Gray Leaf Spot of Perennial Ryegrass

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Disease
Gray leaf spot.

Primary Host
Perennial ryegrass (Lolium perenne).

Pathogen
Magnaporthe oryzae.

Taxonomy
Magnaporthe oryzae Couch (anamorph: Pyricularia oryzae Cavara) was described in 2002 from Magnaporthe grisea (Herbert) Barr based on a multilocus gene genealogy concordant with host preference (2). Hosts of M. oryzae include perennial ryegrass (Lolium perenne L.), rice (Oryzae sativa L.), and most other grasses. Hosts of M. grisea isolates are the crabgrasses (Digitaria sp.). Prior to the segregation, the gray leaf spot pathogen of perennial ryegrass was referred to as M. grisea or the anamorph as Pyricularia grisea (Cooke) Sacc. (10). A detailed history of the taxonomy of M. grisea was reported by Rossman et al. (13).

Symptoms and Signs
Gray leaf spot causes a range of symptoms including leaf spots and leaf blight that can kill plants. Symptom expression is influenced greatly by environmental conditions and host age (10,14). On juvenile perennial ryegrass, leaf infections first appear water-soaked (Fig. 1) (10,14). Leaf blades are distorted and twisted at the point of infection (Fig. 2a) (20). During extended periods of high relative humidity and warm temperatures, profuse production of conidia gives a gray, felt-like, appearance to blighted leaf blades (Fig. 2b). Individual leaf spots quickly coalesce and leaf blight spreads from the blades to the crown.

Fig. 1. The initial symptom of infection by Magnaporthe oryzae, a water soaked lesion on a juvenile perennial ryegrass leaf blade.
The disease takes its name from the distinctive gray-centered leaf spots. Leaf spots typically have necrotic (sometimes chlorotic) margins (Fig. 3). The presence of these characteristic leaf spots represents a useful diagnostic trait. However, these symptoms tend to be difficult to discern on turfgrass with narrow blades. In many cases, by the time outbreaks are recognized, symptoms including discrete leaf spots already have progressed to leaf distortion, collapse, and death, especially within swards of juvenile perennial ryegrass. On more mature perennial ryegrass with wider leaf blades, leaf spots are observed more readily and seem to persist longer because mature plants blight more slowly (10,14).

Field symptoms first appear as small diffuse clusters of turf with an abnormal appearance. Leaf spots usually are evident in the initial outbreak, but they often resemble those caused by several other less important pathogens of perennial ryegrass that result in little lasting turf damage (Bipolaris sorokiniana (Sacc.) Shoemaker, Leptosphaerulina trifoli (Rostr.) Petr., and Curvularia lunata (Wakk.) Boedijn). As a result, gray leaf spot often is first recognized in the field by turf managers as irregular areas of brown matted turf (Fig. 4) (10,20). Sometimes affected areas of perennial ryegrass appear drought stressed (Fig. 5). The application of additional irrigation to apparently droughty areas results in more extensive disease development.
On golf courses, initial outbreaks often occur in taller mown grass (5 cm to 7.5 cm roughs), because roughs are not normally protected with fungicides and the canopy tends to maintain longer dew periods (Fig. 6). These outbreaks usually are mild and coincide with conditions that are favorable, but suboptimal, for disease development. Taller mown perennial ryegrass is managed less intensively and escapes stress associated with frequent mowing and compaction, but if conditions conducive for disease development persist, blighting will become severe in these areas (Fig. 7a). Severe blighting can occur quickly on fairways that are more stressed, more frequently fertilized, and mowed short (1.25 cm to 2 cm) (Fig. 7b). This is especially true when ample inoculum is present and environmental conditions favorable for disease development coincide with a lapse in fungicide protection or fungicide failure due to pathogen resistance (20).
Fig. 7. (a) Gray leaf spot symptoms can become severe on golf course roughs not protected with fungicides. (b) If fungicide protection lapses on golf course fairways, gray leaf spot can quickly spread to blight and kill large areas of perennial ryegrass.

Host Range

More than 50 grass species have been identified as hosts of *M. oryzae* (2). Other economically important diseases caused by the pathogen include rice blast, gray leaf spot of St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), and gray leaf spot of annual ryegrass (*Lolium multiflorum* Lam.) (1,14,19). Gray leaf spot of maize, however, is not a disease caused by *M. oryzae*, but a completely different fungus, *Cercospora zeae-maydis* Tehon and Daniels (22). Based on recent molecular studies, isolates from perennial ryegrass and tall fescue (*Festuca arundinacea* Schreb) appear to be genetically distinct from other host specific isolates. As a group, isolates that infect perennial ryegrass and tall fescue have limited genetic diversity (4).

Geographic Distribution

Gray leaf spot was first reported as a serious problem of perennial ryegrass in Pennsylvania in 1991 (10). A 1996 report documented that *M. oryzae* was identified in perennial ryegrass samples from a golf course fairway in Maryland as early as 1985 (3). Subsequent accounts confirmed the presence of gray leaf spot in much of the Mid-Atlantic region and Ohio River valley of the United States (8,10,12,16,20). Isolates of *M. oryzae* from perennial ryegrass also have been collected from Rhode Island, Iowa, Kansas, and California (18). The disease has not been reported in northern tier midwestern states such as Michigan, Wisconsin, and Minnesota probably because of fewer and shorter periods of hot, humid conditions. Also, harsh winter conditions greatly reduce survival of the pathogen in infested host residue (7). Generally the disease is not considered a major problem in the southeastern United States, because perennial ryegrass often is used as a winter annual cover for warm season turfgrass species (9). However, gray leaf spot is a serious threat to tall fescue in southeastern states (5). Our estimation of the geographic distribution of gray leaf spot on perennial ryegrass is shown in Fig. 8.
**Pathogen Isolation**

The pathogen can be difficult to isolate from infected leaf tissue, partly due to the concomitant presence of other microorganisms (including pathogens) and the slow-growing, non-competitive growth habit of *M. oryzae* on artificial media. Successful isolation is facilitated by suspending symptomatic perennial ryegrass leaf blades with double-sided tape to the lids of 9-cm Petri dishes containing V8 juice agar (15) (Fig. 9). Conidia are released from the conidiophores, fall to the surface of the agar, and form colonies. Surface sterilization with dilute sodium hypochlorite (10% household bleach) usually is not needed with this method. Lids are transferred to fresh V8 juice agar dishes every 24 hours for 3 days. Exposed culture dishes are covered with new sterile lids and incubated at 23°C under constant fluorescent light. After 2 to 3 days, colonies with characteristics typical of *M. oryzae* should be transferred to fresh agar and incubated as described above for 3 days.

![Fig. 9. The double sided tape method is effective for isolation of *Magnaporthe oryzae.*](image)

**Pathogen Identification**

After 5 to 7 days of growth, colonies of *M. oryzae* typically appear white, light gray, or dark gray in color (Fig. 10). Aerial hyphae, conidiophores, and conidia are common on media with high nutrient content such as V8 agar, but colonies of some isolates also grow appressed to the agar surface (10). Removal of aerial hyphae from colonies of most isolates by scraping with a rubber policeman reveals dark mycelia growing under the surface of the agar. The optimal growth rate of the fungus was reported between 4 and 5 mm/day at 29°C (10). The identity of colonies can be confirmed microscopically by the presence of characteristic pale brown to hyaline, pyriform, mostly three-celled conidia produced from sympodially proliferating conidiogenous cells (21) (Fig. 11).

![Fig. 10. A characteristic colony of *Magnaporthe oryzae* growing on V8 juice agar.](image)

![Fig. 11. Conidia of *Magnaporthe oryzae* are typically three-celled, have a distinct conidiophore attachment at the wide end, and are pyriform (pear-like) in shape.](image)
A polymerase chain reaction (PCR)-based method for rapid identification and detection of *M. oryzae* from infected perennial ryegrass was developed recently (6). The method utilizes a widely-available DNA isolation kit to extract fungal DNA from diseased plant material. If the sample contains *M. oryzae*, a transposon found only in the *M. oryzae* genome, Pot2, is PCR-amplified. The presence of the pathogen is assessed by the observation of a diagnostic amplicon on an agarose gel in less than 8 hours (the time a colony would grow roughly 2 mm on an agar medium). Multiple suspect lesions or blighted leaf blades from a perennial ryegrass sward can be processed together in one sample; multiple samples from different swards can be evaluated on the same agarose gel.

Isolates specific to perennial ryegrass and tall fescue can be distinguished from other isolates of *M. oryzae* by molecular techniques reported by Farman (4).

**Pathogen Storage**

Isolates of the pathogen are commonly stored desiccated on a range of media at 4 to -20°C without significant loss of viability or pathogenicity for as long as 20 years (11). Sterile filter paper (Whatman #1, 7 cm diameter) commonly is placed in the center of a Petri dish with either V8 juice or oatmeal agar (15). A section of a 10-day-old colony is transferred to the center of the filter paper and incubated at 23°C under continuous light until the colony covers the filter paper (about 2 weeks). The filter paper is removed from the agar surface and placed in a new sterile Petri dish to dry. Thoroughly-dried filter paper can be stored for more than 5 years at 4 to -20°C in either a glass vial or sealed paper envelope (Fig. 12) (17). Similar methods have been used to store rice isolates for 10 to 20 years without the loss of viability or pathogenicity (11).

![Fig. 12. Isolates of Magnaporthe oryzae may be stored dried on filter paper, cut into small sections, and maintained in glass vials at 4°C or -20°C.](image)

**Pathogenicity Tests**

Pathogenicity tests should be conducted on the grass species from which the pathogen was isolated. Inoculations with conidial suspensions of *M. oryzae* (7 × 10^3 to 1 × 10^5 conidia per ml) yield best results in controlled environment tests (10,18). Maintaining high relative humidity and extended periods of leaf wetness in saturated or near-saturated environments after inoculation is critical for disease development (Fig. 13). At 28°C, symptoms of gray leaf spot first become evident on inoculated plants at approximately 72 hours after inoculation with or without light. Incubation for extended periods substantially increases disease severity (Fig. 14).
Literature Cited