# The Science (and Art) of Diagnosing Root Diseases

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Anyone who has dropped into the lab and put us on the spot to diagnose a sample has probably heard us say something along the lines of either "there is some fungal activity on the roots, but nothing we're too concerned about" or "there is quite a bit of colonization on the roots." In both cases, whether we suspect a root disease or not, fungal hyphae are usually present on the roots. In fact, on nearly every turfgrass sample that gets submitted to the lab some amount of fungal hyphae can be observed colonizing the roots. So how do we determine which symptoms are caused by a root pathogen and which are not? Well the answer probably doesn't come as too much of a surprise to many of you, but a combination of science and experience (art?) usually lead us to a confident and correct diagnosis.

To simulate the process of diagnosing a possible root-infecting disease let me walk you through a "typical" sample that might come in from a Midwestern golf course. With any sample that comes in, the first thing we look at is the sample submission form. This is critical as it acts like a map describing where the sample has been; from when the symptoms first appeared, to how fast they have progressed, to any pesticides applied. The next step would be to observe the foliar symptoms under a dissecting microscope, which has a lower magnification than a compound microscope and is useful for observing lesions or larger fungal structures.

If nothing of interest is observed on the leaves, we can often rule out a foliar disease and begin to think about root diseases. The most common root-infecting diseases in the Midwest are necrotic ring spot (*Ophiospharella korrae*), summer patch (*Magnaporthe poae*) and take-all patch (*Gaeumannomyces graminis* var *avenae*). Pythium root diseases, most notably Pythium root dysfunction (*Pythium volutum*), have become more common in the last several years and cause the majority of their damage on younger golf course putting greens (Kerns and Tredway, 2008). Complicating matters is the non-pathogenic fungus *Phialophora graminicola* that can appear identical to the other root pathogens under the microscope but does not infect the root's vascular system (Landschoot, 1993).

Infected roots are washed and observed first under the dissecting microscope, which often gives a good idea of the amount of fungal hyphae present on the root surface (Figure 1). More important to correctly diagnosing root diseases then the amount of hyphae present on the surface is the appearance of the vascular cylinder (stele) of the root, or the inner portion that transports water and nutrients up to the plant. A stele that appears discolored likely signals infection by

**Figure 1: Using the dissecting microscope can give us a general idea of the severity of fungal colonization of the root, as observed on this Kentucky bluegrass root exhibiting symptoms of necrotic ring spot.**

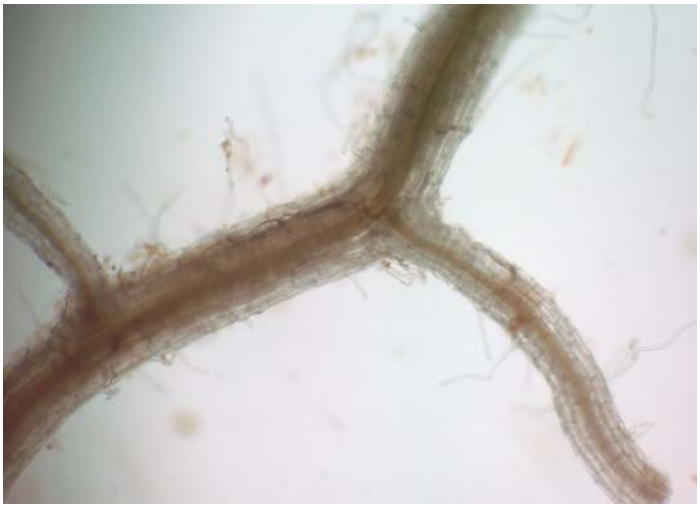

**Figure 2: Once the take-all patch fungus has colonized the root surface it will penetrate into the vascular system and disrupt the flow of water and nutrients in the plant, which will slightly discolor the inner portion of the root.**

the fungus into the root, rather than just a colonization of the surface, and disruption of the plant vascular system (Figure 2).

Next we can transfer a portion of the root onto a glass slide and observe the hyphae under the high-powered compound microscope (Figure 3). Under certain conditions, different root pathogens can produce unique infection structures that may aid in diagnosis. But these structures are often not observed, and morphological differences between pathogenic fungi like *G. graminis* var *avenae* and non-pathogenic fungi like *P. graminicola* may be impossible to find.

Assuming we see all the above signs of a possible root-infecting disease, how can we differentiate between the major diseases mentioned above? Pythium root diseases are somewhat distinct from necrotic ring spot, summer patch, and take-all patch by the cooenocytic (non-septate) hyphae, oospores (sexual spores), and sporangia (asexual spore bearing structure) they produce. But both microscopically and macroscopically it is often very difficult to determine when a sample is take-all patch, NRS, or summer patch. One factor to take into account is the turfgrass species. Take-all patch will only infect creeping bentgrass (*Agrostis stolonifera*), and summer patch is most commonly observed on annual bluegrass (*Poa annua*), Kentucky bluegrass (*Poa pratensis*), perennial ryegrass (*Lolium perenne*), and fescues (*Festuca* spp.). Recent research by Dr. Lane Tredway at North Carolina State has shown evidence of summer patch infecting bentgrass, but more research is needed to determine that relationship (Tredway, 2006). Necrotic ring spot will only infect Kentucky bluegrass, perennial ryegrass, and the fescues while *P. graminicola* has been observed on most common turfgrass species.

If the turfgrass species offers few clues to identify the pathogen, the timing of symptom development can also provide insight. The causal agents of NRS and take-all patch infect when soil temperatures are between 55 and 65°F, yet symptoms develop anywhere from mid-May to late June depending on the weather conditions (Couch, 1995). The causal agent of summer patch becomes increasingly more aggressive as soil temperatures rise throughout the summer, so symptoms that first appear in August or September often are the result of summer patch. The timing method of diagnosing root diseases is where the experience factor comes in, and there are always exceptions to the rule. At this point in the process we can make a confident diagnosis as to the causal agent and will not proceed with further analysis unless there is significant doubt or a specific request has been made by the superintendent.

What more can be done to identify samples that fail to provide a clear cut diagnosis, or where the presence of *P. graminicola* may be suspected? When complications arise, or the superintendent wants proof of identity beyond the previously described methods, there are some further techniques that can be done. Any further analysis usually begins by culturing the pathogen in the lab (Figure 4), which begins by isolating the fungus on a sterile media and then re-isolating until a pure culture without any contaminants is obtained. There are some identifying characteristics of each culture, such as appearance and growth rate at different temperatures, but most often the culture will be used for polymerase chain reaction (PCR)-based analysis. PCR-

**Figure 3: Using a compound microscope allows for the higher magnification of the hyphae on the root surface, which can provide further clues to the identity of the pathogen.**

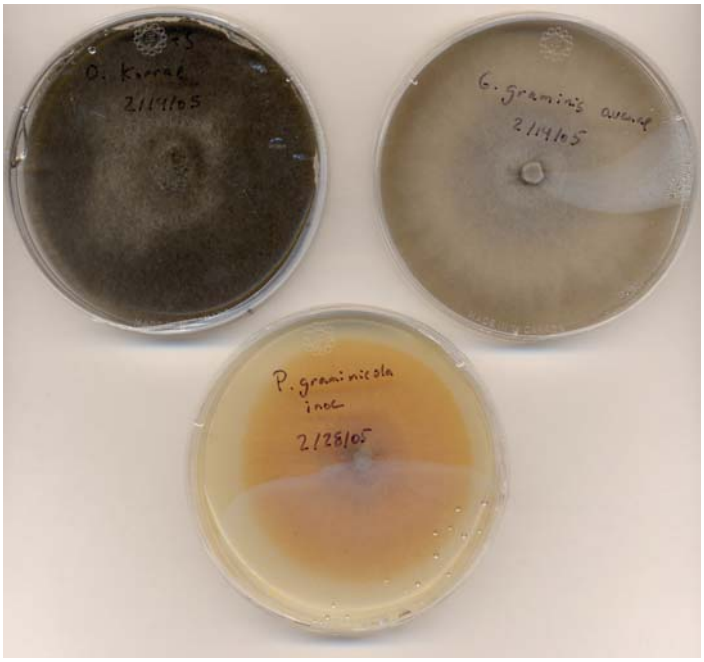


**Figure 4: Though a similar appearance on the root, in culture these pathogens can appear very different. The causal agent of necrotic ring spot is in the upper left, for take-all is in the upper right, and the non-pathogenic Phialophora graminicola is on the bottom. Photo courtesy of Steve Abler of Reinders, Inc**

based diagnostic methods are a species-specific molecular diagnostic method that provide a much more confidant diagnosis of fungal identity. Problems with these methods include time (culturing root pathogens can take weeks), cost, and the cross-reactivity of the species-specific nature of each PCR-based method (Tredway, 2006).

All turfgrass samples that come into the Turfgrass Diagnostic Lab are fully inspected to take into account all possible pathogenic and non-pathogenic causes alike. No pathogen operates in a vacuum, and root pathogens are no different. Environmental conditions, cultural practices, and colonization by non-pathogenic fungal species such as *P. graminicola* and the bacteria *Pseudomonas* spp. will all have an effect on the degree of symptoms observed (Landschoot et al, 1993). The mere presence of fungal hyphae on the roots or of darkened roots or basal regions does not necessarily indicate a root disease, and on the contrary just because at first glance the roots appear healthy does not rule out an infection.

This is where you as the submitter play a crucial role. Proper sample submission and completion of our sample submission form will aid us in providing the fastest and most accurate diagnosis of your sample. More details on sample submission as well as a link to download the sample submission form can be found at our website, www.plantpath.wisc.edu/tdl, which is currently being revamped to provide the maximum benefit to the turfgrass industry of Wisconsin and surrounding states.

**References:**


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