaped (114,116), V-shaped (36), Y-shaped (92), or maze-shaped (56). Single, paired, and multiple choice (54) tests have been used, although paired-choice tests have proven best for detecting repellents. Several assays have distinguished volatiles (and gases) from non-volatiles by separating nematodes from test substances, insect hosts or

bacteria with an air pocket (57,62,66,69,85,92,99), while others have distinguished dialyzable from non-dialyzable substances by separating nematodes from test substances or other organisms with a dialysis membrane (33,51,93).

Ambient stimuli: Guard against evaporative chilling and randomize the orientation of assay plates. Many investigators have blocked light from behavioral arenas even though light has generally not been shown to affect most nematodes at the intensities found in most laboratory settings, yet have ignored the effects of evaporative chilling across arena surfaces. Many nematodes are highly sensitive to temperature gradients. Root-knot nematodes have exhibited responses to temperature differentials of 0.001 C (43) and the author many times has observed *Ditylenchus phyllobius* accumulate toward the same corner of the laboratory within uncovered chemotaxis assay plates in response to room drafts, an effect that is repeatedly reversible by rotating plates 180 degrees, and largely eliminated by covering plates.

Gradient kinetics: Where attempts have been made to control gradient kinetics, the attempt most often has been made (and there are numerous examples) by allowing a solute to diffuse from a point source in the agar or sand following a single or double application 1 to 24 hours before introducing nematodes at another point (25,166). The strategy is to generate a gradient that will decay slowly relative to the time required to assay for nematode responses; the diffusion kinetics of various known compounds through agarose in a linear assay system have been examined in considerable detail (25). Mixing nematodes uniformly with agar or sand before introducing the test substance, however, has often produced strong nematode attraction or repulsion (6,8,14,132,150). It should be noted that in most published studies gradients were not measured in vitro or under natural conditions because the active compounds were unknown.

Stable and measurable non-decaying gradients have been achieved in two ways. One way is to allow solutes to diffuse through a tube (or layer) of agar or sand that is in contact with a test solution reservoir on one end (or side) and in contact with distilled water on the other. This was accomplished by Prot (114) with a U-shaped glass tube and used successfully in a number of his experiments on repulsion of root-knot nematodes by various salts (116,117). Nematodes were introduced at the bottom of the "U". A second way is Dusenbery's countercurrent separation technique (37). This perhaps intimidating but ingenious method utilizes slow, bidirectional flow of two viscous fluids of different specific gravity within a slanted tube to maintain a standing gradient of a test substance added to either one of the fluids; it worked well in several studies on *C. elegans* (38,39,46,47). Two other ways are to release continually a known gas mixture from a point source (83,87,98,128,130,140,150), or create a bilaminar flow of two gas mixtures over the surface of the migration matrix (112).

Gases and volatiles: Recent work on volatile attractants and repellents has sought to identify factors mediating host-finding by entomopathogenic nematodes, primarily *Steinernema* spp. and *Heterorhabditis* spp. Additional work has addressed mechanisms by which the pinewood nematode, *Bursaphelenchus lignicolus* locates the beetle, *M. alternatus*, to obtain transport between host trees. In both of these cases, CO2 appears to be the major attractant involved. Other work on volatiles has addressed pheromones in cyst nematodes (66), the attraction of *C. elegans* to bacteria (69), host discrimination by Steinernematids (172), repulsion of root-knot by entomopathogenic nematodes (173), host exit behavior of pinewood nematode (192), and fundamental sensory physiology of odorant recognition and discrimination in animals, using *C. elegans* as a model (10).

Attraction of *Steinernema carpocapsae* to CO2 was first observed by Gauger et al. (59) by slowly emitting (15-120 l/min) pure CO2 from a finely drawn capillary placed parallel to the surface of a covered agar plate. This technique originally was described by Klingler in his classical studies on *Ditylenchus dipsaci* (83,87,88). Subsequent research on the behavioral sensitivity of entomopathogenic nematodes to CO2 in relation to ambushing and cruising host search strategies (58), utilized placement of wax moth larvae (*G. mellonella*) within pipet tips that were stuck through a sheet of plastic and suspended within a closed agar-filled chamber with the ends of pipet tips just above the agar surface. Placing a KOH-soaked filter paper on the undersurface of the lid to remove CO2 prevented nematode attraction. In further research on the role of volatiles in host searching, a Y-tube choice apparatus was used, with air containing or not containing host volatiles pumped through each half of the "Y" (92). Removal of CO2 with KOH again prevented attraction of *Steinernema glaseri*, as did killing wax moth larvae with chloroform. Studies on vector location by the pinewood nematode, *B. lignicolus*, utilized inline chambers containing insect pupae; gas could be directed either into behavioral arenas or into a gas chromatograph (98,99). Nematodes were attracted to CO2 and to volatiles from insects, but not to volatiles following passage through a CO2-absorbent filter. Release of respiratory CO2 by insect pupae increased during pupal development.

Several additional references to methods for testing responses to CO2 may be useful to the reader. Klingler first observed attraction of *D. dipsaci* to capillaries emitting CO2 at extremely slow flow rates near 15 l/minute (86,87,88). Schmidt and All (141), however, failed in their attempts to attract *S. carpocapsae* to CO2, likely due to anaesthetic effects, when the gas was released into a covered petri dish at 1,000 times this flow rate (20-100 ml/minute). Viglierchio (150), however, did obtain attraction of *Panagrellus silusiae* to pipettes emitting very high flows (500 ml/minute) when the agar surface was covered and protected from the gas flow with a semipermeable membrane, that apparently was included to retard evaporation. Attraction of *R. reniformis* to a subanaesthetic concentration of CO2 (2.5%) in air at approximately 99% relative humidity occurred in petri dishes subjected to a relatively high flow rate (30 ml/minute) from pipets oriented at a 45 angle to the surface (130). These dishes, however, were open, and qualitative characterization of the gradients with the pH indicator bromothymol blue, revealed a V-shaped, isocratic region congruent with the pattern of nematode accumulation, with little or no indication of CO2 absorption in the agar outside the V-shaped region. Nematodes in the V-shaped region were highly motile and controls exposed to an equivalent flow rate of humid air with no added CO2 did not elicit a response. Air in both cases was pumped from a filled 30-liter gas sampling bag containing 100 ml water in one corner, which was suspended in an open pail of water to chill the water in the bag below ambient just enough to prevent condensation in delivery tubes. A modification of this method but with 5% CO2 was used subsequently to study *M. naasi* (167).

Only a few observations are available regarding attraction to CO2 in sand or soil. Dialysis tubing sections filled with CO2-equilibrated water, and laid onto the surface of moist sand (33) elicited attraction of *D. dipsaci*. In another study, when CO2 gradients were manipulated within water-saturated soil on Baermann funnels, only weak responses by *R. reniformis* were observed, compared with responses to temperature (130). Some 30 years previously, Bird (14) had similarly failed to attract *M. incognita* in agar when CO2 was

introduced by bubbling it into the agar prior to solidification. Strong aggregations of *M. incognita* and *R. reniformis* to a controlled point source, however, were achieved within moist (i.e., partially drained) sand at flow rates of 5-20 µl/minute (128). Thus, available evidence suggests that diffusion through the vapor phase may be critical to the achievement of behaviorally effective gradients within soil. For all practical purposes, respiratory gases do not diffuse through saturated soils (24).

The results of Pline and Dusenbery on *M. incognita* (112) indicated that the mean ambient concentration of CO2 can greatly influence the sensitivity of a nematode to the changes in concentration it encounters as it moves. Pline and Dusenbery also provide novel ideas on how to control minute differences in gas gradient magnitude experimentally. In their apparatus a needle from a syringe infusion pump was inserted into a gas flow line, enabling them to "spike" a constant flow of hydrated air passing into the behavioral arena, with relatively minute quantities of CO2. By pumping air from two such sources into a small container through dual jets, bilaminar flow across an agar surface was achieved, permitting the investigators to control and change the gradient rapidly. In future studies, this innovation could be applied to various other soil gases, such as ammonia, oxygen, ethylene, ethane, methane, and sulfur dioxide.

CONCLUSION

Clearly, the numerous studies of nematode behavior that have been conducted during the last several decades have discovered many kinds of responses by highly diverse species to a wide range of environmental stimuli, and the techniques used are nearly as varied and numerous as the species, responses, and stimuli investigated. As our knowledge of nematode behavior expands, new methods are continually being described. The best technique to use greatly depends on the size, speed, and behavioral peculiarities of the nematode to be studied, and general recommendations for any given stimulus are probably unwise. Hopefully, however, sufficient examples of what can be done have been presented to provide prospective investigators of nematode behavior with a basis for selecting and developing methods suited to the nematodes and aspects of nematode ecology they wish to study.

LITERATURE CITED

1. Adamo, J. A., C. P. Madamba, and T. A. Chen. 1976. Vertical migration of the rice white-tip nematode, *Aphelenchoides besseyi*. Journal of Nematology 8:146-152.

2. Azmi, M. I., and M. S. Jairajpuri. 1977. Attraction of plant-parasitic nematodes to host roots. Nematologica 23:119-121.

3. Balakanich, S., and M. R. Samoiloff. 1974. Development of nematode behavior: Sex attraction among different strains of the free-living *Panagrellus redivivus*. Canadian Journal of Zoology 52:835-845.

4. Balan, J. 1985. Measuring minimal concentrations of attractants detected by the nematode *Panagrellus redivivus*. Journal of Chemical Ecology 11:105-111.

5. Balan, J., and N. N. Gerber. 1972. Attraction and killing of the nematode *Panagrellus redivivus* by the predaceous fungus *Arthrobotrys dactyloides*. Nematologica 18:163-173.

6. Balan, J., L. Krizková, P. Nemec, and A. Kolozsváry. 1976. A qualitative method for detection of nematode attracting substances and proof of production of three different attractants by the fungus *Monacrosporium rutgeriensis*. Nematologica 22:306-311.

7. Balanová, J., J. Balan, L. Krizková, P. Nemec, and D. Bobok. 1979. Attraction of nematodes to metabolites of yeasts and fungi. Journal of Chemical Ecology 5:909-918.

8. Balhadère, P., and A. A. F. Evans. 1994. Characterization of attractiveness of excised root tips of resistant and susceptible plants for *Meloidogyne naasi*. Fundamental and Applied Nematology 17:527-536.

9. Barbercheck, M. E., and H. K. Kaya. 1991. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Environmental Entomology 20:582-589.

10. Bargmann, C. I., E. Hartwieg, and H. R. Horvitz. 1993. Odorant-Selective Genes and Neurons Mediate Olfaction in C. elegans. Cell 74:515-527.

11. Beerwinkle, K. R., and P. A. March. 1981. A cam-operated, electronic proportional-control system for laboratory simulation of outside summer temperatures. Southwestern Entomologist 6:53-56.

12. Bilgrami, A. L., I. Ahmad, and M. S. Jairajpuri. 1985. Factors influencing attraction of adult *Hirschmanniella oryzae* towards cabbage seedlings. Revue de Nematologie 8:67-75.

13. Bilgrami, A. L., I. Ahmad, and M. S. Jairajpuri. 1985. Responses of adult *Hirschmanniella oryzae* towards different plant roots. Revue de Nematologie 8:265-272.

14. Bird, A. F. 1959. The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. Nematologica 4:322-335.

15. Bolla, J. A., J. Bramble, and R. I. Bolla. 1989. Attraction of *Bursaphelenchusxylophilus,* Pathotype MPSy-1, to *Monochamus carolinensis* Larvae. Japanese Journal of Nematology 19:32-37.

16. Bone, L. W. 1987. Pheromone communication in nematodes. Pp. 147-152 *in* J. A. Veech and D. W. Dickson, eds. Vistas on Nematology. Hyattsville: Society of Nematologists.

17. Buckley, J. J. C. 1940. Observations on the vertical migrations of infective larvae of certain bursate nematodes. Journal of Helminthology 18:173-182.

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

19. Burr, A. H. 1985. The photomovement of *Caenorhabditis elegans*, a nematode which lacks ocelli. Proof that the response is to light not radiant heating. Photochemistry and Photobiology 41:577-582.

20. Burr, A. H., and C. P. F. Babinszki. 1990. Scanning motion, ocellar morphology and orientation mechanisms in the phototaxis of the nematode *Mermis nigrescens*. Journal of Comparative Physiology A 167:257-268.

21. Burr, A. H., C. P. F. Babinszki, and A. J. Ward. 1990. Components of phototaxis of the nematode *Mermis nigrescens*. Journal of Comparative Physiology A 167:245-255.

22. Burr, A. H., D. K. Eggleton, R. Patterson, and J. T. Leutscher-Hazelhoff. 1989. The role of hemoglobin in the phototaxis of the nematode *Mermis nigrescens*. Photochemistry and Photobiology 49:89-95.

23. Byers, J. A., and G. O. Poinar, Jr. 1982. Location of insect hosts by the nematode, *Neoaplectana carpocapsae*, in response to temperature. Behaviour 79:1-10.

24. Campbell, G. S. 1985. Soil Physics with Basic. Transport models for soil-plant systems. New York: Elsevier.

25. Castro, C. E., N. O. Belser, H. E. McKinney, and I. J. Thomason. 1989. Quantitative bioassay for chemotaxis with plant parasitic nematodes. Journal of Chemical Ecology 15:1297-1309.

26. Castro, C. E., N. O. Belser, H. E. McKinney, and I. J. Thomason. 1990. Strong repellency of the root knot nematode, *Meloidogyne incognita* by specific inorganic ions. Journal of Chemical Ecology 16:1199-1205.

27. Castro, C. E., H. E. McKinney, and S. Lux. 1991. Plant protection with inorganic ions. Journal of Nematology 23:409-413.

28. Caveness, F. E., and J. D. Panzer. 1960. Nemic galvanotaxis. Proceedings of the Helminthological Society of Washington 27:73-74.

29. Clemens, C. D., J. Aumann, Y. Spiegel, and U. Wyss. 1994. Attractant-mediated behaviour of mobile stages of *Heterodera schachtii*. Fundamental and Applied Nematology 17:569-574.

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

31. Croll, N. A. 1970. The behavior of nematodes. New York: St. Martin's Press.

32. Croll, N. A., and J. M. Smith. 1972. Mechanism of thermopositive behavior in larval ookworms. Journal of Parasitology 58:891-896.

33. Croll, N. A., and D. R. Viglierchio. 1969. Reversible inhibition of chemosensitivity in a phytoparasitic nematode. Journal of Parasitology 55:895-896.

34. Davis, D., and J. E. Deak. 1960. An Assay for the Detection of Nematode Repellents. Plant Disease Reporter 44:622-624.

35. DeGuiran, G. 1975. A simple method for maintaining constant soil moisture stresses during experiments on nematodes. Nematologica 21:261-262.

36. Duggal, C. L. 1978. Sex attraction in the free-living nematode *Panagrellus redivivus*. Nematologica 24:213-221.

37. Dusenbery, D. B. 1973. Countercurrent separation: A new method for studying behavior of small aquatic organisms. Proceedings of the National Academy of Science USA 70:1349-1352.

38. Dusenbery, D. B. 1974. Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. Journal of Experimental Zoology 188:41-48.

39. Dusenbery, D. B. 1976. Attraction of the nematode *Caenorhabditis elegans* to pyridine. Comparative Biochemistry and Physiology 53:1-2.

40. Dusenbery, D. B. 1980. Responses of the nematode *Caenorhabditis elegans* to controlled chemical stimulation. Journal of Comparative Physiology A 136:327-331.

41. Dusenbery, D. B. 1985. Video camera-computer tracking of nematode *Caenorhabditis elegans* to record behavioral responses. Journal of Chemical Ecology 11:1239-1247.

42. Dusenbery, D. B. 1985. Using a microcomputer and video camera to simultaneously track 25 animals. Computers in Biology and Medicine 15:169-175.

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

44. 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.

45. Dusenbery, D. B. 1992. Sensory ecology. How organisms acquire and respond to information. New York: W. H. Freeman and Company.

46. 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 A 137:353-356.

47. Dusenbery, D. B., R. E. Sheridan, and R. L. Russell. 1975. Chemotaxis-defective mutants of the nematode *Caenorhabditis elegans*. Genetics 80:297-309.

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

49. Evans, K. 1969. Apparatus for measuring nematode movement. Nematologica 15:433-435.

50. Eveland, L. K., and B. Fried. 1990. Chemoattraction in *Pellioditis pellio* (Nematoda: Secernentia) in the presence and absence of barriers. Journal of Chemical Ecology 16:2859-2863.

51. Eveland, L. K., and M. A. Haseeb. 1993. *Pellioditis pellio*: Substituted phenolic compounds as chemoattractants. Journal of Chemical Ecology 19:2543-2546.

52. Field, J. I., and J. Webster. 1977. Traps of predacious fungi attract nematodes. Transactions of the British Mycological Society 68:467-469.

53. Fraenkel, G. S., and D. L. Gunn. 1961. The orientation of animals. New York: Dover Publications.

54. Futai, K. 1979. Responses of two species of *Bursaphelenchus* to the extracts from pine segments and to the segments immersed in different solvents. Japanese Journal of Nematology 9:54-59.

55. Gans, C., and A. H. Burr. 1994. Unique locomotory mechanism of *Mermis nigrescens*, a large nematode that crawls over soil and climbs through vegetation. Journal of Morphology 222:133-148.

56. García-Rejón, L., S. Verdejo, M. Sánchez-Moreno, and M. Monteoliva. 1985. Some factors affecting sexual attraction in *Ascaris suum* (Nematoda). Canadian Journal of Zoology 63:2074-2076.

57. Gaugler, R., and J. F. Campbell. 1991. Selection for enhanced host-finding of scarab larvae (Coleoptera: Scarabaeidae) in an entomopathogenic nematode. Environmental Entomology 20:700-706.

58. Gaugler, R., J. F. Campbell, and P. Gupta. 1991. Characterization and basis of enhanced host-finding in a genetically improved strain of *Steinernema carpocapsae*. Journal of Invertebrate Pathology 57:234-241.

59. Gaugler, R., L. LeBeck, B. Nakagaki, and G. M. Boush. 1980. Orientation of the entomogenous nematode *Neoaplectana carpocapsae* to carbon dioxide. Environmental Entomology 9:649-652.

60. Georgis, R., and G. O. Poinar, Jr. 1983. Vertical migration of *Heterorhabditis bacteriophora* and *H. heliothidis* (Nematoda: Heterorhabditidae) in sandy loam soil. Journal of Nematology 15:652-654.

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

62. Green, C. D. 1967. The attraction of male cyst-nematodes by their females. Nematologica 13:172-174.

63. Green, C. D. 1977. Simulation of nematode attraction to a point in a flat field. Behaviour 61:130-146.

64. Green, C. D., and D. N. Greet. 1972. The location of the secretions that attract male *Heterodera schachtii* and *H. rostochiensis* to their females. Nematologica 18:347-352.

65. Greet, D. N. 1964. Observations on sexual attraction and copulation in the nematode *Panagrolaimus rigidus* (Schneider). Nature 204:96-97.

66. Greet, D. N., C. D. Green, and M. E. Poulton. 1968. Extraction, standardization and assessment of the volatility of the sex attractants of *Heterodera rostochiensis* Woll. and *H. schachtii* Schm. Annals of Applied Biology 61:511-519.

67. Grewal, P. S., R. Gaugler, and E. E. Lewis. 1993. Host recognition behavior by entomopathogenic nematodes during contact with insect gut contents. Journal of Parasitology 79:495-503.

68. Grewal, P. S., E. E. Lewis, R. Gaugler, and J. F. Campbell. 1994. Host finding behavior as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology 108:207-215.

69. Grewal, P. S., and D. J. Wright. 1992. Migration of *Caenorhabditis elegans* (Nematoda: Rhabditidae) larvae towards bacteria and the nature of the bacterial stimulus. Fundamental and Applied Nematology 15:159-166.

70. Gruner, L., H. Mauleon, and C. Sauve. 1982. Migrations of trichostrongyle infective larvae. Experiments with bovine parasites in soil. Annales de Recherches Veterinaires 13:51-59.

71. Gunn, D. L. 1975. The meaning of the term 'klinokinesis'. Animal Behaviour 23:409-412.

72. 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.

73. Houston, R. S., G. T. Fincher, and T. M. Craig. 1984. Vertical migration of infective larvae of equine strongyles in sandy clay loam. American Journal of Veterinary Research 45:575-577.

74. Huettel, R. N., D. W. Dickson, and D. T. Kaplan. 1982. Sex attractants and behavior in the two races of *Radopholus similis*. Nematologica 28:360-369.

75. Huettel, R. N., and H. Jaffe. 1987. Attraction and behavior of *Heterodera glycines*, the soybean cyst nematode, to some biological and inorganic compounds. Proceedings of the Helminthological Society of Washington 54:122-125.

76. Huettel, R. N., and R. V. Rebois. 1986. Bioassay comparisons for pheromone detection in *Heterodera glycines*, the soybean cyst nematode. Proceedings of the Helminthological Society of Washington 53:63-68.

77. Imperia, P. S., B. Fried, and L. K. Eveland. 1980. Pheromonal attraction of *Schistosoma mansoni* females toward males in the absence of worm-tactile behavior. Journal of Parasitology 66:684-686.

78. Ishibashi, N., and E. Kondo. 1990. Behavior of infective juveniles. Pp. 139-150 *in* R. Gaugler, and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton: CRC Press.

79. Jansson, H.-B., A. Jeyaprakash, R. A. Damon, Jr., and B. M. Zuckerman. 1984. *Caenorhabditis elegans* and *Panagrellus redivivus*: Enzyme-mediated modification of chemotaxis. Experimental Parasitology 58:270-277.

80. Jansson, H.-B., A. Jeyaprakash, N. Marbán-Mendoza, and B. M. Zuckerman. 1986. *Caenorhabditis elegans*: Comparisons of chemotactic behavior from monoxenic and axenic culture. Experimental Parasitology 61:369-372.

81. Jansson, H.-B., and B. Nordbring-Hertz. 1979. Attraction of nematodes to living mycelium of nematophagous fungi. Journal of General Microbiology 112:89-93.

82. Johnson, P. W., and C. D. McKeen. 1973. Vertical movement and distribution of *Meloidogyne incognita* (Nematoda) under tomato in a sandy loam greenhouse soil. Canadian Journal of Plant Science 53:837-841.

83. Jones, J. T., R. N. Perry, and M. R. L. Johnston. 1991. Electrophysiological recordings of electrical activity and responses to stimulants from *Globodera rostochiensis* and *Syngamus trachea*. Revue de Nematologie 14:467-473.

84. Khalil, M. 1922. Thermotropism in ankylostome larvae. Proceedings of the Royal Society of Medicine 15:16-18.

85. Kiyohara, T. 1982. Sexual Attraction in *Bursaphelenchus xylophilus*. Japanese Journal of Nematology 11:7-12.

86. Klingler, J. 1961. Anziehungsversuche mit *Ditylenchus dipsaci* unter Berücksichtigung der Wirkung des Kohlendioxyds, des Redoxpotentials und anderer Faktoren. Nematologica 6:69-84.

87. Klingler, J. 1963. Die Orientierung von *Ditylenchus dipsaci* in gemessenen künstlichen und biologischen CO2-Gradienten. Nematologica 9:185-199.

88. Klingler, J. 1972. The effect of single and combined heat and CO2 stimuli at different ambient temperatures on the behavior of two plant-parasitic nematodes. Journal of Nematology 4:95-100.

89. Klink, J. W., V. H. Dropkin, and J. E. Mitchell. 1970. Studies on the host-finding mechanisms of *Neotylenchus linfordi*. Journal of Nematology 2:106-117.

90. Kramer, P. J. 1983. Water relations of plants. Orlando: Academic Press.

91. Kurppa, S., and T. C. Vrain. 1985. Penetration and feeding behavior of *Pratylenchus penetrans* in strawberry roots. Revue de Nematologie 8:273-276.

92. Lewis, E. E., R. Gaugler, and R. Harrison. 1993. Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Canadian Journal of Zoology 71:765-769.

93. Lownsbery, B. F., and D. R. Viglierchio. 1960. Mechanism of accumulation of *Meloidogyne incognita acrita* around tomato seedlings. Phytopathology 50:178-179.

94. Lucker, J. T. 1936. Extent of vertical migration of horse strongyle larvae in soils of different types. Journal of Agricultural Research, Washington, D.C. 52:353-361.

95. McCallum, M. E., and D. B. Dusenbery. 1992. Computer tracking as a behavioral GC detector: nematode responses to vapor of host roots. Journal of Chemical Ecology 18:585-592.

96. McInnes, K. J., R. W. Weaver, and M. J. Savage. 1994. Soil water potential. Pp. 53-58 *in* R. W. Weaver, ed. Methods of soil analysis. Part 2. Microbiological and biochemical properties. Madison: Soil Science Society of America.

97. Miller, P. M., and J. L. McIntyre. 1976. Activated charcoal inhibits the entry of *Pratylenchus penetrans* and *Heterodera tabacum* into tomato and tobacco roots. Journal of Nematology 8:297-298.

98. Miyazaki, M., A. Yamaguchi, and K. Oda. 1978. Behaviour of *Bursaphelenchus lignicolus* in response to carbon dioxide. Journal of the Japanese Forestry Society 60:203-208.

99. Miyazaki, M., A. Yamaguchi, and K. Oda. 1978. Behaviour of *Bursaphelenchus lignicolus* in response to carbon dioxide released by respiration of *Monochamus alternatus* pupa. Journal of the Japanese Forestry Society 60:249-254.

100. Mok, M., D. Abraham, R. B. Grieve, and C. B. Thomas. 1986. Thermotaxis in third and fourth-stage *Dirofilaria immitis* larvae. Journal of Helminthology 60:61-64.

101. Monoson, H. L., A. G. Galsky, J. A. Griffin, and E. J. McGrath. 1973. Evidence for and partial characterization of a nematode attraction substance. Mycologia 65:78-86.

102. Moyle, P. L., and H. K. Kaya. 1981. Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida: Steinernematidae), in sand. Journal of Nematology 13:295-300.

103. Nultsch, W., and D.-P. Häder. 1988. Photomovement in motile microorganisms - II. Photochemistry and Photobiology 47:837-869.

104. Overgaard, C. 1947-48. An apparatus for the quantitative extraction of nematodes and rotifers from soil and moss. Natura Jutlandica 1:271-277.

105. Parker, J. C., and A. J. Haley. 1960. Phototactic and thermotactic responses to the filariform larvae of the rat nematode *Nippostrongylus muris*. Experimental Parasitology 9:92-97.

106. Payne, F. K. 1922. Investigations on the control of hookworm disease. XI. Vertical migration of infective hookworm larvae in the soil (preliminary report). American Journal of Hygiene 2:254-263.

107. Peacock, F. C. 1959. The development of a technique for studying the host/parasite relationship of the root-knot nematode *Meloidogyne incognita* under controlled conditions. Nematologica 4:43-55.

108. Peters, B. G. 1928. On the bionomics of the vinegar eelworm. Journal of Helminthology 6:1-38.

109. Peters, B. G. 1952. Toxicity tests with vinegar eelworms. I. Counting and culturing. Journal of Helminthology 26:97-110.

110. Pinkerton, J. N., H. Mojtahedi, G. S. Santo, and J. H. O'Bannon. 1987. Vertical migration of *Meloidogyne chitwoodi* and *M. hapla* under controlled temperature. Journal of Nematology 19:152-157.

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

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

113. Pollock, C., and M. R. Samoiloff. 1976. The development of nematode behaviour: stage-specific behaviour in *Panagrellus redivivus*. Canadian Journal of Zoology 54:674-679.

114. Prot, J.-C. 1978. Influence of concentration gradients of salts on the movement of second stage juveniles of *Meloidogyne javanica*. Revue de Nematologie 1:21-26.

115. Prot, J.-C. 1978. Vertical migration of four natural populations of *Meloidogyne*. Revue de Nematologie 1:109-112.

116. Prot, J.-C. 1978. Behaviour of juveniles of *Meloidogyne javanica* in salt gradients. Revue de Nematologie 1:135-142.

117. Prot, J.-C. 1979. Influence of concentration gradients of salts on the behaviour of four plant parasitic nematodes. Revue de Nematologie 2:11-16.

118. Prot, J.-C. 1979. Horizontal migrations of second-stage juveniles of *Meloidogyne javanica* in sand in concentration gradients of salts and in a moisture gradient. Revue de Nematologie 2:17-21.

119. Prot, J.-C. 1980. Migration of plant-parasitic nematodes toward plant roots. Revue de Nematologie 3:305-318.

120. Prot, J.-C., and S. D. Van Gundy. 1981. Effect of soil texture and the clay component on migration of *Meloidogyne incognita* second-stage juveniles. Journal of Nematology 13:213-217.

121. Rees, G. 1950. Observations on the vertical migrations of the third-stage larva of *Haemonchus contortus* (Rud.) on experimental plots of *Lolium perenne* S24, in relation to meteorological and micrometeorological factors. Parasitology 40:127-142.

122. Rende, J. F., P. M. Tefft, and L. W. Bone. 1982. Pheromone attraction in the soybean cyst nematode *Heterodera glycines* race 3. Journal of Chemical Ecology 8:981-991.

123. Riddle, D. L., and A. F. Bird. 1985. Responses of the plant parasitic nematodes *Rotylenchulus reniformis, Anguina agrostis* and *Meloidogyne javanica* to chemical attractants. Parasitology 91:185-195.

124. Roberts, T. M., and R. E. Thorson. 1977. Chemical attraction between adults of *Nippostrongylus brasiliensis*: Description of the phenomenon and effects of host immunity. Journal of Parasitology 63:357-363.

125. Robinson, A. F. 1989. Thermotactic adaptation of two foliar and two root-parasitic nematodes. Revue de Nematologie 12:125-131.

126. Robinson, A. F. 1992. Cambios en la atracción de *Ditylenchus phyllobius* hacia tejidos de *Solanum elaeagnifolium* durante el desarrollo de la planta. Nematropica 22:37-45.

127. Robinson, A. F. 1994. Movement of five nematode species through sand subjected to natural temperature gradient fluctuations. Journal of Nematology 26:46-58.

128. Robinson, A. F. 1995. Optimal release rates for attracting *Meloidogyne incognita, Rotylenchulus reniformis*, and other nematodes to carbon dioxide in sand. Journal of Nematology 27:42-50.

129. Robinson, A. F., G. L. Baker, and C. M. Heald. 1990. Transverse phototaxis by infective juveniles of *Agamermis* sp. and *Hexamermis* sp. Journal of Parasitology 76:147-152.

130. Robinson, A. F., and C. M. Heald. 1991. Carbon Dioxide and temperature gradients in Baermann funnel extraction of *Rotylenchulus reniformis*. Journal of Nematology 23:28-38.

131. Robinson, A. F., and C. M. Heald. 1993. Movement of *Rotylenchulus reniformis* through sand and agar in response to temperature, and some observations on vertical descent. Nematologica 39:92-103.

132. Robinson, A. F., and G. Saldaña. 1989. Characterization and partial purification of attractants for nematode *Orrina phyllobia* from foliage of *Solanum elaeagnifolium*. Journal of Chemical Ecology 15:481-495.

133. Rode, H. 1965. Über einige Methoden zur Verhaltensforschung bei Nematoden. Pedobiologia 5:1-16.

134. Rode, H. 1962. Über das Thermopräferendum von Larven des Kartoffelnematoden (*Heterodera rostochiensis* Woll.) unter Berücksichtigung ihrer Wanderaktivität im Reizgefälle. Pedobiologia 9:405-425.

135. Rode, H. 1970. Über das Verhalten von Larven des Kartoffelnematoden in kombinierten chemisch-thermischen Reizgefällen. Pedobiologia 10:207-223.

136. Ronald, K. 1960. The effects of physical stimuli on the larval stage of *Terranova decipiens* (Krabbe, 1878) (Nematode: Anisakidae) I. Temperature. Canadian Journal of Zoology 38:623-642.

137. Ronald, K. 1963. The effects of physical stimuli on the larval stage of *Terranova decipiens* III. Electromagnetic spectrum and galvanotaxis. Canadian Journal of Zoology 41:197-217.

138. Rutherford, T. A., and N. A. Croll. 1979. Wave forms of *Caenorhabditis elegans* in a chemical attractant and repellent and in thermal gradients. Journal of Nematology 11:232-240.

139. Sandstedt, R., T. Sullivan, and M. L. Schuster. 1961. Nematode tracks in the study of movement of *Meloidogyne incognita incognita*. Nematologica 6:261-265.

140. Schmidt, J., and J. N. All. 1978. Chemical attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to insect larvae. Environmental Entomology 7:605-607.

141. Schmidt, J., and J. N. All. 1979. Attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to common excretory products of insects. Environmental Entomology 8:55-61.

142. Schroeder, W. J., and J. B. Beavers. 1987. Movement of the entomogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. Journal of Nematology 19:257-259.

143. Steiner, G. 1925. The problem of host selection and host specialization of certain plant-infesting nemas and its application in the study of nemic pests. Phytopathology 15:499-534.

144. Stirling, G. R. 1991. Biological control of plant parasitic nematodes. Wallingford: CAB International.

145. Stringfellow, F. 1984. A unique bioassay: *Haemonchus contortus* adult male spicule sheaths attract conspecific larvae. Proceedings of the Helminthological Society of Washington 51:163-165.

146. Sukul, N. C., P. K. Das, and S. K. Ghosh. 1975. Cation-mediated orientation of nematodes under electrical fields. Nematologica 21:145-150.

147. Sukul, N. C., S. K. Ghosh, and P. K. Das. 1977. The influence of pre-incubation of *Labronema digitatum* at different ionic concentrations to subsequent electrotaxis. Nematologica 23:24-28.

148. Tominaga, Y., A. Nagase, Y. Kuwahara, and R. Sugawara. 1983. Behavioral responses of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) to bitter and pungent substances. Applied Entomology and Zoology 18:106-110.

149. Tominaga, Y., M. Yamamoto, Y. Kuwahara, and R. Sugawara. 1984. Behavioral responses of the pine wood nematode to terpenes. Agricultural and Biological Chemistry 48:519-520.

150. Viglierchio, D. R. 1990. Carbon dioxide sensing by *Panagrellus silusiae* and *Ditylenchus dipsaci*. Revue de Nematologie 13:425-432.

151. Viglierchio, D. R., and P. K. Yu. 1983. On nematode behavior in an electric field. Revue de Nematologie 2:171-178.

152. Wallace, H. R. 1958. Movement of Eelworms I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. Annals of Applied Biology 46:74-85.

153. Wallace, H. R. 1958. Movement of eelworms II. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and of *Ditylenchus dipsaci* (Kuhn) Filipjev. Annals of Applied Biology 46:86-94.

154. Wallace, H. R. 1958. Movement of eelworms III. The relationship between eelworm length, activity and mobility. Annals of Applied Biology 46:662-668.

155. Wallace, H. R. 1959. Movement of eelworms IV. The influence of water percolation. Annals of Applied Biology 47:131-139.

156. Wallace, H. R. 1960. Movement of eelworms VI. The influences of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. Annals of Applied Biology 48:107-120.

157. Wallace, H. R. 1961. The orientation of *Ditylenchus dipsaci* to physical stimuli. Nematologica 6:222-236.

158. Westermann, P. R. 1995. Comparative vertical migration of twenty-one isolates of the insect parasitic nematode *Heterorhabditis* spp. in sand at 20 oC. Fundamental and Applied Nematology 18:149-158.

159. Wieser, W. 1955. The attractiveness of plants to larvae of root-knot nematodes. I. The effect of tomato seedlings and excised roots on *Meloidogyne hapla* Chitwood. Proceedings of the Helminthological Society of Washington 22:106-112.

160. Wieser, W. 1956. The attractiveness of plants to larvae of root-knot nematodes. II. The effect of excised bean, eggplant, and soybean roots on *Meloidogyne hapla* Chitwood. Proceedings of the Helminthological Society of Washington 23:59-64.

161. Wyss, U., F. M. W. Grundler, and A. Münch. 1992. The parasitic behaviour of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica 38:98-111.

162. Zur, B. 1966. Osmotic control of the matric soil-water potential: I. Soil-water system. Soil Science 102:394-398.

ADDITIONAL LITERATURE CITED (added March 2000)

163. Abou-Setta, M. M. and L. W. Duncan. 1998. Attraction of *Tylenchulus semipenetrans* and *Meloidogyne javanica* to salts in vitro. Nematropica 28:49-59.

164. Anderson, A. R. A., B. D. Sleeman, I. M. Young, and B. S. Griffiths. 1997. Nematode movement along a chemical gradient in a structurally heterogeneous environment. 1. Experiment. Fundamental and Applied Nematology 20:157-163.

165. Anderson, A. R. A., B. D. Sleeman, I. M. Young, and B. S. Griffiths. 1997. Nematode movement along a chemical gradient in a structurally heterogeneous environment. 2. Theory. Fundamental and Applied Nematology 20:165-172.

168. Bansemir, A. D., and M. V. I. Sukhdeo. 1996. Villus length influences habitat selection by *Heligmosomoides polygyrus*. Parasitology 113:311-316.

169. Bessho, K., S. Yamada, T. Kunitani, T. Nakamura, T. Hashigushi, Y. Tanimoto, S. Harada, H. Yamamoto, and R. Hosono. 1995. Behavioral responses in *Caenorhabditis elegans* to high magnetic fields. Experientia 51:284-288.

170. Campbell, J.F., and H. K. Kaya. 1999. How and why a parasitic nematode jumps. Nature 397:485-486.

171. Dhawan, R., D. B. Dusenbery, and P. L. Williams. 1999. Comparison of lethality, reproduction, and behavior as toxicological endpoints in the nematode *Caenorhabditis elegans*. Journal of Toxicology and Environmental Health - Part A 58:451-462.

172. Grewal, P. S., E. E. Lewis, and R. Gaugler. 1997. Response of infective stage parasites (Nematoda: Steinernematidae) to volatile cues from infected hosts. Journal of Chemical Ecology 23:503-515.

173. Grewal, P. A., E. E. Lewis, and S. Venkatachari. 1999. Allelopathy: A possible mechanism of suppression of plant parasitic nematodes by entomopathogenic nematodes. Nematology a:735-743.

174. Grundler, F. M. W., M. Sobczak, and S. Lange. 1997. Defense responses of *Arabidopsis thaliana* during invasion and feeding site induction by the plant-parasitic nematode Heterodera glycines. Physiological and Molecular Plant Pathology 50:419-429.

175. Hewlett, T. E., E. M. Hewlett, and D. W. Dickson. 1997. Response of *Meloidogyne* spp., *Heterodera glycines*, and *Radopholus similis* to tannic acid. Journal of Nematology 29(s):737-741.

176. Huang, X., and J. O. Becker. 1999. Life cycle and mating behavior of *Belonolaimus longicaudatus* in gnotobiotic culture. Journal of Nematology 31:70-74.

177. Ishibashi, I., S. Taku, and E. Kondo. 1995. Infectivity of nictating juveniles *of Steinernema carpocapsae* (Rhabditida: Steinernematidae). Japanese Journal of Nematology 24:20-29.

178. Jaffe, H., R.N. Huettel, A.B. DeMilo, D.K, Hayes, and R.V. Rebois. 1989. Isolation and identification of a compound from soybean cyst nematode, *Heterodera glycines*, with sex pheromone activity. Journal of Chemical Ecology 15:2031-2043.

179. Kanagy, J. M. N., and H. K. Kaya. 1996. The possible role of marigold roots and a-terthienyl in mediating host-finding by Steinernematid nematodes. Nematologica 42:220-231.

180. Lewis, E. E., J. F. Campbell, and R. Gaugler. 1997. The effects of aging on the foraging behavior of *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Nematologica 43:355-362.

181. Neves, J. M., N. Simões, and M. Mota. 1998. Evidence for a sex pheromone in *Steinernema carpocapsae*. Nematologica 44:95-98.

182. Orion, D., and M. Bar-Eyal. 1995. Observations on the life cycle and behavior of *Helicotylenchus multicinctus* in monoxenic culpture of *Arabidopsis thaliana*. Nematropica 25:67-70.

183. Perry, R. N. 1994. Studies on nematode sensory perception as a basis for novel control strategies. Fundamental and Applied Nematology 17:199-202.

184. Perry, R. N. 1996. Chemoreception in plant parasitic nematodes. Annual Review of Phytopathology 34:181-199.

185. Perry, R. N., and J. Aumann. 1998. Behaviour and sensory responses. Pp. 75-102 in R. N. Perry and D. J. Wright, eds. The physiology and biochemistry of freeliving and plant parasitic nematodes. Wallingford, Oxon, UK: CABI Publishing.

186. Read, A. F., and A. Skorping. 1995. The evolution of tissue migration by parasitic nematode larvae. Parasitology 111:359-371.

187. Ricci, M., I. Glazer, J. F. Campbell, and R. Gaugler. 1996. Comparison of bioassays to measure virulence of different entomopathogenic nematodes. Biocontrol Science and Technology 6:235-245.

188. Riga, E., D. R. Holdsworth, R. N. Perry, J. Barrett, and M. R. L. Johnston. 1997. Electrophysiological analysis of the responses of males of the potato cyst nematode *Globodera rostochiensis* to fractions of their homospecific sex pheromones. Parasitology 115:311-346.

189. Riga, E., R. N. Perry, J. Barrett, and M. R. L. Johnston. 1997. Electrophysiological responses of male potato cyst nematodes, *Globodera rostochiensis* and *Globodera pallida*, to some chemicals. Journal of Chemical Ecology 23:417-428.

190. Robinson, A.F., and B. A. Jaffee. 1996. Repulsion of *Meloidogyne incognita* by alginate pellets containing hyphae of *Monacrosporium cionopagum*, *M. ellipsosporum*, or *Hirsutella rhossiliensis*. Journal of Nematology 28:133-147.

191. Shapiro, D. I., and I. Glazer. 1996. Comparison of entomopathogenic nematode dispersal from infected hosts versus aqueous suspensions. Environmental Entomology 25:1455-1461.

192. Stamps, W. T., and M. J. Linit. 1998. Chemotactic response of propagative and dispersal forms of the pinewood nematode *Bursaphelenchus xylophilus* to beetle and pine derived compounds. Fundamental and Applied Nematology 3:243-250.

193. Steiner, W. A. 1996. Dispersal and host-finding ability of entomopathogenic nematodes at low temperatures. Nematologica 42:243-261.

194. Stromberg, B. E. 1997. Environmental factors influencing transmission. Veterinary Parasitology 72: 247-264.

195. Thomas, J. H. 1998. Social life and the single nucleotide: foraging behavior in *Caenorhabditis elegans*. Cell 94:549-553.

196. Von Mende, N. 1997. Invasion and migration behaviour of sendentary nematodes. Pp. 51-64 in C. Fenoll, F. M. W. Grundler, and S. A. Ohl, eds. Cellular and molecular aspects of plant-nematode interactions. Dordrecht: Kluwer Academic Publishers.

197. Westerman, P. R. 1997. Comparative vertical migration of insect parasitic nematodes *Heterorhabditis* and *Steinernema* spp. in sand at 9 °C. Fundamental and Applied Nematology 20:405-408.

198. Young, I. M., B. G. Griffiths, and W. M. Robertson. 1996. Continuous foraging by bacteria feeding nematodes. Nematologica 42:378-382.

Techniques for Measuring Nematode Development and Egg Production

J. D. Eisenback

Professor, Department of Plant Pathology, Physiology, and Weed Science,
Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

jon@vt.edu

Life cycles of nematodes are not complex, but measuring nematode development is often difficult. There are six stages including the egg, four juvenile stages, and the adult. All stages, except the development of the egg into the first-stage juvenile, are separated by a molt (Fig. 1). Life cycles vary from less than 5 days to more than a year depending upon the species, and can be greatly affected by temperature and substrate quality. Generations of most soil-borne nematodes overlap because egg hatch and penetration occur throughout the growth of the plant. However, in some species generations may not overlap (9).

Egg production by nematodes is easily determined in species that deposit eggs in a sac, within plant tissues, or retain them within the female body. Quantifying egg production by the species that deposit them singly in the soil is extremely difficult and time consuming. Eggs are not only difficult to extract, but they are also difficult to identify (9); however, several techniques have been developed that are effective for some genera.

Measuring Nematode Development

Pot cultures (10): Inoculate small plants growing in sterile soil with 100 freshly hatched second-stage juveniles. Allow 24 hr for penetration and remove plants from the pots, thoroughly wash the root system to remove nematodes that have not penetrated, and replant in sterile soil. Extract two plants daily and stain them in acid fuchsin (5).

Staining includes a 4 min soak in 10% chlorine bleach (5.25% NaOCl), followed by a 45 sec rinse in running tap water and a 15 min. soak in tap water to remove residual bleach that interferes with the stain. Drain the roots and transfer them to a beaker with 30-50 ml of tap water. Add 1 ml of stock acid fuchsin (3.5 gm acid fuchsin in 250 ml of acetic acid and 750 ml of distilled water). Boil the solution for 30 sec on a hot plate or in a microwave oven. Cool to room temperature, drain, rinse in running tap water. Transfer the stained roots to 20-30 ml of acidified glycerine (a few drops of 5N HCl), and heat to destain.

Following destaining, the roots may be stored in acidified glycerine until they are examined. Nematodes are examined by dissecting them from the plant tissue and mounting them in glycerine on glass slides. It may be necessary to support the coverslip with small glass rods or with rings of Zut or nailpolish. Use the occurrence of each molt to delineate the time periods for each development stage (Fig. 1).

Monoxenic cultures (7,8): Inoculate axenic root explants with a 0.1 ml sterile aqueous suspension containing approximately 50 freshly hatched second-stage juveniles. Leave unsealed overnight to eliminate excess moisture and to allow penetration into the roots. Prevent additional penetration by transferring intact root explants to new petri dishes containing fresh media. Seal the plates with Parafilm® and incubate them in a temperature controlled growth chamber. Remove the root explants from the petri dishes daily and stain them with acid fuchsin as described previously. Mount the specimens on slides and examine with a light microscope. Use the occurrence of each molt to delineate the time periods for each developmental stage (Fig. 1).

in vitro observation (11): Surface-sterilize seeds of suitable host (with thin roots) for 60 min. in 4% Chloramine T and 1% streptomycin sulphate. Thoroughly rinse the seeds in sterile distilled water and place on 0.8% distilled water agar for germination in the dark (3-4 days at 25 C). Under sterile conditions, transfer a seedling (with a 1-2 cm long radical) to an observation chamber made with an 8 cm diameter coverslip (0.13 mm thick) placed in a 14 cm diameter plastic petri dish (Fig. 2). For ectoparasitic species, coat the coverslip with a thin layer of 0.8% distilled water agar (about 7 ml) and add a few drops of sterile Hoagland's solution. Use nutrient agar for sedentary species (2) and do not add the Hoagland's solution. Seal the petri dishes with Parafilm and place them in the light at 25C until several lateral roots develop.

Surface sterilize nematodes in 0.1% HgCl for 4 min (*Trichodorus* spp. in 0.03% NaN3) and rinse in distilled water. Inoculate the seedling with 25-50 nematodes and place in the dark or low light (less than 1000 Lux). Observe the nematodes directly through the petri dish at low magnification. For observations at high magnification, remove the coverslip from the petri dish and top it with an 8 cm diameter coverslip (0.13 mm thick) (Fig. 2). Be careful to avoid the formation of air bubbles in the sandwich. Place the entire sandwich of coverslips under the microscope objective for observation, even under oil. After observation, return the sandwich to a petri dish containing moist cotton wool to prevent desiccation. Use the occurrence of each molt to delineate the time periods for each developmental stage (Fig. 1).

Measuring Egg Production

Egg sac with chlorine bleach extraction (6) (*Meloidogyne*, *Rotylenchulus*, *Sphaeronema*, and others): Wash pot cultures under a gentle stream of water to remove all soil particles. Place the root system into a solution of 10% commercial bleach (0.5% sodium hypochlorite solution) and shake vigorously for 5 min. Pour the solution through a 500 mesh sieve (25 µl pore size) and rinse the eggs

in running tap water until the smell of chlorine is gone. Rinse the eggs into a beaker and add water to make 1000 ml. If the number of eggs is extremely high, a subsample can be diluted to reduce the density. Count the number of eggs in 10 ml to determine the total number of eggs produced. Egg production is usually reported as the number of eggs per gram root tissue.

Eggs retained in the female body (*Globodera*, *Heterodera*, and others)

Blender extraction: Place cysts into blender with 300-500 ml of tap water and blend for 1 min. at a moderately high speed. Pour through a 200 mesh sieve (74 µm pore opening) and catch the eggs on a 500 mesh sieve (25 µm pore opening). Rinse the eggs into a beaker and add water to make 1000 ml. Count the number of eggs in 10 ml to determine the total number of eggs produced. If the number of eggs is too high to count accurately, then a small subsample can be diluted to reduce the number of eggs per unit volume. Egg production is usually reported as the number of eggs per gram of root tissue.

Glass tissue homogenizer (Ten-Broeck) (1): Transfer cysts to a drop of water in the glass tube of the homogenizer with a forceps or camel's hair brush, and place the piston in the tube. Carefully rotate the piston by hand, remove the piston, and pour ground material containing the eggs and juveniles into a bottle. Add water to make 100 ml, suspend the eggs and juveniles in the water by mixing with air, and quantify them after pipetting a 10 ml aliquot into a counting dish.

Chlorine bleach extraction (1): Place the cysts into a solution of 10% commercial bleach (0.5% sodium hypochlorite solution) and shake vigorously for 8-10 min. Pour the solution through a 500 mesh sieve (25 µm pore size). Rinse the eggs in running tap water until the chlorine is gone, pour the eggs into a beaker, and add water to make 1000 ml. A subsample can be diluted to reduce the density if the number of eggs is extremely high. Count the number of eggs in a 10 ml aliquot to determine the total number of eggs present.

Eggs deposited in plant tissue (*Ditylenchus*, *Pratylenchus*, and others)

Sucrose gradient extraction (3): Blend plant tissue containing nematodes in 200 ml of 30% sucrose solution for 30 sec in a blender and pour the suspension into a 250 ml round bottom centrifuge tube. Spin down for 8 min at 1500 g in a swing-bucket head. Pour the supernatant through a 325 mesh sieve (43-µm pore size) over a nylon mesh sieve with 15 µm openings (Nitex© nylon mesh; Tobler, Ernst & Traber, Inc., 420 Sawmill River Road, P. O. Box 112, Elmsford, N. Y. 10523) to remove large plant debris. Rinse under running tap water to remove the sucrose. Wash the residues into a beaker with less than 25 ml of water and layer the suspension on 20 ml of 30% sucrose solution within a 50 ml centrifuge tube. Pour the suspension with the receiving tube tilted in as near a horizontal position as possible. Centrifuge the gradient for 4 min at 1500 g. The eggs and vermiform nematodes will be concentrated in a band at the interface of the sugar gradient. Remove the eggs from the interface with an aspirator bottle and rinse them on the 15 µm mesh.

Eggs deposited singly in the soil (*Longidorus*, *Trichodorus*, *Xiphinema*, and others)

Sugar flotation (4): Mechanically stir or shake soil samples for 2 min. in a sugar solution of 484 g sucrose in a liter of water with 6 ppm of ethoxy ethyl mercury chloride (0.02% aretan) added to reduce microbial growth. Approximately 100 ml of soil can be processed with 200 ml of sucrose in a 250 ml flask. Let the suspension stand for 30 sec to allow the heavier particles to settle. Decant the supernatant into centrifuge flasks, add sucrose solution, and spin for 6 min at 1,700 g for *Xiphinema* and *Longidorus* or 3 min at 1,150 g for *Trichodorus*. After centrifugation immediately pour the supernatant into a 45 cm tall one-liter cylinder filled with tap water. Let settle for 1-1.25 hrs. Remove all of the water except the bottom 2 cm and pour it into a boiling tube. Allow it to settle for 1-1.25 hrs to reduce the volume to 5 ml for microscopic examination.

Literature Cited

1. Barker, K. R. 1985. Nematode extraction and bioassays. Pp. 19-35, *in*, K. R. Barker, J. N. Sasser, and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, Vol.II Methodology. North Carolina State University Graphics: Raleigh, NC.

2. Dropkin, V. H., and W. R. Boone. 1966. Analysis of host-parasite relationships of root-knot nematodes by single-larva inoculations of excised tomato roots. Nematologica 12:225-236.

3. Dunn, R. A. 1973. Extraction of eggs of *Pratylenchus penetrans* from alfalfa callus and relationship between age of culture and yield of eggs. Journal of Nematology 5:73-74.

4. Flegg, J. J. M., and D. G. McNamara. 1968. A direct sugar-centrifugation method for the recovery of eggs of *Xiphinema*, *Longidorus*, and *Trichodorus* from soil. Nematologica 14:156-157.

5. Hussey, R. S. 1990. Staining nematodes in plant tissues. Pp. 190-193, *in*, B. M. Zuckerman, W. F. Mai, and L. R. Krusberg, eds. Plant Nematology Laboratory Manual. The University of Massachusetts Agricultural Experiment Station: Amherst, MA.

6. Hussey, R. S. and K. R. Barker. 1973, A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reptr. 57:1025-1028.

7. Lauritis, J. A., R. V. Rebois, and L. A. Graney. 1982. Technique for gnotobiotic cultivation of *Heterodera glycines* Ichinohe on *Glycine max* (L,) Merr. Journal of Nematology 14:422-424.

8. Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1983. Life cycle of *Heterodera zeae* Koshy, Swarup, and Sethi on *Zea mays* L. axenic root explants. Journal of Nematology 15:115-119.

9. Norton, D. C., and T. L. Niblack. 1991. Biology and ecology of nematodes. Pp. 47-72, *in*, W. R. Nickle, ed. Manual of Agricultural Nematology. Marcell Dekker, Inc. New York.

10. Raski, D. J. 1950. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40:135-152.

11. Wyss, U., and U. Zunke. 1985. *In vitro* feeding of root parasitic nematodes. Pp. 91 96, *in*, B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant Nematology Laboratory Manual. The University of Massachusetts Agricultural Experiment Station: Amherst, MA.

Legends for Figures

Fig. 1. Diagram of the life cycle of a typical plant-parasitic nematode. (Redrawn from Lauritis et al., 1983.).

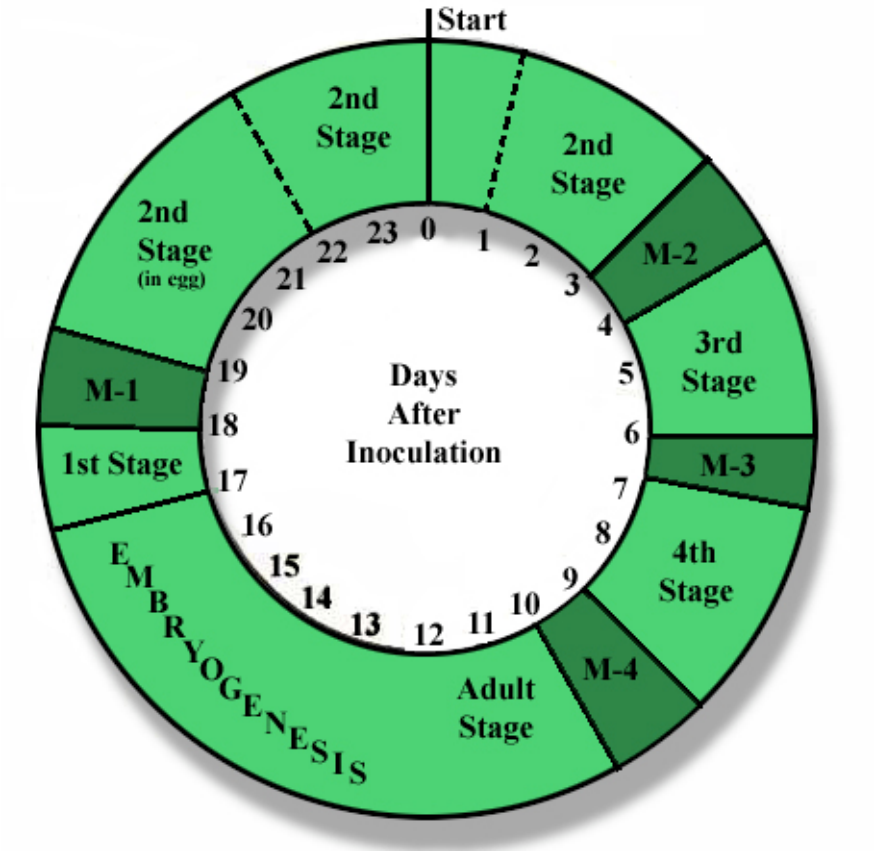
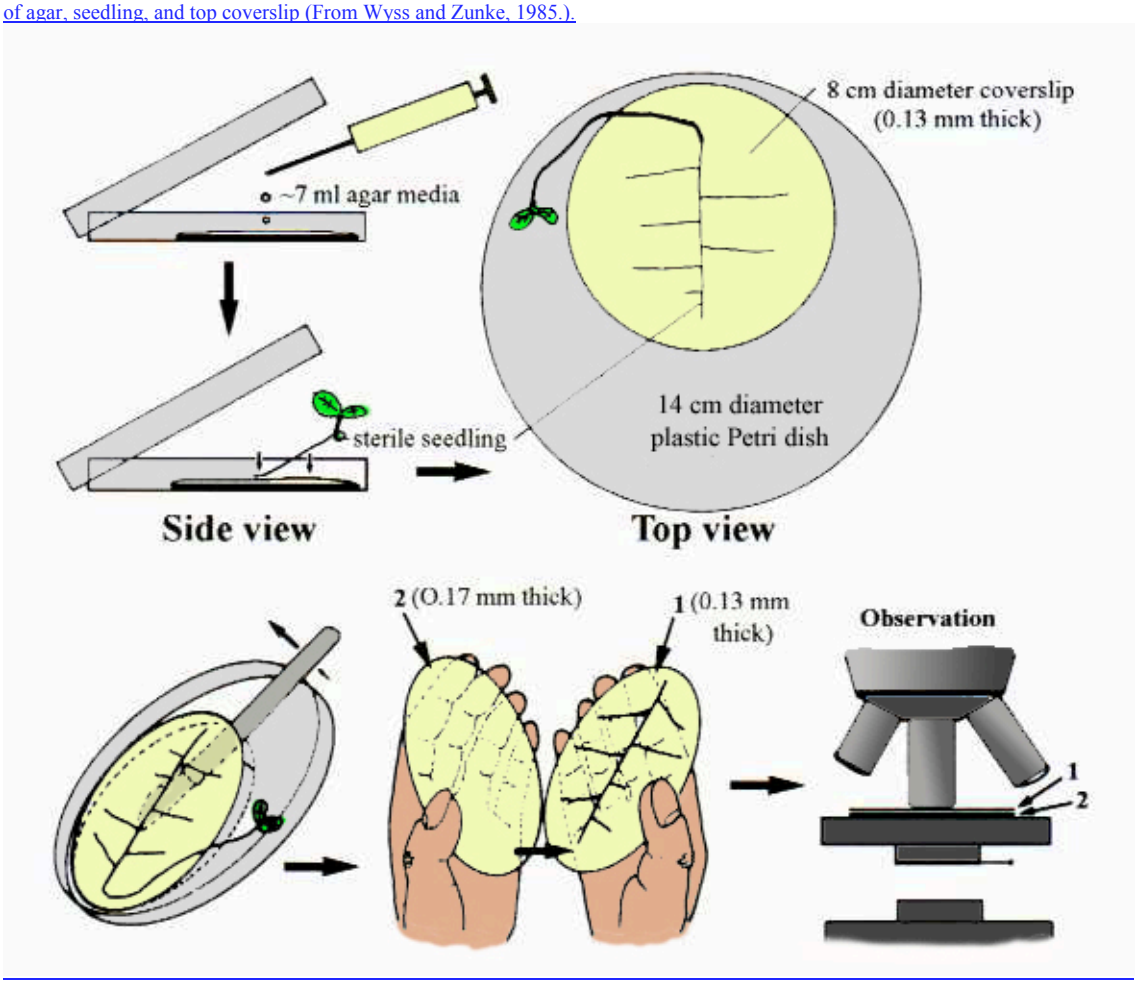


Fig. 2. Drawings of the chamber for in vitro observation of vermiform plant-parasitic nematodes feeding on plants showing placement of agar, seedling, and top coverslip (From Wyss and Zunke, 1985.).



Methods for studying the response of nematodes to extreme environmental conditions.

Thomas A. Forge

Lakehill Applied Soil Ecology
PO Box 274, Kaleden, BC V0H 1K0

tforge@vip.net

There are two general goals of research on the response of nematodes to extreme environmental conditions. One goal is to quantify the relationships between extreme environmental conditions and mortality for the purposes of understanding species distributions and population dynamics. Another goal is to describe and understand the life-history, behavioral, and physiological adaptations that permit certain species to survive periods of extreme environmental conditions. These two goals are related in that knowledge of the adaptations evolved in nematodes to survive extreme environmental conditions is critical for the development of ecologically relevant procedures for accurately quantifying their limits of tolerance. This chapter will focus primarily on the methods used to assess limits of survival rather than on the adaptations that enable some species to survive extreme conditions.

Field-vs-laboratory studies

Because nematode populations in the field are influenced by natality, migration, predation, parasitism and competition in addition to mortality caused by abiotic factors, field data are generally not useful for quantifying the effects of environmental conditions on nematode mortality. However, correlations between extreme environmental conditions and nematode mortality in the field can be useful for determining whether a species is adapted to survive such conditions. For example, in two years of a four-year study of winter mortality of a field population of *Pratylenchus scribneri* in Wisconsin, U.S., substantial nematode mortality occurred before the soil froze (23). These results indicated that initial physiological adjustments to the dropping temperatures or some other factor associated with the onset of winter, rather than frozen conditions *per se*, were likely responsible for much of the cumulative overwinter mortality. Careful analyses of environmental conditions in the field are also extremely valuable to determine the range of environmental conditions that are relevant for laboratory studies.

Life-stage distributions

When experiments are conducted using mixed-stage nematode populations, it is critical to document the life-stage distribution of the population and, if possible, to quantify survival for each stage separately. Different nematode life-stages generally differ greatly in their tolerance of freezing, heat, and desiccation (9,28,38,43,45,48,49). When working with mixed-stage populations it is also important to ensure that egg hatch or reproduction does not occur during the test exposures or post-exposure recovery and extraction processes. This is an especially important consideration when doing research on free-living nematodes because many of the common bacteriophageous and mycophagous species have generation times of three to four days (44).

Assessment of survival

Methods for assessing nematode survival should be considered carefully. The most commonly used method for assessing nematode survival is either spontaneous or induced movement. In most cases movement is a good method for assessing the survival of vermiform nematodes. However, nematodes exposed to sublethal stresses may lack movement even though they are still viable. For example, Heald & Robinson (15) found that juvenile *Rotylenchulus reniformis* exposed to sublethal high temperatures required up to ten days to regain mobility. Similarly, nematode motility may be inhibited by exposure to sublethal doses of the nematicide phenamiphos (24). Other methods for assessing survival include stains (5,17,26,36), enzymatically induced fluorescence (4) and autofluorescence (9). Vital stains require cuticle permeability to work and are therefore dependent on the nature of the event causing nematode death and the amount of time since death. Autofluorescence does not depend on cuticle permeability, but it does require a microscope equipped with epifluorescent illumination. As a result of the diversity of nematode responses to extreme environmental conditions, it is not possible to recommend a single best procedure for assessing survival. The choice of a method must be based on careful evaluation of the physiology and life-history of the species and the type of environmental extremes being studied. Ideally, the chosen method for assessing survival should be checked against at least one other method.

Statistical treatment of survival data

For any given exposure duration, the relationship between dose of an environmental stress (e.g. low temperature, low water potential) and percent mortality usually follows a sigmoidal curve (10,11,28,50). The sigmoidal relationship occurs when the frequency distribution of tolerances of individuals in the population is normally distributed. Probit analysis, which is based on the assumption of a normal distribution of tolerances for the population (8), is often used to analyze such survival data. Probit analysis involves transforming data on percent survival to probits and computing linear regressions of the relationship between dosage and probits. Regression equations are then used to estimate dosage causing a set percent mortality, usually 50 or 90 percent. Dosage causing these set percent mortalities are usually referred to as LD50's of LD90's, respectively. If survival data does not follow a sigmoidal pattern (21) then the probit model would not be appropriate.

The relationship between any dosage and percent mortality depends on the duration of exposure. Preliminary experiments or logistical constraints are often used to choose a single, standard exposure time that is used for assessing the effects of all doses. An alternative but more extensive approach is to assess mortality at a number of exposure times for each dose, use regression to calculate the exposure times necessary to cause some percent mortality (e.g. LT90) for each dose, and then analyze the relationships between doses and LT90 values. For any given dose of an environmental stress, the relationship between exposure duration and percent mortality is

also sigmoidal, and probit analysis may be used to estimate the LT90's (19). The cumulative inverse normal distribution has also been used to estimate LT90's (15). If the experimental dosage is sufficiently harsh that there is no apparent lag time before mortality begins (21,41) then an asymptotic model of the form $Y = a(1\text{-}e\text{-}bx)$, where Y denotes percent mortality, x is dose, *b* is slope, and *a* is a constant, has been used successfully to estimate LT90's from such data (21).

II. Low Temperatures

Supercooling points, which are the temperatures at which nematode body contents freeze, have been frequently used to assess the ability of nematodes to survive subzero temperatures. Supercooling temperatures are generally determined by observing nematodes as they are being cooled on a temperature-controlled microscope stage. A detailed description of the materials and methods used for measuring supercooling temperatures can be found in Wharton and Rowland (46).

Most nematode species are killed by internal freezing (2,22,28,35,45,47,48,50), whereas some are able to survive (1,29-31,47). Supercooling temperatures are sometimes determined using nematodes removed from contact with water to prevent contact with exogenous ice formation (29-31,47). However, contact with exogenous ice increases the temperature of internal freezing of most nematode species and life-stages (28,35,45,48-50). Because nematode cuticles are hydrophilic, nematodes are likely to be in contact with ice in all but very dry soils. Depending on the ecological setting, supercooling temperatures of nematodes in contact with ice may be more ecologically relevant than supercooling temperatures of nematodes exposed to subzero temperatures in the absence of contact with ice.

The determination of ecologically realistic supercooling temperatures should also be based on exposure times relevant to field conditions. Short exposure durations could result in lower estimates of supercooling temperatures (and greater perceived tolerance) than if exposure times on the order of days had been used. Wharton (45) reported that the percentage of *Panagrolaimus davidi* eggs that froze did not change in the first sixty minutes after freezing of the surrounding water. Mortality of *Meloidogyne hapla* second-stage juveniles (J2) in frozen soil (10) and *Pratylenchus penetrans* (19) occurred for several days or weeks after the initiation of exposure.

Desiccation caused by freezing of soil solution around nematodes can cause significant mortality at temperatures above supercooling temperatures, indicating that measuring survival of populations in frozen conditions may be more ecologically relevant than supercooling points. The formation of ice in soil generates extremely low water potentials, which are directly related to temperature at -1.12 MPa/C. Survival of *M. hapla* J2 after freezing in sucrose solutions didn't differ from survival after exposure to ice-free combinations of subzero temperature and water potential equivalent to those of the frozen solutions, indicating that the low water potentials generated by freezing can cause substantial mortality (10).

Studies to assess the potential for survival in soil at subzero temperatures should include soil moisture as a variable in the experimental design. The survival of nematodes in frozen soil is generally greater in moderately dry soil than in moist soil (10,43). Low water potentials increased survival of *M. hapla* J2 in frozen soil directly, probably by reducing the pore space filled with ice, and indirectly by causing physiological changes before freezing that increased the nematode's tolerance of frozen conditions (10). When exposed to more extreme desiccating conditions, some nematode species enter a state of anhydrobiosis (6,7,13,25,34,39,42,51). Because they are already desiccated, anhydrobiotic nematodes are very tolerant of subzero temperatures (25,39,42).

Studies on tolerance of nematodes to frozen conditions should also consider whether the nematodes have been exposed to ecologically relevant acclimation regimens. The ability of nematodes to survive subzero temperatures varies seasonally (29-31), and changes in supercooling points (22) and the ability to survive in frozen solutions (11) have been induced by acclimation to low temperatures in the laboratory.

III. High Temperatures

Most studies on nematode tolerance to high temperatures involved placing test tubes containing either nematodes in water or small volumes of nematode infested soil into water baths at desired temperatures (3,20,21,32,37,40). As with cold/freezing tolerance, pretreatment exposure to sublethal high temperatures can cause significant increases in heat tolerance (40). The moisture content of soil is also of critical importance to nematode survival at high temperatures. For example, the heat tolerance of vermiform *Xiphinema bakeri* was decreased by low water potentials (37), contrasted with the heat tolerance of encysted J2 of *Globodera rostochiensis* was greatest at low water potentials (20).

IV. Desiccation

As functionally aquatic organisms, terrestrial nematodes are more often challenged by occasional periods of desiccating conditions than by excess water. Studies to assess the ability of nematodes to survive desiccation must include careful analysis of drying rates. When faced with reductions in soil moisture, many nematode species are able to enter a state of anhydrobiosis (6,7,13,25,34,39,42,51), in which the loss of cellular water results in a reduction of metabolism, in some cases to undetectable levels (51). Once induced into the anhydrobiotic state, some nematode species are able to survive complete desiccation for years (39,51). Although some nematode species appear to be able to survive rapid desiccation, many soil-dwelling species appear to require a period of slow drying before they are capable of surviving more extreme dehydration (6,13,39,51). To assess the ability of a nematode species to survive at low water potential, first determine a rate of drying that influences the ability to survive at some fixed exposure, then quantify survival at a range of low water potentials and exposure durations.

Methods to manipulate water potential of the environment include placing nematodes on filter papers suspended over salt or glycerol solutions in desiccator jars (6,13,25,34). The water potential of air in equilibrium with a salt solution is directly related to the solution concentration according to this formula:

$\Psi = \phi MRT$

where M is the concentration of the solute (moles/kg), R is the universal gas constant (0.0831 bar kg mole-1K-1), T is the Kelvin temperature, ϕ is the osmotic coefficient (usually near 1), and ν is the number of osmotically active particles per molecule of solute (e.g. 2 for KCl). One

criticism of the desiccator jar method is that temperature gradients within the jars create non-equilibrium conditions and variable water potentials. A method which reduces the problem of temperature gradients, and has not been used in nematode research, is to place nematode samples on the lid of an inverted petri dish and cover the samples with the bottom of the petri dish containing salt-amended agar at the desired water potential (14). Robinson et al. (33) also used the approach of equilibrating the water potential of air with a solution, and exposed *Orrina phyllobia* to variable water potentials by placing the nematodes on cover slips in streams of air that had been passed through glycerol solutions of variable concentrations.

Much previous research on survival of nematodes at low water potentials has involved drying nematode-infested soil. Most soil-dwelling nematodes assumes a coiled position when anhydrobiotic and it is possible to extract and observe nematodes from dry soil without first rehydrating the soil (12). However, the lack of coiling should not be taken as an indication of the failure to survive low water potentials and, conversely, it should not be assumed that all coiled nematodes are viable.

The most commonly used methods for manipulating soil water potentials are air-drying soil to specific moisture contents (39,41), use of ceramic pressure plates (7,18,38) and hanging water columns (18,38). Air-drying soil results in pockets with different soil moisture contents, which are difficult to document. Pressure potential is the measurement of interest generally, not water content, which can change for every soil type (16,27). If water content is measured, then it is necessary to relate soil moisture to soil tension by the use of pressure plates or thermocouple psychrometers (39,41). Ceramic pressure plates may be the most ecologically relevant method for studying nematode survival at low water potentials, since this method can generate water potentials in the range of 0 to -1.5 MPa. Demeure et al. (7) used the ceramic pressure plate to demonstrate that three species of nematodes began to coil and perhaps enter the anhydrobiotic state at between -0.05 and -0.6 MPa. One potential problem with pressure plates is that the pressures will change the partial pressures of oxygen and carbon dioxide in the sample. Hanging water columns generate water potentials from saturation to conditions considerably wetter than field capacity. It is difficult to maintain a column over 2 m (10.33 m H20 = -1 MPa). This range is not useful for studying extreme desiccation, however, these columns can be maintained for long periods of time and do not affect gas pressure within soil.

Conclusion

The ability of nematodes to survive low temperatures, high temperatures and desiccation all depend on preconditioning regimens (i.e. pretreatment acclimation or acclimatization), and the evaluation and accurate reporting of preconditioning regimens is critical to all research on tolerance of environmental extremes. Supercooling points have often been used to assess nematode cold tolerance. Soil moisture, and perhaps other factors, interact with the freezing process in soil to cause mortality at temperatures above supercooling points. Consequently, supercooling points may not give ecologically relevant estimates of the potential survivorship of populations in soil at subzero temperatures. The survival of nematodes exposed to high temperatures is also dependent on soil moisture. Many soil-dwelling nematodes appear to be able to survive extreme desiccation for extended periods provided they have been exposed to an adequately slow drying rate. Because soils have different moisture characteristic curves, a potentially interesting area for future research would be to consider the effects of soil type on dynamics of the induction of anhydrobiosis and relationships between water potential and survival at low and high temperatures.

Literature Cited

1. Asahina, E. 1959. Frost resistance in a nematode *Aphelenchoides ritzemabosi*. Low Temperature Science Series B 17:51-62.

2. Ash, C. P., and H. J. Atkinson. 1986. *Nematodirus battus*: development of cold hardiness in dormant eggs. Experimental Parasitology 62:24-28.

3. Barbercheck, M.E., and S. L. Von Broembsen. 1986. Effects of soil solarization on plant-parasitic nematodes and *Phytophthora cinnamommi* in South Africa. Plant Disease 70:945-950.

4. Bird, A. F. 1979. A method distinguishing between living and dead nematodes by enzymatically induced fluorescence. Journal of Nematology 11:103-105.

5. Chadhouri, N., R. I. Dick, R. S. Englebrecht, and J. H. Austin. 1966. Staining of free-living nematodes by eosin-y dye. Nematologica 12:337-342.

6. Crowe, J. H., and K. A. C. Madin. 1975. Anhydrobiosis in nematodes: evaporative water loss and survival. Journal of Experimental Zoology 193:323-334.

7. Demeure, Y., D. W. Freckman, and S. D. Van Gundy. 1979. Anhydrobiotic coiling of nematodes in soil. Journal of Nematology 11:189-195.

8. Finney, D. J. 1971. Probit analysis, third edition. London: Cambridge Univ. Press.

9. Forge, T. A., and A. E. MacGuidwin. 1989. Nematode autofluorescence and its use as an indicator of viability. Journal of Nematology 21:399-403.

10. Forge, T. A., and A. E. MacGuidwin. 1992a. Effects of water potential and temperature on survival of the nematode *Meloidogyne hapla* in frozen soil. Canadian Journal of Zoology 70:1553-1560.

11. Forge, T. A., and A. E. MacGuidwin. 1992b. Impact of thermal history on tolerance of *Meloidogyne hapla* second-stage juveniles to external freezing. Journal of Nematology 24:262-268.

12. Freckman, D. W., D. T. Kaplan, and S. D. Van Gundy. 1977. A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils. Journal of Nematology 9:176-181.

13. Glazer, I., and D. Orion. 1983. Studies on anhydrobiosis of *Pratylenchus thornei*. Journal of Nematology 15:333-383.

14. Harris, R. F., W. R. Gardner, A. A. Adebeyo, and L. E. Sommers. 1970. Agar dish isopiestic equilibration method for controlling the water potential of solid substrates. Applied Microbiology 19:536-537.

15. Heald, C. M., and A. F. Robinson. 1987. Effects of soil solarization on *Rotylenchulus reniformis* in the lower Rio Grande Valley of Texas. Journal of Nematology 19:93-103.

16. Hillel, D. 1980. Fundamentals of soil physics. New York: Academic Press.

17. Jatala, P. 1975. Efficiency of potassium permanganate in differentiating between live and dead nematodes. Annals of Applied Biology 80:109-113.

18. Kable P. F. and W. F. Mai. 1968. Influence of soil moisture on *Pratylenchus penetrans*. Nematologica 14:101-122.

19. Kimpindki, J., and R. A. Dunn. 1985. Effect of low temperatures in the field and laboratory on survival of *Pratylenchus penetrans*. Plant Disease 69:526-527.

20. LaMondia, J. A., and B. B. Brodie. 1990. The effects of moisture on the thermosensitivity of *Globodera rostochiensis* (Nematoda). American Potato Journal 67:349-356.

21. Lazarovitis, G., M. A. Hawke, Th. H. A. Olthof, and J. Coutu-sundy. 1991. Influence of temperature on survival of *Pratylenchus penetrans* and of microsclerotia of *Verticillium dahliae* in soil. Canadian Journal of Plant Pathology 13:106-111.

22. Mabbett, K., and D. A. Wharton. 1986. Cold tolerance and acclimation in the free-living nematode *Panagrellus redivivus*. Revue de Nematologie 9:167-170.

23. MacGuidwin, A. E., and T. A. Forge. 1991. Winter survival of *Pratylenchus scribneri*. Journal of Nematology 23:198-204.

24. Marban-Mendoza, N., and D. R. Viglierchio. 1980. Behavioral effects of carbofuran and phenamiphos on *Pratylenchus vulnus*. I. Motility and dispersion. Journal of Nematology 12:102-113.

25. O'Dell, S. J., and J. H. Crowe. 1979. Freezing in nematodes: the effects of variable water contents. Cryobiology 16:534-541.

26. Ogiga, I. R., and R. H. Estey. 1974. The use of meldola blue and nile blue A for distinguishing dead from living nematodes. Nematologica 20:271-276.

27. Papendick, R. I., and G. S. Campbell. 1981. Theory and measurement of water potential. Pp. 1-22 *in* J. F. Parr, W. R. Gardner, and L. F. Elliott, eds. Water Potential Relations in Soil Microbiology, SSSA special publ. 9. Madison, WI.: Soil Science Soc. of America.

28. Perry, R. N., and D. A. Wharton. 1985. Cold tolerance of hatched and unhatched second-stage juveniles of the potato cyst-nematode, *Globodera rostochiensis*. International Journal for Parasitology 15:441-445.

29. Pickup, J. 1990. Seasonal variation in the cold-hardiness of a free-living predatory nematode, *Coomansus gerlachei* (Mononchidae). Polar Biology 10:107-315.

30. Pickup, J. 1990. Seasonal variation in the cold hardiness of three species of free-living antarctic nematodes. Functional Ecology 4:257-264.

31. Pickup, J. 1990. Strategies of cold-hardiness in three species of Antarctic dorylaimid nematodes. Journal of Comparative Physiology B 160:167-173.

32. Porter, I. J., and P. R. Merriman. 1983. Effects of solarization of soil on nematode and fungal pathogens at two sites in Victoria. Soil Biology and Biochemistry 15:39-44.

33. Robinson, A. F., C. C. Orr, and C. E. Heintz. 1984. Some factors affecting survival of desiccation by infective juveniles of *Orrina phyllobia*. Journal of Nematology 16:86-91.

34. Saeed, M., and J. Roessner. 1984. Anhydrobiosis in five species of plant associated nematodes. Journal of Nematology 16:119-124.

35. Sayre, R. M. 1964. Cold-hardiness of nematodes I. Effects of rapid freezing on the eggs and larvae of *Meloidogyne incognita* and *M. hapla*. Nematologica 10:168-179.

36. Shepard, A. M. 1962. New blue R, a stain that differentiates between living and dead nematodes. Nematologica 8:201-207.

37. Sutherland, J. R., and L. J. Sluggett. 1974. Time, temperature, and soil moisture effects on *Xiphinema bakeri* nematode survival in fallow soil. Phytopathology 64:507-513.

38. Townshend, J. L. 1973. Survival of *Pratylenchus penetrans* and *P. minyus* in two Onterio soils. Nematologica 19:35-42.

39. Townshend, J. L. 1984. Anhydrobiosis in *Pratylenchus penetrans*. Journal of Nematology 16:282-289.

40. Towson, A. J., and B. Lear. 1982. Control of nematodes in rose plants by hot water treatment preceded by heat hardening. Nematologica 28:339-353.

41. Towson, A. J., and W. J. Apt. 1983. Effect of soil water potential on survival of *Meloidogyne javanica* in fallow soil. Journal of Nematology 15:110-115.

42. Tsai, B. Y., and S. D. Van Gundy. 1989. Tolerance of prot-anhydrobiotic citrus nematodes to adverse conditions. Revue of Nematologie 12:107-112.

43. Vrain, T. C. 1978. Influence of chilling and freezing temperatures on infectivity of *Meloidogyne incognita* and *M. hapla*. Journal of Nematology 10:177-180.

44. Wharton, D. A. 1986. A functional biology of nematodes. Baltimore: Johns Hopkins Univ. Press.

45. Wharton, D. A. 1994. Freezing avoidance in the eggs of the antarctic nematode *Panagrolaimus davidi*. Fundamental and Applied Nematology 17:239-243.

46. Wharton, D. A., and J. J. Rowland. 1984. A thermoelectric microscope stage for the measurement of the supercooling points of microscopic organisms. Journal of Microscopy 134:299-305.

47. Wharton, D. A., S. R. Young, and J. Barrett. 1984. Cold tolerance in nematodes. Journal of Comparative Physiology B 154:73-77.

48. Wharton, D. A., and G. S. Allan. 1989. Cold tolerance mechanisms of the free-living stages of *Trichostrongylus colubriformis* (Nematoda: Trichostrongylidae). Journal of Experimental Biology 45:353-370.

49. Wharton, D. A., and I. M. Brown. 1991. Cold tolerance mechanisms of the antarctic nematode *Panagrolaimus davidi*. Journal of Experimental Biology 155:629-641.

50. Wharton, D. A., and M. R. Surrey. 1994. Cold tolerance mechanisms of the infective larvae of the insect parasitic nematode, *Heterorhadditis zealandica* Poinar. Cryo-letters 15:353-360.

51. Womersley, C. 1987. A reevaluation of strategies employed by nematode anhydrobiotes in relation to their natural environments. Pp. 165-173 *in* J. A. Veech and D. W. Dickson, eds. Vistas on Nematology: a commemoration of the twenty-fifth anniversary of the Society of Nematologists. Hyattsville, Md.: Society of Nematologists.

Sampling and Extraction Techniques for Nematodes

Robert McSorley

Dept. of Entomology and Nematology
P.O. Box 110620
University of Florida
Gainesville, FL 32611-0620
Phone: (904)-392-1901 ext. 137
Fax: (904)-392-0190
rmcs@gnv.ifas.ufl.edu

Quantification of nematode population densities is critical for many ecological studies. Numerous methods and modifications of methods are available for estimating nematode densities (1, 3-5, 7, 17, 20, 21, 26, 31, 38). General guidelines for sampling and extracting nematodes, particularly from soil samples, are presented here, but the purpose of a particular study may dictate the methods or modifications actually used. In any case, the sampling and extraction methods used will greatly affect the quality of the nematode population data obtained.

SAMPLING METHODS

Soil samples for nematode analysis can be collected with many types of tools, such as shovels, soil augers, trowels, or cylindrical tubes (1, 4, 17, 38). A cylindrical tube 20-25 mm in diameter or similar modification is often used to provide cores of relatively consistent shape (4).

If vertical stratification of the nematode population is not a concern, then a sample collected through the soil profile to a depth of 20 to 30 cm (e.g., a core 20 to 30 cm long) is probably adequate (26). If data are desired only on specific soil horizons or depths, then the core must be appropriately subdivided or sampling restricted to those zones. Deeper sampling may be required to collect rhizosphere samples from trees or other deep-rooted plants (5).

Horizontal distribution of nematodes is usually very uneven and clumped (5,18). This irregular spatial distribution is typical of nematode populations even in sites that appear to be relatively homogeneous, such as fallow fields or agricultural monocultures (4,18,26). Therefore, a sample usually consists of cores collected from a number of locations within the site, to represent the entire area (4,26). A systematic sampling plan, or collection of cores at regular intervals, is often recommended over random sampling because it gives more reliable results (13) and is easier to implement (19).

Recommendations on sample size (i.e., how many cores to collect) vary, depending on the nematode species and size of the area sampled (26). For diagnostic and advisory samples, a sample size of 10 to 30 cores is often used to estimate nematode population densities in sites up to about 2.0 ha in size (4,5,26). Sampling error increases as the size of the area sampled increases (26,29), so areas larger than 2.0 ha should be subdivided and sampled separately. If the sampling program is conducted for quarantine purposes or for detection of rare species, then a much more intensive sampling plan is required (24,26). When sampling a nematode community or several species simultaneously, the sampling plan for the most difficult species to sample should be used (19,29), although sample size recommendations are not available for most species. In one study (27), clumping was similar for nematode genera in several orders, implying that a similar sampling plan could be used for most of them. Anticipated seasonal fluctuations in population densities and availability of specific life stages should be considered in planning when to collect a sample that will be most informative (4,5,26).

The above guidelines pertain to relatively homogeneous sites. Areas with obvious heterogeneity due to differences in soil type, plant species, previous crop, topography, or other factors should probably be sampled separately (4,5,26). Row crops can be sampled consistently within the plant rows, if maximum densities are desired (3). Variation in nematode densities with differences in plant cover causes particular difficulty when sampling forests, old fields, or other highly heterogeneous sites. It is possible to establish and sample very small plots (about 1 m2) to measure nematode densities associated with small patches of plant cover (41), or somewhat larger plots (up to 100 m2) could be sampled and dominant vegetation within the plots described (25). In the latter case, 5 cores from 5-m x 5-m plots (8) or 12 cores from 10-m x 10-m plots (25) have been used.

SAMPLING METHODS: ADVANTAGES AND DISADVANTAGES

Although many factors affect the quality of a soil sample, the relationship between sample size (number of cores collected) and precision is of particular concern (4,19,26). As the number of cores collected increases, sample precision increases and sample error shows a corresponding decrease (26,29). Thus there is a trade-off between sample precision and labor involved in collecting the sample (19). Precision may increase relatively rapidly as the number of cores is increased from one to 10 per sample, but increases relatively little if numbers of cores are increased above 30-50 per sample (19,26,29). To increase precision beyond these limits, multiple samples (of multiple cores each) can be considered (19,26,28). On the other hand, a small number of cores or even a single core per sample may be necessary if plot size is so small that destruction of the site is a concern. If background information on nematode distribution is available from preliminary samples, the precision of population estimates associated with given sample sizes can be calculated (4,15,26,39). Coefficients of variation of 50-100% can be quite common, even for carefully-collected nematode samples (28,33).

EXTRACTION METHODS

The cores comprising a single sample are typically bulked and mixed well. If the sample must be transported or stored prior to extraction, it should be kept cool (10-15C) but not frozen (4,6). From the sample, a subsample of 50, 100, 250, or 500 cm3 can be removed for extraction of nematodes. Extraction from smaller volumes is often more efficient (26,35), and so a recent laboratory manual (3) uses a 100-cm3 subsample.

Although many different methods exist for extracting nematodes from soil (1,5,20,21,26,38) most can be divided into active methods, which depend on nematode movement, and passive methods, which do not (26,31). Most active methods are based on the Baermann funnel (2) technique, in which a wet soil sample is placed on a porous filter (tissue, cloth) supported by a wire screen at the top of a water-filled funnel. Nematodes move out of the soil through the filter and fall into the stem of the funnel, from which they can be collected (1,20,21,38). Many modifications of this method exist (20,21,26,38); some of the more common modifications include replacing the funnel with a more efficient bowl (36) or tray (40), or concentrating the soil sample by sieving (12).

Common passive methods for separating nematodes from soil include sieving, centrifugation (11,23), use of flocculating agents (9), elutriation (10), or a combination of these methods. In the sieving and centrifugation procedure (23), the soil sample is first concentrated by sieving, and then centrifuged in water causing nematodes and soil particles to settle while organic debris remains suspended. The pellet is resuspended in sucrose or other solute and centrifuged, causing soil particles to settle while nematodes remain suspended (1,3).

Soil extraction procedures must be modified or other specialized methods used for extraction of nematodes from roots, stems, bulbs, seeds, or other plant material (1,14,20-22,26,31,38). The Baermann funnel or its modifications can be used to extract nematodes from forest litter, but other more specialized methods have been developed as well (32,37).

EXTRACTION METHODS: ADVANTAGES AND DISADVANTAGES

Numerous studies are available comparing the efficiencies of various soil extraction methods (5,6,20,21,26,38). In some cases, passive methods such as centrifugation can be more efficient than active methods, particularly with inactive genera such as *Criconemella* or immobile life stages such as eggs (26,31). However, dead nematodes will also be recovered by passive methods, and the use of sugar and other solutes may distort features of some specimens. Performance of some methods may be affected by soil type, and in general active methods are probably more flexible with substrates other than soil (26,38). Active methods often require only simple materials and their major advantage is low cost (31). A major disadvantage of active methods is dependence on temperature, which greatly influences efficiency of recovery (5,26,31,34,38). In addition, active methods require an incubation period of at least 24-48 hr, during which time egg hatch may affect numbers recovered (16). Passive methods can provide a more accurate description of age structure of a population at the time of sampling (31).

Because nematological laboratories use many different extraction and sampling methods, nematode numbers reported can vary widely. While comparative population data can usually be obtained within a laboratory using consistent extraction and sampling methodology, it can be risky to compare population data obtained by different laboratories or methods. If this must be done, it is possible to submit divided or duplicate samples to each laboratory or method and develop relationships relating the counts obtained by each (26,30,35).

LITERATURE CITED

1. Ayoub, S. M. 1980. Plant nematology, an agricultural training aid. Sacramento, CA: Nema Aid Publications.

2. Baermann, G. 1917. Eine einfache Methode zur Auffindung von *Ancylostomum* (Nematoden) Larven in Erdproben. Geneeskundig Tijdschrift voor Nederlandsch Indie 57:131-137.

3. Barker, K. R. 1985. Soil sampling methods and procedures for field diagnosis. Pp. 11-20 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant nematology laboratory manual. University of Massachusetts Agricultural Experiment Station, Amherst, MA.

4. Barker, K. R., and C. L. Campbell. 1981. Sampling nematode populations. Pp. 451-474 *in* B. M. Zuckerman and R. A. Rohde, eds Plant parasitic nematodes. Volume III. New York, NY: Academic Press.

5. Barker, K. R., and C. J. Nusbaum. 1971. Diagnostic and advisory programs. Pp. 281-301 *in* B.M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes. Volume I. New York, NY: Academic Press.

6. Barker, K. R., C. J. Nusbaum, and L. A. Nelson. 1969. Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. Journal of Nematology. 1:240-247.

7. Barker, K. R., D. P. Schmitt, and J. P. Noe. 1985. Role of sampling for crop-loss assessment and nematode management. Agriculture, Ecosystems and Environment 12:355-369.

8. Blair, J. M., R. W. Parmelee, and R. L. Wyman. 1994. A comparison of the forest floor invertebrate communities of four forest types in the northeastern U.S. Pedobiologia 38:146-160.

9. Byrd, D. W., Jr., C. J. Nusbaum, and K. R. Barker. 1966. A rapid flotation-sieving technique for extracting nematodes from soil. Plant Disease Reporter 50:954-957.

10. Byrd, D. W., Jr., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and C. A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206-212.

11. Caveness, F. E., and H. J. Jensen. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proceedings of the Helminthological Society of Washington 22:87-89.

12. Christie, J. R., and V. G. Perry. 1951. Removing nematodes from soil. Proceedings of the Helminthological Society of Washingtong 18:106-108.

13. Church, B. M., H. Gough, and J. F. Southey. 1959. Soil sampling procedures for potato root eelworm cysts. Plant Pathology 8:146-151.

14. Coolen, W. A., and C. J. D'Herde. 1972. A method for the quantitative extraction of nematodes from plant tissue. Ministry of Agriculture, State Agricultural Research Centre, Ghent.

15. Ferris, H. 1984. Probability range in damage predictions as related to sampling decisions. Journal of Nematology 16:246-251.

16. Freckman, D. W. 1982. Parameters of the nematode contribution to ecosystems. Pp. 81-97 *in* D. W. Freckman, ed. Nematodes in soil ecosystems. Austin, TX: University of Texas Press.

17. Goodell, P. B. 1982. Soil sampling and processing for detection and quantification of nematode populations for ecological studies. Pp. 178-198 *in* D. W. Freckman, ed. Nematodes in soil ecosystems. Austin, TX: University of Texas Press.

18. Goodell, P., and H. Ferris. 1980. Plant-parasitic nematode distributions in an alfalfa field. Journal of Nematology 12:136-141.

19. Goodell, P. B., and H. Ferris. 1981. Sample optimization for five plant-parasitic nematodes in an alfalfa field. Journal of Nematology 13:304-313.

20. Hooper, D. J. 1990. Extraction and processing of plant and soil nematodes. Pp. 45-68 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, UK: CAB International.

21. Hooper, D. J., and K. Evans. 1993. Extraction, identification and control of plant parasitic nematodes. Pp. 1-59 *in* K. Evans, D. L. Trudgill, and J. M. Webster, eds. Plant parasitic nematodes in temperate agriculture. Wallingford, UK: CAB International.

22. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025-1028.

23. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

24. Jones, F. G. W. 1955. Quantitative methods in nematology. Annals of Applied Biology 42:373-381.

25. McSorley, R. 1993. Short-term effects of fire on the nematode community in a pine forest. Pedobiologia 37:39-48.

26. McSorley, R. 1987. Extraction of nematodes and sampling methods. Pp. 13-47 *in* R. H. Brown and B. R. Kerry, eds. Principles and practice of nematode control in crops. Orlando, FL: Academic Press.

27. McSorley, R., W. H. Dankers, J. L. Parrado, and J. S. Reynolds. 1985. Spatial distribution of the nematode community on Perrine marl soils. Nematropica 15:77-92.

28. McSorley, R., and D. W. Dickson. 1991. Determining consistency of spatial dispersion of nematodes in small plots. Journal of Nematology 23:65-72.

29. McSorley, R., and J. L. Parrado. 1982. Estimating relative error in nematode numbers from single soil samples composed of multiple cores. Journal of Nematology 14:522-529.

30. McSorley, R., and J. L. Parrado. 1982. Relationship between two nematode extraction techniques on two Florida soils. Soil and Crop Science Society of Florida Proceedings 41:30-36.

31. McSorley, R., and D. E. Walter. 1991. Comparison of soil extraction methods for nematodes and microarthropods. Agriculture, Ecosystems and Environment 34:201-207.

32. Minderman, G. 1956. New techniques for counting and isolating free living nematodes from small soil samples and from oak forest litter. Nematologica 1:216-227.

33. Noe, J. P., and C. L. Campbell. 1985. Spatial pattern analysis of plant-parasitic nematodes. Journal of Nematology 17:86-93.

34. Robinson, A. F., and C. M. Heald. 1989. Accelerated movement of nematodes from soil in Baermann funnels with temperature gradients. Journal of Nematology 21:370-378.

35. Rodríguez-Kábana, R., and P. S. King. 1975. Efficiency of extraction of nematodes by flotation-sieving using molasses and sugar and by elutriation. Journal of Nematology 7:54-59.

36. Rodríguez-Kábana, R., M. H. Pope. 1981. A simple incubation method for the extraction of nematodes from soil. Nematropica 11:175-185.

37. Schouten, A. J, and K. K. M. Arp. 1991. A comparative study on the efficiency of extraction methods for nematodes from different forest litters. Pedobiologia 35:393-400.

38. Southey, J. F., ed. 1986. Laboratory methods for work with plant and soil nematodes. London: Her Majesty's Stationery Office.

39. Southwood, T. R. E. 1978. Ecological methods. Second edition. New York, NY: Chapman and Hall.

40. Whitehead, A. G., and J. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55:25-38.

41. Yuen, P. H. 1966. The nematode fauna of the regenerated woodland and grassland of Broadbalk Wilderness. Nematologica 12:195-214.

DIVERSITY INDICES

Gregor W. Yeates

Landcare Research,
Private Bag 11052,
Palmerston North,
New Zealand
YeatesG@landcare.cri.nz

A nematode sample may contain in excess of fifty taxa in varying proportions. In order to compare samples, in time or space, it is often necessary to summarise the complexity of the fauna in a single value or index. As an index is a summary of information and its calculation results in a loss of information care is necessary in selecting the most appropriate index for a particular use.

In addition to traditional `information statistics' and relative *r* - *K* strategies, application of various ratios between nematode feeding groups (e.g. 6, 12, 16, 18, 21, 22) and Bongers' (3, 4) `Maturity Index' (*see* The Nematode Maturity Index, R. G. M. de Goede and T. Bongers), there are various mathematical analyses of population structure (e.g. *k*-dominance, correspondence analysis, multi-dimensional scaling) which fall outside the scope of this section.

Standard texts describing the application of various indices in biology include Pielou (13) and Magurran (9). Overviews of the application of indices to nematodes have been given by Bernard (2) and Yeates & Bongers (20); applications to various ecosystems include-

terrestrial ecosystems: Freckman and Ettema (6), McSorley (10), McSorley and Frederick (11), Wasilewska (16), Yeates (17, 18), Yeates and Bird (19), Yeates et al. (21),

freshwater: Anderson (1), *marine:* Boucher (5), Hodda and Nicholas (7), Lambshead et al. (8), Tietjen (14), Vincx (15).

Using the basic information on specimens identified:

N the number of individuals identified

s the number of taxa to which individuals have been allocated; to reduce confusion between an *N* and an *n* a given taxon is regarded as the *i*th taxon

p the proportion of individuals in the *i*th taxon

Basic indices that can be calculated are:

diversity $H' = -\sum_{i=1} pi \log_e pi$

evenness $J' = H' / H'max$ where $H'max$ is $\log_e s$

richness $SR = (s-1)/\log_e N$

dominance $\lambda = \sum p2i$

diversity $H2 = -\log_e \lambda$

The Shannon-Weaver index *H'* is commonly used to assess *diversity* but as it may be dominated by abundant taxa or the overall number of taxa both *evenness* and *richness* (Margalef index) are often calculated. The Simpson index (*D*) or it's reciprocal ($\lambda = 1/D$) can be used to assess *dominance* and its loge transformation offers an alternative measure of *diversity* (*H2*)

Results of these and a wide range of indices appear in the literature. It is important to understand the differences between the various indices and the influence of the base of logarithms used (log10, loge), and the level of identifications made (family, genus, species, feeding group). According to Magurran (9), provided replicate, independent determinations (i.e. on independent field samples) of such indices are made on each population of interest it is possible to apply normal, parametric statistics to test differences between values.

Great care must be taken when comparing values of indices between studies. In addition to differences in calculation, sediment texture (e.g. exclusion of larger forms from finer sediments), seasonal patterns (e.g. migratory infective juveniles of Heteroderidae), vegetation type and rotation (*via* host specificity and litter quality), predator:prey interactions (e.g. reduction of prey by predators) and microsites (e.g. row and inter-row samples) are among factors which affect index values.

LITERATURE CITED

1. Anderson, R.V. 1992. Free-living nematode associations in Pool 19, Mississippi River. Journal of Freshwater Ecology 7:243-250.

2. Bernard, E.C. 1992. Soil nematode biodiversity. Biology and Fertility of Soils 14:99-103.

3. Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

4. Bongers, T., Ferris, H. 1999. Nematode community structure as a bioindicator in environmental monitoring. Tree 14:224-228.

5. Boucher, G. 1990. Pattern of nematode species diversity in temperate and tropical subtidal sediments. Marine Ecology 11:133-146.

6. Freckman, D.W., Ettema, C.H. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agriculture, Ecosystems and Environment 45:239-261.

7. Hodda, M., Nicholas, W.L. 1996. Nematode diversity and industrial pollution in the Hunter River estuary, NSW, Australia. Marine Pollution Bulletin 17:251-255.

8. Lambshead, P.J.D., Platt, H.M., Shaw, K.M. 1983. The detection of differences among assemblages of benthic species based on an assessment of dominance and diversity. Journal of Natural History, London 17:859-874.

9. Magurran, A.E. 1988. Ecological diversity and its measurement. London, Croon Helm.

10. McSorley, R. 1997. Relationships of crop and rainfall to soil nematode community structure in perennial agroecosystems. Applied Soil Ecology 6:147-159.

11. McSorley, R., Frederick, J.J. 1996. Nematode community structure in rows and between rows of a soybean field. Fundamental and Applied Nematology 19:251-261.

12. Neher, D.A., Campbell, C.L. 1994. Nematode communities and microbial biomass in soils with annual and perennial crops. Applied Soil Ecology 1:17-28.

13. Pielou, E.C. 1975. Ecological diversity. New York, Wiley.

14. Tietjen, J.H. 1989. Ecology of deep-sea nematodes from the Puerto Rico Trench area and Hatteras Abyssal Plain. Deep-sea Research 36:1579-1594.

15. Vincx, M. 1990. Diversity of the nematode communities in the southern bight of the North Sea. Netherlands Journal of Sea Research 25:181-188.

16. Wasilewska, L. 1994. The effect of age of meadows on succession and diversity in soil nematode communities. Pedobiologia 38:1-11.

17. Yeates, G.W. 1984. Variation in soil nematode diversity under pasture with soil and year. Soil Biology and Biochemistry 16:95-102.

18. Yeates, G.W. 1999: Effects of plants on nematode community structure. Annual Review of Phytopathology 37:127-149.

19. Yeates, G.W., Bird, A.F. 1994. Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. Fundamental and Applied Nematology 17:133-145.

20. Yeates, G.W., Bongers, T. 1999. Nematode diversity in agroecosystems. Agriculture, Ecosystems and Environment 74:113-135.

21. Yeates, G.W., Newton, P.C.D., Ross, D.J. 1999. Response of soil nematode fauna to naturally elevated CO2 levels influenced by soil pattern. Nematology 1:285-293.

22. Yeates, G.W., Wardle, D.A., Watson, R.N. 1993. Relationships between nematodes, soil microbial biomass and weed-management strategies in maize and asparagus cropping systems. Soil Biology and Biochemistry 25:869-876.

THE ROLE OF NEMATODES IN THE MINERALIZATION OF NUTRIENTS FROM TERRESTRIAL ECOSYSTEMS

B. S. Griffiths

Soil Plant Dynamics Unit,
Scottish Crop Research Institute,
Invergowrie, Dundee DD2 5DA, UK.
bgriff@scri.sari.ac.uk

Although nematodes contribute 1% or less to total soil respiration (29), they consume 5-25% of the microbial standing crop (6), and contribute 4-22% of total net nitrogen mineralization (15). The effect of nematodes on nutrient mineralization results directly from the excretion of ingested nutrients that are not required for production (14) and indirectly from the modification of the microbial community, accelerated turnover of microbial cells, and inoculation of new substrates with microorganisms (17). The techniques described here have been used to elucidate the extent and mechanism of nematode involvement in nutrient mineralization at a range of scales (i.e. from single species interactions under gnotobiotic conditions through to ecosystem level food web analyses). The large diversity of techniques available precludes the extensive detailing of individual methods, so the reader is encouraged to follow-up on particular techniques from the literature list. All the techniques have a role to play in furthering our understanding of nematode ecology, but the important point is to choose the most appropriate scale for any particular study.

MICROCOSMS (LABORATORY STUDIES)

Increases in the accumulation of nutrients in mixed cultures of microorganisms plus nematodes, compared with pure cultures of microorganisms, have commonly been used to demonstrate nematode effects on net mineralization. In its simplest form this requires a culture of nematodes growing on a food microorganism. Nematodes collected from soil by funnel extraction can be cultured readily on agar plates (11), using the mixed bacterial flora on the plate as food. It is important to prevent contamination by protozoa, as these out compete nematodes in simple systems and would make the results of any experiments difficult to interpret (13). Ideally nematodes would be reared axenically (26), so that when nematodes are inoculated in an experiment there is no extra inoculation of microorganisms. However, as it is not practical to develop the stringent growth media required for each species of nematode good working practice is to maintain nematodes monxenically on a single food microorganism. Nematodes can be sterilized to remove contaminating microorganisms (8) before transfer to a pure culture of the food microorganism. Regular subculturing of the nematode onto fresh media, using standard aseptic microbiological techniques, should ensure the supply of nematodes for experimental purposes. They can be washed from the surface of the agar plates (in sterile saline solutions) to remove the vast majority of adhering microorganisms, and used to inoculate microcosms.

The experimental media in which the nematodes and microorganisms are allowed to interact can be, in order of increasing complexity: liquid media (13), solid agar media (11), sterile glass beads (3), sterile sand (2, 31), or sterile soil (9). Replicate samples of the media are inoculated with known numbers of microorganisms and nematodes, together with control treatments without nematodes. Sequential samples are then analyzed to determine the time-course of nutrient mineralization and the population dynamics of the organisms. These simple systems can be used to accommodate increasingly complex mixtures of organisms, for example: bacteria, bacterial-feeding nematodes, fungi and fungal-feeding nematodes both with and without plants (24, 25, 33); bacteria, bacterial-feeding nematodes and nematophagous mites (7); or bacterial-feeding nematodes and isopods (1). These references also give details of analyzing nutrients (CO_2, N, P), enumeration of different populations and preparation of sterile microcosms.

Laboratory microcosms are easy to set up and give results that are relatively straightforward to interpret. They do, however, contain a much simplified microbial community, and the results of any microbe/fauna interaction can be strongly influenced by the choice of organism. It is also possible to inoculate with a more representative mixed microbial community, thereby avoiding the painstaking addition of separate species to create complex communities (12, 19). The use of field soil in laboratory microcosms (14) is more realistic in terms of microbial population structure and soil physico-chemical conditions, but makes the interpretation of nematode effects more difficult.

MESOCOSMS (LABORATORY AND FIELD STUDIES)

Mesocosm studies usually involve the collection of undisturbed soil blocks, which are treated and incubated in the laboratory or placed back in the field. This means that the heterogeneous nature of the soil is retained and that, in the field at least, ambient fluctuations of temperature, moisture and substrate input are maintained. Teuben and Verhoef (32) concluded that field mesocosms are preferable to laboratory microcosms for studying soil ecosystem processes. It has been common to remove/inactivate nematodes in order to determine their effect on ecosystem processes. Biocides can be used to selectively remove components of the soil biota, i.e. bacteria, fungi, mycorrhyzal fungi, nematodes, and microarthropods (21). The application of nematicides, such as carbofuran, has few direct effects on non-target organisms. This is not the case for biocides applied against other groups of organisms. A nematicide is usually applied at a rate recommended by the manufacturer, and soil populations and processes are then compared with an untreated control (35). Soil blocks can be returned to the field after application (37). The effect of nematodes can also be derived by applying biocides against other taxonomic groups, and relating changes in nematode population to changes in other populations and rates of mineralization (22). Great care must be exercised in the interpretation of biocide experiments because of the initially increased C and N mineralization from dead nematodes and microorganisms and from the addition of extra nutrient (particularly N) with the biocidal agent.

Microwaving and freeze/thaw cycles can also be used to defaunate soil blocks, and were more effective than a carbofuran/napthalene mixture in eliminating nematodes (20). They did, however, have large effects on soil physico-chemical properties, so the authors recommended that a control treatment that has been defaunated and reinoculated was included in the design of the experiment. Brucker *et al*. (5) designed apparatus for collecting soil blocks (250 mm3) and defaunating them by deep-freezing in the field. The blocks were then returned to the field, but could be separated from the surrounding soil by nylon mesh to prevent recolonization. The advantages of defaunation as an experimental technique are that: (i) the treatment can be maintained indefinitely if the defaunation is complete and recolonization is prevented; (ii) selected taxonomic and trophic groups can be inoculated singly or in combinations to test particular hypotheses. The disadvantages are that: (i) it may be difficult or expensive to achieve; (ii) it may cause undesirable changes in soil physical or chemical characteristics (20); (iii) the microbial community that develops after defaunation may be functionally different from the initial population.

FIELD STUDIES

Biocides are commonly used in field studies, in exactly the same way as in mesocosms apart from their application to marked plots in the field (4, 23). Nematode populations still need to be monitored in biocide treated plots as control is never 100% and re-inoculation can occur relatively rapidly (36,37).

The other strategy to determine the role of nematodes in nutrient cycling in the field, is to infer effects from the concomitant changes in nematodes, microflora and nutrients observed over time. The scale of these studies can range from the specific experiment or observation, through to the all-encompassing food web analysis and simulation modeling approach. Wasilewska and Biénkowski (34) used the litter bag technique to demonstrate the dynamics of nematode populations and nematode community structure on different substrates, this was then related to the rates of substrate decomposition. Similarly focussed studies using small field plots have been used to show nematode responses to: rainfall (28); soil organic matter (27); and manure type (18). Such approaches give strong evidence for nematode involvement when linked to measures of decomposition or nutrient cycling (30). Complete food web analyses of an ecosystem represents the definitive means of determining nematode (or any other taxonomic group) involvement, but it is also the most technically demanding. Examples to date have shown that nematodes have an important role to play in nutrient mineralization (see introduction). The application of models which include aspects of the interactions between nematodes and other organisms have improved the quantification of the role of nematodes in nutrient cycling, and identify the most important links and interactions between nematodes and other soil organisms (10)

LITERATURE CITED

1. Abrams, B.J., and M.J. Mitchell. 1980. Role of nematode-bacterial interactions in heterotrophic systems with emphasis on sewage sludge decomposition. Oikos 35:404-410.

2. Anderson, A.R.A., I.M.Young, B.D.Sleeman, B.S.Griffiths, and W.M.Roberstson. 1997. Nematode movement along a chemical gradient in a structurally heterogeneous environment. 1. Experiment. Fundamental and Applied Nematology 20:157-163.

3. Anderson, R.V. and D. C. Coleman. 1977. The use of glass microbeads in ecological experiments with bacteriophagic nematodes. Journal of Nematology 9:319-322.

4. Beare, M.H., R.W. Parmelee, P.F. Hendrix, W. Cheng, D.C. Coleman, and D.A. Crossley. Jr. 1992. Microbial and faunal interactions and effects on litter nitrogen aand decomposition in agroecosystems. Ecological Monographs 62:569-591.

5. Brucker. A., J. Wright, C. Kampichler, R. Bauer, and E. Kandeler. 1995. A method of preparing mesocosms for assessing complex biotic processes in soil. Biology and Fertility of Soils 19:257-262.

6. Brussaard, L., L.A. Bouwman, M. Geurs, J. Hassink, and K.B. Zwart. 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. Netherlands Journal of Agricultural Science 38:282-302.

7. Brussaard, L., J.P. Kools, L.A. Bouwman, and P.C. de Ruiter. 1991. Population dynamics and nitrogen mineralization rates in soil as influenced by bacterial grazing nematodes and mites. pp. 517-523 *in*: G.K. Veeresh, D. Rajagopal, and C.A. Viraktamath, eds. Advances in management and conservation of soil fauna. Oxford and IBH Publishing Co. PVT. Ltd., New Delhi.

8. Chantanao, A., and H. J. Jensen. H.J. 1969. Techniques for controlling bacterial flora of *Pristionchus iheritieri.* Journal of Nematology 1:277-278.

9. Coleman, D.C., R.V. Anderson, C.V. Cole, E.T. Elliott, L. Woods, and M.K. Campion. 1978. Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. Microbial Ecology 4:373-380.

10. de Ruiter, P.C., J. Bloem, L.A. Bouwman, W.A.M. Didden, G.H.J. Hoenderboom, G. Lebbink, J.C.Y. Marinissen, J.A. de Vos, M.J. Vreeken-Buijs, K.B. Zwart, and L. Brussaard. 1994. Simulation of dynamics in nitrogen mineralization in the below-ground food webs of two arable farming systems. Agriculture, Ecosystem and Environment 51:199-208.

11. Ferris, H., S. Lau, and R. Venette. 1995. Population energetics of bacterial-feeding nematodes: respiration and metabolic rates based on CO2 production. Soil Biology & Biochemistry 27:319-330.

12. Frey, J.S., J.F. McClellan, E.R. Ingham, and D.C. Coleman. 1985. Filter-out-grazers (FOG): A filtration experiment for separating protozoan grazers in soil. Biology and Fertility of Soils 1:73-79.

13. Griffiths, B.S. 1986. Mineralization of nitrogen and phosphorous by mixed cultures of the ciliate protozoan *Colpoda steinii*, the nematode *Rhabditis* sp. and the bacterium *Pseudomonas fluorescens*. Soil Biology & Biochemistry 18:637-641.

14. Griffiths, B.S. 1989. The role of bacterial-feeding nematodes and protozoa in rhizosphere nutrient cycling. Aspects of Applied Biology 22:141-145.

15. Griffiths, B.S. 1994. Microbial-feeding nematodes and protozoa in soil: Their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. Plant and Soil 164:25-33.

16. Griffiths, B.S., and S. Caul. 1993. Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. Biology and Fertility of Soils 15:201-207.

17. Griffiths, B.S., and R.D. Bardgett. 1997. Interactions between microbial-feeding invertebrates and soil microorganisms. pp. 165-182 *in* J.D. van Elsas, E.M.H. Wellington, and J.D. Trevors, eds. Modern Soil Microbiology. Marcel Dekker, New York.

18. Griffiths, B.S., K. Ritz, and R.W. Wheatley. 1994. Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system. Soil Use and Management 10:20-24.

19. Griffiths, B.S., M. Bonkowski, G. Dobson, and S. Caul. 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. Pedobiologia 43:297-304.

20. Huhta, V., D.H. Wright, and D.C. Coleman. 1989. Characteristics of defaunated soil. I. A comparison of three techniques applied to two different forest soils. Pedobiologia 33:415-424.

21. Ingham, E.R. 1985. Review of the effects of 12 selected biocides on target and non-target soil organisms. Crop Protection 4:3-32.

22. Ingham, E.R., J.A. Trofymow, R.N. Ames, H.W. Hunt, C.R. Morley, J.C. Moore, and D.C. Coleman. 1986. Trophic interactions and nitrogen cycling in a semi-arid grassland soil. II. System responses to removal of different groups of soil microbes or fauna. Journal of Applied Ecology 23:615-630.

23. Ingham, E.R., D.C. Coleman, and J.C. Moore. 1989. An analysis of food-web structure and function in a shortgrass prairie, a mountain meadow, and a lodgepole pine forest. Biology and Fertility of Soils 8:29-37.

24. Ingham, R.E., J.A. Trofymow, E.R. Ingham, and D.C. Coleman. 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecological Monographs 55:119-140.

25. Mikola, J., and H. Setälä. 1999. Interplay of omnivory, energy channels and C availability in a microbial-based soil food web. Biology and Fertility of Soils 28:212-218.

26. Patel, T.R., and McFadden. B.A. 1978. Axenic and synchronous culturesof *Caenorhabditis elegans*. Nematologica 24:51-62.

27. Schnürer, J., M. Clarholm, and T. Rosswall. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biology & Biochemistry 17:611-618.

28. Schnürer, J., M. Clarholm, S. Boström, and T. Rosswall. 1986. Effects of moisture on soil microorganisms and nematodes: A field experiment. Microbial Ecology 12:217-230.

29. Sohlenius, B. 1980. Abundance, biomass and contibution to energy flow by soil nematodes in terrestrial ecosystems. Oikos 34:186-194.

30. Steinberger, Y., D.W. Freckman, L.W. Parker, and W.G. Whitford. 1984. Effects of simulated rainfall and litter quantities on desert soil biota: nematodes and microarthropods. Pedobiologia 26:267-274.

31. Sundin, P., A. Valeur, S. Olsson, and G. Odham. 1990. Interactions between bacteria-feeding nematodes and bacteria in the rape rhizosphere: effects on root exudation and distribution of bacteria. FEMS Microbiology Ecology 73:13-22.

32. Teuben, A., and H.A. Verhoef. 1992. Relevance of micro- and mesocosm experiments for studying soil ecosystem processes. Soil Biology & Biochemistry 24:1179-1183.

33. Venette, R.C., F.A.M. Mostafa, and H. Ferris. 1997. Trophic interactions between bacterial-feeding nematodes in plant rhizospheres and the nematophagous fungus *Hirsutella rhossiliensis* to suppress *Heterodera schactii*. Plant and Soil 191:213-223.

34. Wasilewska, L., and P. Biénkowski. 1985. Experimental study on the occurrence and activity of soil nematodes in decomposition of plant material. Pedobiologia 28:41-57.

35. Wright, D.H., and Coleman. D.C. 1988. Soil faunal vs. fertilization effects on plant nutrition: results of a biocide experiment. Biology and Fertility of Soils 7:46-52.

36. Yeates, G.W. 1985. Effects of two nematicides on biological activity in a Typic Haplaquoll at Castlepoint. 2. Nematodes. New Zealand Journal of Agricultural Research 28: 141-150.

37. Yeates, G.W., S.S. Bamforth, D.J. Ross, K.R. Tate, and G.P. Sparling. 1991. Recolonization of methyl bromide sterilized soils under four different field conditions. Biology and Fertility of Soils 11:181-189.