

COLLECTING AND SHIPPING TURFGRASS SAMPLES FOR DISEASE DIAGNOSIS



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Collecting and Shipping Turfgrass Samples For Disease Diagnosis

Following these procedures
Will greatly increase your chances
Of receiving a prompt, accurate diagnosis.

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There are a multitude of fungi, plant parasitic nematodes, bacteria and viruses that attack turfgrasses. Most disease problems are caused by fungi and plant parasitic nematodes, but there are a few known bacterial or viral turfgrass pathogens.

Many fungal diseases can be distinguished easily because of the unique signs or symptoms that accompany their activity. For example, the red thread fungus produces a pink-gelatinous mycelium and characteristic red, antler-like sclerotia that are almost always present, making this disease easy to diagnose. Unfortunately, many diseases do not always produce textbook symptoms. Certain signs, such as foliar mycelium for instance, can be deceiving. During early infection stages, the fungi that cause dollar spot, brown patch and Pythium blight may produce large amounts of foliar mycelium. Most turfgrass managers equate foliar mycelium with Pythium blight. But in many situations, this is an error in judgement. Though sometimes difficult to distinguish by on site inspection, these three diseases can be easily separated by microscopic observation of the mycelium.

Other pathogens, particularly those that attack roots, can be extremely difficult to identify. Take-all, summer patch, dead spot, and spring dead spot are root and stem diseases. These pathogens cannot be identified accurately by mycelial characteristics alone. To be absolutely certain, a pathologist must isolate the pathogen on a specialized medium, but this process is very time consuming and not consistently successful. For the aforementioned diseases, the pathologist likely will base his/her diagnosis on hyphal characteristics, host, field symptoms and other factors. Pythium induced root dysfunction and root rot are especially difficult and time consuming to diagnose since roots have to be visually inspected for the presence of spores.

Many fungal diseases, or the activity of parasitic nematodes, may produce disease symptoms that mimic environmental stress or insect pest damage. A combination of overlapping symptomatology of numerous diseases, as well as environmental stresses and insect pests, makes diagnosis a challenge for even the experts. When perplexed or exasperated, superintendents normally send samples to a laboratory to obtain a precise answer for unknown maladies.

Collecting and Shipping Samples For Fungal Diseases and Bacterial Wilt

Land Grant Universities provide plant disease diagnostic services, and like private labs, they charge a testing fee, which can be as high as \$100 per sample. Because of this, it is not only prudent for superintendents to choose a laboratory in which they have confidence, but also to ensure that samples have been properly collected and shipped.

Most turf pathologists at universities have few or no daily responsibilities in plant disease diagnostic labs or clinics run by the cooperative extension service. Therefore, if you wish for a particular specialist to handle your testing, it is wise to contact that individual by telephone or email prior to shipping samples. Otherwise, the turf disease specialist may be out of town or have other commitments that preclude a rapid handling of your sample. Because time always is an essential factor in treating turf diseases, samples must be transported as rapidly as possible. Either direct transport or overnight express shipment is preferable.

When using express mail, never ship on Friday or Saturday, because samples arriving over a weekend or holiday will not be processed until the following Monday or workday. Furthermore, there may not be adequate

time for diagnosticians to handle and properly process samples received on Friday; therefore, **it is best for your samples to arrive early in the week.**

The pathologist should receive fresh samples in good condition. For best results, they must be collected while the disease is actively injuring or causing a decline of the turf. Samples collected even a few days after disease activity has subsided may yield negative or misleading information. Golf course cup cutters provide an ideal sample size. **Do not use ¾" or 1.0" soil probe cores.** Using a cup cutter, choose an area that will contain about one third each of old damage, new damage and healthy turf. The majority of the sample should contain various stages of unhealthy, declining, blighted tissue and even some dead tissue (if present). The sample need only be two to three inches deep. Sending an eight inch deep cup cutter sample is not only a waste of money, but large amounts of soil are messy to handle in the lab. Wrap each plug in an inner damp paper towel, followed by an outer wrapping in aluminum foil. Aluminum foil is preferred because it helps ensure that soil and roots remain intact during transport, thus avoiding mixing soil with the turfgrass canopy. **Pack the box as if you were sending glass** to avoid tumbling of the sample. Improper packaging results in the mixing of soil with the turfgrass canopy, which can either ruin the sample or greatly impede a proper diagnosis.

Avoid sending mostly dead plants or samples from turf areas recently treated with a fungicide. Samples should be clearly marked on the **outside** of the wrapped turf plugs. It is extremely helpful to provide photo's for the pathologist since he/she cannot be on site to see the actual symptoms. Additional site information also is needed. The sample should be accompanied by a site information form from the lab or a letter. The form or letter should be placed in a separate plastic bag and should contain the following information:

1. Turf Species and Site Affected

- green, tee or fairway
- full sun, partial shade, heavy shade
- soil often wet or dry
- name of cultivar and age of turf (especially putting greens), if known
- mowing height and frequency



Cup cutter plugs (3.0 inch diameter x 2.0 inches deep) wrapped in a moist towel and wrapped again in aluminum foil are best.

2. Symptoms and Environmental Conditions

- describe symptoms (e.g., circular patches, diffuse thinning or color changes) and distribution (e.g., widespread, localized, scattered)
- describe weather conditions just prior to and during the period when injury became evident
- note date symptoms first appeared

3. Symptoms and Environmental Conditions

- describe symptoms (e.g., circular patches, diffuse thinning or color changes) and distribution (e.g., widespread, localized, scattered)
- describe weather conditions just prior to and during the period when injury became evident
- give date symptoms first appeared

4. Cultural and Chemical

- list all pesticides, fertilizers and other chemicals applied to the site within 14 days prior to appearance of symptoms
- note the total amount of nitrogen fertilizer applied in the last 12 months and soil pH
- irrigation frequency, amounts and time of day

5. Email Photographs

- close-up photos and photos taken from a standing position are particularly useful

SUMMARY OF KEY POINTS FOR SENDING FUNGAL/BACTERIAL WILT SAMPLES

- Call the lab to ensure a turf pathologist is available to receive your sample(s).
- Ship by overnight express early in the week to ensure arrival by Thursday; do not ship samples on Friday or Saturday.
- Using a cup cutter, select an area that displays early and advanced stages of the disease. **Cup cutter plugs are inappropriate for parasitic nematode assays.** See the discussion below on proper sample collection for plant parasitic nematodes.
- Avoid sending samples that contain only dead tissue, fungicide residues or excess soil.
- Wrap the sample in an inner damp paper towel and an outer wrap of aluminum foil.
- Identify site location of sample (e.g., Green 2) on the outside of the foil wrap.
- Pack samples as if they were glass to avoid tumbling in the box.
- Note turf species, symptoms and site information.
- Email or mail close up and standing position photographs of symptomatic turf.

Collecting and Shipping Samples For Plant Parasitic Nematodes

All too often a superintendent will send a cup cutter plug or shovelful of soil from a single site to the laboratory and request a nematode assay. This type of sample is unacceptable because the results will likely provide meaningless information.

A good sample from a single green will require several minutes of work. It is best to collect soil a day after rain or irrigation when it is moist, but not wet or muddy. About one pint of soil from each site is necessary, rather than a composite sample from two or more greens, tees, fairways, sports fields, sod fields, etc. In areas where parasitic nematodes are a chronic problem, soil should be collected in late spring or early summer (about mid-June) and again in late summer (about mid- to late September). Nematode counts from monitored sites will provide baseline information on population densities of parasitic nematodes for each sample area. This baseline information is particularly useful for interpreting problems with putting greens that are poorly rooted and tend to wilt rapidly each summer.

Samples should be collected in the upper 3 inches of soil because this area represents the zone where most turfgrass roots are found. If roots are confined only to a 1 to 2 inch soil depth, this would be the appropriate zone from which to sample. The best tool for the job is the common "soil probe", which extracts cores that range from 0.75 to 1.0 inch (2.0-2.5 cm) in diameter. **Soil cores should be taken randomly from the interface between damaged or declining turf and healthy turf.** The thatch layer, and plant stems and leaves should be discarded—only soil and roots are needed. Samples from dead areas, or areas in where few plants persist, will provide false population density information. This is because plant parasitic nematodes are obligate parasites that feed only on living plant tissue. Consequently, parasitic nematodes naturally migrate outwards from severely thinned areas, and populations generally are highest in the region where unhealthy and healthy plants meet. About 15 to 20 randomly selected soil cores, or one pint (480 cc) of soil, will provide a satisfactory sample size. Collected soil cores should be uniformly mixed and placed in a plastic bag or specially-lined bags provided by the testing laboratory. Avoid using thin-paper lunch bags because the moist soil is likely to cause the bags to fall apart during transport. It is very important that site information be marked on the outside of the bag with a water-proof marker. Do not place small scraps of paper that identify the collection site inside the bag. These paper scraps deteriorate rapidly, and will probably be unreadable within a few hours of contact with moist soil.

Once collected, the soil should be kept out of direct sunlight. Samples placed on the dashboard or in the trunk of a car will heat-up rapidly, killing the nematodes. If samples must be held for a few days, they should be refrigerated or placed in a cool and dark room. Extremes in temperature, high or low, will kill nematodes. This must be avoided because only living nematodes can be effectively extracted from soil.

Unfortunately, there are no reliable data correlating parasitic nematode number per sample and expected degree of turf injury in the field. For example, a count of 800 root knot nematodes/250 cc of soil may cause no visual damage to a putting green that is well drained and deeply rooted. But, 100 root knot nematodes/250 cc soil from a green in which rooting is shallow may result in severe thinning of the stand during hot, dry or windy periods. The nematologist, however, generally will be able to provide a good management recommendation based

not only on the total number of plant parasitic nematodes detected, but also on the species of nematodes that are present.

SUMMARY OF KEY POINTS FOR SENDING SOIL FOR A PLANT PARASITIC NEMATODE ASSAY

- Collect moist soil samples to root zone depth using a 0.75” soil probe.
- Sample areas randomly and at the interface between declining and healthy turf. Do not take samples from extremely thin or dead areas.
- Soil from 15 to 20 cores, or one pint of soil, should be mixed and thatch, stem and leaves should be discarded.
- Place soil in specially lined bags and keep samples in a cool place until being shipped by express mail.
- Identify the site of collection on the outside of the bag using a waterproof marker.