Soil-Inhabiting Nematodes

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NEMATODES CONSTITUTE A MAJOR phylum of animals, exhibiting a great diversity of forms, habitats, and food sources. The study of animal-parasitic nematodes is called helminthology; the study of plant-parasitic, soil, and marine forms is nematology. These exercises are concerned with microbivorous (microbe-feeding) and plant-parasitic soil nematodes.

Microbivorous nematodes are an extremely heterogeneous group, consisting of numerous families, genera, and species. These nematodes are rarely considered to be harmful to crops, although some may enter a diseased plant part and aggravate the injury. Most of the microbivorous forms feed on bacteria or fungi, but protozoans, micrometazoa, and other nematodes may serve as food. Soil nematodes, on the other hand, are preyed upon by tardigrades, arthropods, certain fungi, and other animals and may be parasitized by protozoa and bacteria. Relatively little is known of these interactions and the ways in which they influence the fauna and flora of soils.

The plant-parasitic nematodes are soil-inhabiting microscopic roundworms (fig. 1) that feed on plant roots (Agrios 1969). All plant-parasitic nematodes characteristically have a feeding apparatus known as a stylet (Bird 1971). The stylet, which resembles a hypodermic needle, enables the nematode to puncture plant cell walls, secrete enzymes into the cells, and withdraw digested cell contents. The feeding activities of these nematodes usually result in stunting and unthrifty plant growth, reduced crop yields, and, occasionally, plant death. One recent estimate of crop losses due to nematode parasitism is $1 billion/year in the U.S. alone (Anon. 1968). Equally important, nematodes can attack plants in combination with other disease-causing microorganisms (fungi and bacteria), and some nematodes transmit plant viruses. The combined effects are more destructive to plants than the damage caused by the parasites separately.

All plants are susceptible to attack by one or more species of plant-parasitic nematodes. The major damage done by nematodes is caused by their feeding activity on plant root systems. Nematode species, however, vary in their preference for plant food. Some feed on relatively few kinds of plants, whereas others feed on many different plant species. Plant-parasitic nematodes are obligate parasites and must feed on living plants to obtain the necessary nutrients to develop and reproduce.

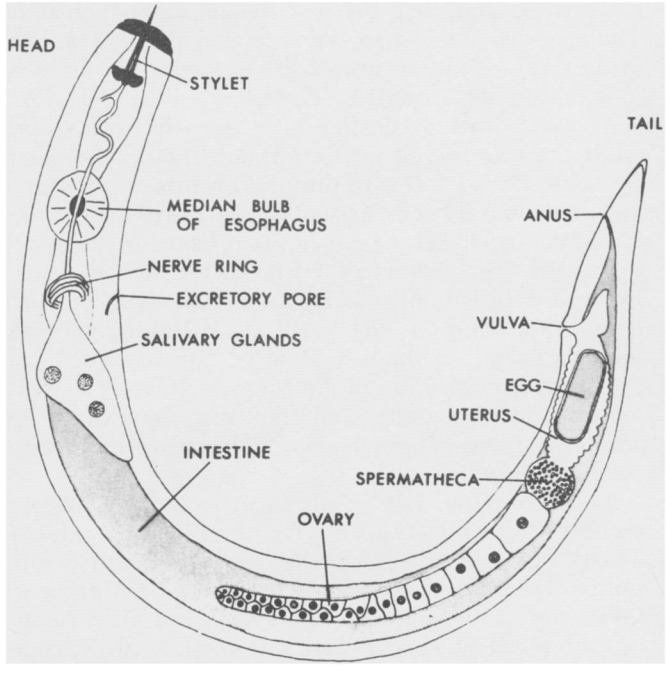

Fig. 1. A plant-parasitic nematode.

In general, the life cycle of a plant-parasitic nematode is simple and direct. Females lay eggs, either singly or in clusters, depending on the nematode species. Juvenile forms (larvae) are differentiated and hatch from eggs. Four juvenile stages are recognized, each stage separated by a molt. In many species the first molt occurs within the egg and second-stage juveniles hatch from the eggs. Adult males and females are differentiated during the final molt. (Most nematodes reproduce by cross fertilization; however, some are parthenogenetic.)

Extraction and Culture

A Baermann funnel is often used to separate nematodes from soil, roots, or organic detritus (Southey 1970). It consists of a conical glass funnel (100 mm in diameter) with a short piece of rubber tubing attached to the stem and closed by a spring-action clamp (fig. 2). A circular piece of wire screen is placed inside the top of the funnel, resting about an inch below the top rim. A wet-strength facial tissue, to retain soil, is placed on the screen, sloping up the funnel sides but not overlapping the rim. The funnel is filled with tap water to a level above the screen.

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Microbivorous nematodes are most easily obtained from decaying or rotting refuse, such as from a compost pile or garbage dump. Collect a soil sample from such a source and place 3 tablespoons gently into the water on top of the facial tissue. The active nematodes in the sample will migrate downward through the tissue and settle to the bottom of the funnel stem. After one or two days, 5–10 ml of water, which will contain most of the nematodes, should be collected from the funnel. Several Baermann funnels can be set up with soil from different sources. Examine the nematode suspension under a stereoscopic microscope. Notice the abundance and the different forms of nematodes recovered from the small sample. Microbivorous nematodes are readily distinguished from plant-parasitic ones by their very rapid movement. If any plant-parasites are present in the sample they can be recognized by their very sluggish movement and possession of a stylet in the anterior end (fig. 1).

Certain of the microbivorous nematodes (for example, *Rhabditis* species) can be temporarily cultured on plain water agar (15 g agar per liter distilled water). Add 1 ml of the nematode suspension obtained from the Baermann funnel to a petri dish containing water agar. When nematodes are added to the plates they carry along bacteria that readily proliferate on the agar and serve as a food source for the microbivorous nematodes. In three or four days the nematodes should have increased in numbers and be depositing eggs on the agar surface. Observe the feeding behavior of these nematodes under the stereoscopic microscope as they ingest bacteria.

Development of Root-Knot Nematodes

Root-knot nematodes *(Meloidogyne* species) are the most important plant-parasitic nematodes in the U.S. and are widespread in every state. Many home gardens are infested with this particular plant-parasitic nematode, which will attack such plants as tomato, bean, okra, squash, cucumber, carrot, and potato. Root-knot nematode inoculum for this experiment can usually be obtained from the nematologist in the plant pathology department at the land grant university in each state. Write to the nematologist and request a quart of root-knot nematode infested soil.

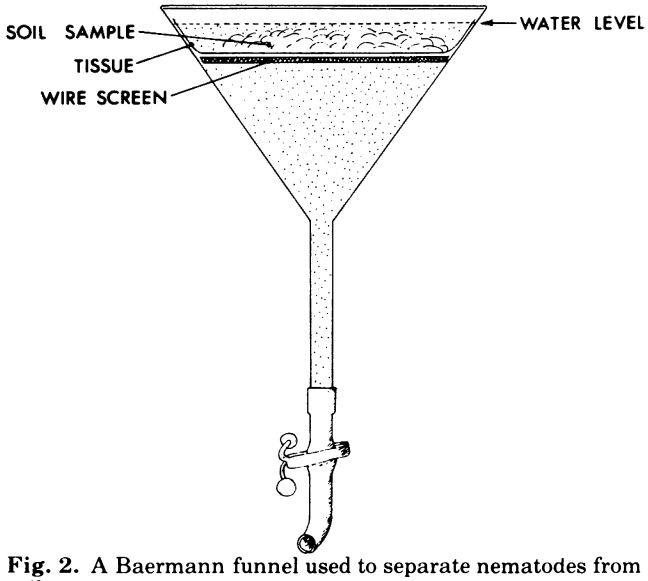

Fig. 2. A Baermann funnel used to separate nematodes from soil.

Fig. 3. A severely galled tomato root system infected with root-knot nematodes. Arrows indicate egg masses deposited on the surface of the galls by female nematodes.

Prior to receiving the nematode-infested soil, tomato plants, *Lycopersicon esculentum* Mill., should be grown to an appropriate size for inoculation. This will require approximately 28 days. First, plant seeds of a root-knot nematode susceptible tomato variety (for example, Rutgers, Manapal, Beefeater, or Springset) in vermiculite and keep moist. Seedlings will be ready to transplant in seven to nine days to 7.5-cm pots (small Dixie cups with small holes in the bottom work just as well) containing sterilized sandy loam soil. Soil can be sterilized by autoclaving or heating in an oven until it has remained for at least 30 minutes at 82 °C or above. The plants should be kept in an area with ample sunlight at 25–32 °C and will need to be fertilized biweekly with Ra-Pid-Gro or another water soluble complete fertilizer. In approximately 20 days the tomato plants will be of sufficient size (15 cm) for inoculation.

If the plants are not ready for inoculation when the infested soil is received, the soil can be stored at 10 °C for ten to fifteen days without loss of nematode viability. When the plants are ready, divide the quart of nematode-infested soil into six parts and add one part to each of six 15-cm pots containing sterilized sandy loam soil. Transplant one tomato plant to each pot. Plant three tomatoes in separate pots containing only sterilized soil to serve as control plants. The tomato plants will need abundant sunlight, daily watering and biweekly fertilizing for the duration of the experiment.

One week after inoculation, harvest two plants by washing the soil from the root system. Nematodes that

have penetrated the roots can be observed only when stained by incubating the roots in an acid fuchsin-lactophenol staining solution in an oven at 80 °C for approximately 2 hours (Southey 1970). Make the stain by adding 5 ml of a 1% stock solution of acid fuchsin to each 100 ml of lactophenol. The lactophenol consists of liquid phenol (500 ml), lactic acid (500 ml), glycerin (1000 ml), and distilled water (500 ml). Pour off the acid fuchsin-lactophenol stain, rinse the roots with tap water, and destain by incubating at room temperature in clear lactophenol until the roots become almost transparent. This will probably require two to three days and will be facilitated by changing the lactophenol solution periodically. As the root tissue destains, nematodes will retain the stain. After sufficient destaining, observe the nematodes in the roots by pressing sections of the stained roots between two glass slides and examine under a stereoscopic microscope. Compare the root systems of the inoculated plants with the root system of a noninoculated control plant at each harvest.

Three weeks after inoculation, harvest two more tomato plants and stain as indicated above. Roots penetrated by the root-knot nematode should now have begun to enlarge into galls; therefore, select the swollen roots for staining and examination.

At *eight weeks* after inoculation, stain the roots of one of the remaining inoculated plants as outlined above. The last plant should be used in obtaining nematode eggs, which will have been deposited in a mass in a gelatinous matrix on the surface of the galled roots (fig. 3). The egg masses can be easily seen when stained reddish pink by incubating the entire root system in a .0015% (15 mg/1000 ml) aqueous solution of phloxine B for 15 minutes followed by a rinse in tap water.

Nematode embryogenesis can be studied by removing an egg mass with forceps and teasing it apart with dissecting needles in water in a depression slide. Examine under a compound microscope. Notice eggs in various stages (2-celled, 4-celled, and so on) of development and some containing fully developed juveniles (fig. 5). Second-stage juveniles will hatch from the eggs if kept in water at room temperature.

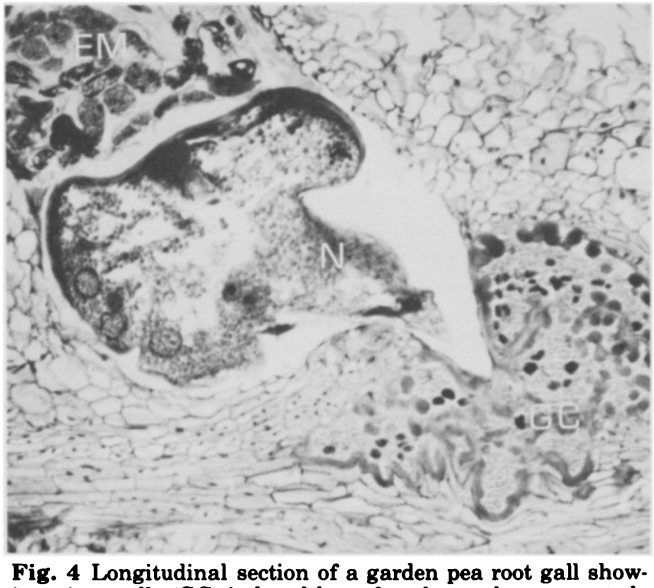

Fig. 4 Longitudinal section of a garden pea root gall showing giant cells (GC) induced by a female root-knot nematode (N). Note egg masses (EM) at the posterior end of the female nematode.

At this time additional tomato plants can be inoculated with 10 egg masses or infested soil can be stored in plastic bags at 10 °C for several months in order to maintain the nematode culture.

Expected Results

Microbivorous nematodes are most likely to grow and reproduce on agar plates having a minimum of fungal growth and a maximum of bacterial growth. In observation of the feeding nematodes, the observer should see the rapid movements of the lower esophageal region and the simultaneous intake of both bacteria and air. For closer observation of the esophageal region of this nematode, mount a few specimens on a depression slide and observe the nematodes under the low or medium power of a compound microscope.

Certain additional experiments can be tried with the microbivorous nematodes. Obtain several different bacterial cultures and transfer some nematodes to them to ascertain the specificity of this nematode for different food sources. (Other unicellular organisms, for example, yeast, can be used.) One can also study some elements of this nematode's life cycle by isolating a single nematode and observing the rate of egg production.

Root-knot nematode juveniles usually penetrate the root at sites near the root tip, and examination of the week-old plants should be concentrated in that area. After three weeks, definite nematode-induced symptoms should be present in the form of root enlargements. Each of these enlargements is a result of one or more infective second-stage juveniles penetrating the root and settling at that site and feeding. Stained nematodes will have enlarged to some degree from those observed in the week-old plants. The nematode causes hypertrophy (abnormal enlargement of some cells) and hyperplasia (abnormally rapid cell division) to produce the galls (Godfrey and Oliviera 1932; Bird 1974). A cross-section of an infected root showing the internal structure of a root-knot nematode induced gall is shown in fig. 4. The hypertrophied cells are called

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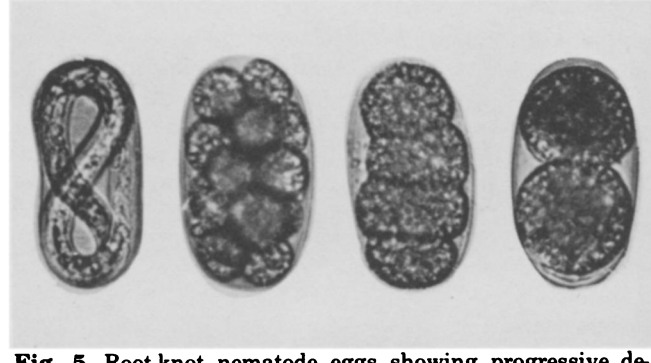

Fig. 5. Root-knot nematode eggs showing progressive development from the two-celled stage to the mature juvenile ready to hatch.

more specific readings; (iii) providing more structure in discussions; and (iv) meeting more often at the beginning of the semester and then less often as TAs become more aquainted with their tasks.

The fact that 83% of the students were satisfied with what they had learned in the seminar provides motivation for the faculty to maintain the effort. Course directors have been well pleased with the performance of TAs who have completed the course. With these encouraging results, the seminar is being continued.

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Undergraduate Research Projects

NSF grants totaling $2.8 million will support 222 projects involving 1,765 college students in apprenticeships in scientific research. Participating students are full-time undergraduates, usually between the junior and senior year, who have demonstrated promise while completing a substantial part of their college science preparation. Students in URP projects are selected to work in specific projects closely matched to their interests and background. URP grants were made in all fields of science, but highest priority was given to energy-related general research.

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"giant cells" and are the principal cellular response of susceptible plants infected by root-knot nematodes. These cells are an integral part of this highly specialized host-parasite relationship, serving as the permanent feeding sites for these nematodes.

On plants inoculated eight weeks before, galling will be conspicuous (fig. 3). When extensive galling occurs, the efficiency of the root system in absorption and translocation of nutrients and water is greatly impaired, resulting in reduced plant vigor and eventual stunting of the plants. On the plant stained with acid fuchsin-lactophenol, the greatly enlarged globose females will be visible within the galls. Sometimes the giant cells mentioned above will also be visible as a dark-stained mass located in the center of the root near the head of the nematode. On many of the galls a gelatinous mass containing 500–1,000 eggs laid by the female nematode will be visible. The phloxine B stain will greatly facilitate locating the egg masses on the second plant. Many of the eggs observed in the depression slide will show progressive development from one cell to the juvenile stage (fig. 5). Most root-knot nematode species are parthenogenetic, with males rarely being present in a population.

Additional experiments can be done on the pathogenicity of this nematode by adding varying numbers of egg masses to new soil and planting with healthy tomato seedlings. The pathogenicity can be estimated by both a reduction in plant height and vigor, and the amount of galling present on the roots. Other plant species can be inoculated to determine the host range of the root-knot nematode. A host plant is one on which the nematode can feed, reproduce, and produce symptoms (galls).

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Ancient Proverb

Whoever fears to submit any question to the test of free discussion loves his own opinion more than the truth.