MICROSCOPIC IDENTIFICATION OF COMMON TURFGRASS PATHOGENS*

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INTRODUCTION

The clinical diagnosis of turfgrass diseases is presently carried out in a rather peculiar fasion. The turf manager, who is on-site, knows the environmental and management history of his particular piece of diseased turf. When a disease appears, he attempts to make a diagnosis from the appearance of the symptoms on the grass. This method of diagnosis is fine if the complex of symptoms is classical, that is, if it looks just like it's supposed to. If it is unusual because of environmental factors or because of the presence of more than one pathogen, then the fun begins.

A grass plant has only a limited number of ways of responding to pathogen attack. It can get spots, it can turn yellow, or it can just drop dead. Sometimes, dollarspot can look like Pythium blight or red thread, or brown patch can look like fairy ring, or Typhula snow mold can look like Fusarium patch. So the person in charge of "grass beautiful" makes an educated guess and runs for the fungicide shelf, because he usually has nothing more to go on than what the symptom pattern looks like and what the weather has been. Sometimes he's lucky and the weather changes or the chemical really works, and calm returns--until the next onslaught. If the first chemical he applies doesn't check the disease, he tries another and another and another--usually with rising panic as his grass disappears.

Finally in desperation, he takes a cup cutter, removes a 4-inch plug of his suffering sod, and sends it to an "expert" for diagnosis. Now, this "expert" is usually miles away and has none of the on-site manager's knowledge about how this grass has been managed or what it has been subjected to by the elements. He doesn't even know what the whole symptom pattern looks like. All he has is a 4-inch plug of suffering sod, which by now is really suffering, since it has spent up to a week in a dark, dank box on its journey to the "expert."

If the "expert" is lucky and there's any grass left, he may recognize the symptom just by looking and can bail out the poor waiting manager. More often, though, an attempt must be made to culture the pathogen from the diseased grass (2 or 3 more days). Now the black magic really starts! Any grass plug--whether showing symptoms or not--will probably yield on culture, at least three turf pathogens. So even after all of this examination, the "expert" still must make an educated guess about what is ailing this poor grass.

As a sometimes "expert," I know how easy it is to be one. I think that with a minimum of equipment and a few sign-posts to go by, any turf manager can become his own "expert." Indeed, he can be better than someone off-site, because he knows the history of his turf and he sees the symptom pattern. With a little bit of know-how, he can often make a diagnosis within a few minutes by examining a few blades of his ailing grass under a microscope. It is really quite easy, much quicker than the distant "expert," much surer than just looking at the symptom pattern, and, in the long run, probably a lot cheaper.

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There are three very important aspects to the identification of turfgrass diseases:

(1) Knowing of the ENVIRONMENT under which the disease developed. Has it been hot or cold? Wet or dry? What management practices have been used? Nitrogen? Fungicides? Insecticides? Herbicides?
(2) Careful observation of the SYMPTOMS ON THE GRASS. This involves getting down on your knees, preferably with a magnifying lens of some sort, and closely examining the diseased grass. Does it have spots on the leaves? What do they look like? Are the leaves blighted? Is there crown or root rot? Can you see the cottony growth of fungi on the affected grass?
(3) What kinds of FUNGUS STRUCTURES can be seen by examining the diseased grass under microscope?

NECESSARY EQUIPMENT

The only items that are truly necessary to do microscopic examination and diagnosis of turf diseases are an adequate compound microscope, some microscope slides and cover slips. You can get set up with these for as little as $200 to $300.

The most expensive piece of equipment needed is, of course, a microscope. An adequate microscope with three objectives (different magnifications) and a built-in, substage light can be purchased for $200 to $250. This may seem expensive, but you'll probably save that much very quickly in fungicides which you don't use unnecessarily. This price is for a single-eyepiece scope. For a compound binocular scope (two eyepieces for easier looking), you will probably have to pay about $250 more. A mechanical stage, if you want one, is an additional $100. A mechanical stage is a gadget which moves the slide with knobs, so you don't have to slide it around with your fingers. Neither of these additional-cost items is really necessary, but they do make slide examination easier and more convenient. Since you will only be examining an occasional slide, they should be considered luxury items. If you are really on a tight budget and want to try a cheaper microscope to start, there are some available for under $100. Before buying one of them, get one on a trial basis to see if you feel it is adequate for your purposes.

A box of glass microscope slides ($4.00/gross), a box of cover slips ($1.00/100), and a dropper-bottle of water complete your lab, and you are ready to examine your first slide.

An additional help, but also an additional expense, is some sort of magnifier to examine your turf plug so that you can select a blade of grass with fungal mycelium or lesions for microscopic examination. This can be anything from a simple 10X magnifying lens for $10-$15 to a stereo-microscope for $150. Again, this is not necessary, but can be a great aid.

For nematode counts and identifications, some very simple and inexpensive equipment is needed to extract the nematodes from your turf sample: several 4-inch-diameter glass or plastic funnels ($1.50 each), a wooden funnel support for 2 funnels ($4.00), 2 pinch clamps and rubber tubing ($4.00), some small centrifuge tubes ($4.00/10), a 1/4 cup measure, some formaldehyde, and 2 small pieces of screen.

FUNGAL STRUCTURE AND APPEARANCE

The important infectious diseases of turfgrass which are recognized at this time are caused almost exclusively by fungi. Technically, fungi are plants, but one would be hard-pressed to see much similarity between a fungus and a tree.

Compared with higher plants, fungi are very simple in structure. They are composed of a much-branched system of thin tubes, which resemble branching roots or tree limbs. One of these thin tubes is called a hypha, several are called hyphae, and a mass of them is called a mycelium. Sometimes these terms are used interchangeably. Individual hyphae are not visible without magnification, but a mycelium can be seen with the naked eye. Under conditions of high moisture, mycelium of the fungi which cause Pythium blight, Fusarium patch, dollar spot, brown patch, and other turfgrass diseases can often be seen on infected grass. These masses of hyphae on grass, when observed with the naked eye, look very similar, regardless of which fungus is involved. However, when a blade of grass which has mycelium growing on it is put under a microscope and examined, there are often noticeable differences. It is these differences, together with careful observation of the symptom pattern and the environment during symptom development, which make it possible to identify certain fungi.

A word of caution--these microscopic aids to identification are meant to be used in conjunction with symptom and environmental observation, and are by no means fool-proof. There is much more to the accurate scientific identification of fungi than these simple observations. If used as intended, however, they will greatly increase your chances of accurately identifying a particular disease.

Pythium may often be distinguished from Sclerotinia (the dollar spot fungus) or Rhizoctonia (the brown patch fungus) by the appearance of the hyphae under the microscope. The hyphae of some fungi have cross walls which separate the hyphae into individual cells. Sclerotinia and Rhizoctonia both have such cross walls. Other fungi have no cross walls in their hyphae. Pythium is an example of a fungus with no cross walls.

The way the hyphae branch can often give clues to help identify fungi. In most fungi, the hyphae branch in V's much like tree branches. Rhizoctonia, however, usually has a characteristic branching which helps to identify it. The branches in Rhizoctonia are usually at right angles, and, in addition, the hyphae have little constrictions or pinched-in places at the origin of the branches. As you can see, just by making a simple microscopic examination for the presence or absence of cross walls and the type of branching, one can make an educated guess about whether the fungus in question is Pythium, Sclerotinia, or Rhizoctonia.

Another word of caution--these observations of hyphal structure provide CLUES to the identity of fungi. ALL fungi without cross walls in their hyphae are NOT Pythium--so just because you see hyphae without cross walls, you cannot say you are looking at Pythium. All you can say is that fungus you see MAY be Pythium. You must put together all of your 'clues'--microscopic, environmental, and symptom observation. The same is true of the branching type which you observe. ALL fungi with right-angle branching are NOT Rhizoctonia, nor are ALL fungi with V-branching Sclerotinia. Do not make the mistake of believing you can positively identify fungi so easily.

Another feature of the mycelium of certain fungi which can aid in identification is a structure called a clamp connection. These unique, donut-like structures occur in hyphae which have cross walls. They appear as little branches which originate on one side of a cross wall, bend around, and attach to the hypha on the other side of the cross wall, so that the "clamp" forms a little bridge between the two cells on either side of the cross wall. These structures are very distinctive, and once you know what they look like, there is little difficulty in spotting them. Three common pathogens of turfgrass have clamp connections: Typhula (the gray snow mold fungus), Corticium (the red thread fungus), and most fairy ring fungi. Here, again, symptoms and environment can serve to separate these three fungi from one another.

There are two other mycelial structures which can be seen with the naked eye and which are quite diagnostic. These are the brown to black sclerotia of Typhula

(the gray snow mold fungus) and the coral red stroma of Corticium (the red thread fungus). The sclerotia of Typhula are found embedded in leaf tissue, and are hard, resistant structures which enable the fungus to survive unfavorable conditions. The red stroma of Corticium are masses of hyphae adhering together, and appear as red threads on the ends of the grass leaves. The disease gets its name from these red stroma. Both of these structures are easily seen with the naked eye, but often don't appear until the late stages of the disease. In these cases, they aren't too helpful in early diagnosis, since damage may be severe before these structures appear. As you will recall, both of these fungi form clamp connections, which can be observed with a microscope long before the sclerotia or "red threads" may appear.

Many fungi form some kind of spores. Spores are somewhat like the seeds of higher plants and serve much the same purposes. They help the fungus to survive periods of unfavorable environment that may kill the mycelium, and they serve to multiply and spread the fungus from place to place. Spores are found in a great variety of sizes and shapes, and are often quite distinctive. A distinctive spore can be quite valuable as an identification aid. Such spores are produced by four common turfgrass pathogens, Helminthosporium (the leaf spot/melting-out fungus), Curvularia (the fading-out fungus), and Fusarium (the Fusarium blight and Fusarium patch fungi).

The spores of Helminthosporium and Curvularia look somewhat alike, but with some practice you can learn to tell them apart. They are large, dark, cigar-shaped spores with three or more cells. Helminthosporium spores are uniformly dark and are generally straighter than Curvularia spores. Curvularia spores may be slightly curved and the middle cell in the spore is sometimes keystone-shaped. In addition, the cells on either end of the Curvularia spore are usually lighter in color than the center cells.

Fusarium spores are also quite distinctive. They are long, slender canoe- or crescent-shaped spores, with 2 or more cells. It can be a little tricky to distinguish the spores of the Fusarium blight fungus from the Fusarium patch fungus, but you won't have to do this since the environments under which these two diseases occur are very different.

The diseases of turfgrass which have leaf spot phases or typical leaf lesions are usually fairly easy to identify from the leaf symptoms. These include Helmintohsporium leaf spot, dollar spot, rust, powdery mildew, and strip smut. If the leaf lesions are typical, then there should be no need to use microscopic examination for diagnosis of these diseases. Sometimes, though, Helminthosporium leaf spot or dollar spot may not present the typical symptom pattern. In these cases, microscopic examination of affected leaves can usually resolve the problem.

There is another fungus which produces crescent-shaped spores with only one cell which may be confused with Fusarium spores. These spores are produced by Collectotrichum graminicola, the fungus which causes anthracnose on turfgrasses. Anthracnose is most common during periods of excess moisture and temperatures of 80 to 90 F. Anthracnose can be recognized, however, by the presence on blighted and killed leaves of numerous, tiny, black spore-bearing bodies (acervuli) with prominent black spines (setae). These can usually be seen in abundance with the aid of a 10X magnifying lens or a steromicroscope.

PREPARATION OF SAMPLES FOR MICROSCOPIC EXAMINATION

When selecting diseased grass specimens to examine under the microscope, do not select completely dead grass. There are all kinds of fungi which grow on dead grass, and this can make finding the fungus which actually killed the grass very difficult. Try to find areas where the disease is working, and the grass is just beginning to show symptoms. If you can, select blades which have mycelium on

them. Early morning or humid, overcast days are good times to select blades which have mycelium on them. This is the point at which a magnifyng lens can be very helpful. With it, you can see the symptom close up, and may be able to see strands of fungal hyphae, or even insects, which you have not seen without magnification.

When you have selected some grass which you think may have your culprit on it, put a drop or two of water on a 1 x 3 inch microscope slide. Place several pieces of grass which show symptoms or mycelium in the water and cover the whole business with a cover slip. Don't just drop the cover slip on the water and grass, because this will trap air bubbles around the grass blades. It is very difficult to see properly when a slide is full of air bubbles, so try to avoid as many as possible. Holding the cover slip at about a 45° angle with the slide, place on edge in the water and gently lower it until it is totally in contact with the water and grass. Now you can examine the grass under the microscope for the presence of spores and the features of the hyphae. You may have to make several slides before you get a good one where you can really see the mycelium and spores which may be there. I usually make two or three to begin with. If you have a lot of mycelium on the grass, and it tends to stick together when it gets in the water, take the corner of the cover slip and tease the mycelium apart so that you can examine individual strands of hyphae for structure.

Examine your slide thoroughly and carefully. Don't stop as soon as you have identified your first spore or piece of mycelium. Begin at one corner of your slide and move back and forth until you have covered the entire slide. Do this back and forth scanning with a low power objective, and, when you see hyphae or spores, switch to a higher power objective to examine the structure carefully. It is not uncommon to find two or three different fungal pathogens present in a turf sample. Your problem may be due to all of them, one of them, or none of them! Part of you job as a manager is to put all your evidence together, and make your best estimate about what is causing your problem. Remember, that's what the "experts" do too. They are very seldom completely sure, either!

WHAT TO DO WITH YOUR FINDINGS

There will be times when, no matter how long or how carefully you examine grass from certain symptoms, you will not be able to find anything which will help you decide what is ailing your grass. This is particularly true when symptoms are a result of root injury caused by fungi. This happens to the "experts," too. Sometimes there just isn't anything obvious to pin the problem on. Depending on the season, though, you should be able to come up with an answer with your microscopic examination at least 50% of the time. When you can't, this is the time to seek the help of the distant "expert." When "expert" microscopic examination turns up nothing, your grass will be cultured. To do this, little bits of grass are placed on various kinds of growth media. In about a week, the troublemakers which have been hiding inside the grass will grow out onto the media, and we can see who they are. As I said in the introduction, however, fungus pathogens will grow out of almost any turf sample--even if it's "healthy." So you can see, even the "expert" has to try to put together information on symptoms, environment, and fungi to come up with an educated estimate about what is ailing your grass. IN OTHER WORDS, HE DOES JUST WHAT YOU DO! The important thing is to make the diagnosis as "educated" or sensible as possible, using all the information available.

Let us assume that you have found one or more pathogenic fungi or a high count of parasitic nematodes in your turf sample. Does this mean that you have found the cause of the symptom which is present on your grass? It may--but it is by no means certain. You must now put together <u>all</u> of your information about (1)

the ENVIRONMENT under which the disease developed, (2) the appearance and severity of the SYMPTOMS, and (3) the PATHOGENS which you have seen. You can then make a more educated decision about whether or not to use a fungicide or nematicide, and which one to use.

If you have a choice of several materials to use, it may be advisable, and, in the end, more economical to run a small field trial of your own to find out which one may control the disease best. This is not difficult to do. Apply strips of your test materials across a small plot of diseased grass, always leaving an untreated check area. Fortunately, many times a symptom will disappear just because the weather changes, and the pathogen is no longer able to attack the grass. Your untreated check will tell you whether this has happened. Without the untreated check, you might think your materials had caused the symptoms to disappear, and apply chemicals which you don't need. If the materials in your trial are going to control the symptom, you should be able to see some response within a short time. You can then pick the best material from the ones which you have tested, and treat the entire affected area with the best material.

This may seem like a lot of time to invest when something is chewing on your grass, but remember, it's probably a lot faster to do your own examination and on-site testing of control chemicals, than to wait for your sample to reach some "expert" by mail, have them do what you could have done, and then mail the results back to you. <u>You</u>, the on-site manager, are in a position to do the job much more quickly, and, with a little practice, much more accurately than the distant "expert." After all, you are there where the action is.