PART III

What Kind of a Toad is a Nematode?

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Although our random probing with a Hoffer tube of 142 greens on 36 golf courses back in 1969 and 1970 did work to provide documentation that just about every established green (139/142) that we sampled was infested with plant nematodes, I am not necessarily comfortable in recommending such "randomness" with regard to sampling. Plant pathogens (organisms that cause plant disease) of all types (and plant nematodes really can be the cause of plant disease) rarely are evenly distributed across any piece of "plantscape" including a green. Soil cores possibly collected from "hot spots" as well as "cold spots" across a green may provide an "average" that may mask a significant potential stress with which the turfgrass manager may have to deal. Or, in other words, averages include "extremes" which, in the case of plant nematodes, may be growth-limiting or "predisposing" populations. That fact became very evident back in 1995 when two soil samples cut with a cup-cutter arrived at the Plant Disease Clinic (PDC) in Stakman Hall. The samples were taken from two of the USGA greens at Keller Golf Course that had been rebuilt in 1991 and sodded in either the fall of 1991 or the spring of 1992. The sod that was used had been grown on sand and theoretically should have grown well when laid on the sand of the USGA greens. By the summer of 1995 several but not all of those 12 greens had become essentially unplayable during the heat of a summer that Charlie Pooch (Superintendent at Les Bolstad course) described as being "a beast of a year for growing turfgrasses." The Keller superintendent at that time felt that he had explored all of the chemical and cultural options other than application of a nematicide that existed to him. And so in early September he collected a soil sample from each of the two worst greens to learn if plant nematodes could be "at the root of his problem." Ms. Sandee Gould, Chief Diagnostician of the PDC at that time, asked for advice about how she should proceed to process those samples for plant nematodes. Essentially she was asking: "How do I proceed with this bag of loose sand topped with a very thin disc of sick plants with unhealthy roots?" We decided that she should try to get 100 cm-3 of sand from each "thin disc" and process it for nematodes. One sample in particular turned out to be from what some plant pathologists would call a very "hot spot" on Green #8. It contained in excess of 5,000 plant nematodes. Most of which were stunt nematodes, (tylenchorhynchus spp.) which is the most common plant nematode inhabiting Minnesota's golf greens. No other sample from Keller has ever been found to contain anywhere close to that many nematodes. The other cup-cutter sample from Green #18 also came from a "bad" area because it contained over 1,100 stunt nematodes. How did the superintendent find such "hot spots"? Was it luck, skill, chance? Could he have repeated his sampling prowess if he had gone out a second time? I believe that the "hot spot" sample came from the left rear portion of Green 8 where the turfgrass may have been a bit more healthy than it was in the center of the green. But why there? If the superintendent had a specific reason for sampling there that reason has been lost. At any rate the plant nematode populations present in those two samples from greens that were visually "in trouble" dramatically re-aroused our interest in the plant nematodes that can parasitize turfgrasses. It seemed to be a classic example of a disease that could be caused by plant nematodes: 1) the grass started off OK in spring when plant stresses that adversely affect the growth of turfgrasses tend to be minimal and plant

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nematodes in the cool soil tend to be sluggish. 2) The amount and quality of plant growth declined as the various plant stresses including nematode activity built-up with the heat of June. 3) Nothing that the experienced superintendent did with fungicides and watering had any beneficial effect on the diseased turf. 4) The grass started to come back in September with cooler conditions that are more favorable for the growth of turfgrasses and which slow down the activities of plant nematodes. And 5) the entire recently published issue of TurfGrass Trends (Volume 4, Issue 10, October, 1995) was devoted to "Nematode Disorders of Turfgrasses: How Important are They?" by Eric B. Nelson. Table 4 in that article listed "Damage Thresholds for Various Nematodes on Representative Cool-season and Warm-season Turfgrasses". The threshold for damage by the "Keller Nematode," a stunt nematode, was listed as 300/100 cm-3 of soil. The stunt nematode populations detected by two "cup-cutter" samplings thus exceeded a published threshold for damage by a factor of at least 3 to about 17. Our interest in plant nematodes in putting greens probably would not have been "jump-started" if the Keller superintendent had done his collecting by combining 10 randomly-selected one-inch diameter "Hoffer tube" cores in one bag. More intensive sampling (one sample from each of the 18 greens) in the fall of 1995 revealed that the average Keller "Hoffer tube" sample contained about 409 stunt nematodes/100 cm-3 of soil which could be considered by some as being sort of a "Ho-Hum" population. And the randomly collected Hoffer tube samples from those two worst greens (#8 and #18) only contained 831 and 233 stunt nematodes/100 cm-3 soil, respectively. In defense of the cup-cutter sampling technique, it did provide data documenting what could happen in terms of multiplication and survival of plant nematodes in two Keller greens as affected by the environment, the management practices and amount of play that existed in 1995. And

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if the superintendent had collected 10 "Hoffer tube cores" in a random fashion, "What Kind of a Toad is a Nematode?" would have been concluded with Chapter 2.

Paul Diegnau came to Keller as Keller's new superintendent in 1996 convinced that plant nematodes had not been responsible for the poor playing conditions of several of the greens in 1995. My first meeting with Paul was polite, civil and definitely chilly. Dr. Stienstra and I visited Paul in his office in the old Quonset hut maintenance building that Keller had at that time. He describes his 1996 attitude best on page 20 of the October, 2005, issue of *Hole Notes* by saying "I was of the same mind set" (nematodes are a 'southern' problem and not an issue in the northern climes) ten years ago. Nematodes??? Get serious!" Paul uses the term "holistic" to describe his approach to culturing quality turf. And that approach with no direct attention paid to plant nematodes seemed to work because in the early summer of 1996 a sports columnist for the St. Paul Pioneer Press described Keller as "never looking better." And so what could I do but retreat back to my once again tarnished Ivory Tower at the "U"! Don White continued to include a lecture and laboratory devoted to plant nematodes in his fall quarter course. And Paul turned out to be a fairly agreeable fellow after all. Every fall we made a pilgrimage to Keller so that the students could learn first-hand what Paul was doing with the golf course that by all rights should have had a nematode problem but really(?) didn't. Paul explained (again as stated in October, 2005 *Hole Notes)* to the students in each of those classes that: "My theory has always been that any damage inflicted by nematodes can be overcome with proper cultural programs and by maximizing plant health." He always allowed the students to use Hoffer tubes to try to find the progeny of those infamous Keller nematodes. And they always found them even though some of the student-designed sampling protocols that were followed such as taking the soil cores only from ball marks were more than a bit questionable.

And then came the summer of 2005, another "beast of a year for growing turfgrasses" when Paul and 6 other superintendents who responded to Paul's survey (*Hole Notes*, October 2005) acknowledged that there had been outbreaks of Take All Patch on their courses that summer. Paul wrote that "I have a feeling that the current golf season brought nematode damage to the forefront and under the spotlight. I find it hard to believe that the quantity and variety of chemistries that we - and others - threw at our TAP outbreak were so ineffective in arresting this pest. There must be other factors at work here. Instead of 'grasping at straws', I am hopeful this study (more intensive, less extensive nematode survey #2) will provide some answers." Please do not consider Paul Diegnau to be "a lone voice crying in the wilderness." He has some pretty impressive supporters like Mr. Oscar L. Miles, the recently retired superintendent of The Merit Club of Libertyville, Illinois. Mr. Miles has given us permission to share the following information that he provided to Paul on August 29, 2005. "Back in 2001 we had a similar problem and found TAP and very high numbers of parasitic nematodes, both stunt and ring. For us, these two problems seem to work together. Nematodes weakening the plant with TAP becoming the primary problem." On September 1, 2005, Mr. Miles supplied additional information about his TAP and plant nematode experiences: He had two labs confirm that he had plants collapsing from TAP. He went on to say: "I couldn't believe this was the complete answer to our problem so we literally dug deeper and took another set of samples to the University of Wisconsin to have a nematode check done. Several weeks later I heard from the UW Nematologist. She was very excited. She had never seen Tylenchorhynchus - stunt (nematode) numbers this high this far north. They were at 940 per 100 cc's of soil sample. Remember, this is the following year after the 2000 United States Women's Open at the Merit Club, where we had subjected our PennLinks greens to stressful growing practices to maintain the degree of ball roll distance and smoothness required for championship play. That summer and fall after the Open and into 2001 we attempted to maintain the Open speeds. THIS WAS A BIG MISTAKE. I am sure that we had TAP and harmful Nematodes before the greens were subjected to this stressing maintenance but, we were able to mask it with additional leaf tissue from higher HOC and fall and following spring TAP protectants drenched in to protect the roots."

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Take All Patch, the real reason why this "What Kind of Toad is a Nematode?" series came into being, is caused by a soil-inhabiting, root-rotting fungus called Gaeumannomyces graminis. The fungus causes a root rot that kills the epidermal and cortical cells of the infected root. Presumably its activities create an environment that is detrimental to most if not all plant nematodes because those nematodes have to feed on living plant cells and the root rot deprives them of such cells. Although various kinds of plant nematodes have been shown to be able to survive for a period of months without a food source, most of them can be expected to perish quite soon particularly in the heat of summer when 1) their heat-enhanced activities will cause them to rapidly utilize their stored food reserves and 2) the decomposition products released by the diseased roots could be expected to stimulate the activity of competing and perhaps antagonistic microorganisms and/or result in the release of natural nematicides from decomposing plant tissues. Although we all recognized that starting an intensive plant nematode study in September-October, 2005, had many similarities to the message conveyed by that old saying about "Locking the barn door after the horse has been stolen," three superintendents who had fought TAP in 2005 (Forest Hills, Keller, and Mankato) agreed to participate in the

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study. Although the TAP-affected greens were slowly on the way back to the quality levels expected by the superintendents, those superintendents attempted to identify locations where TAP had been present and other areas that had remained healthy. They collected soil samples from specific sites that could be found again. They collected 5 one-inch diameter Hoffer tube cores from small areas of about 64 square inches. Soil from the root-zone at a given sampling site went into one plastic bag as a sample. Soil from below the root-zone to a depth of 6 inches was combined in another plastic bag as another sample. And, if it was physically feasible, soil cores from the 6 to 12 inch deep layer were combined in another plastic bag as a third sample from a given sampling site. The samples from 18 sampling sites at Forest Hills, 36 at Keller, and 19 at Mankato were stored in a refrigerator at St. Paul until they could be processed for nematode extraction by the Cornell piepan method.

Well, what did we prove by sampling TAP-infested greens for nematodes after the disease had run its course? At Forest Hills there did not seem to be any relationship between the numbers and kinds of plant nematodes that were present AFTER THE EPIDEMIC and the susceptibility of the turfgrass to TAP. Each of the three severely-afflicted greens that were sampled for plant nematodes has its own built-in weaknesses. Green #16 is an elevated green with good drainage that is shaded and does not receive much morning sunshine. Soil samples collected from 6 locations on Green #16, which Superintendent Marlow Hansen identified as being the green that was most severely-affected by TAP, contained very few plant nematodes. None contained enough stunt (300/100 cm-3) or any other kind of plant nematode to qualify as having met the population densities necessary to cause damage (all by themselves) to cool-season turfgrasses (Nelson, 1995). TAP was estimated to be equally severe on Greens #5 and #6. Green # 5 has been a "good green" although it is poorly drained. Green #6 is disease-prone, low-lying and shaded. Three of the 6 sampling sites on Green #5 were found to contain large (910-1825) and potentially significant root-zone populations of stunt nematodes. The other 3 sampling sites contained "unimportant" root-zone populations (0-264) of stunt nematodes. Two of the 6 sampling sites on Green #6 were found to have potentially significant root-zone stunt nematode populations of 445 and 997/100 cm-3 of soil. The other 4 sampling sites each contained "theoretically unimportant" root-zone populations of 37-98 stunt nematodes.

Although Superintendent Hansen has never seen any evidence in 18+ years that: 1) plant nematodes have adversely affected the growth of turfgrasses at Forest Hills and 2) no direct relationship between the incidence and severity of TAP and plant nematodes was discovered in 2005, he has chosen to continue with and significantly increase the scope of nematode study there in 2006. Each of the 3 greens for which we now have "nematode data sites" will be resampled in September and up to an additional 6 sampling sites per green will be added. An additional green will also be intensively sampled as described above. "Why," you say, "for what purpose?" Well, this is our current thinking: If the summer of 2007 turns out to be another "doosy" as Paul Diegnau chooses to describe a "tough year for

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Fall colors at Rutgers Bay Lake Lodge

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growing turfgrasses", then hopefully we will be in the position to determine if there is any kind of correlation between plant nematode populations and the incidence and severity of TAP or any other kind of plant disease that might appear. The TAP story from Keller in 2005 was quite similar to the one from Forest Hills. At least 3 greens were severely affected and TAP was also recognized as being present on additional greens. Paul Diegnau has already written about his efforts to deal with the disease. He tried a variety of different fungicidal chemistries with no effect. He increased the level of TLC that each infected green received. It was an extremely stressful summer for him, a "doosy" as he described it.

Superintendent Diegnau selected 12 sampling sites on each of 3 of Keller's greens that had been most severely affected by TAP. He selected sites where either symptoms were still visible or where he remembered that the disease had been present. He selected other sites where the bentgrass had remained healthy. The TAP status of the locations where the soil samples (root-zone, below root-zone to 6 inches, and 6 to 12 inches) were collected was not identified to the Plant Nematology Laboratory. Root quality-quantity at each sampling site was estimated on the basis of soil volume when the samples were processed. Five root-zone cores from an area of reasonably healthy golf green grasses will yield something more than 100 cm-3 (4/5th cup) of soil. On that basis, the turfgrasses of Keller Green #6 were the weakest (only one of 12 root-zone samples yielded more than 100 cm-3 of soil) of those of the 3 greens that were sampled. The turfgrasses of Keller Green #9 had considerably better roots than those of #6 with 7/12 root-zone samples yielding at least 100 cm-3 soil. The plants sampled on Green #18 (a seriously-affected green in 1995) were the strongest with 10/12 root-zone samples yielding at least 100 cm-3 soil. The total number of plant nematodes extracted from the root-zone samples from greens 6 and 9 were about equal at 4165 and 4332, respectively. Fewer plant nematodes, 3428, were extracted from the "better" root-zone samples from Green #18. Potentially significant populations of stunt nematodes of 300 or more/100 cm-3 soil were present in 5/12 and 6/12 root-zone samples from Greens #6 and #9, respectively. Only 2/12 root-zone samples from Green #18 were estimated to be infested with 300 or more stunt nematodes. The largest population of stunt nematodes (830) was estimated to be present in a root-zone sample from Green #6. It may be appropriate and useful to use the information given above to recognize a relationship that seems to exist for many plant nematodes. Severely stressed plants eventually can only support small populations of nematodes. Moderately stressed plants (Greens #6 and #9) will support larger populations. And less stressed or more vigorous plants (Green #18) will support fewer plant nematodes. Plant nematodes have to feed on living plant cells. But how, biochemically or otherwise, do the cells of a moderately stressed plant differ from those of either a severely stressed or especially a less stressed one? It is an intriguing but unanswered question with implications for future management of plant nematodes!

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Please continue to remember that we were in a "reactive mode" in 2005 as we tried to extrapolate back to the role that undefined plant nematode populations might have had that subsequently facilitated the development of the symptoms of TAP later in the summer. This is what Paul Diegnau wrote after he had time to compare the plant nematode data with the incidence and severity of TAP for the three Keller greens that were sampled in September, 2005: "I just looked at my mapping and labeling system for our sample taking adventure last September. I was rather excited after looking at the first 6 sample sites as the high nematode populations correlated with the Take-All-Patch. Then everything went 'willy nilly,' so to speak. I think the TAP vs. non-TAP sampling site selection process was entirely too subjective. We most likely sampled the TAP sites at various stages of the disease (mostly later) on into various degrees of recovery. As I recall, SOME of the TAP sites I was selecting were not definitive; I was taking my best guess. I would assume that there are some ongoing population dynamics occurring with the nematodes as the TAP infections run their course through those localized bentgrass communities and their food sources decline??? So the $10,000 question becomes: "How much does the timing of the sampling relative to the progression of the TAP impact the sampling results? How quickly do these populations shrink once the bentgrass roots are no longer a viable food source? Or do they simply shift over to the Poa annua roots?"

Fred Taylor, Superintendent at the Mankato Golf Club, believes that Take-All-Patch was probably present on his course in 2004, and its presence was the reason why he first became interested in plant nematodes. The fungus was identified as being present in 2005 on several of Mankato's greens causing cosmetic effects that concerned him but apparently did not concern (no complaints) the players. The symptoms persisted and were still very apparent to him in early November. The three greens at Mankato for which we now have nematode data all seem to be both rather uniformly (in distinct contrast to Forest Hills) as well as heavily infested with plant nematodes. Mankato's Green #3 with 425-1599 stunt nematodes, #6 with 551-1188 stunt nematodes, and #14 with 637-3885 stunt nematodes/100 cm-3 of soil all seemed to be especially vulnerable to outbreaks of Take All Patch or any other disease caused by a fungus whose causal agent might benefit from the biochemical-mechanical damage caused by plant nematodes. Of those greens, #14 with approximately 39% more total plant nematodes than #3 and 78% more total plant nematodes than #6 would seem to be most vulnerable.

In 2006, Take All Patch was judged to be entirely absent from Forest Hills and Keller and of very limited and only an early season occurrence at Mankato. Mankato's Green #3 was the only one where its symptoms were observed. Those three superintendents controlled TAP in 2006 with a preventative spray program that began with applications in the fall of 2005 and continued with additional applications early in the spring of 2006. Although any correlation between the incidence and severity of TAP and abundance of plant parasitic nematodes has not been strengthened and may have been weakened by the observations made during the past 12 months, we are enthusiastic about having the opportunity to make some further progress toward obtaining a better understanding of the possible significance of the "northern" turfgrass nematodes.

Chapter IV of "What Kind of a Toad is a Nematode?" will conclude the series with additional information that was obtained from the soil samples collected in 2005. We will also address a new concern about the health of turfgrass roots that has surfaced as the result of our being able to learn something about what is going on in the plant root-zones of so many different greens.