

Microscopic Identification of Turfgrass Diseases



Alan Windham
Frank Wong
Brandon Horvath

Microscopy 101

The use of a microscope can be fascinating or in some cases frustrating if you have limited experience with microscopy. Ideally, if you wish to become proficient at identifying turf diseases, it's best to have a **dissecting microscope** (6-40X) and a **compound microscope** (40-400X). Each microscope is valuable and has particular strengths.

Dissecting Microscope

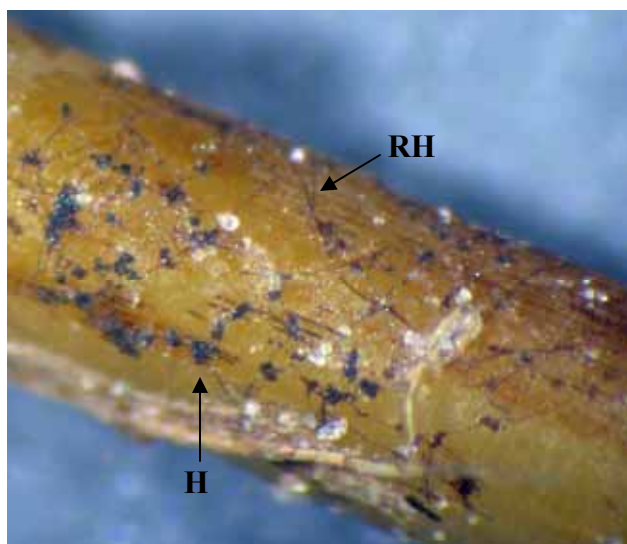
A dissecting microscope is quite useful in disease diagnosis. It's a good place to start with a sample. In many cases you may put all or a portion of a plug from a green on the stage of the microscope and examine the foliage, stolons and roots for the presence of fungal hyphae, fruiting bodies and, in some instances, masses of spores. A dissecting microscope is very useful to pinpoint foliage or fungal structures that can be transferred to a glass slide for examination with a compound microscope.



A Meiji trinocular dissecting microscope. The photo tube accommodates a camera for photography.



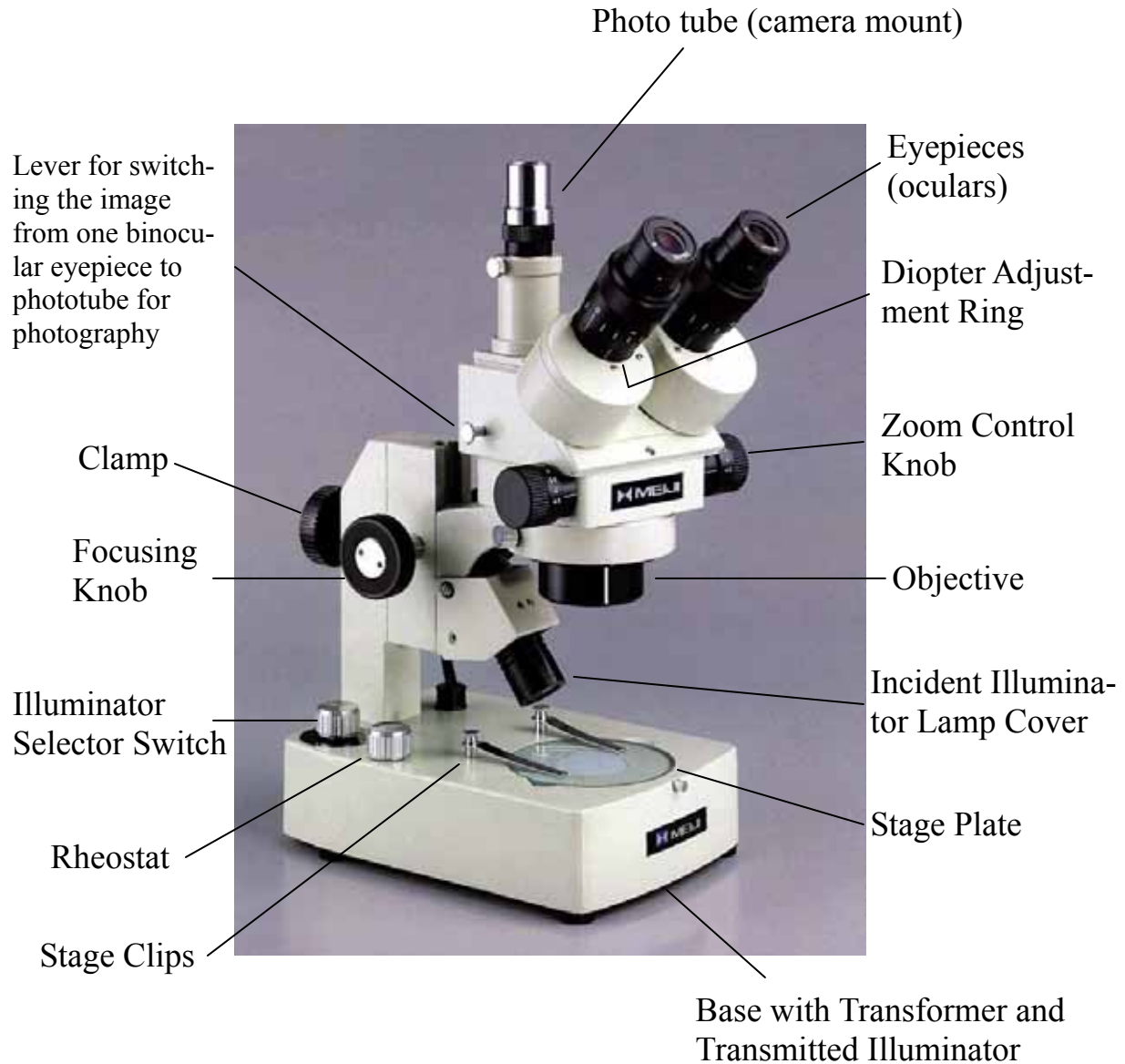
Mycelium of *Sclerotium rolfsii* as viewed with a dissecting microscope (40X). Mycelium may be retrieved with forceps or a probe and placed on a glass slide for examination with a compound microscope.



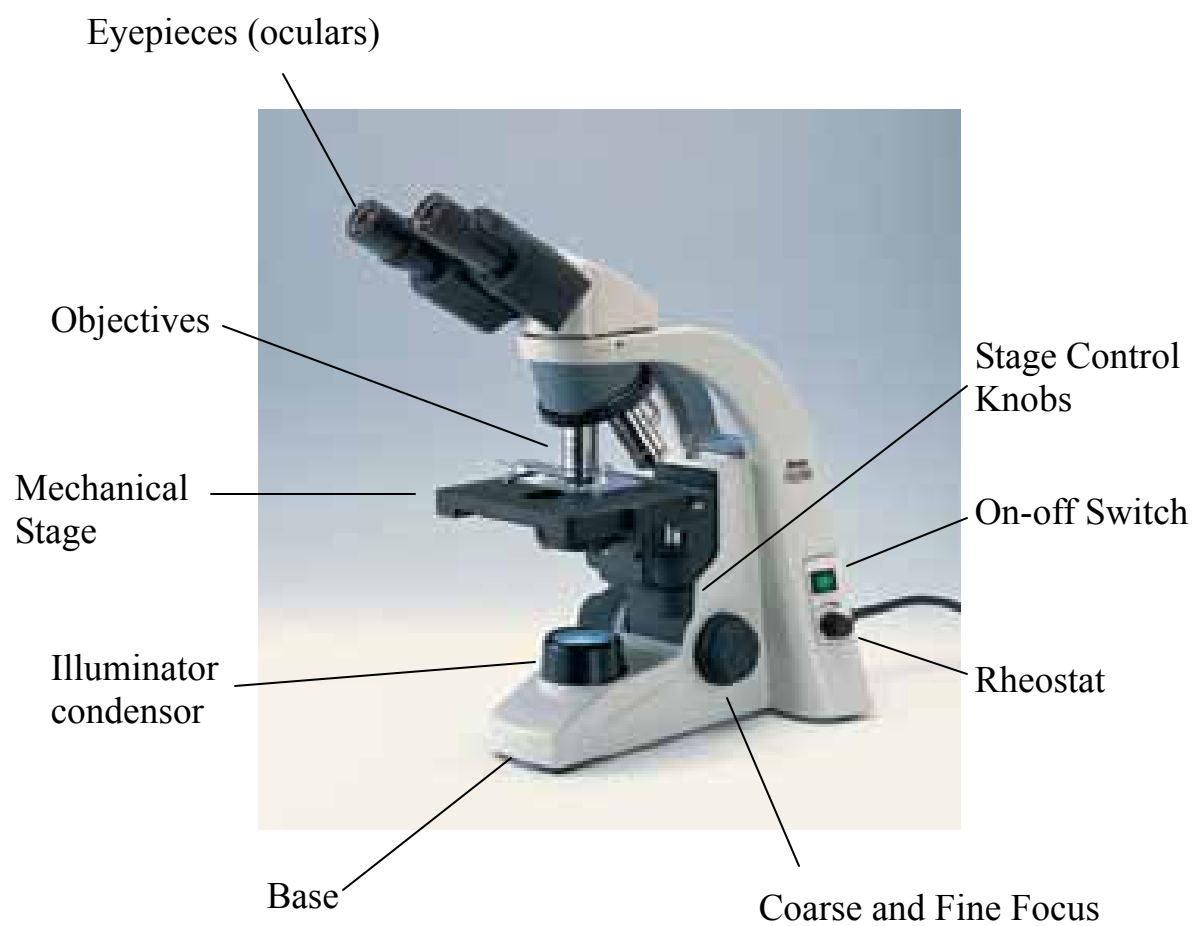
Runner hyphae(RH) and hyphopodia (H) of *Gaemannomyces graminis var graminis* on a ultradwarf bermudagrass stolon (40X).

A dissecting microscope may also be used to scan plant material for mites and small insects. You can also observe galled roots infected with root knot nematodes. Other nematodes are difficult to observe without special preparation of a soil or plant sample. Leave most nematode assays to the experts.

Dissecting Microscope



Compound Microscope

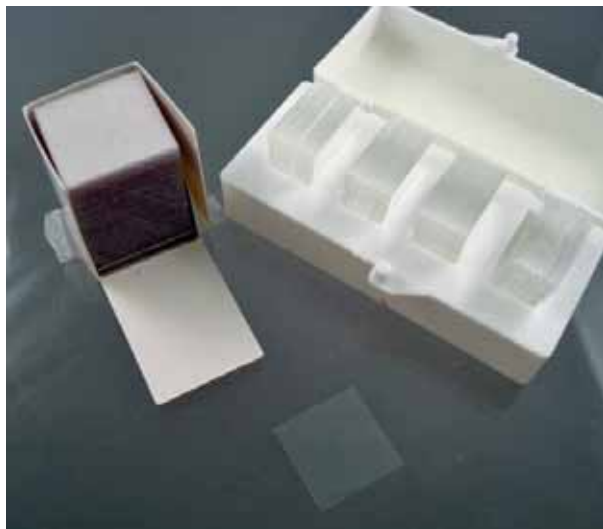


Lab Supplies

There are a few lab supplies that you will need in addition to microscopes. Microscope slides, cover slips and a dropper-bottle of water are the basics. It also helpful to have a scalpel, a pair of forceps, a probe and some mycological stains or reagents such as lactophenol.



Glass microscope slides are used to examine plant tissue or fungal structures with a compound microscope. The dimensions of most slides are 1x3 inches.



Glass or plastic cover slips (glasses) are placed over the specimen once it is mounted on a microscope slide. Cover slips are available in various dimensions. Common sizes are 15 mm-to-22mm².



Water (A) is the most common mounting medium. Lactophenol (B) can be used to preserve specimens for short periods of time. Specimens mounted in lactophenol will not dry out as quickly as those mounted in water. Cotton blue in lactophenol, (C) is a common mycological stain.

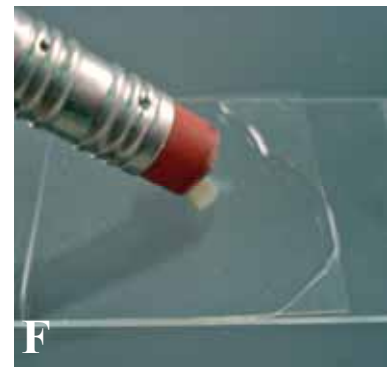
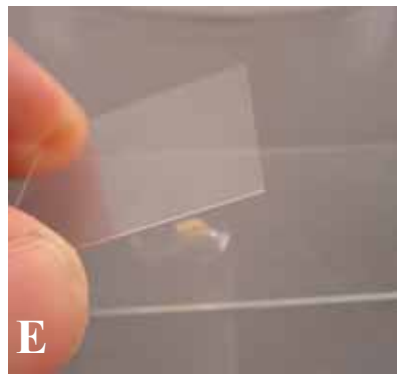
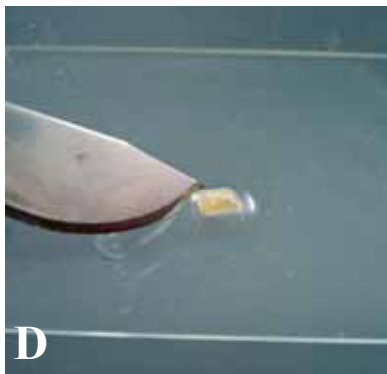
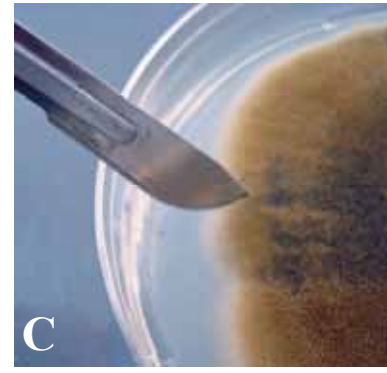
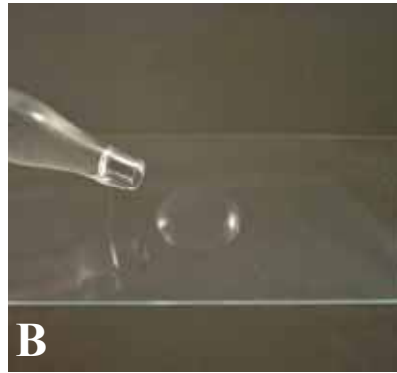
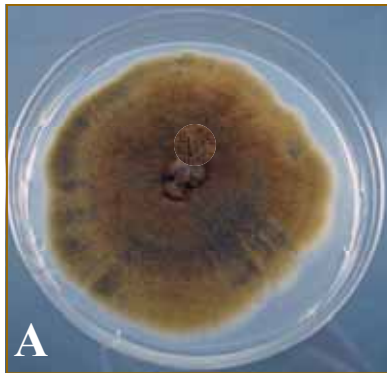


A permanent marker for labeling slides, a scalpel for cutting tissue, a pair of forceps for moving tissue from specimen to slide and a probe are helpful instruments used in preparing specimens for microscopy.



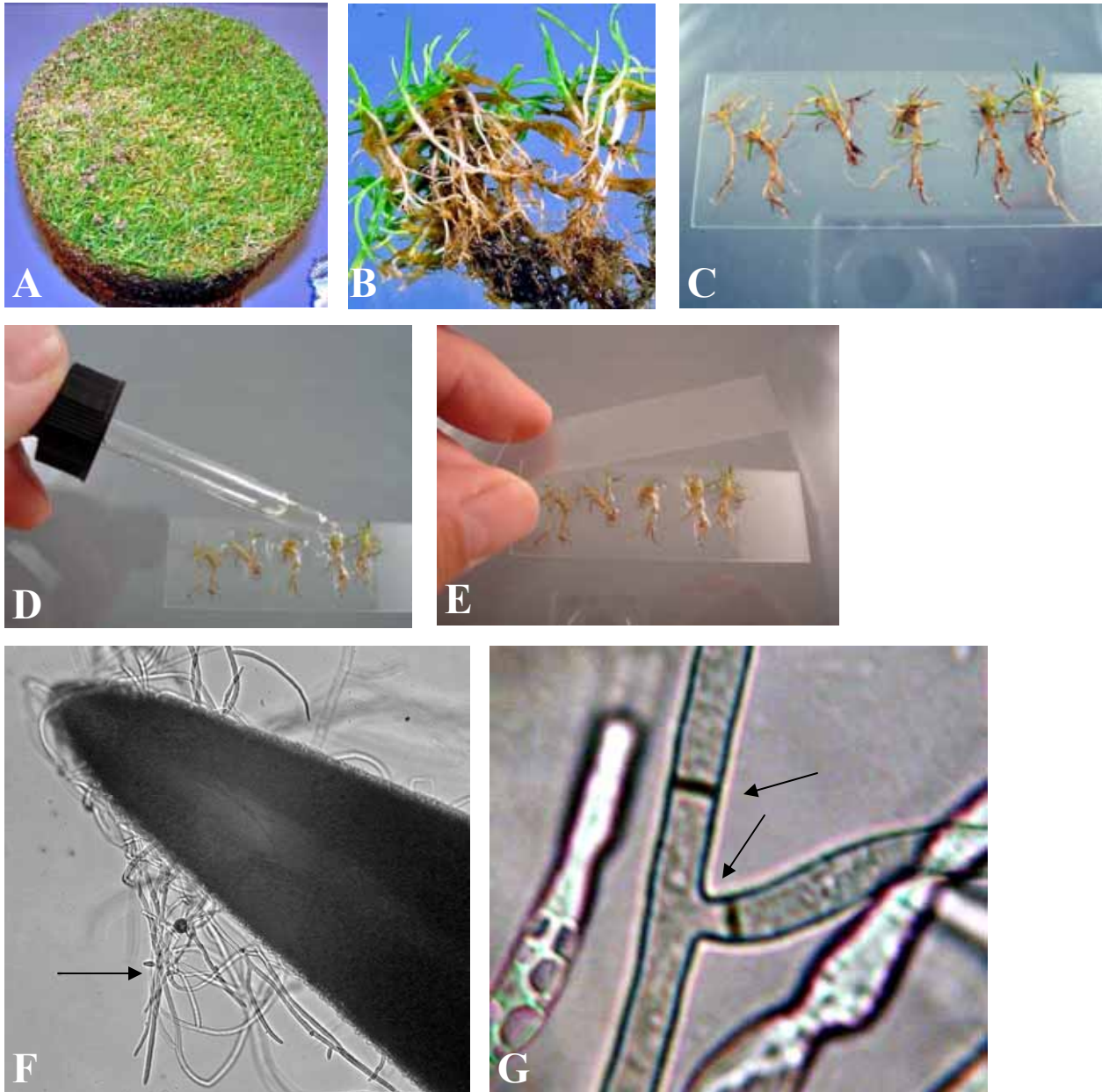
Clear fingernail polish can be used as a sealant to preserve prepared slides. Use lactophenol to mount the specimen. Place a cover slip over the specimen and seal the edges of the cover slip with fingernail polish. Semi-permanent slides may last from 6 months to 2 years.

Preparing a slide from a fungal culture



Steps to prepare a slide mount from a fungal culture for viewing with a compound microscope: (A) Fungal culture growing on potato dextrose agar, (B) Place one drop of water on glass slide, (C) With a scalpel, cut a very small piece of agar and mycelium from the edge of the colony, (D) Place the agar piece in the drop of water, (E) Place a cover slip at a 45 degree angle over the agar piece and water to minimize air bubbles in the mount, (F) Gently flatten the agar piece with the eraser of a pencil, (G) Mycelium is dispersed and ready to view

Preparing a slide from a grass plug



Steps to prepare a bentgrass specimen and view with a compound microscope: (A) bentgrass plug taken from the margin of a patch disease, (B) a portion of the plug from the symptomatic area was collected and washed to remove sand (you may also prepare an unwashed specimen), (C) bentgrass plants are placed on a glass slide, (D) the slide is flooded with water, (E) cover slip is gently placed on plants, (F) slide is viewed first with the 4X and 10X objective, hyphae is observed along the edge of the leaf, (G) once fungal structures are observed, increase magnification to the 20X or 40X objective so that you may clearly see morphological characteristics which aid in identification of the fungus. In this case, the pathogen in *Rhizoctonia* which has right angle branching, septations and hypha that is slightly constricted where branching occurs.

Rhizoctonia

Diseases: brown patch, large patch, yellow patch, leaf and sheath spot



Diagnostic tips:

- Hyphae generally large diameter, usually consistent diameter
- Septate hyphae
- Right angle branching of hyphae
- Hyphae may be constricted near point of branching
- Identification by hyphal characteristics only; no spores associated with *Rhizoctonia* with only rare exceptions
- Start with a dissecting microscope and scan foliage of hyphae
- Remove foliage with hyphae and mount in water on a slide.
- Examine with 4 or 10X objective to find hyphae
- View at 20 or 40X to clearly see hyphal morphology
- Mycelium may be easier to see if leaf tissue is mounted in a stain such as cotton blue in lactophenol

Common species:

Rhizoctonia solani

Rhizoctonia cerealis

Rhizoctonia zeae

Binucleate *Rhizoctonia*-like fungi

Figures

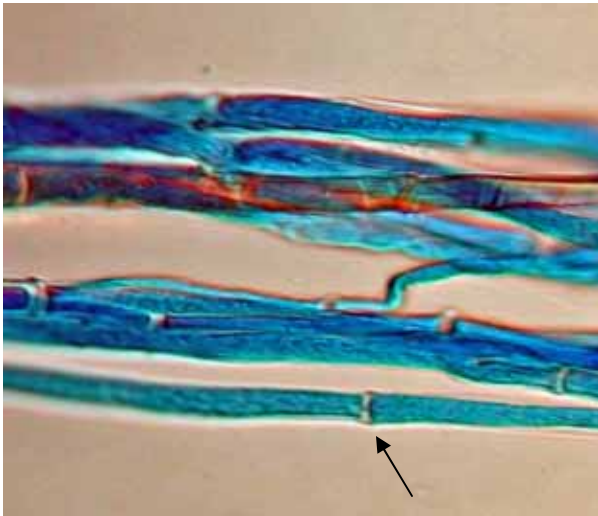
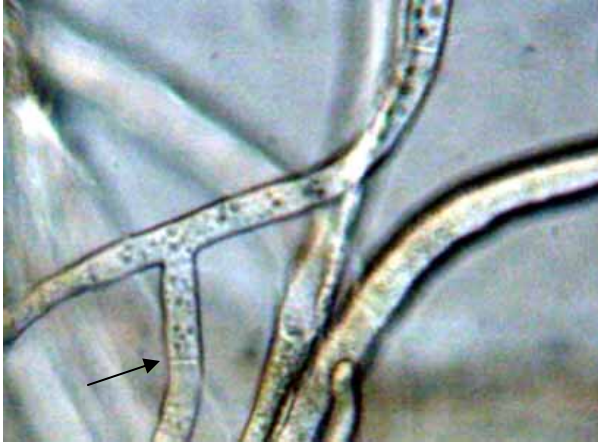
Top: *Rhizoctonia* hyphae in a water mount (400X)

Middle: *Rhizoctonia solani* growing among bentgrass leaves (60X)

Bottom: Brown patch on a creeping bentgrass green; smoke ring is visible at edge of patch

Sclerotinia

Diseases: dollar spot



Diagnostic tips:

- Septate hyphae
- Hyphae vary in diameter
- Mycelium white
- Cytoplasm in hyphal cells may be grainy
- Septations may be difficult to spot in water mounts, but easily spotted after staining with cotton blue or aniline blue
- Mycelium is often observed on grass when dew is present
- If mycelium is not present incubate plug in a moist chamber for 12-48 hrs
- Observe hyphae in a water mount at 40, 100 and 400X

Common species:

Sclerotinia homeocarpa

Figures

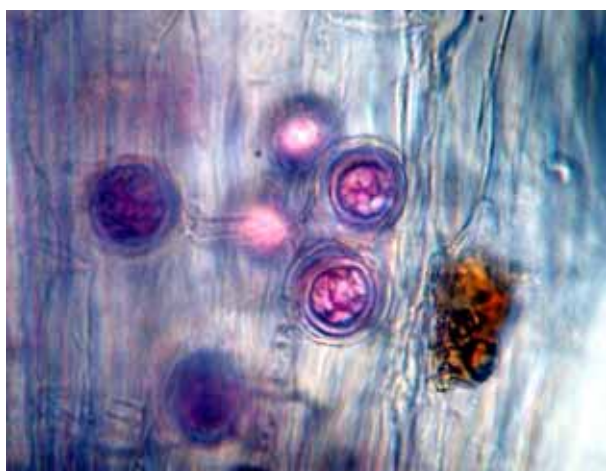
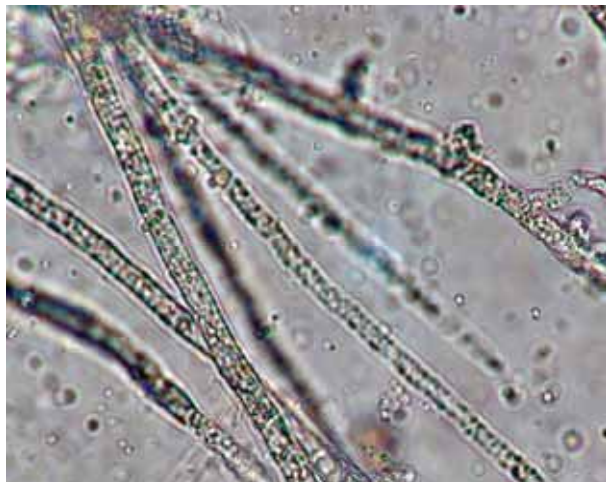
Top: *Sclerotinia* hyphae mounted in water (400X)

Middle: *Sclerotinia* hyphae mounted in aniline blue (400X)

Bottom: *Sclerotinia* hyphae visible in a dollar spot patch on a creeping bentgrass green.

Pythium

Diseases: pythium blight, cottony blight, pythium crown rot, pythium root rot, pythium patch



Diagnostic tips:

- Branching of hyphae may be sparse
- Coenocytic hyphae (no septations or extremely rare). Observe hyphae at 400X.
- Spherical, doublewalled oospores may be observed in leaf or root tissue. Oospores may be spotted with a compound microscope in infected tissue at 40-100X.
- If pythium foliar blight is very active, lobulate sporangia may be observed among hyphae.
- Infected roots may or may not be discolored.
- Foliage may appear water soaked and slimy with a foliar blight

Common species:

Pythium aphanidermatum

Pythium graminicola

Pythium volutum

Pythium ultimum

Many other species have been assoc with turf.

Figures

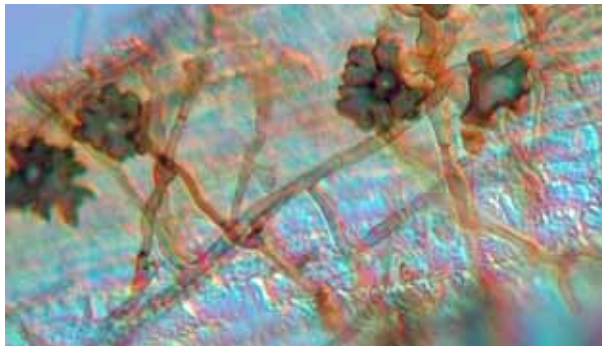
Top: Pythium hyphae mounted in water at 400X; hyphae is coenocytic (no septations)

Middle: Double walled oospores embedded in bentgrass root tissue at 400X; stained with acid fuchsin

Bottom: Sporangia (lobulate) at 400X; stained with acid fuchsin

Gaeumannomyces graminis var graminis

Diseases: bermudagrass decline, take-all patch (St. Augustine), zoysia decline, root decline



Diagnostic tips:

- Runner hyphae is darkly pigmented, septate and well branched
- Hyphopodia are darkly pigmented, deeply lobed
- With a dissecting microscope look for runner hyphae on stolons, rhizomes and roots (10-60X)
- With a scalpel remove a thin slice of the epidermis of the stolon containing runner hyphae and hyphopodia and mount in water; observe with a compound microscope at 40, 100 and 400X.

Common species:

Gaeumannomyces graminis var graminis

Gaeumannomyces graminis var avenae (take-all patch on bentgrass)

Gaeumannomyces incrustans

Figures

Top: Runner hyphae and hyphopodia of Ggg on a bermudagrass stolon (60X)

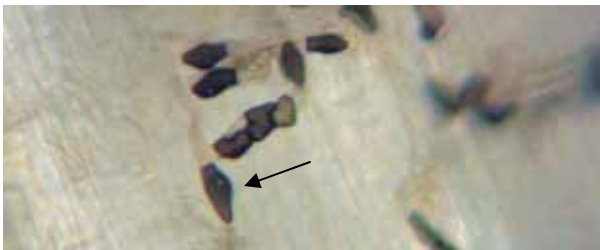
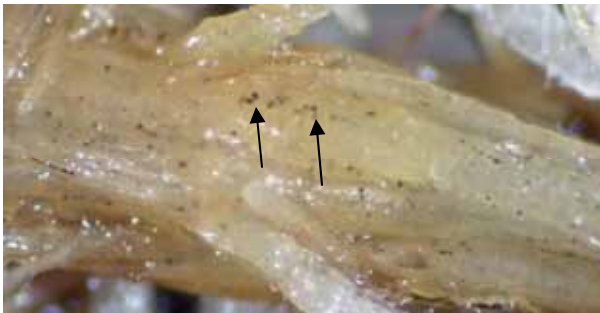
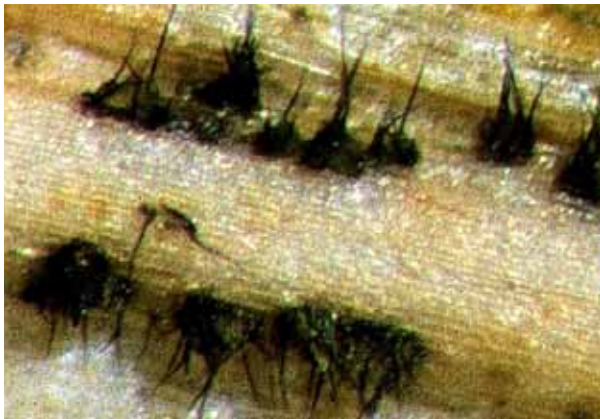
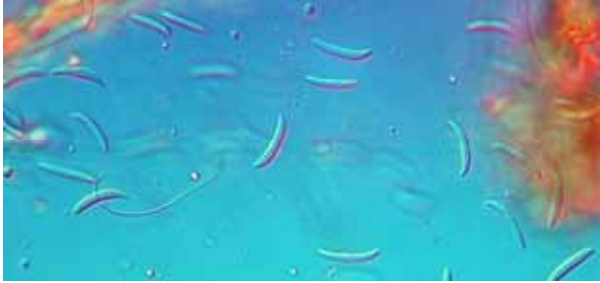
Upper middle: Deeply lobed hyphopodia and runner hyphae of Ggg (400X)

Lower middle: Lobed hyphopodia (1000X)

Bottom: Root decline of bermudagrass

Colletotrichum cereale

Disease: anthracnose (foliar blight, basal rot)



Diagnostic tips:

- Observe diseased plants with a dissecting scope at 10-60X to observe fruiting bodies (acervulii) with black, hair-like structures (setae)
- Infection mats (dark brown to black) may also be visible on the leaf sheath at 10-60X
- Wash specimen and observe leaf sheath, crown of plant and upper roots for appressoria with a dissecting microscope 40-60X (dark gray, charcoal); appressoria anchor the fungus on plant tissue
- Mount leaf sheath tissue and/or whole plants in water and observe at 40-100X with a compound microscope
- Setae, appressoria, infection mats and spores may be observed on some samples
- Spores (one celled, crescent shaped) are best viewed at 400X

Common species:

Colletotrichum cereale

Colletotrichum graminicola

Figures

Top: Spores (conidia) 400X

Upper middle: Fruiting bodies (acervulii) with black, hair-like setae (60X)

Middle: Appressoria visible on bentgrass crown (40X)

Lower middle: Appressoria 400X

Lower: Infection mats on bentgrass leaf sheath tissue (60X)

Bipolaris

Diseases: leaf blight, leaf spot, crown and root rot of warm season grasses; some cool season grasses (formerly Helminthosporium complex)



Images courtesy of M. Tomaso-Peterson



Image courtesy of R. Martin

Diagnostic tips:

- *Bipolaris* may cause a leaf spot, leaf blight, stem and crown rot of warm season grasses
- Observe diseased tissue with a dissecting scope 10-60X for dark fungal growth which may indicate sporulation
- Mount thin portions of diseased tissue in water mounts and observe the edge of the tissue for conidia (spores) and conidiophores (stalks) 40-100X
- Observe conidia at 400X
- *Bipolaris* conidia are generally light to dark brown; ellipsoidal, straight

Common species:

Bipolaris spicifera
Bipolaris cynodontis
Bipolaris sorokiniana

Figures

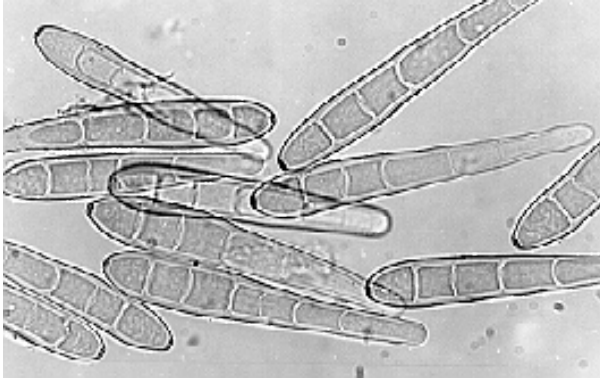
Top: *Bipolaris* conidia (spores) and conidiophores (stalks) on bermudagrass 100X

Middle: *Bipolaris spicifera*, *B. cynodontis* and *B. sorokiniana* conidia 400X

Bottom: *Bipolaris spicifera* conidia 1000X

Drechslera

Diseases: leaf spot, leaf blight, crown rot; generally on cool season grasses



Images courtesy of Maria Tomaso-Peterson



Diagnostic tips:

- Usually found on cool season grasses
- Sporulation may be difficult to observe on lesions with a dissecting scope, but attempt at 40-60X
- Cut and remove lesions from leaf blades and mount in water
- Conidia (spores) may be observed adjacent to plant tissue at 40-100X
- Conidia are mostly tapered and have 4-9 septations

Common species:

Drechslera nobleae

Drechslera catenaria

Drechslera dictyoides

Figures

Top: *Drechslera* conidia

Middle: *Drechslera* conidia attached to a conidiophore

Bottom: *Drechslera* leaf spot on ryegrass

Pyricularia

Disease: gray leaf spot (ryegrass, tall fescue, St. Augustine)



Image courtesy of P. Harmon



Image courtesy of R. Latin

Diagnostic tips:

- Leaf spots may be visible to the naked eye
- Observe leaf spots with a dissecting microscope at 40-60X
- Remove leaf tissue with spots and mount in water
- Observe with compound microscope at 40-100X; look for conidia
- Increase magnification to 400X once conidia are observed and look for colorless, conidia that are rounded on one end and acutely tapered on the other
- Conidiophores (stalks) may be observed on diseased tissue.
-

Common species:

Pyricularia grisea

Figures

Top: *Pyricularia* conidia (400X)

Middle: *Pyricularia* conidia (1000X)

Bottom: Gray leaf spot of ryegrass

Microdochium

Diseases: pink snow mold (cool season grasses); pink patch (bermudagrass)



Diagnostic tips:

- Patches may have pink colored mycelium at the periphery
- Observe plug with a dissecting microscope 10-60X
- Look for fruiting bodies (sporodochia) on the leaves with dissecting microscope 60X; sporodochia may be pink or white
- Mount leaf tissue with sporodochia in water and observe with a compound microscope
- Look for sporodochia along the edge of the leaf at 40-100X; conidia (spores) should be visible adjacent to sporodochia
- Conidia are crescent shaped and 1-3 septate.

Common species:

Microdochium nivale

Figures

Top: *Microdochium* conidia (400X)

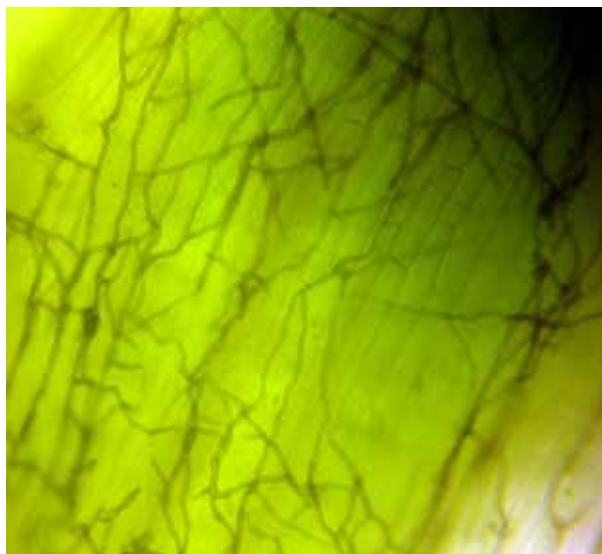
Upper middle: Pink snow mold on perennial ryegrass

Lower middle: Pink mycelium visible in patch on perennial ryegrass

Bottom: A pink patch disease caused by a *Microdochium*-like fungus on ultradwarf bermuda

Curvularia

Disease: dog print disease, *Curvularia* is often found on weak, stressed turf



Diagnostic tips:

- Observe plug with dissecting microscope at 40-60X for sporulation
- Conidia (spores) are borne on conidiophores (stalks)
- Remove diseased tissue from plug and mount in water
- Observe the edge of leaf tissue, stolons, roots for pigmented hyphae, conidiophores and conidia; use compound microscope at 40-100X
- Observe conidia with compound microscope at 100-400X
- Look for curved, pigmented conidia; conidia may have one to two enlarged central cells

Common species:

Curvularia lunata

Figures

Top: *Curvularia* conidia (400X)

Middle: *Curvularia* conidia attached to a conidiophore

Bottom: *Curvularia* hyphae growing on stressed bentgrass leaf tissue