Nematodes that Eat Plants

George: why does the root of that sick celery look peculiar?

A healthy celery root system on the left and one infested with root-knot nematodes, *Meloidogyne* sp., on the right. Which one do you believe would compete better for water and soil nutrients? Taken from the slide collection of Dr. George Bird, Michigan State University.

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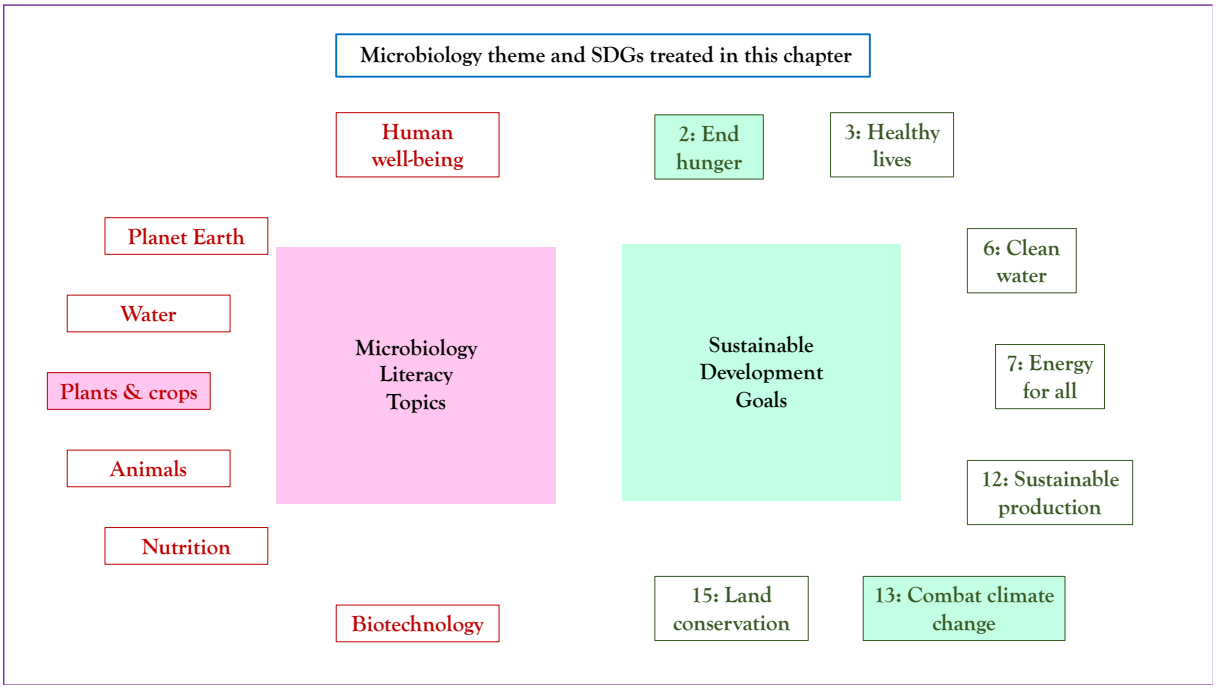
Storyline

Many animals will consume plants but most of them can be seen with the naked eye. These animals are usually referred to as pests because they injure plants and have no long associations with them. Pests are often mammals, insects as well as other arthropods, and mollusks such as snails and slugs. Sometimes, we do not see them feeding, we just observe the damage they do. Plant-parasitic nematodes are different because they are all microscopic animals, so they cannot be seen with the naked eye. They are pathogens which are organisms that cause infectious diseases, in this case those of plants. Parasites are organisms that obtain nutrients from other organisms (their hosts), typically have long associations with their hosts and usually reproduce in or on their hosts. This interaction with their hosts often results in the production of symptoms which are clues used by disease diagnosticians in their diagnoses. I am a plant disease diagnostician at Michigan State University and work almost exclusively with nematodes. Plant-parasitic nematodes are really the only animals (there are exceptions depending on the definitions you use) that cause diseases of plants.

Every species of plant presently known to biologists has at least one nematode parasite. Most have a half dozen or more. Plant-parasitic nematodes are frequently referred to as "hidden enemies" since plant producers never see them but suffer the consequences of their existence in reduced profits on their farms, their plant nurseries or greenhouses. Really, it is unusual not to find any plant-parasitic nematodes in sites/fields with long histories of plant production. They are very common animals. So, it is very likely nematodes are feeding on the crops grown on your farm or in the garden, lawn, greenhouse, etc.

The Microbiology and Societal Context



The microbiology: plant disease. *Sustainability issues:* food crop yields and food security; soil carbon metabolism and global warming.

Nematodes that Eat Plants

1. ***Nematodes, I've Never Heard of Them!*** If this statement applies to you, don't feel bad because it's true of most people. Even my wife dislikes telling people what I do because it all seems quite bizarre or esoteric. However, what I find amusing is that many children have heard of nematodes where adults have not. Fans of the "SpongeBob SquarePants" cartoons are somewhat familiar with nematodes as very thirsty and hungry animals. https://www.youtube.com/watch?v=lCoq4DFlSaU.

2. ***They live in damp places.*** Nematodes are members of the animal kingdom and are non-segmented roundworms. Zoologists place them in their own major group (Phylum Nematoda) and they share some characteristics with flatworms, earthworms (annelids, the true segmented worms) and arthropods. They possess most of the same systems as humans except for a circulatory system. Since our circulatory system plays a major role in delivering oxygen to our tissues and nematodes lack one, the oxygen they use must diffuse through their skins (the outer most surface of a nematode is known as its cuticle, and it must be shed for a nematode to grow). Since oxygen does not diffuse through tissues as easily as it does air or water, nematodes must be long and thin so that O_2 can reach their internal organs. Since nematodes are quite susceptible to desiccation (any animals that obtain oxygen by diffusion must constantly keep their surfaces wet in order to respire), they are usually found in aquatic environments. Lakes, oceans, ponds, rivers, streams and soil are great environments for nematodes.

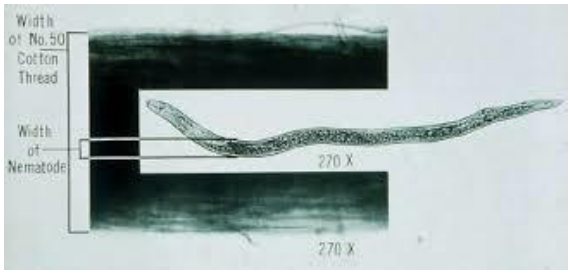
3. ***They exploit active and passive modes of dispersal.*** Since nematodes are worms, they lack legs and wings. They possess muscles, so they can move on their own often in a snake-like (serpentine) fashion. However, long distance dispersal is an issue for nematodes so some use other animals to move them around or in the case of plant-parasitic nematodes, they are often transported long distances in their hosts or in soil. Since they can't be seen, gardeners often purchase plants with unwelcome guests if the propagators of those plants ignored nematodes. As I often tell my children, "Ignoring a problem usually doesn't make it go away." If you purchase nematode-infested transplants, a problem someone else ignored can become your headache.

4. ***There are lots of them around!*** One fact about nematodes that the population at large is unaware of is they are the most abundant or prominent animals on our planet. Scientists currently estimate that 7 to 8 out of 10 animals on Earth are nematodes. Some estimates of nematode abundance include 9 billion per acre of good farmland, 20 million in one square meter of marine sediment and 90,000 in one rotting apple lying on the soil surface. Most people accept the fact that insects are probably the most abundant animals on Earth but although there are far more insect species known than nematodes, the worms have one big advantage. Nematodes survive very well in marine environments, whereas insects do not (except for a couple of species, insects are not truly marine), and we all know that saltwater covers approximately 70% of our planet.

5. ***Nematodes are highly diverse: some are parasites of plants and animals, whereas others are beneficial.*** Nematologists have described about 25,000 species to date and roughly 15% of them are plant parasites. Approximately, 50% are parasites of animals including invertebrates. One such animal parasitic nematode is the dog heartworm which uses mosquitoes to move from host to host (the mosquitoes in this case are biological vectors or intermediate hosts). The remaining nematodes are beneficial and consume algae, bacteria, fungi, protozoans as well as other nematodes. They play integral roles in aquatic and soil food webs and evidence suggests the bacterial-feeding nematodes found in the soil are important in plant nutrition due to their abilities to mineralize the elements found in their food and release them on the surfaces of plant roots, so they are readily accessible to plants.

6. ***Plant-Parasitic Nematodes.*** All plant-parasitic nematodes share three common characteristics.

a. They are all microscopic, the longest being about 8 mm (*ca.* 1/3 inch) in length.



A female root-lesion nematode, *Pratylenchus* sp., superimposed over a cotton thread to illustrate the small size of the worm. Taken from the slide collection of Dr. George Bird, Michigan State University.

b. They all possess stylets. A stylet is an apparatus found in the head of a nematode used to puncture plant cells and to obtain nutrients.

c. They are all *obligate* parasites of plants, meaning they require living hosts on which to feed so they can grow and reproduce.

As a caveat, all plant-parasitic nematodes possess stylets but not all stylet-bearing nematodes are plant parasites.

The cephalic region of a lance nematode. The stylet is conspicuous.
Photo presumably taken by H. H. Lyon, Cornell University.

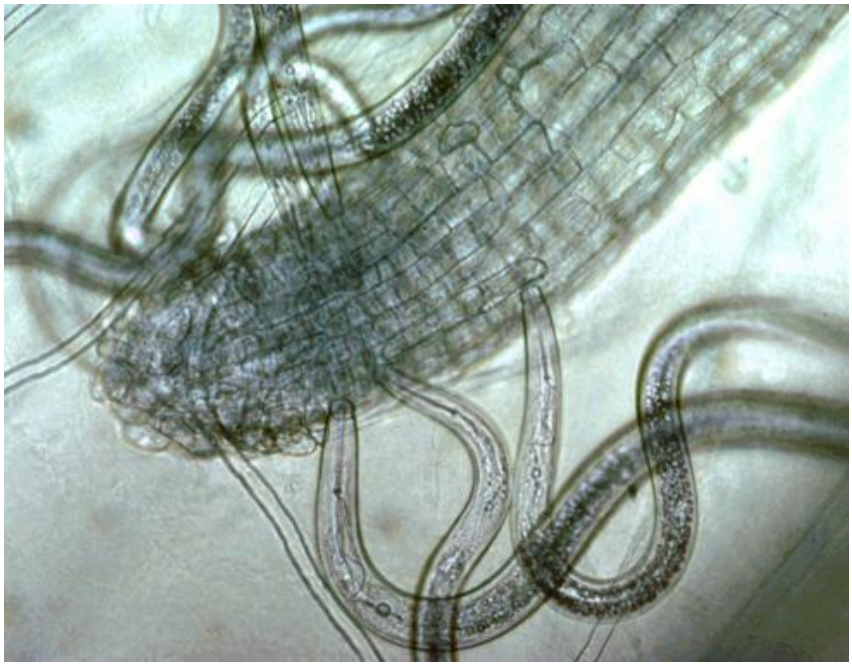
Plant-parasitic nematodes are important in Plant Pathology for three major reasons. Firstly, they directly cause infectious diseases of plants. Secondly, some nematodes can serve as

predisposition agents, providing infection courts (wounds) for other invading plant pathogens with which they cooperate. Since many plant-pathogenic bacteria and fungi occur in the soil and invade roots, I believe this is a seriously underrated aspect of the importance of nematodes in agriculture. The third is that some nematodes can transmit viruses, like mosquitoes vector dog heartworms.

7. ***Parasitic styles.*** Plant-parasitic nematodes exhibit different parasitic habits. Most species (*ca.* 95%) feed on or within roots, but some feed on leaves or modified leaf tissues. The foliar-feeding nematodes are a scourge of glasshouse and greenhouse growers, but these nematodes do exist and prosper outdoors. Nematodes that feed within plant tissues are separated into two primary groups as either migratory endoparasites or sedentary endoparasites. Migratory endoparasitic nematodes penetrate tissues and migrate throughout them as they feed and lay eggs. These nematodes kill cells in their wake.

In contrast, sedentary endoparasitic nematodes remain sessile within their hosts after they establish feeding sites. In a weird twist, the nematodes sort of hijack their hosts so that the plants provide specialized cells to keep the nematodes alive. If the host dies, any sedentary endoparasitic nematodes die along with it because they are "trapped" inside. It is important to keep in mind the goal of most parasites is to keep their hosts alive. Some parasites, in animals, including humans, are known to work in concert with their hosts to modulate their immune systems so both the host and parasites survive for extended periods of time (up to 30 years or more).

The third group of nematodes feed as ectoparasites. With ectoparasites, the bodies of the nematodes remain outside the host, only their stylets are inserted into tissues. Most plant-parasitic nematodes feed as ectoparasites. All the plant-parasitic nematodes that vector plant viruses are ectoparasites. Also, almost 100% of plant-parasitic nematodes spend at least part of their life histories in the soil.



Sting nematodes, *Belonolaimus longicaudatus*. Sting nematodes are ectoparasites.

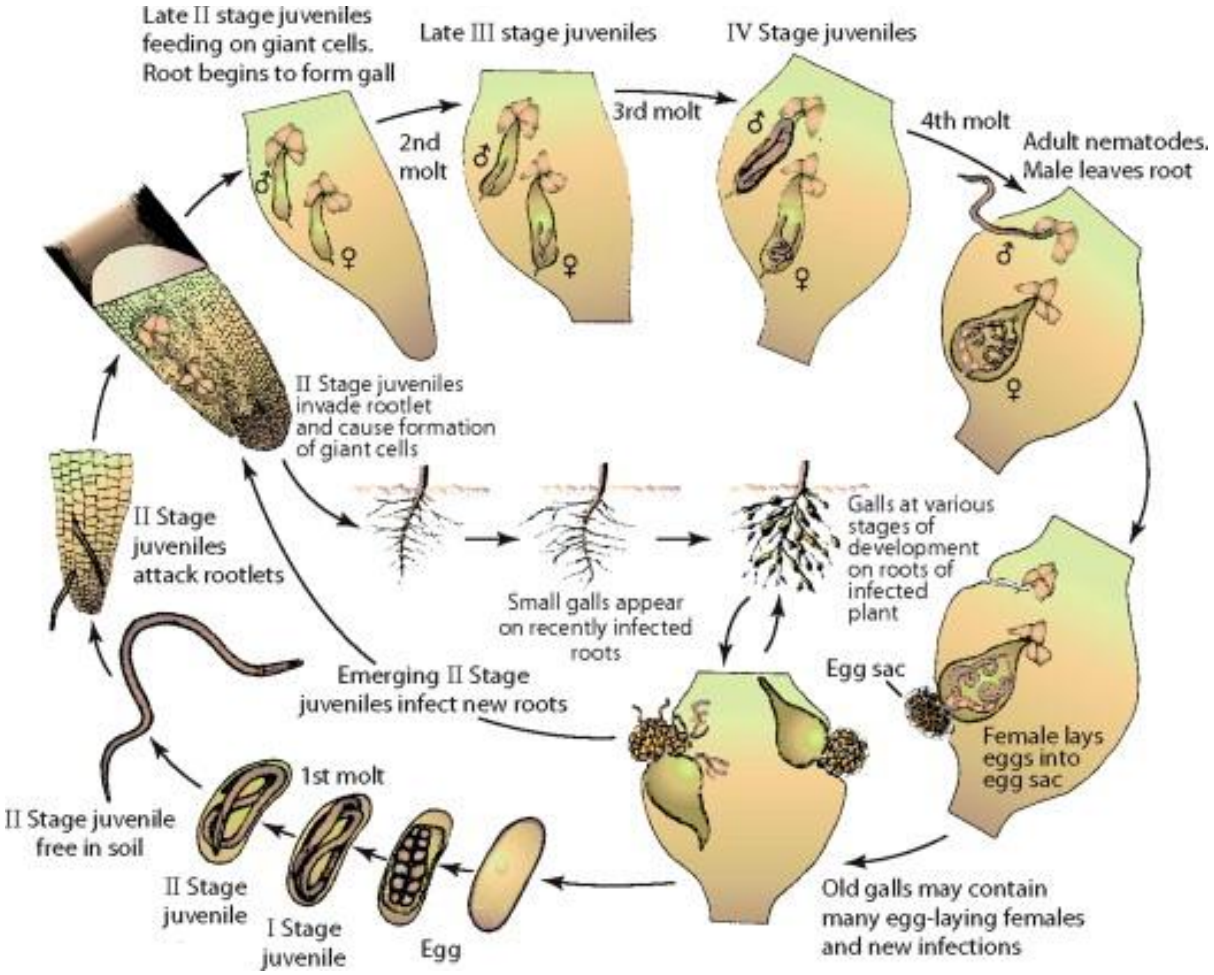
Photo by UC Riverside, Center for Invasive Species Research.

8. ***Symptoms of disease.*** As mentioned earlier, every species of plant produced for fiber or food has at least one nematode parasite. Some plant species are far more susceptible to nematode feeding than others and differences within cultivars of plants exist. Parasitism by root feeding nematodes often results in the production of no characteristic above ground symptoms. Feeding by nematodes can result in chlorotic (yellowing), stunted or wilted plants. Yields of agricultural crops are reduced and 100% crop loss can occur. Symptoms as the result of nematode feeding resemble those caused by many abiotic or biotic stressors. Since infections by nematodes often result in plants with reduced root volumes or weights, it makes sense that plants wilt or exhibit symptoms of nutrient deficiencies, since these plants don't compete well for water or soil nutrients. For this reason, among others, nematode problems often go undiagnosed. As a Nematologist, I believe people (even farmers, physicians and veterinarians) have some inherent biases against nematodes and some of that bias is due to a lack of knowledge and understanding of these important animal and plant parasites. For instance, when was the last time someone shared a story about nematodes on Facebook or you saw something in the popular press or heard a tidbit on the news? Have you ever heard a story about a mass migration of nematodes? I'd venture to say never or close to it. Toss in the fact that nematodes are the most abundant animals on Earth, it's almost mind boggling to me most people are completely unaware of their existence.

9. ***Root-knot nematodes (Meloidogyne spp.) are the most important plant-parasitic nematodes on a Worldwide basis.*** These nematodes account for roughly a quarter of all the crop loss caused by plant-parasitic nematodes. There are 80 – 90 described species but there are four major ones. I can't think of any plant species that are not touched by at least one species of root-knot nematode. For instance, coffee is reported to be a host to 17 species of root-knots. Root-knot nematodes tend to be subtropical or tropical in their distributions. For example, in Michigan we have a temperate climate and routinely only encounter two species of root-knot nematodes, although we do have a root-knot nematode "dinosaur." This nematode is the Michigan grape root-knot nematode, *Meloidogyne nataliei*. Its current known Worldwide distribution is two townships in Southwest MI. Its fossil remains were discovered in 1977 (just joking about the fossil remains part).

10. ***Root-knot nematode life cycle and sex reversal.*** Root-knot nematodes are sedentary endoparasites. A young nematode hatches from an egg present in the soil (there are four pre-adult stages known as juveniles, the first molt occurs in the egg and a second-stage juvenile emerges), locates a root and penetrates it. These nematodes are attracted to a region of a root known as the zone of elongation, an area in which the vascular tissue (phloem and xylem) has not yet differentiated. A video showing the penetration of the roots of *Arabidopsis thaliana* by the Southern root-knot nematode, *M. incognita*, can be found here https://www.youtube.com/watch?v=BTXW7OHxL-Q. The nematode begins to feed in pro-vascular tissue on specialized cells called giant cells produced by its host. If the nematode cannot initiate this response within the host, it dies. As the infected roots begins to grow, the nematode is now embedded within the vascular tissue of the plant and can rob its host of the sugars it produces through photosynthesis. The juvenile nematode swells as it feeds. If the nematode is a female, she becomes balloon-shaped and lays eggs (often around 500) in a gelatinous material she

produces on the surface of her body. If the juvenile nematode is a male, it is worm-shaped, exits the root so he can mate with females. Female root-knot nematodes are gluttons, but males do not feed. In an amazing ability to communicate within invertebrates, root-knot nematodes can regulate their own populations so as to not to overburden their hosts and kill them. They do this by sex reversal. Juvenile nematodes, genetically programmed to be females, sex reverse and become males. Since males don't feed, the plant gets to keep more of its sugars. How do Nematologists know this has happened? Sex-reversed males have two testes whereas "normal" males (no sex reversal) have one. Female root-knots have two ovaries. Almost, mind blowing, I contend! Can you think of any other animals that can change their sex? Fans of the *Finding Nemo* movie might have a clue.



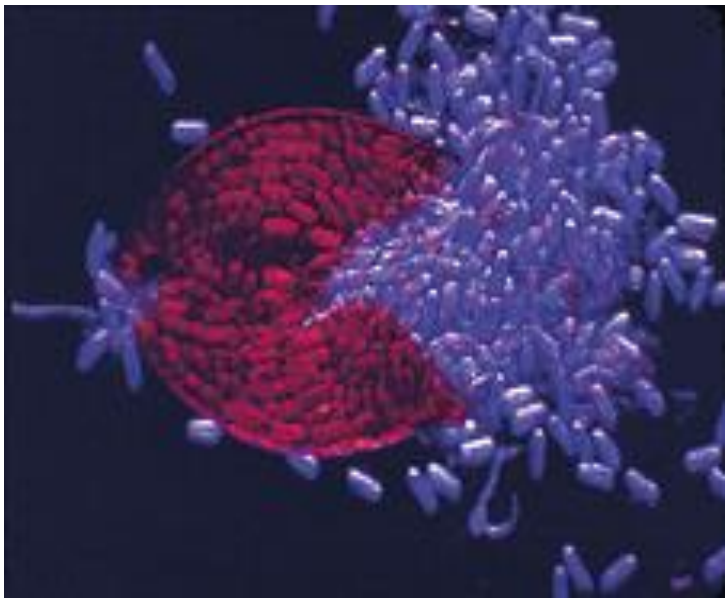
Life cycle of root-knot nematodes, *Meloidogyne* sp. The life cycle of a plant-parasitic nematode typically begin with an egg, followed by 4 juvenile stages (pre-adults) and a female or male. This image was taken from APS.net.

11. ***Host plant protection of pregnant female nematodes.*** The host also provides another service to nematodes. As root-knot nematodes feed, knots or galls are produced which encase the swollen females providing them some protection from their enemies that exist in the soil. Therefore, a characteristic symptom of a root-knot infestation is the presence of galls on roots. The size of the galls will vary depending on the species of root-knot nematode and the number

of nematodes within each gall. Since the second-stage juveniles are attracted to a specific region of a root, it makes sense that nematodes become somewhat clustered. Knotted roots do not compete well especially for water and root-knot nematode-infested plants often seem "starved" for water.

12. ***Cyst nematodes.*** Another group of destructive plant-parasitic nematodes are the cysts. There are multiple genera of cyst nematodes but the two most important are the *Globodera* spp. and the *Heterodera* spp. The golden cyst nematode, *G. rostochiensis*, is the most regulated of all plant-parasitic nematodes, as over 100 countries enacted quarantines aimed at this important pathogen of potato. Containment of this nematode to upstate New York in the 1900s may represent the most successful regulatory program in the history of U.S. agriculture.

Like root-knots, cyst nematodes are sedentary endoparasites. The life cycles of the two groups are somewhat similar but feeding by cyst nematodes does not result in the formation of galls on roots. Cyst nematodes get their name from the fact that the cuticles of the females become hardened when their bodies are filled with eggs so that they form "protective houses" for the developing eggs and juveniles (pre-adults).



A cyst broken open to reveal hundreds of eggs.
This image was taken from the slide collection of Dr. George Bird, Michigan State University.

Cyst nematodes are formidable opponents for three primary reasons:

a. they are destructive parasites and cause serious crop losses;

b. they have high fecundity rates as each female can produce several hundreds of eggs depending on the species, and

c. cysts can lie dormant in the soil for a decade or slightly longer in the absence of host crops.

I frequently tell growers, when talking to them about cyst nematodes, that if their fields become infested with them, they are going to have to learn how to maximize yields in the presence

of these worms. For the most part, eradication is not a reality. Cyst nematodes appear better suited to survive in temperate climates than most species of root-knots.

13. ***SCN: the soybean destroyer!*** The most notable species of *Heterodera* is probably *H. glycines*, commonly known as the soybean cyst nematode (SCN). It is regarded as the most serious pathogen of soybeans anywhere it exists, and in just the U.S. alone causes crop losses in excess of two billion dollars a year. This species originated in Asia and has spread to all the major soybean producing areas of the World. SCN feeds on other crops too (mostly in the bean family) but soybean is its most important host. Farmers ignore SCN at their own peril. If they choose not to control SCN, they typically will lose 50% or more of their soybean yields in heavily infested fields. For much more information on SCN and some educational videos, please visit the following website, https://www.thescncoalition.com/lets-talk-todes/research-collection. This YouTube video also provides some pertinent information on SCN accompanied by some music https://www.youtube.com/watch?v=G1IKzS7hw8Q.

14. ***Geographic preferences.*** Estimates suggest there are approximately 200 genera and 4300 species of plant-parasitic nematodes. It shouldn't come as a surprise that some species are important in some geographic regions but not others, reflecting temperature (nematodes are cold-blooded invertebrates) and host species. For instance, virulent nematodes of rice are really of no concern to growers in regions like MI where we don't grow the crop.

15. ***How do we know if we have nematodes?*** Since plant-parasitic nematodes are microscopic animals, to detect their presence and quantify them, collection of plant tissues and soil is necessary. Sampling for nematodes usually falls under two objectives, problem avoidance and problem diagnosis. After the collection of samples, they should be delivered or sent to any local Plant Diagnostic Lab(s) with a Nematologist on staff, such as the one we have here at Michigan State University.

Plant producers should take steps to avoid nematodes. Since they are the unseen enemy, this is easier said than done. Fields should always be sampled for nematodes before sowing any susceptible annual crops or planting any perennial ones. If chemical control is an option, it needs to be understood that many nematicides (substances that kill nematodes) are often toxic to plants and thus cannot be applied to existing crops. In addition, the numbers of nematicides are limited and for some crops, none are registered for use. If a farmer plants a vineyard, for example, and learns he/she has a nematode problem 3 or 4 years after planting I often ask, "The horse is now way out in the pasture and now you want to close the barn door?" The window for controlling nematodes may have already shut.

Often symptoms of undetermined cause(s) become evident over the course of a growing season. To ascertain or rule out nematodes as the causal agents it is necessary to collect samples. Plant-parasitic nematode numbers are typically highest in the margins of diseased areas especially if some of the plants in the center of these areas are dying. I typically recommend the collection of two samples, one from diseased areas and one from apparent healthy areas for comparison purposes.

Plant-parasitic nematodes tend to be clumped in their distributions, so the symptoms caused by their feeding tend to be in clumps. When crops are present and symptoms are evident,

locating these spots is often quite easy. However, the same cannot be said after plants have been removed from fields. Many scientific articles exist detailing sampling for nematodes. Sampling and interpreting results can be the source of frustration for plant producers. A tremendous amount of variability is usually the result of multiple samples taken from nematode-infested locations. I will not cover any of this in any more detail.

16. ***Oops, we have a Nematode Problem. What do we do?*** Don't fret, you're not the first or the last to learn you have damaging levels of plant-parasitic nematodes. I always like to tell my clients to be informed and learn as much as possible about the nematodes present in their fields. Plant-parasitic nematodes differ tremendously in their virulence (the amount of damage they do), so focus your efforts on the more destructive species (genera). If you want to maximize profits and optimize plant health, it is often necessary to focus on the major limiting factors in a crop's production.

If you need to reduce population densities (numbers) of damaging species of nematodes, there are a six main tactics you can employ: biological, biotechnological, chemical, cultural, genetic and physical are the primary controls.

Some tactics are more sustainable than others. For instance, cultural controls are very effective against most nematodes, especially those with limited host ranges. Rotations including non-host crops will reduce population densities of most nematodes. However, to effectively utilize crop rotations, it is important to understand the host preferences of the nematodes on your farm. Unfortunately, some nematodes have extremely wide host ranges exceeding 300 – 400 species of plants, so these pathogens are difficult to control with rotations alone.

Genetic controls typically consist of using cultivars/varieties of plants with resistance to certain species of plant-parasitic nematodes. Resistance is often used against cyst and root-knot nematodes. Resistance is often effective, but nematodes often overcome it, so it's not a cure all forever.

My best advice is, if do learn you have a nematode problem, consult local experts. In our lab, in addition to diagnosing and identifying nematode problems, we also advise our clients on what we believe are the optimal tactics for management. Not all labs function as such. Some will just return numbers, leaving you to research how to control the nematodes in question.

17. ***I'm Intrigued, I Want to see Some Nematodes!*** Well, if you haven't ascertained it by now, you're going to need a microscope. I can identify plant-parasitic nematodes to genus using 40X magnification but then as I joke with my students, "I'm a trained expert. It takes 8 or 9 hours to get to my level" To see nematodes in detail, 100 – 1000X is necessary. I use a dissecting scope to count and identify nematodes to genus. If I need to identify any to species, I move them to a compound light microscope and view them at 400, 1000 or 1500X. We also use molecular procedures in our lab to identify nematodes to species.

To see nematodes, it is necessary to extract them from plant tissues or soil. In our lab, to remove nematodes from the soil, we use sieves and a procedure called centrifugation/flotation. The University of Florida has a video demonstrating this process https://www.youtube.com/watch?v=x_X08MdV5qk. However, nematodes can be extracted from soil inexpensively using Baermann pans. To construct Baermann pans, collect two pie pans or tins and cut the bottom out of one. Place the cut one inside the intact one with a section of screen

over it (use flexible screen so it molds to the contour of the pie pan). Place two tissue papers on the screen and pour some soil on the tissues. Fill the bottom pan with enough water that it touches the tissues. Fold the tissues over the soil and place the pans somewhere for 48 - 72 hours. Remove the top pan with the screen and pour the liquid into a beaker or any container that holds water. Allow the nematodes to settle to the bottom and remove some using a pipette. Put two to four drops on a microscope slide and look for nematodes. Chances are, you'll have an "ah ha" moment watching them swim.

18. ***Not all nematodes are microscopic.*** As a final note, although most, if not all, soil inhabiting nematodes are microscopic, there are many animal-parasitic nematodes that can be seen with the naked eye. Since most of these remain within their hosts and just pass their eggs, you will never see them unless you're observing a necropsy in a veterinary clinic or hospital. As a young impressionable scientist, I witnessed many necropsies in the veterinary clinic in which I was employed and saw many canine hearts full of heartworms. The adults were clearly visible to me as they measure 12 – 14 inches (25 – 30 cm) in length. However, on occasion, if you have a pet dog infected with roundworms (ascarids), they may pass some in their vomit. Gross.

19. ***Our learned society.*** The Society of Nematologists is a group whose members primarily conduct research on plant-parasitic nematodes. The address for the SON website is https://nematologists.org/.

20. ***Join us!*** The fields of Nematology and Parasitology are always in need of some expertise. I find parasites quite fascinating. I hope you found this article informative and somewhat enjoyable to read. Do you think a career in Nematology might be of interest to you?

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 2. End hunger.** Arable land on our planet is finite, so plant producers are going to need to maximize productivity on that land in order to feed and clothe (cotton and hemp) a growing population. Since plant-parasitic nematodes reduce crop yields, minimizing their impacts will result in increased productivity. The goal is to do it with minimal chemical inputs because many nematicides (chemicals that kill nematodes) are often highly toxic, persist in the soil and are environmental (groundwater contamination) and human health (carcinogens) risks.

On the other hand, most nematodes present in high quality soils feed on bacteria and fungi. The feeding by bacterial-feeding nematodes results in the recycling of nutrients, particularly nitrogen as the waste products of nematodes are ammonia and, to a lesser extent, urea (there are others). Both ammonia and urea can be utilized by plants for growth and bacterial-feeding nematodes excrete these substances on the surfaces of roots where bacteria often colonize. Steps should be taken to increase the numbers of these types of nematodes in the soil if possible.

The above will require an understanding of the biology and ecology of these nematodes. What conditions favor them to flourish? As plant producers, what steps can be taken to augment them in soil? Nematologists have made tremendous strides in the past 50 or so years understanding the impacts of these nematodes but much more needs to be learned in order to

harness their abilities to fertilize plants naturally. The training and hiring of more nematologists are essential for education and research.

- **Goal 13. Combat climate change.** The release of carbon into the atmosphere from the soil contributes to the warming of the planet. Nematodes are the most abundant animals in most soils, so it seems obvious they play a role in soil food webs through the impacts of their interactions with plants and other organisms. Disturbance of the soil through cultivation and tillage often results in the release of carbon dioxide into the atmosphere. As farmers increasingly adopt no-till and reduced tillage practices, they will depend more on the naturally occurring soil organisms to maintain productive soils, especially if these practices are coupled with reduced synthetic fertilizer and pesticide inputs. It will be increasingly important to understand the roles all nematodes play in the soil ecosystem if farmers implement more sustainable farming practices by depending less on fossil fuels.

And: carbon sequestration by soils is presently a topic of considerable interest in seeking solutions to global warming. Is it possible soil-inhabiting nematodes play a role (primary, secondary, tertiary) in the removal of CO2 from the atmosphere?