

Epidemiology of Gray Leaf Spot of Perennial Ryegrass

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Objective

The continuing objective of this research is to investigate the environmental factors that influence the winter survival of *Magnaporthe oryzae* and to determine the role of primary inoculum in the development of gray leaf spot epidemics on perennial ryegrass (*Lolium perenne*). In addition, we are developing a method for rapid identification of gray leaf spot that utilizes polymerase chain reaction (PCR)-based detection of *M. oryzae* in symptomatic perennial ryegrass turf.

Rationale

Gray leaf spot of perennial ryegrass is caused by *M. oryzae*. Past reports and publications have referred to this pathogen as *Magnaporthe grisea*. Isolates of the pathogen from crabgrass (*Digitaria* spp.) are still referred to as *M. grisea*, and isolates of the pathogen from other grass hosts, including perennial ryegrass, are now referred to as *M. oryzae*. No morphological characters separate the two fungi. This species-segregation stems from a genealogy of three genes: actin, beta-tubulin, and calmodulin. Several base substitutions in each of the three gene loci and mating infertility distinguish *M. grisea* from *M. oryzae*. These distinctions were found to be concordant with host preference of the tested isolates.

The first reported outbreak of gray leaf spot on perennial ryegrass occurred in 1985 in Maryland. Since the next published report in 1991, outbreaks have been reported in the Mid-Atlantic, Midwest, and New England regions of the United States. The disease was first confirmed in Indiana in 1999.

Pathogen Survival: An epidemic occurs when a pathogen and a susceptible host interact under favorable environmental conditions. Our knowledge of gray leaf spot epidemics is still incomplete. The disease occurs sporadically, although perennial ryegrass is commonly grown and disease-favorable conditions occur each year. Our research efforts focus on the survival and early summer portion of the pathogen life cycle. Previous researchers have conducted studies with *M. oryzae* on other grasses (rice and bamboo grass) and have found that the amount of surviving fungus can impact disease development the following season. *M. oryzae* does not produce specialized survival structures but survives as mycelia in infested host residue. It is thought that the surviving fungus produces conidia (spores) on the infested residue in spring or early summer to initiate the next season's epidemic. This research is designed to investigate the likelihood of overwinter survival of *M. oryzae* in the Midwest and to estimate the contribution of this initial inoculum to gray leaf spot epidemics in the summer.

Pathogen Detection: Symptoms of gray leaf spot can be easily overlooked or misdiagnosed in the early stages of an epidemic. By the time the disease causes extensive turf damage, remedial fungicide applications are often not effective and perhaps cost-prohibitive. Early detection of *M. oryzae* in symptomatic tissue using polymerase chain reaction (PCR) methods could provide accurate and timely disease diagnosis and improve the chances for effective and efficient management with fungicides.

Procedures

Pathogen Survival: Survival experiments were conducted at the Wm. H. Daniel Turfgrass Research and Diagnostic Center (DANL) and in laboratory facilities at Purdue University. Survival of the fungus was addressed by exposing infested perennial ryegrass residue to varying environmental conditions. Multiple samples of the exposed debris were collected over time, and the viability of the pathogen was assessed.

Aerial densities of *M. oryzae* conidia were monitored during the summers of 2000, 2001, and 2002 at the DANC. A volumetric air sampler was positioned in an established sward of perennial ryegrass. In 2000, the site was inoculated with perennial ryegrass residue infested with *M. oryzae* to establish the disease. No other inoculation was made. The air sampler collected particles from the air and deposited them into a small, plastic, collection tube. The collection tube was exchanged with a new tube every 3-5 days throughout the spring and summer months. After the tubes that contained the collected particles were retrieved, their contents were microscopically examined for the characteristic three-celled conidia of *M. oryzae*. The quantity of the conidia per tube and the conidial density for the collection period were calculated.

Pathogen Detection: The detection procedure utilized a commercial DNA isolation kit and a PCR protocol with primers designed to amplify a DNA nucleotide sequence found only in the *M. oryzae* genome. Suspect lesions were ground in an extraction buffer. The buffer with the extracted DNA was then added to a PCR reaction mixture. If the pathogen was present, the *M. oryzae* nucleotide sequence was PCR-amplified. The PCR reaction product was subjected to electrophoresis in an agarose gel, and stained with ethidium bromide. PCR products were visualized with ultraviolet illumination. If the pathogen was present and the sequence was amplified, a DNA band of the appropriate size was visible.

Results

Pathogen Survival: *M. oryzae* survived in dehydrated residue subjected to a range of temperatures from -4 C to 23 C for extended periods of time (Fig. 1). Two dehydrated treatments are shown in Fig. 1, LAB and VAR. Residue subjected to the LAB treatment was stored at a constant 23 C. The temperature regime for the VAR treatment was maintained in an incubator and approximated ground temperatures during Indiana winter months. Temperatures were adjusted at each sample date so that on 4 Dec, 13 Jan, 14 Feb, 14 Mar, and 17 Apr, the temperature was adjusted to 4 C (initial), 0 C, -4 C, 4 C, 8 C, and 12 C, respectively. *M. oryzae* did not survive well in hydrated perennial ryegrass residue subjected to similar conditions (Fig. 2; ALT, +4C, and -20C). Hydrated residue exposed to the ALT treatment was exposed to alternating 24 h-periods of 4 C and -20 C temperatures. Hydrated residue exposed to the +4C and -20C treatments were maintained at 4 C and -20 C respectively. Alternating freeze-thaw cycles and extended periods of extreme cold temperatures reduced the numbers of conidia produced by surviving *M. oryzae*. During the winters of 2000-2001 and 2001-2002, the pathogen population that survived in residue located in a stand of perennial ryegrass at the DANL was drastically reduced

from initial levels of 50,000 and 25,000 conidia produced per gram of residue, respectively, to near zero (Fig. 1; DAN) (Fig. 2; DAN).

Conidia of the pathogen were collected by the air sampler in the summer of 2000 approximately one week after inoculation with pathogen-infested residue. The numbers of observed conidia were very low then gradually increased to peak in September (Fig. 3; 2000). In 2001, conidia were again collected, however, the numbers of sampled conidia remained low (Fig. 3; 2001) compared to levels recorded in 2000. *M. oryzae* was not isolated from perennial ryegrass at the DANC in 2001. In 2002, a similar trend to 2001 was observed (Fig. 3; 2002), but more conidia were collected than in 2001. Again, *M. oryzae* was not isolated from perennial ryegrass at the DTC in 2002.

Pathogen Detection: Several perennial ryegrass leaf blades with gray leaf spot lesions were processed using the PCR-based detection protocol. The protocol efficiently detected the pathogen from diseased perennial ryegrass. Several other pathogenic fungi capable of causing leaf spots on perennial ryegrass were also tested with the protocol. None of the other fungi were detected with the method (Fig. 4).

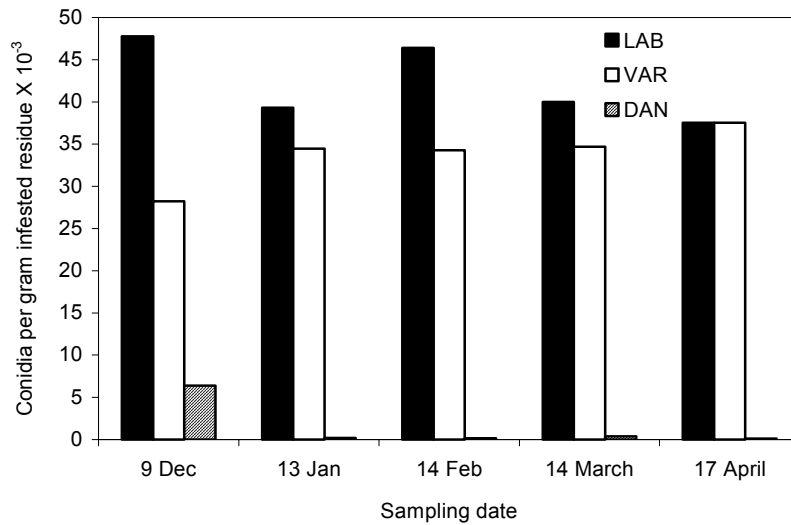


Fig. 1. Conidia of *M. oryzae* produced per gram of infested perennial ryegrass residue exposed to three environmental regimes. Residue subjected to the LAB regime was dehydrated and stored at 23 C. Residue subjected to the VAR regime also was dehydrated but temperatures were maintained between -4 C and 8 C to mimic ground temperatures in Indiana between Nov and May. Residue in the DAN treatment was exposed to ambient conditions in a perennial ryegrass research plot at the DANL in West Lafayette, Indiana. Initial conidiation was approximately 50,000 conidia/g. The experiment was initiated on 10 Nov, 2000.

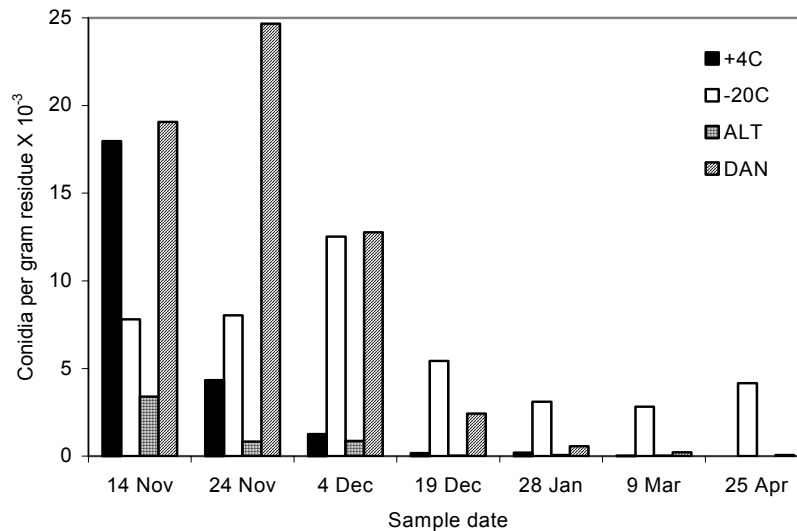


Fig. 2. Conidia of *M. oryzae* produced on infested perennial ryegrass residue subjected to four environmental regimes. Residue was exposed to 4 C, -20 C, and alternating (ALT) 24-hour periods of 4 C and -20 C temperatures. Also, residue in the DAN treatment was exposed to ambient conditions from Oct to Jun in a perennial ryegrass research plot at DANL in West Lafayette, Indiana. The initial conidiation of the residue prior to exposure to different environmental regimes was recorded at 42,000 conidia/g on 4 Nov, 2001.

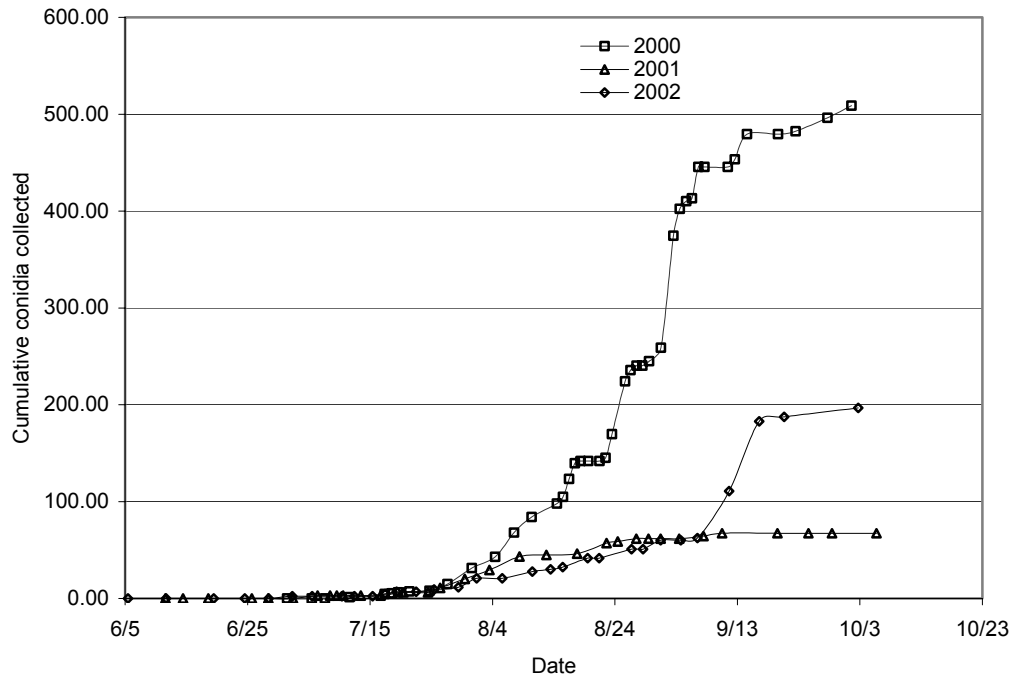


Fig. 3. Cumulative *Magnaporthe oryzae* conidia collected during three growing seasons. Conidia were collected with a Burkard volumetric air sampler that was located in a perennial ryegrass research plot at the DANL in West Lafayette, Indiana.

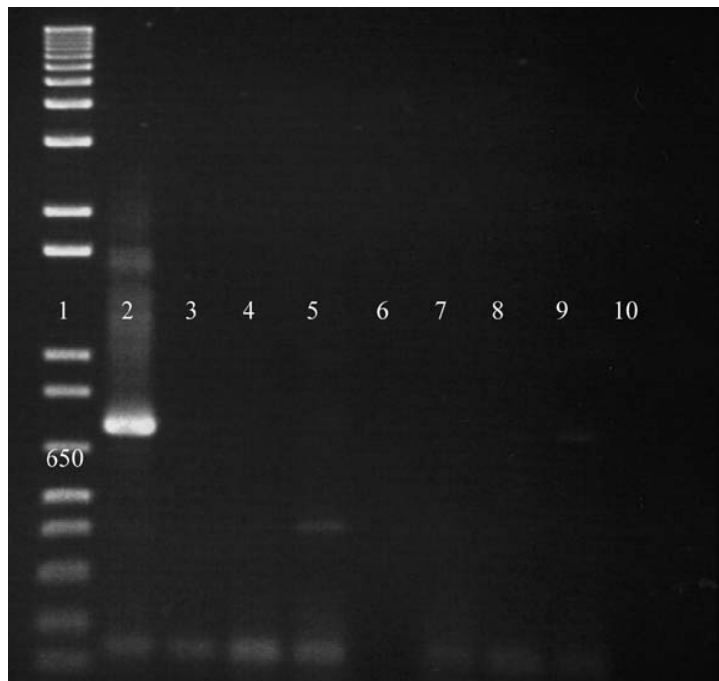


Fig. 4. The white band of DNA (lane 2) is PCR product amplified from *Magnaporthe oryzae*. Primers were developed to detect the presence of the Pot2 transposon in the *M. oryzae* genome. Other fungi tested from lanes 3 through 9 were *Bipolaris sorokiniana*, *Sclerotinia homoeocarpa*, *Leptosphaerulina trifolii*, *Rhizoctonia solani*, *Curvularia lunata*, *Laetisaria fuciformis*, and *Colletotrichum graminicola*, respectively. A 1 Kb+ ladder is shown in lane 1 for size reference.