PART II

What Kind of a Toad is a Nematode?

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The study of plant nematodes by members of the Department of Plant Pathology at the U of M in St. Paul officially began in 1956 when Dr. Don Taylor was assigned to lead the effort. During the next several summers he and his students traveled extensively in the state collecting soil samples from around the roots of nine different field crops. The members of his research team identified a number of different kinds of recognized plant parasitic nematodes as well as some that in their opinions were "maybe" parasites. Of those that were commonly accepted at that time as being parasites, members of only 6 different genera were commonly present in the samples that they had collected. The common names of those nematodes are dagger, lance, lesion, pin, spiral, and stunt. All of these with the exception of the dagger will be or have been considered in detail in this series of papers dealing with nematodes that may affect cool season turfgrasses. Taylor's efforts dispelled the commonly-held and partially valid belief that plant nematodes could not tolerate the low temperatures to which they would be exposed when snow cover was minimal and Minnesota's soils froze deeply. Such conditions are definitely trying and for some plant nematodes at least only the most resistant stages, perhaps the egg or the adult female, actually do survive.

Efforts to prove that plant parasitic nematodes could do more than just feed on plants and could actually cause plant disease began shortly after the survey phase was completed. Data documenting the ability of Minnesota's indigenous plant nematodes to cause significant losses have been hard to obtain. Progress has been slow because plant nematodes often need the help of a combination of stresses including a crop-hostile environment in order to cause disease. For example, lesion nematodes are often abundant in the fertile corn-soybean soils of Southern Minnesota. If the nematodes are abundant beginning early in the season and the growing season is dry, then those lesion nematodes can reduce grain yields by as much as 20 bu/acre without conspicuously affecting plant growth. But if it is a good growing season with generous and timely rains, then the effects of the root rot that the nematodes cause are overcome or masked because the plants growing in such fertile soils have ready access to all the nutrients and water that they need. As the result, progress in the process of proving that plant nematodes can actually cause plant disease has typically been slow with numerous dead-ends. That was very definitely the situation in 1969 when Don White suggested that the two of us study the plant nematodes that he presumed infested the greens of Minnesota's golf courses. His offer could have been considered enlightened, far-sighted, or perhaps even ludicrous depending upon a given person's point of view. I should have been "on the fence" when he made his offer. On one hand Don was suggesting that we study a plant-nematode system that provided the stability of a monoculture that many plant nematodes require. On the other hand, the superintendents were, as part of their job descriptions, providing the tender loving care that could mask any stresses that the nematodes could cause. The response of many of the superintendents whom Don approached was probably a bit more critical. The prevailing attitude then and perhaps even now is that plant nematodes are pathogens that are recognized as being of concern to superintendents of golf courses located in Florida and other really warm places but certainly not of concern for Minnesota's superintendents. As we considered what we wanted to say in these "What Kind of a Toad is a Nematode" chapters, Dr. White recalled that he proposed the golf green-nematode survey partially because he really did not know much about plant nematodes. His graduate training at Iowa State University had not dealt with plant nematodes. But he felt that he needed to devote a portion of one of his classes to plant nematodes since some of his students would sooner or later end up in a warmer part of the world where plant nematodes can be a significant plant stress. And he felt that the best way to learn about plant nematodes was to study them!

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We began in September, 1969, to evaluate the suitability of Minnesota's greens as habitats for the progeny of the indigenous plant nematodes. We are using the phrase "progeny of the indigenous nematodes" to emphasize the concept that most plant nematodes have wide host ranges. Although human activity has unfortunately been very successful in spreading plant pathogenic organisms about, we believe that some of the nematodes that were originally present in association with the native prairie vegetation and forest trees have been capable of changing hosts from the diversity of those native plants to a turfgrass monoculture. Don talked with the superintendents about the green and/or the course and evaluated root quality as revealed by the soil cores that I collected. I used a Hoffer soil sampling tube to collect one-inch diameter soil cores that were usually 8 to 10 inches long. Eight to 10 soil cores were collected in a very random fashion from each green with many of them coming from locations that were fairly close to the collar of that green. We had decided to sample in this manner to minimize any damage to the playing surface to which the players might object. And when my "large aerification holes" were carefully filled with sand, the wounds that I had made were less objectionable than the ubiquitous (for some courses) ball marks.

The survey began in Northern Minnesota and worked to the south. I started out by putting all of the soil cores from a given green in a single plastic bag. I did this primarily as a compromise to speed up the sampling process and to minimize our "in the way of play" presence on a given green. Even though we did our sampling after Labor Day when play had greatly diminished, it still was more than a little disconcerting to be standing unprotected in the target area looking at soil cores when we probably should have been looking out for incoming projectiles. But starting from Day 1 at the Cloquet Country Club we did take the time to tease apart each soil core to determine how deep the grass roots had been able to penetrate. By this procedure we were able to determine that the average depth of rooting in the fall and usually after aerification was 3.68" and that the rooting depths of the grasses growing on 142 greens on 36 golf courses ranged from as little as one inch to as much as a rather amazing 10 inches.

As you have probably gathered from at least the apologetic tone of the previous paragraph if not from your understanding of basic plant pathology, I quickly acknowledged and was concerned that my "start-up" sampling procedure, which was based to an excessive degree upon convenience, violated two very important principles of plant nematology. The First Principle was and is that plant nematodes have to have access to living plant roots. And since plant nematodes are small and sluggish, it seemed reasonable to expect that most of the nematodes that are present in a green would be found in the root zone. To mix the "Root-zone soil" with the "Below root-zone soil" would seem to be adding a "dilution factor error" to our results. Eventually we slowed down enough to put the portion of the soil cores that contained most of the roots into a "root-zone" (RZ) bag while the remainder of each core went into a "below root-zone" (BRZ) bag. At any rate, all of the bags regardless of whether they contained combined RZ and BRZ samples or just the preferred RZ or BRZ soil were kept cool as they were transported to St. Paul where they were refrigerated until they could be processed by the Cornell piepan procedure. Although nematodes are to-a-point heat-loving organisms, they are easily inactivated at temperatures of around 124 F which may be reached in a closed vehicle on a sunny summer day. And nematodes must have, in addition to being alive, sufficient food reserves so that they can move if they are going to be separated from the soil by an "Active" extraction procedure such as the Cornell piepan. Although some nematologists have insisted that soil samples must be processed within 24 hours of their collection, it was impossible for us to follow that regime. So we refrigerated the samples so that the well-fed "greens" nematodes would be sluggish if not totally inactive and could not use-up the food reserves that they would need to separate themselves from the soil layer in the screen-bottomed piepan when their sample was eventually processed.

The "hot-spot" concept that will come to the fore in Chapter 3 is basic to plant disease diagnosis. If a plant disease is caused by a living entity (a pathogen), then one should expect to find that the pathogen or its effects (symptoms) are unevenly distributed across the "plantscape" or, for turfgrasses, the green, tee box, or fairway. It is a premise that is typically verbalized during the first meetings of an introductory plant pathology course and is often readily visible on golf courses. My failure to deal with infection center concept of plant pathology was The Second Principle that was violated. I was sampling "in the once-over lightly" convenience mode as a guest on some of the most high-value vegetation in the State. I was taking samples that might provide an "average" nematode population estimate for a given green. What might that "average" population estimate tell us and what might it hide? Would that population estimate have been most useful if I had been lucky and had hit the hot spots (infection centers) in the green where the potential of a given nematode to develop on those plants growing in that soil under those management and environmental conditions was maximum? Or what if I had missed the "hot spots" and the "nematode report" would have come back to the superintendent with words to the effect that the green was not infested with potentially significant populations of plant nematodes? What additional information would we have gained from our efforts and the indulgences of so many cooperating superintendents if we had perhaps collected a set of cores from each of 5 very localized areas (a 5-6 inch diameter circle perhaps) at locations such as the front, cup-placement areas (left, center, and right) and the back of each green? Or maybe the traffic patterns on to, on and off a green should have dictated the locations of our samples? The health of the grass plant as affected by the stress that it is under can be expected to affect the number of nematodes that will develop in and around its roots. But now, with considerable remorse and my usual 20-20 hindsight, I can only wonder if we had gone to that extra bother if a pattern would have developed that would have helped us develop a truly appropriate protocol for sampling a green for plant nematodes?

RESULTS of the SURVEY: We collected soil samples from a total of 142 greens. All but three of them proved to be infested with plant parasitic nematodes. One of those three "plant nematode-free" greens was an old one. It should have been infested because plant nematodes typically can at least exist wherever plants can grow. But "apparently nematode-free" samples do occur and I do not have an explanation for why plant nematodes were not detected. The other two greens were new ones only about a year old.

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Any plant nematodes that may have been associated with the soil components from which those greens had been formed could have died-out if that soil had lain fallow for a while before the green was constructed. Or maybe the greens were constructed of nematode-free sand from a gravel pit and nematode-free peat from a bog. As many readers of *Hole Notes* are aware, the MGCSA recently provided funds to the U's Horticulture Department that helped facilitate the construction of a USGA sand green as well as a green constructed from the existing pasture soil. The USGA research green was seeded in the fall of 2002 and the "push-up" green was seeded in 2003. The USGA green was sampled for plant nematodes in the autumn of 2004, again in early October, 2005, and in between by a few students who were trying to fulfill a plant pathology course requirement during the 2004 and 2005 spring semesters. The "push-up" green was sampled for nematodes for the first time in October, 2005.

The U of M's USGA green at three years of age in October 2005 was not "nematode-free" since predaceous nematodes that feed on nematodes and other small animals and other kinds of non-plant nematodes were already established. The main type of "non-plant nematode" feeds on soil bacteria, multiplies very rapidly, and may become very numerous and possibly significant in the root-zone soil of a golf green. But plant nematodes, which are typically more delicate than their predaceous and bacteria-ingesting relatives, have not yet been detected in any soil sample collected from the USGA green. On one hand you would be justified if you found this to be somewhat surprising since equipment that might move infested soil from the older research green on the U of M campus is also used on the new green. But on the other hand, however, the sand and peat mix used to build the green undoubtedly was free of plant nematodes when it arrived at the construction site. And most plant nematodes can be somewhat more finicky when it comes to getting established as "drop-ins" in a new site than are other kinds of soil nematodes. The "Push-up green", however, has not been able to enjoy a plant nematode-free "grace period". Pin nematodes, a common nematode that has long been present in the old turfgrass research area and presumably also was in the former pasture where the new research greens are located, and a few spiral nematodes have been detected in the samples collected from that push-up green. Several of the pin nematode populations were substantial if not significant two years after the green was constructed.

Five of the six genera of plant nematodes that Taylor had determined a decade earlier to be commonly present in association with the roots of 9 different field crops were found in Minnesota's golf greens. The stunt nematode, Tylenchorhynchus spp. (Figure 2, Chapter 1). was most commonly found in association with bentgrass and Poa annua in our 1969-70 survey and remains Minnesota's most common turfgrass nematode still today. We found it to be present in 84.5% of the greens that were sampled. This nematode feeds as a migratory ectoparasite. That means that it penetrates epidermal cells of plant roots with its stylet but does not physically enter the root with the rest of its body. Its pathogenic effects must therefore be primarily due to the secretions produced in its pharynx or esophagus that are introduced into the root

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through the parasitized cell that it penetrated with its stylet. Nelson (Nelson, Eric B. 1995. Nematode disorders of turfgrasses: How important are they? TurfGrass Trends 4:12) listed the threshold population for damage as being 300 stunt nematodes/100 cm-3 soil.

The ring nematode (Figure 3, Chapter 1) was in second place as a parasite of Minnesota's golf green grasses based on its frequency of occurrence. It was present in 64.8% of the greens that were sampled. It, like the stunt nematode, feeds as a migratory ectoparasite and does not enter root cells with any part of its body other than a portion of its stylet. It is regarded as being a much weaker pathogen of turfgrasses with 1500 ring nematodes/100 cm-3 of soil needed to cause measurable damage. It is a much more sluggish nematode than the stunt nematode and that presumably is part of the reason why it is less pathogenic. It was detected by Taylor in his survey and but did not find its way into his "commonly present" list of plant parasites associated with 9 different field crops.

In third place in terms of frequency of occurrence was the spiral nematode, Helicotylenchus spp., which was present in 59.2% of the 142 greens that were sampled. This nematode can feed as a migratory ectoparasite, a migratory ecto-endoparasite penetrating partway into the root cortex, or as a migratory endoparasite entering all the way into the root's cortical layer. Although it would seem to have a pathogenic advantage over the stunt nematode because it can also cause mechanical damage as it moves into and through the cortex, Nelson lists its threshold for damage at 600 spiral nematodes/100 cm-3 of soil.

In fourth place in terms of frequency of occurrence was the lance nematode, Hoplolaimus spp., which was present in 54.9% of the 142 greens that were sampled. This nematode functions primarily as a migratory endoparasite moving, living, feeding, and reproducing within the cortical layer of the root. It is a rather large nematode and a potent pathogen. Nelson listed its threshold population for damage at 150 nematodes/100 cm-3 of soil.

In fifth place in terms of frequency of occurrence was the pin nematode, Paratylenchus spp., which was present in 45.1% of the 142 greens. Although this nematode will be reintroduced a bit later as causing turfgrasses on a portion of at least one green at the Bemidji Country Club to go off-color (wilt), it really is a very weak pathogen. It feeds as a migratory ectoparasite on root hairs and may do so at one location for a considerable period of time. It did not even "make" Nelson's thresholds for damage to cool-season turfgrasses list. I think that pin nematode populations in excess of 1000/100 cm-3 of soil need to be present if its feeding is going to be pathogenic (cause damage) to certain kinds of plants such as greenhouse roses and soybeans in farm fields.

In sixth place in terms of frequency of occurrence was the lesion or meadow nematode, Pratylenchus spp., which was present in only 19.7% of the 142 greens that we sampled. This nematode resembles the lance nematode in many ways. It is a pathogenic migratory endoparasite that, although smaller than the lance nematode, has been determined to be just as pathogenic to cool season turfgrasses having a 150 nematodes/100 cm-3 soil threshold for damage. It possibly should be called the "horticultural nematode" because it infects so many horticultural species and does so much damage to them.

Other plant nematodes such as the dagger, needle, and stubby-root were detected infrequently. All of them are potent pathogens and 2 of them, the dagger and stubby-root nematodes made Nelson's list with thresholds for damage to cool-season turfgrasses of 200 and 100 nematodes/100 cm-3 soil, respectively. These are nematodes that often are more efficiently separated from soil by sugar flotation extraction methods than by with the Cornell piepan procedure that was the primary technique used in our study. Maybe they are more common and significant in Minnesota's golf greens than our results to date suggest.

Well, for the 6 different kinds of plant nematodes that were most commonly detected as inhabiting Minnesota's golf greens, is one more of a Northern Minnesota Nematode or a Southern Minnesota Nematode or a Twin Cities Nematode than any of the others? Maybe the lance nematode, Hoplolaimus spp., is less of a Northern nematode than it is a Southern or Cities Nematode. And maybe the pin nematode, Paratylenchus spp., is less of a Southern Nematode than it is a Cities or Northern Nematode. But other than that there seems to be only one more thing that I should mention as being an outcome of our 1969-70 greens X plant nematode survey. And that is this: in 1969-70, despite perceived weaknesses in my sampling protocol, about one green in 10 turned out to be infested with a potentially (Nelson's Thresholds) damaging population of plant nematodes.

Positions often do or should change with time. It seems to us now that it was easier to be a successful superintendent when we first began studying the plant nematode populations inhabiting golf greens than it is now. Superintendents then used the available mercury-based fungicides to control the snow molds and other kinds of diseases caused by fungi were not of much concern. As Don White remembers it, the stress diseases of turfgrasses in particular were not very important then. Over the intervening years reductions in the height of cut probably have produced the most severe stress experienced by turfgrasses today. That stress was largely absent in 1969-70. And it seems to us now that some if not many of the diseases to which turfgrasses may succumb are really complexes brought on by a variety of plant stresses. We currently are of the opinion that plant nematodes can provide just one of those several stresses that turfgrasses may experience and which, in combination with other stresses, may tip the balance in favor of plant disease.

My hypothesis back in 1969-72 was that plant nematodes might stunt the growth of turfgrasses and by that mechanism make it a bit more difficult for the superintendent to maintain turf quality. I saw it then as a simple one stress-one vulnerable plant cause-and-effect relationship. For example, the following simplistic relationship seemed perfectly reasonable and straight-forward to me at that time. Large populations of pin nematodes were found to be present in portions of at least one of Bemidji's Town and Country Club greens that had the tendency to go off-color on a hot summer afternoon unless the superintendent came to the aid of those stressed grass plants with a light application of water. Although the pin nematode is only weakly pathogenic, if there are enough of them and they are feeding on the root hairs of the host plant, then it seemed reasonable to expect that the host plant was going to be limited in its ability to take up needed moisture. As the result I reached the excessively narrow conclusion that plant nematodes would do "their own thing" all by themselves and by so-doing limit the quality and quantity of turfgrass roots. And if we could reduce the size of the potent populations of plant

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nematodes by applying nematicides, then maybe we could measure an improvement in plant growth. Maybe if the grass roots were healthier the superintendent would not have to provide the extra TLC that a green or a portion of a green required on a hot summer afternoon.

And so we secured the cooperation of 5 superintendents (Bemidji, Cloquet, Duluth, Glencoe, and Rochester) who had greens that were found as the result of our survey to be adequately infested with either lesion (Pratylenchus spp.) or lance (Hoplolaimus sp.) nematodes. Greens infested with either or both of those two nematodes were chosen because, as explained earlier, they are migratory endoparasitic nematodes. Such nematodes penetrate into the cortex of plant roots where their activities cause biochemical as well as mechanical damage to the plant cells. Those nematodes seemed to be the ones that would have the best chance of being pathogenic, of causing disease, if any kind of plant nematode could adversely affect the growth of turfgrasses. And much later, in October, 1995, threshold levels for damage to cool season turfgrasses as presented by Nelson confirmed that other nematologists also considered the lesion and lance nematodes to have enhanced potential to be pathogens of turfgrasses. Only 150 nematodes/100 cm-3 of soil of either type of nematode had been determined as having the potential to damage cool season turfgrasses while 300 stunt nematodes or 1500 ring nematodes/100 cm-3 soil had to be present to have the same disease-causing potential. The cooperating superintendents, apparently with the blessing of their greens committees (a simpler time then than now, perhaps), allowed us to temporarily stake out 16 large plots (4 by 10 feet) along the margins of each infested green. The experimental design that we used was a compromise; if something went wrong with a treatment, at least the center of the green would be "out of harm's way". Often the hot spots with regard to nematode population densities were in the center of the green that was "off limits" to us. As a result some of our plots contained rather mediocre and quite variable plant nematode populations. In those long ago days there were many more nematicides, both registered and experimental, available than there are today. Our experiments consisted of three treatments and a control each replicated 4 times on a given green. The chemicals that were used were applied within the string-defined confines of the appropriate plots. Some were granular or liquid formulations that were sprinkled over the area of the appropriate plot and then watered in. One was a liquid that was injected with a very large "hypodermic needle" called a "Nemagun". We removed the strings and stakes once the treatments had been applied and the green went back into play. We returned periodically to make visual observations and collect soil samples so that the nematode populations could be monitored.

The nematode populations in the plots were not appreciably affected by any of the chemicals that we used. That was not an unexpected result since once a soil becomes infested with plant nematodes it will remain infested. And treatments like we used could have been expected to reduce plant nematode populations by no more than 45-55%. Those efforts went on for 2 summers with no improvement in plant growth being detectable. And so by the fall of 1972 all we could say was: "Yes, plant nematodes are frequently abundant in Minnesota's golf greens. Potentially damaging populations can be detected as readily in northern greens as they can in greens located in Southern Minnesota or the Twin Cities area. We believe that some of those nematode populations are of sufficient size and pathogenic composition to be able to damage plant roots to the extent that the plants will be stunted or would benefit from extra tender, loving care. But No, we have not been able to prove that plant nematodes measurably affect the growth of the turfgrasses that are growing on Minnesota's golf greens."

Professor Ward Stienstra is reported to have taken the "nematode ball" and run a little ways further with it. He determined, under greenhouse conditions, that turfgrasses growing in plant nematode-free soil grew better than did turfgrasses growing in infested soil. But as he acknowledged, what happens to turfgrasses growing under greenhouse conditions will most likely be far different from what happens on a putting green that is under play. Differences in growth in the greenhouse that might be visible when grass is allowed to grow for a period of days before being clipped would be invisible in a situation where the grass is cut on a daily basis.

And so Don White and I went our separate ways with Don getting interested in perennial strains of Poa annua and I being "rescued" by the arrival of the soybean cyst nematode (a genuine, bonafide plant pathogen) and the opportunity to teach introductory plant pathology.

(Editor's Note: Part III of the "What Kind of a Toad is a Nematode?" trilogy will deal with some additional turfgrass-related nematological happenings that have occurred since our interest in plant nematodes was abruptly reawakened in September, 1995.)

MGCSA Fall Mixer

Monday, October 9

Oakdale Golf Club

Buffalo Lake

Host Superintendent:

Mike Knodel

See www.mgcsa.org for more information