

What Kind of a Toad is a Nematode?

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That was a question that I really was asked years ago by a lawyer who, to his credit, was trying to make polite conversation while trapped at his wife's Paulson family reunion. How would you have answered that question? Would it have been sufficient to have stopped after saying something about the large roundworms that parasitize insects and animals? Would Jerry have been interested in hearing that nematodes are really very abundant organisms occupying second place after insects in terms of the number of different kinds? Could I have kept his attention long enough to tell him that if the physical and all other biological characteristics except nematodes of Planet Earth were to disappear, that an image of the planet would still be present? That image would be composed of nematodes had inhabited the seas, fresh waters, other organisms including plants, and soils of the planet. Would he have been interested in learning that one type of plant nematode, the root-knot nematode, has been identified as being one of the top five pathogens that is limiting food production at least in the warmer parts of the world? Well, I suspect that any response of any substance would just have elicited a big yawn from Jerry.

Nematodes have been and remain pretty much "second class pathogens" even in Departments of Plant Pathology and certainly with northern golf course superintendents for whom the fungi have typically been considered to be the most important causes of plant diseases. So why did the University of Minnesota choose to hire its first plant nematologist rather than a turfgrass pathologist back in the 1950's? There weren't any plant diseases in the 1950's in Minnesota that were recognized as being caused by plant nematodes! And there would not be any until 1978 when the presence of the soybean cyst nematode in the state was finally recognized. The answer to that question at that time was based primarily on the premise that every well-rounded graduate of the U's Plant Pathology Department really needed to have had the opportunity to receive hands-on instruction in the relatively new academic area of plant nematology. And by hiring someone who had

been trained in plant nematology and who could provide that kind of instruction it was assumed that that person would also eventually uncover some aspect of the subject that would be an appropriate area for study even here in Minnesota where plant nematodes really were believed to be unimportant. In Chapter II you will see how that belief helped provide some of the motivation that got Dr. White and me started with our studies dealing with turfgrass nematodes. And the answer to the second question is related to a recurring theme that permeates these "What Kind of a Toad is a Nematode?" discussions.

Compared to the present, it seemed like it was easier to take care of turfgrasses back then in the 1950's, 60', and 70's when the need to achieve adequate green speed was not such a big factor and mercury compounds took care of the snow molds and perhaps other fungi as well. The question about why a Turfgrass Pathologist was not hired at that time is at least partially answered at this point in Chapter I because that answer hopefully describes the situation in the 1950s and beyond that opens the door for us to try to build a case for a special kind of worm, the plant parasitic nematode. And finally, why did two members of the MGCSA give us the opportunity to fill more than a few pages of *Hole Notes* with information about "worms"? Has something pertaining to or affecting plant nematodes changed or is something possibly changing?

Plant nematodes are non-segmented roundworms totally unrelated to "toads."

They have inhabited the soils of our planet for a very long time. This past spring a former student took the time to contact me to say that a program recently shown on Public Television contained a segment dealing with plant parasitic nematodes that had been trapped and preserved in plant resins some 40 million years ago. We can recognize a nematode that has or had the potential to be able to feed on living plant cells because it has a protrusible and typically hollow ("a micro-hypodermic needle") mouth spear or stylet. (Figure 1) Presumably that is what the student saw or possibly even what the narrator tried to call the viewers' attention to in that PBS presentation. Not all nematodes that pos-

sess stylets are plant parasites but many that do have stylets must feed on living plant cells. The anterior portion of the nematode, officially its stoma, that is "armed" with a stylet is said to exhibit the greatest amount of morphological diversity. Stylets may be "short", "long" or something in between.

The nematode pictured as Figure 1, which would be much more likely to be present around the roots of woody plants in foundation plantings around the clubhouse than it would in a golf green, would be described as having a long stylet. Stylets may also be "strong", "weak" or something in between. The plant nematode that is the most often found in Minnesota's golf greens is commonly called the "stunt" nematode even though it has a moderately weak stylet (Figure 2). The vast majority of the plant nematodes have stylets that terminate in three enlargements called knobs. Muscles attached to the stylet knobs enable the organism to protract and retract its stylet. The nematode pictured as Figure 1 has rather prominent stylet knobs while the stunt nematode (Figure 2) has somewhat weaker ones. A person who identifies and counts the plant nematodes that have been separated from a soil sample will use stylet shape and size to help in the identification process. And although the ability of a plant nematode to mechanically and perhaps biochemically penetrate plant cell walls and plant membranes is essential to its survival, there does not seem to be much of a relationship between the "rigor" of a nematode's stylet and its ability to cause plant disease. The stunt nematode (Figure 2) with its rather weak stylet

Figure 1

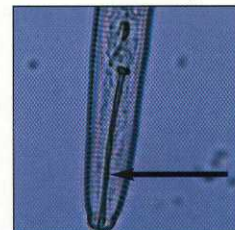
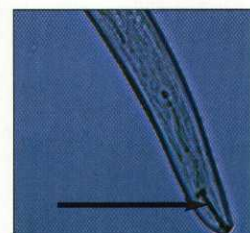


Figure 2



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may be able, according to Nelson (1995), to damage cool-season turfgrasses when there are 300 or more of them present in about four-fifths of a cup (100 cm³) of soil. But 1,500 ring nematodes despite their rather impressive stylets and larger body diameter, need to be present in 100 cm³ of soil before they can damage cool-season turfgrasses. Or, in other words, a plant nematode has to have something else going for it before it can cause plant disease. And that "something else going for it" is the "chemistry lab" that is part of the nematode's pharynx or esophagus. The pharynx or esophagus (Figure 2) occupies the remainder of the clear area of the nematode's body posterior to the stylet knobs and anterior to the typically dark and granular-appearing intestine. A gland or perhaps more than one gland produce secretions that are introduced into plant cells through the stylet that can alter

the functioning or even cause the death of those cells. And if the mechanical capacity of the organism's stylet coupled with its even more important biochemical potential were not enough to be able to cause plants to "become sick", some authors even call our attention to the ability of some plant nematodes to release waste products that can elicit unwanted plant growth responses. I know some of you who are reading this article well enough that I can almost hear you saying: "Oh, come on! I have never, ever seen bentgrass, *Poa annua*, or any other kind of

grass plant that was SICK due to the activities of plant nematodes! And furthermore, I don't expect to ever see such plants!" Okay, let's end this paragraph by rephrasing that "sick bit" for bentgrass and *Poa annua* by asking you to accept the fact that plant nematodes are one of the important reasons why plants can not live up to their genetic potential or, more simply, why plants can be stunted. But what about the corn plants visible in Figure 3. You would not have to wander too far on to the Anoka Sand Plain in May or June of most years to find grass (corn) plants that are "sick" due to the feeding of plant parasitic nematodes! And while we are concentrating on those sick plants, please note that healthy plants can be present right next to the "sick" ones. The needle nematode, which "all by itself" was the cause of the "sick" corn plants pictured in Figure 3, feeds on the root tips of seedling plants causing characteristic "stubby-root" symptoms (Figure 4). Plant nematodes range in size from about 0.3 mm (.01") to a maximum of 5 mm (0.2") in length. The vast majority of them remain wormlike through their entire 30 day life cycle. A thirty day life-cycle is just a starting point. During a beast of a summer when you may be having an awful time even maintaining somewhat healthy grass, the average plant nematode is probably in its ideal environment being more active, feeding more intensively, and with its life-cycle shorter than it would be during a summer when "growing grass is just a piece of cake." Their body diameters are measured in microns (1/1000 of a millimeter) making them essentially microscopic even when suspended in clear water where they will appear somewhat analogous to "flecks of dust". Some important plant nematodes like the cyst and root-knot become sedentary and enlarge. The living female cyst nematode breaks through the epidermis of an infected root becoming

visible as a pin-head size lemon-shaped white body (Figure 5: five of them attached to a rotten soybean root are visible). The cyst nematodes, one species of which has been considered to be one of the most significant pathogens of soybean, are not of significance as parasites of turfgrasses in the Minnesota-Western Wisconsin environment. Although the root-knot nematode (Figure 6: the forked carrot root with the little galls or knots is diagnostic for the presence of the Northern root-knot nematode) is not one of our most common nematode turfgrass pathogens, it may be one of the more damaging. The female nematodes are present in the galls or knots. And those succulent galls that are stimulated to form by the nematode are very suitable for colonization by other kinds of soil-inhabiting organisms. If our ideas about the significance of plant nematodes on northern golf courses are in fact changing, then it is, I believe, appropriate to recognize that root-knot and other plant nematodes can and do interact with other causes of plant disease to facilitate the development of those diseases. But how can anything that small that takes so long to complete its life-cycle be significant to you, a plant person, if not to Jerry, the criminal

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Figure 3



Figure 4



163 Yard Par 3 eighth hole at the Refuge Golf Club in Oak Grove, Minnesota.



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lawyer? I would like you to consider plant nematodes to be just one more stress to which plants are exposed. A plant nematode is of interest to a plant nematologist because of how and where it feeds and what those physical and biochemical activities do to the plant root cells that provide the nutrients that sustain it. But individually their effects are usually not too impressive. Yes, one root-knot nematode can conceivably cause a carrot or parsnip's taproot to fork. And the feeding of one needle nematode can conceivably cause a corn seedling to develop a "stubby-root condition" (Figures 3 and 4). But usually, as indicated by the threshold for damage populations of stunt and ring nematodes, plant nematodes need to be abundant. If it were a straw that broke the camel's back, then maybe enough plant nematodes can combine with other stresses to adversely affect the growth of turfgrasses. And for reasons that are not at all clear at this time, the nematode "straws" that can possibly break the "back" of the perennial bentgrass and the *Poa annua* can become very numerous in some

greens or just portions of some greens. Fred Taylor, the superintendent at Mankato Golf Club, has been interested in plant nematodes for the past three seasons. He initially became interested in them because the technician at a laboratory in New York where Fred had sent a sample to have a root-rotting fungus identified remarked that the soil contained a lot of nematodes and that maybe Fred should have those nematodes identified. He accepted that advice and had our lab and another laboratory in Florida confirm that stunt nematodes were indeed present and possibly significant at the Mankato Golf Club. In October, 2005, he sampled three of the Mankato Golf Club's greens intensively by collecting soil samples from a total of 19 locations on those greens. It turned out that each of those locations contained an average of 994-1527 plant nematodes/100 cm³ (4/5th cup) of soil. So, you say, what do such numbers possibly mean? Well, how about expressing those population estimates in another way? What if an influential member of Mankato Golf Club's Greens Committee wearing size 12 golf shoes that are about 30 cm long and 9 cm wide is standing on a spot where Superintendent Taylor sam-

pled last fall and where one of those "average" (1260/100 cm³) root-zone popu-



lations of nematodes existed? If the rooting depths of the turfgrasses growing at that location were 5 cm, then the golfer is standing on 2,700 cm³ of nematode habitat. And that "prime" plant nematode habitat contains about 33,000 plant nematodes that are all trying to feed on any available and suitable grass root epidermal and cortical cells. Luckily plant nematodes are too small and too weak to be able to move soil particles. What kinds of complaints would Fred Taylor get if our influential golfer with the size 12 shoes three-putted that hole because of the wave-like undulations of the putting surface caused by the movements of all of those plant nematodes? The plant nematodes in the root-zone "horizon" actually often represent only a part of the nematode population that is present there. Nematodes that feed on

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bacteria, fungi, and various soil animals including other nematodes often are even more abundant in the root-zone and typically more active than are the plant parasites.

I would like to use the title of an article that appeared in a gardening magazine many years ago to try to introduce a concept that I think is quite important to remember about plant nematodes. The title of that article went something like this: "If you do everything right and still your garden fails you, maybe its nematodes!" That author did not say: "If you do everything right and still your garden fails you, well then nematodes are what ails it!" Soil samples have to be collected, processed, and the plant nematode populations described before one should even begin to think about making such a statement. Sampling for plant nematodes might be considered by some to be both an art and a science. At any rate, quite a bit was learned as the result of the sampling efforts that eight different superintendents made last autumn. What they did and the information that was gained as the result of their efforts seem to fit best in Part III of "What Kind of a Toad is a Nematode?" Please look there for our thoughts about that important aspect of plant nematology.

It seems appropriate to repeat a true "Once Upon a Time" story at this point in an effort to introduce you to some of the characteristics of the procedures that are used in nematology laboratories or plant disease clinics to separate plant nematodes from soil. We received a soil sample at St. Paul from an Iowa agronomist who asked that its plant nematode content be determined. This was done and our estimate of the plant nematode population that it contained was sent to him. Not many days later we received a rather critical response back from that agronomist who pointedly told us that he did not have very much faith in what we were doing in our "Ivory Tower" at St. Paul. He explained that we had received only half of the soil sample that he had collected. The other and, in his mind, identical soil sample had been sent to another plant nematology laboratory or plant disease clinic. He claimed that the two labs had sent him very different plant nematode population estimates both in terms of kinds of nematodes that were present and as well as in numbers. Why, he asked, should he believe an apparently erroneous



Ms. Amy Holm, chief diagnostician for the U of M's Plant Disease Clinic.

laboratory report from Minnesota when the report from the lab in Iowa was so different (and undoubtedly more accurate)? I tried to explain to him that there are two main ways of separating nematodes from soil. I went on to state that there is no one best method for separating all of the different kinds of plant nematodes from soil. We used one method that we had found through testing to be best for separating the nematodes that we routinely encountered in Minnesota's soils. It is quite likely that the other lab used a very different procedure. And probably the two laboratories processed different volumes of soil. I tried to convince him that both reports were accurate and informative when each was evaluated on the basis of how the sample was processed.

But let's return to the basics. Most of the nematode population data that are reported in the three chapters of this article were obtained by using an "active" method to separate nematodes from soil. A nematode that is successfully going to exit a soil sample has to be able to move if the extraction method is an "active one". The plant nematology laboratory and the Plant Disease Clinic at the U of M at St. Paul use the Cornell pan (piepan) modification of an earlier technique that is known as the Baermann Funnel Procedure. Ms. Amy Holm, chief diagnostician for the University of Minnesota's Plant Disease Clinic, is shown above using the Cornell piepan equipment. The bottom of the top piepan has been removed and replaced with a coarse screen. Three layers of porous filtering materials (milk filter and 2 layers of facial tissue) are placed on the screen. A thin layer of 50 cm-3 of sieved and mixed soil is placed on top of the filtering materials. The modified screen-bottom piepan that contains the filters supporting the soil sample is placed in an intact piepan that contains cool tap water. The layer of soil becomes saturated

and open spaces between soil aggregates and particles are flooded. Plant nematodes are sluggish animals and do not move fast enough to swim. They can move by pressing against solid surfaces. When they reach water-filled spaces they slip, slide, and settle under the influence of gravity. e more active plant nematodes like the stunt nematode (Figure 2) begin to exit the soil layer and pass through the filtering materials into the bottom or intact piepan within about 12 hours after the "system is activated". More sluggish individuals like the ring nematode may take several days to exit the system. Plant nematodes are given a total of 7 days to make their exit from the layer of soil through the layers of filtering materials into the bottom piepan in our laboratory. The liquid in the bottom piepan containing soil-inhabiting nematodes and other animals is then decanted into a beaker where the nematodes are concentrated. I then take a representative 8-milliliter subsample from the liquid in the beaker to provide the nematodes that are to be identified and counted.

A passive method of extraction is used by many nematology laboratories and plant disease clinics. Nematodes contained in a soil sample are suspended in a quite concentrated (about 3/4th pound of sucrose/quart of liquid) sugar solution. Nematodes are only slightly heavier than water and will remain suspended in the sugar solution while heavier soil components may be "pulled down" by centrifugation or by the actions of a flocculating agent. The nematodes that remain in the clear "supernatant" are then separated from that toxic sugar solution (it would plasmolize them if they remained in it too long) by sieving. Sieves that are used to "catch" the plant nematodes have openings of about 35-54 microns. I call these rapid procedures that take only 10-15 minutes to complete, passive methods because dead or inactive as well as live nematodes may be "floated-out" of soil without their having to move at all. There are a number of procedural variations of these two main (active and passive) methods and/or combinations of the two that various nematologists have developed to meet their own particular needs. It is not surprising that different estimates of the kinds and numbers of nematodes that are present in even "identical" soil samples can be obtained when such different procedures are used.

(Editor's Note: Part II of "What Kind of a Toad is a Nematode?" will summarize what we did beginning in 1969 when we first attempted to learn something about the plant nematodes that inhabit Minnesota's golf greens.)