A Scanning Electron Microscope Peek at Poa annua and a Fungus

By Gary Gaard, TDDL Staff Member
Department of Plant Pathology, University of Wisconsin-Madison

A mission goal of the Turfgrass Disease Diagnostic Lab is to "provide rapid, accurate and specific disease diagnostic information". Rapid, accurate and specific is not attainable with current technology when it comes to diagnosis of the patch diseases caused by ectotrophic root-infecting (ERI) fungi-necrotic ring spot, take-all and summer patch. The microorganisms that incite turfgrass patch diseases are fungi with dark pigmented hyphae (thread-like fungal structures) that can be seen with the light microscope. These hyphae grow externally (ectotrophic) on roots and crowns and occasionally attach to an epidermal cell with a structure (hyphopodia) comparable in attachment and function to the mouth of the fresh water leech. This study is the beginning of our search for better ways to rapidly and accurately diagnose your ERI fungal patch diseases.

The accompanying micrographs are of thirty-two day old *Poa annua,* which may have the disease necrotic ring spot caused by the fungal pathogen *Leptosphaeria korrae.* The pathogen was isolated from TDDL Kentucky bluegrass sample 96-152 from Milwaukee County. *Poa* was infected with the pathogen simply by growing *Poa* from seed in soil that contained the pathogen. The micrograghs are presented here to give those of you that have *Poa* annua and an occasional disease on your golf courses an "understanding creates possibilities" image of host and pathogen.

To be certain that a turfgrass disease is present, in addition to symptoms and environmental conditions, some distinct feature must be identified. The distinguishing feature can be associated with the host, as the distinct "hour glass" tan leaf lesion with reddish-brown borders of dollar spot. Or it can be associated with the pathogen, for example the characteristic spores of pink snow mold. Unfortunately an easily observed distinguishing feature for Wisconsin ERI patch diseases has not been identified. This winter Dr. Maxwell's lab is evaluating molecular methods for identifying the causative pathogens for ERI patch diseases.

With the light microscope, I can look at a grass root that is suspected to be infected with a patch disease and say, "There's the fungus". WRONG!!!!! What may look like the fungal pathogen that causes the disease may be any of several saprophytic fungi. Even though hyphopodia are a size that can be seen with the light microscope, it is generally not possible to distinguish them from other fungal structures such as germinating spores.

This summer the TDDL received samples of the ERI diseases necrotic ring spot and take-all, and it became evident that better diagnostic methods were needed to identify the fungi that cause these diseases. This fall I looked at some specimens of patch diseases in the Scanning Electron Microscope (SEM) to see if this instrument could be used as a diagnostic tool. I wanted to see if I could diagnose a patch disease rapidly and easily by visualizing hyphopodia. *Poa annua* was inoculated with necrotic ring spot fungus and prepared for viewing. Preliminary results are not encouraging, as no identifying morphological characteristic for this pathogen was observed. No distinguishing feature was observed in the host, *Poa annua.* Additionally, root hairs are too close in size to the fungal hyphae and too numerous for positive identification of the pathogen.

Biological specimens for the SEM are killed chemically, dehydrated in a solvent, and then they are dried in an auxiliary instrument to remove all solvents and stabilize the specimen. Finally a thin layer of gold is deposited on the specimen surface with another auxiliary instrument. The advantages of the SEM compared to the light microscope are a three dimensional view of the specimen surface, higher magnifications, and the ability to see particles one thousand times smaller.

Dr. Gayle Worf first described the disease necrotic ring spot. Normally necrotic ring spot is a disease of Kentucky bluegrass, but the pathogen will also infect *Poa annua.* There are many reasons for infecting another host. One of Dr. Worf's reasons was investigation of the possibility of using the necrotic ring spot disease as a biological control of *Poa.* My reason was to produce a disease/host association that would be easier to interpret in the microscope because 1). there should be fewer non-pathogen microorganisms, 2). *Poa* is easier to look at than Kentucky bluegrass because with Kentucky bluegrass crown elongation and rhizomes make interpretation of results more difficult, and 3) I wanted to be sure I was looking at a young, actively growing pathogen.

Kentucky bluegrass (necrotic ring spot) and creeping bentgrass (take-all) were also viewed with the scanning electron microscope. No distinguishing features were observed for these pathogens. However, there are indications that there may be some very distinct host responses. These two perennial grasses are much more complex to look at than *Poa annua.* Crown elongation and root dynamics add a new dimension to interpreting results, and there is most likely a seasonal/soil moisture differential response.

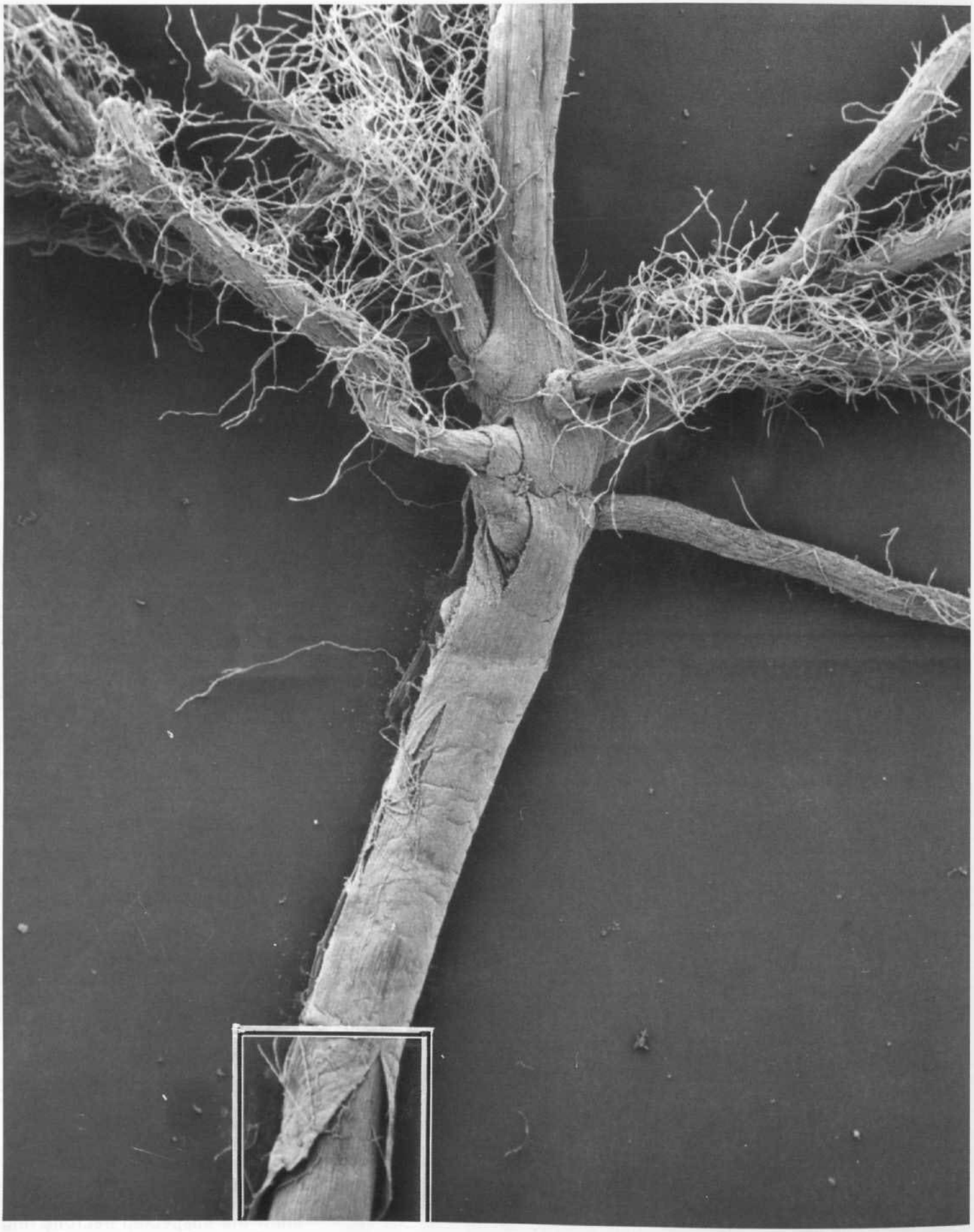
ACKNOWLEDGMENTS:

I wish to thank Heidi Barnhill for assistance with the SEM, S.A. Vicen for preparation of the photographs, Chris Wendorf from L.L. Olds Seed Company for *Poa annua* seed, and Dr. Maxwell and Dr. Worf for editorial comments. This project was supported by gift funds to D.P. Maxwell.

FIGURES:

The scanning electron micrograph on the next page is *Poa annua* magnified 55X. Roots, root hairs and the leaf sheath are obvious. The rectangle is enlarged on page 48.

Poa annua magnified 350X to show the suspected necrotic ring spot fungus. H = hyphae, and arrows point to structures that could be hyphopodia on page 49.



***Poa annua* magnified 55X.**

***Poa annua* magnified 350X**