

A Semiquantitative Method for Enumerating and Observing Parasites and Predators of Soil Nematodes

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Abstract: A laboratory method was developed to count and observe antagonists of soil nematodes and simulate their relationships in the soil. A 10- to 25-cc soil sample is suspended in water and washed through a series of small standard sieves. Residues are washed into a small beaker and collected on a 24-mm filter paper disk in a filter holder under vacuum. The disk is placed on corn meal agar in a petri dish. Microfauna and flora present in the sample colonize the organic matter on the disk and move onto and into the agar where they can be observed easily. Distinct successions of organisms usually occur and within 6-18 days or more, parasites and predators of nematodes are often abundant, especially nematode-trapping fungi. Counting predation events and parasitized nematodes in replicate dishes after specific incubation periods allows quantitative comparisons between soil samples. The method has distinct advantages over others for enumerating organisms which attack nematodes. *Key Words:* microflora, microfauna interrelationships, technique, predacious fungi, *Arthrobotrys conoides*, *A. dactyloides*, *Monacrosporium gephyropagum*.

Common plating methods for observing and isolating nematode-trapping fungi from soil have been those in which soil was

sprinkled into a petri dish, into which cool, but still liquid, agar with dilute nutrient concentrations is dispersed (2), where soil or associated organic matter is sprinkled directly onto the surface of corn meal agar in petri dishes (1), or where soil crumbs, root

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fragments, and pieces of organic matter have been placed onto and/or pressed into the surface of corn meal agar (6). The above techniques often leave heterogeneous, opaque material on the plates, requiring laborious searching, often over extended periods of time, for indications of fungi. Replicate plates usually do not give uniform results and only empirical data can be obtained.

A few semi-quantitative techniques have been devised for detecting predacious fungi in soil, but all require the addition of suspensions of live nematodes (3, 4, 7). They have not worked as well, nor are they as

simple to perform, as the method herein reported. An abstract has been published (5).

MATERIALS AND METHODS

Replicate 10-g samples are taken from soil, passed through a dry No. 9 or 10 standard sieve, soaked in water for 10 min, and suspended in about 500 ml of tap water in beakers. (Up to 25-g samples can be analyzed from mineral soils containing little organic matter; but in soil with an abundance of light organic debris, sample size may have to be limited to 5 g.) Samples are mixed with an electric or magnetic stirrer, or by agitation in two Erlenmeyer flasks with ground glass necks joined by rubber tubing. The soil suspension is washed through a graded series of sieves, depending upon the soil type. The most commonly used are: No. 20 or 32, No. 100, and No. 325 or 400. The suspension is decanted through each sieve separately into a flask (or tall beaker) (Fig. 1) and allowed to stand briefly so that heavy mineral particles will settle. The sieves can be supported in funnels over flasks which receive the suspension passed through the sieves. Each sieve used is rinsed separately with a small stream of water into a small beaker. With practice, the material from each of three sieves can be pooled into a 25-ml beaker; these are set aside to allow fine suspended material to settle. The suspensions are poured into a split-funnel, filter holder (Pyrex microanalysis filter holder, No. XX1002500), Millipore Corp., Bedford, MA, containing a 24-mm diameter filter paper disk (fast-type) on a sintered glass base with vacuum applied. It is important not to clog the filter paper initially and slow the passage of the suspension. Simultaneous filtration of a number of samples can be accomplished on a multi-place vacuum manifold (Fig. 2). Filtration is stopped when water leaves the mat. The filter paper disk is removed and placed on the surface of one-quarter strength corn meal agar (Difco water agar, 3 parts: Difco corn meal agar, 1 part) in the center of a petri dish (Fig. 3). Plastic typing grid dishes are most convenient to use (Falcon Plastics, Los Angeles, CA) and facilitate precise scanning of the dishes. It is possible to increase the amount of soil processed and the size of the filter disk used (4.25 cm), as well as the area for observation by utilizing large (150 × 25 mm), round, phage-typing dishes.



FIG. 1. Equipment for sieving and decanting soil samples after agitation in joined flasks.



FIG. 2. Equipment used for collecting residues on filter paper disks for placement onto agar plates.

RESULTS AND DISCUSSION

The microflora and microfauna collected on the filter paper disk colonize the organic matter on the disk, and move out on and into the agar where they can be observed easily (Fig. 3). Distinct successions of organisms can be observed. In the first few days, fungi and bacteria develop sparsely, followed by microphagous organisms, particularly protozoa and nematodes. In about 6-12 days, parasites and predators of nematodes, particularly nematode-trapping fungi appear. The latter can be isolated into pure culture by removing one or more conidia from erect conidiophores with a finely sharpened, sterile transfer needle moistened with agar and then transferring them to slants of corn meal agar. Bacterial-feeding nematodes develop rapidly, and often fungal-feeding forms also develop. Populations of predacious nematodes, when present, develop slowly after many weeks. The succession of organisms which develop in the dishes approximates that observed in the colonization of natural substrates in the soil. Quantitative comparisons between soil samples are made by counting predation events and parasitized nematodes or species of organisms present in replicate dishes after a specific incubation period. The dishes are kept at room temperature and can be observed for a year or more if suitably stored to prevent desiccation. The organic matter on the paper disk can be observed using overhead illumination but usually only the conidiophores of predacious fungi can be seen clearly. Observations on the habits of predacious nematodes and certain soil microfauna such as mites, collembola and enchytraeid annelid worms, when present, are also possible with extended incubation. An abundant development of Enchytraeidae is usually detrimental to later observations because of extensive burrowing and disturbance of the agar caused by such worms.

The closed plastic petri dishes can be scanned initially under a dissecting microscope for the onset of events of interest. Later, the lids may be removed and the dishes examined under higher powers of a dissecting microscope or with lower power objectives of a compound microscope. High power water immersion objectives can be used. Non-water immersion high power objectives frequently fog from the condensed moisture of the agar

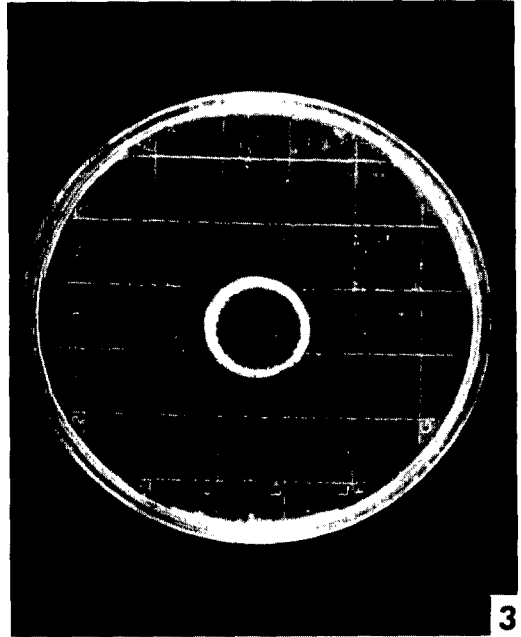


FIG. 3. Agar plate after several days of incubation. Sectors surrounding the disk contain numerous trapping organs of predacious fungi.

unless the objectives are warmed. Dishes can be observed from the bottom with a dissecting microscope and also with a X10 or lower power objective of a compound microscope, or with some microscopes, a X20 objective. Observations through the bottom surface reveal, particularly, the presence of parasitic fungi such as *Catenaria* spp. and *Harposporium* spp. A plexiglass cradle for the plastic petri dishes can be made easily and attached in place of the usual slide holder on the mechanical stage of a microscope to facilitate accurate searching of the plates.

A comparative test was made between my method and the one described by Klemmer and Nakano (4), since theirs also utilized a relatively large soil sample and could be performed without the subsequent addition of colonized nematodes. Three separate citrus grove soil samples known to have substantial populations of predacious fungi were examined by each method. With their method, five replicate plates were prepared for agar strips from each 10 g sample; while with my method, three 10-g portions of each of the three soil samples were processed. All petri dishes were observed for nematode-trapping fungi after 12, 18, 26, and 33 days. None was observed on any of the 15 petri dishes prepared by Klemmer and Nakano's

method while by my method I observed *Arthrobotrys conoides* and *A. dactyloides* in all replicates of sample 1; and *A. conoides*, *A. dactyloides* and *Monacrosporium gephyropagum* in all replicates of sample 2, and in two of three replicates of sample 3.

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