If Disease Hits, Then What?

By AMY HOLM
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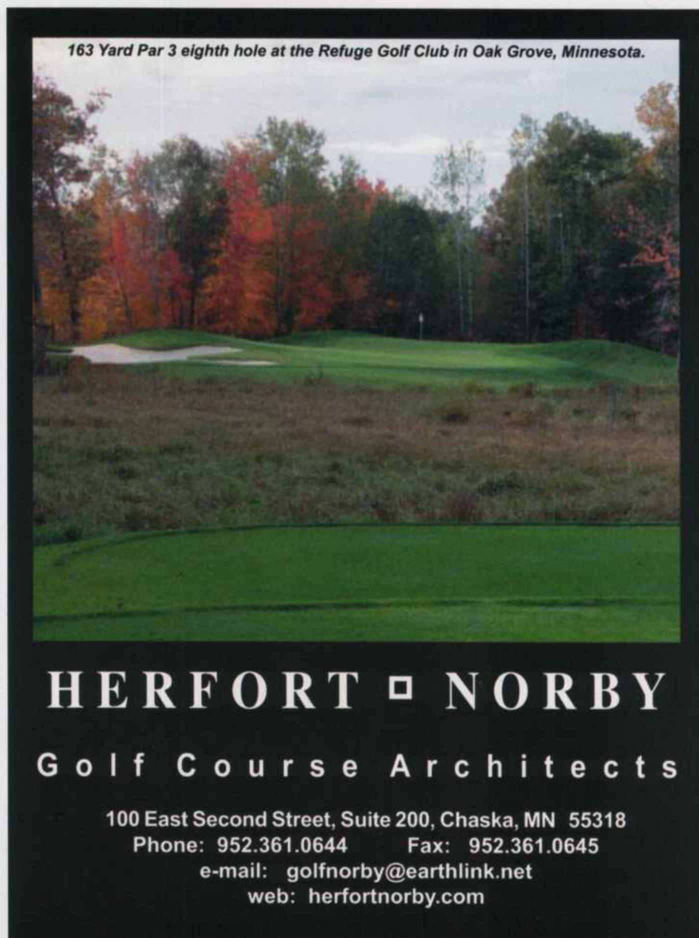
Hello, my name is Amy Holm. I am the plant disease diagnostician at the University of Minnesota's Plant Disease Clinic and identifying plant maladies is my passion. In 2001 I received my M.S. degree in Plant Pathology at North Dakota State University and then spent three years as a Research and Development Plant Pathologist at Syngenta Crop Protection prior to starting at the U of M in 2005. This article is intended to 1) help you understand what the U's Plant Disease Clinic can and what it can not do, 2) describe the help that ANY plant disease diagnostician needs from YOU if you are going to send in a sample of diseased plant tissue, 3) describe what goes on at our or any other clinic when it receives YOUR sample of diseased plant tissue and 4) provide current information about the services provided by some of the clinics that currently specialize in the diagnosis of diseases of turfgrasses.

The University of Minnesota's Clinic is a self-supporting function of the Department of Plant Pathology. The U's Plant Disease Clinic is the equivalent of a General Practitioner in that it can diagnose plant diseases caused by both living (biotic) as well as non-living (abiotic) stresses. It currently does not have sufficient personnel to be able to specialize in the diagnosis of diseases of any particular type of plant. It is housed in Room 105 of Stakman Hall on the St. Paul Campus. The Clinic is open from 8 a.m. until 4:30 p.m., Tuesday through Friday. Diseased plant material may be sent to the Plant Disease Clinic, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108.

Diseases of turfgrasses and other kinds of plants that are caused by a biotic agent cannot develop unless there is an interaction between a susceptible plant species or cultivar, a virulent pathogen and an environment within a favorable range for disease development. Or more succinctly, the components of The Plant Disease Triangle (causal agent, susceptible plant and suitable environment) have to be present for a disease to occur.


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

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So what do you do if a disease that you do not recognize "strikes"? To facilitate rapid, accurate diagnoses, a diagnostician at ANY clinic needs to receive a properly prepared sample along with the requested information on the submission form. An adequate sample is at least a 6" x 6" piece of turf, including the root system and soil. If the samples are collected using a golf course cup cutter, at least two plugs should be submitted. IMPORTANT: Samples need to be collected from the border between healthy and diseased turf, so that two-thirds of the sample is diseased and one-third is healthy. The soil and roots should be wrapped in aluminum foil and placed in a cardboard box along with packing material to hold the samples in place. Samples should not be stored or transported in plastic bags. If possible, include digital images along with physical samples. Pictures of the symptoms from a distance of 6 feet or more are often very useful for diagnosis of disease and insect problems. The sample, digital photos, and completed submission form should be sent by overnight mail.

Thermal Cycler Machine

The appearance of disease symptoms on affected plants is an important source of diagnostic information but other specific details are needed in order to make an accurate diagnosis. For example, information on disease patterns, affected grass species, disease injury (e.g. small circular spots, irregular concentrated patches, or large rings/circles) and environmental conditions must be provided to the diagnostician. Water drainage, soil conditions, terrain slope, age of the stand, traffic patterns around affected area and proximity of competing trees may all be significant factors in turf disease development. Chemical applications and the method of application also may provide valuable diagnostic clues.

The diagnostician must rely on visual symptoms, microscopic features and

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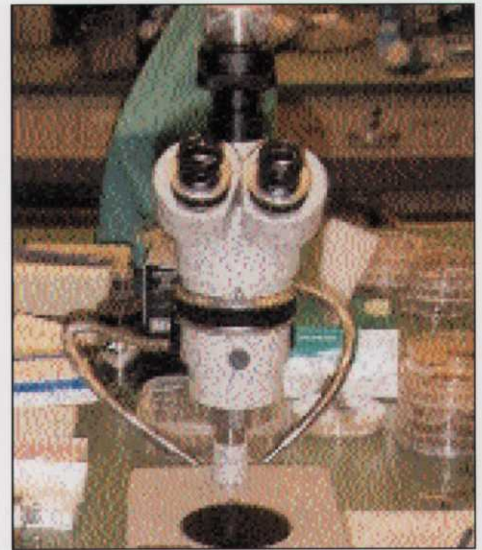
laboratory assays (e.g. isolation method and polymerase chain reaction assays) to efficiently detect and identify turfgrass pathogens. For certain disease organisms such as smut, powdery mildew, anthracnose and rust, fungal fruiting bodies may be visible without magnification or at low magnification. When a fungal organism is not macroscopically visible, it must be identified based on microscopic characteristics of its spores, hyphae (basic structural unit of a fungus) and mycelium (the body of a fungus). For example, some root-infecting fungi can be distinguished from other darkly-pigmented fungi (e.g. Bipolaris, Curvularia, Drechslera, Epicoccum, etc.) based on their hyphal characteristics. The root-infecting fungi may produce dark brown, runner ectotrophic (food "gathering") hyphae.

Diagnosticians often incubate symptomatic (diseased) plant tissue in a moisture chamber for 24-48 hours at room temperature. Under such conditions, fungal organisms usually grow well (produce hyphae) and may reproduce (sporulate). This procedure is most useful when the plant's foliage has been directly infected. The spores and mycelium that form can then be mounted in water on a glass slide for microscopic examination. However, one problem inherent with this technique is that any fungus present on the infected tissue may grow in this environment, and this can make it difficult to determine which was the primary pathogen responsible for causing the symptoms. Also, recognize that the process takes time (24 to 48 hours under ideal conditions).

Many root-rotting pathogens (living cause of plant disease) need to be cultured on an agar medium for positive identification. For each sample, excess soil is removed from the root layer, and the roots and stolons are washed for at least several minutes under running tap water to remove all remaining soil. Roots and stolons are cut into 2 cm pieces and rinsed thoroughly again in tap water. The tissue pieces are surface disinfected in sodium hypochlorite and washed in sterile distilled water. Sectioned tissue is dried on sterile paper towels and placed on an agar medium which is composed of a gelatin-like material on which microorganisms are grown. Five pieces of root that each contains both diseased as well as healthy tissue are aseptically placed in each Petri dish. Several dishes containing different kinds of media are typically used. The plates are incubated in a growth chamber maintained at 20 - 30° C. After a few days, the morphology of the dominant fungal colony is examined visually and microscopically. The color and morphology of fungal colonies in culture are useful taxonomic features. The culture plate isolation



Stereo Microscope

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technique is often utilized to identify pathogens of agricultural crops, trees and shrubs. But because a golf course superintendent often needs an answer within 72 hours, this method is not always practical for certain turfgrass diseases. Several turf grass pathogens, including some of the root-rotting fungi, are relatively slow-growing in culture.

The difficulties encountered when attempting to distinguish between different turfgrass pathogens using the methods described above has created a need for improved detection methods. Recent advances in biotechnology have resulted in the development of molecular techniques that may assist in the detection of turfgrass diseases. For instance, the polymerase chain reaction (PCR) has been used to detect and produce "patterns" (bands in agarose gel) that can be used to identify turf pathogens. PCR is a method that is used to amplify DNA for analysis. The PCR technique allows amplification (multiplication) of minute amounts of pathogen DNA in the sample by using DNA primers (short pieces of DNA) specific to the particular pathogen. PCR assays allow diagnosticians to process samples in three to four hours. In addition, this technique is very sensitive, enabling diagnosticians to detect a pathogen within plant tissue in the early stages of infection. Continued research on molecular techniques could result in improved, reliable and rapid diagnostic tests with practical applications in turfgrass pathology.

I hope this article helps you gain a more comprehensive understanding of the methods used to detect and identify turfgrass pathogens. The U's Plant Disease Clinic is not able to meet the current demand for rapid turn-around of turfgrass samples with our current staffing. If you experience a significant disease-related problem, feel free to contact our clinic and we can help direct your efforts to the best of our ability. We may be reached at (612) 625-1275 or at holmx157@umn.edu.

(Editor's Note: The following is contact information for laboratories that specialize in turfgrass diagnostics:

Plant Diagnostic Laboratory
Rutgers NJAES
Ralph Geiger Turfgrass Education Center
20 Indyk-Engel Way
North Brunswick, NJ 08902
www.rcre.rutgers.edu/plantdiagnosticlab/
Phone: (732) 932-9140
E-mail: clinic@rcre.rutgers.edu
Fee: $95/sample for out-of-state samples

NC State Turfgrass Diagnostics Lab
Campus Box 7211
1227 Gardner Hall
100 Derieux Place
Raleigh, NC 27695-721
www.ces.ncsu.edu/pdic
Phone: (919) 513-3878
E-mail: lee_butler@ncsu.edu
Fee: $100/sample for out-of-state sample
Contact Lee Butler to inquire about packages

Texas Plant Disease Diagnostic Laboratory
1500 Research Parkway, Suite A130
Texas A&M University Research Park
College Station, TX 77845
http://plantpathology.tamu.edu
Phone: (979) 845-8032
Fee: $30/sample for out-of-state samples

Turfgrass Diagnostic Lab
University of Wisconsin-Madison
O. J. Noer Research Facility
2502 Hwy M
Verona, WI 53593-9537
www.plantpath.wisc.edu/tdl
Phone: (608) 845-2535
E-mail: plk@plantpath.wisc.edu
Fee: $100/sample for out-of-state sample

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