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Gray leaf spot is a newly emerging disease of perennial ryegrass in several regions of the United States. Scientists at a number of universities including Penn State University and the University of Kentucky are conducting comprehensive studies to unravel the complexities of this potentially devastating disease.

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The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 215 projects at a cost of \$21 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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# Gray Leaf Spot of Perennial Ryegrass Turf: An Emerging Problem for the Turfgrass Industry

Wakar Uddin, Gnana Viji, and Paul Vincelli

## SUMMARY

In recent years, severe outbreaks of gray leaf spot have resulted in extensive damage to perennial ryegrass golf course fairways and athletic fields, particularly in the mid-western and northeastern United States. Plant pathologists at a number of universities including Pennsylvania State University and the University of Kentucky, are conducting comprehensive studies to unravel the mysteries of this devastating disease. Among their findings:

- Since the first report of gray leaf spot on perennial ryegrass in 1991, outbreaks of gray leaf spot have occurred sporadically, resulting in serious loss of turf in 1995, 1998, and 2000 in the mid-Atlantic region. The disease has recently been reported from the Midwest, New England, and the western United States.
- DNA analysis showed the vast majority of pathogen isolates were distinct from wheat blast isolates, but closely related to isolates from tall fescue.
- Gray leaf spot is often observed first in turf in golf course rough maintained at higher mowing heights. These areas may be partially shaded and have extended leaf wetness periods and high humidity that are more conducive for infection.
- Gray leaf spot normally develops from early August to mid-October. Environmental conditions prevailing during this late summer period and availability of inoculum are major determinants in the development of gray leaf spot epidemics. Studies showed that temperatures between 26-29 C (79-84 F) were optimal for disease development.
- Among currently labeled fungicides, the most effective materials for gray leaf spot control are azoxystrobin, trifloxystrobin, and thiophanate methyl. Timely use of fungicides can help prevent the disease epidemic from reaching its logarithmic phase.
- Increasing the amount nitrogen increases gray leaf spot severity. Source of nitrogen also influences gray leaf spot development. A recent study has shown that gray leaf spot severity was lower when controlled-release forms of nitrogen were applied compared to fast-release nitrogen sources.

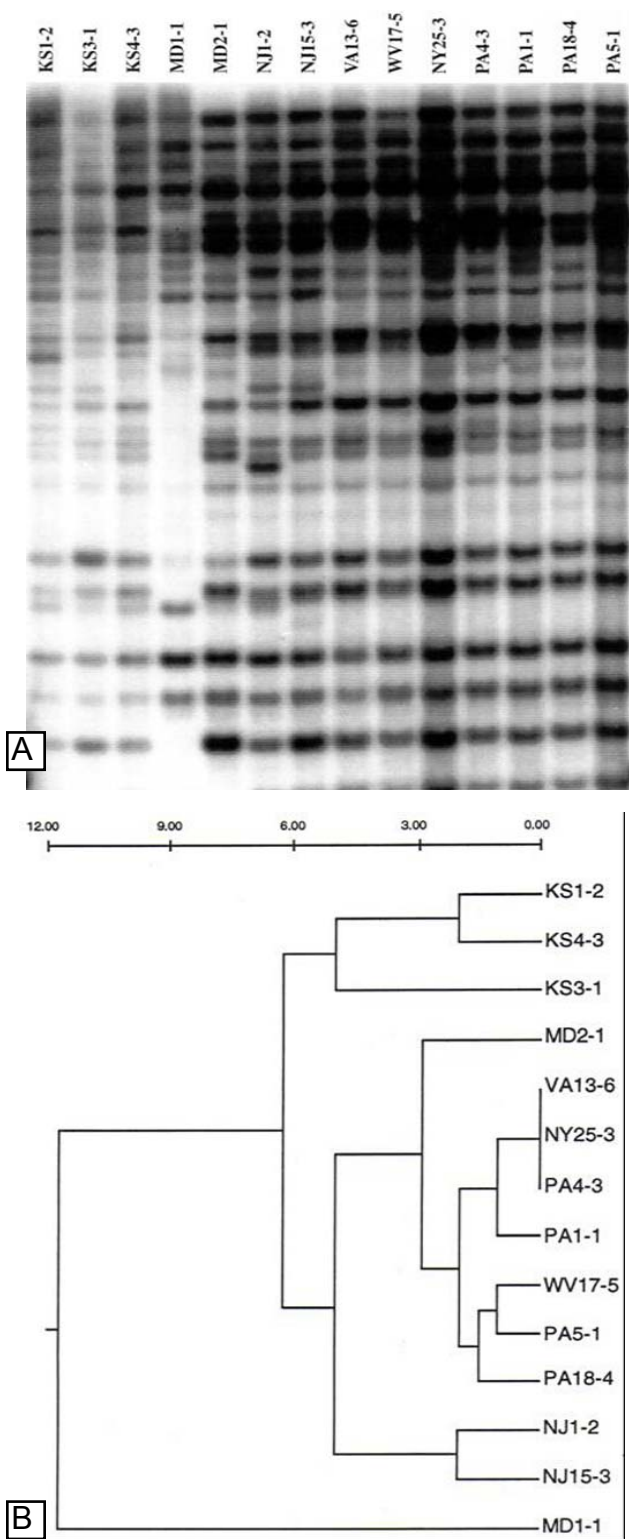
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Perennial ryegrass (*Lolium perenne* L.) is a cool-season grass originated from open areas and forest fringes of southern Europe and western Asia. It was first used in England in 1677 as a forage grass. It is widely used in the turf industry, especially on golf course fairways, due to its superior agronomic attributes such as turf color, upright and bunch growth habit, rapid germination and coverage, tolerance to close-mowing and soil compaction, and absence of thatch (25). Additionally, tolerance to cold weather has led to the use of perennial ryegrass for over-seeding dormant bermudagrass golf tees and fairways during the fall in the southern United States.

Gray leaf spot, or blast, caused by *Magnaporthe grisea* (Hebert), anamorph, *Pyricularia grisea* (Cooke) Sacc, is a newly emerging disease of perennial ryegrass in several regions of the United States. Recently, Couch and Kohn (8) proposed dividing *M. grisea* into two species, *M. grisea* and *M. oryzae*, for isolates causing disease on several gramineous hosts including perennial ryegrass. However, *M. grisea* is likely to remain as the standard name for the teleomorph of *P. grisea* until a formal name is



Figure 1. Extensive damage of perennial ryegrass on a golf course fairway caused by gray leaf spot.



**Figure 2.** Comparison of genetic similarity among *Magnaporthe grisea* isolates from perennial ryegrass using Pot2 fingerprinting probe. A, Fingerprints of representative isolates from Kansas (KS), Maryland (MD), New Jersey (NJ), Virginia (VA), West Virginia (WV), New York (NY), and Pennsylvania (PA). B, Phenogram derived by UPGMA (unweighted pair group method, arithmetic mean) based on Pot2 restriction fragment length polymorphism data.

adopted by the International Committee on Fungal Nomenclature.

In recent years, severe outbreaks of gray leaf spot have resulted in extensive damage to perennial ryegrass golf course fairways and athletic fields, particularly in the midwestern and northeastern United States. Under favorable conditions, the disease develops rapidly, and entire ryegrass swards can be killed within a few days, leaving only annual bluegrass and other grassy weeds that are not affected by the disease. Since gray leaf spot was first detected on perennial ryegrass in 1991, the disease commonly has resulted in increased fungicide costs of \$20,000 to \$25,000 per year on courses with perennial ryegrass fairways, and certain resorts have reported losses of up to \$500,000 in revenue due to severe outbreaks of the disease (Vincelli, unpublished).

Turfgrass managers are now considering replacing perennial ryegrass with other turfgrass species such as creeping bentgrass and Kentucky bluegrass due to the extensive and unpredictable damage caused by gray leaf spot. However, replacement of perennial ryegrass with these turfgrass species does not provide the best solution because of the superior agronomic characters of perennial ryegrass, and rapid thatch build-up and patch disease problems in other turfgrass species.

### Hosts

*Magnaporthe grisea* is pathogenic to more than 50 gramineous hosts including small grains, and forage and turfgrasses (1, 2, 16, 28, 31, 35, 42, 75). The fungus is probably best known for the devastating losses it can cause on rice (*Oryza sativa* L.). It may infect at any growth stage of the rice plant causing rapid leaf, nodal, neck and panicle blighting that is referred to as blast. The term blast has also been used to describe the disease in other cereal grains such as wheat (*Triticum aestivum* L.) and finger millet (*Eleusine corocana*) (1).

The disease in broadleaf hosts such as species of *Ctenenthe*, *Marantha*, and *Stromanthe*, has been referred to as Pyricularia leaf spot (29). In 1957, Malca and Owen (24) reported a leaf spot disease of St. Augustinegrass (*Stenotaphrum*



*secundatum* Walt.) in Florida caused by *M. grisea*. They named the disease gray leaf spot rather than blast because it did not result in rapid leaf blighting or plant death.

Gray leaf spot is a common foliar disease of St. Augustinegrass in the southeastern United States (33, 57); however, it generally does not cause damage to this grass species to the extent reported in perennial ryegrass (49). Severe outbreaks of a disease caused by *M. grisea* were observed on annual ryegrass (*Lolium multiflorum* Lam.) used for forage in the southern United States during the early 1970s (2,6). The disease was referred to as blast because of the resemblance of foliar symptoms (leaf spot and blighting) to leaf blast symptoms on rice (2,40).

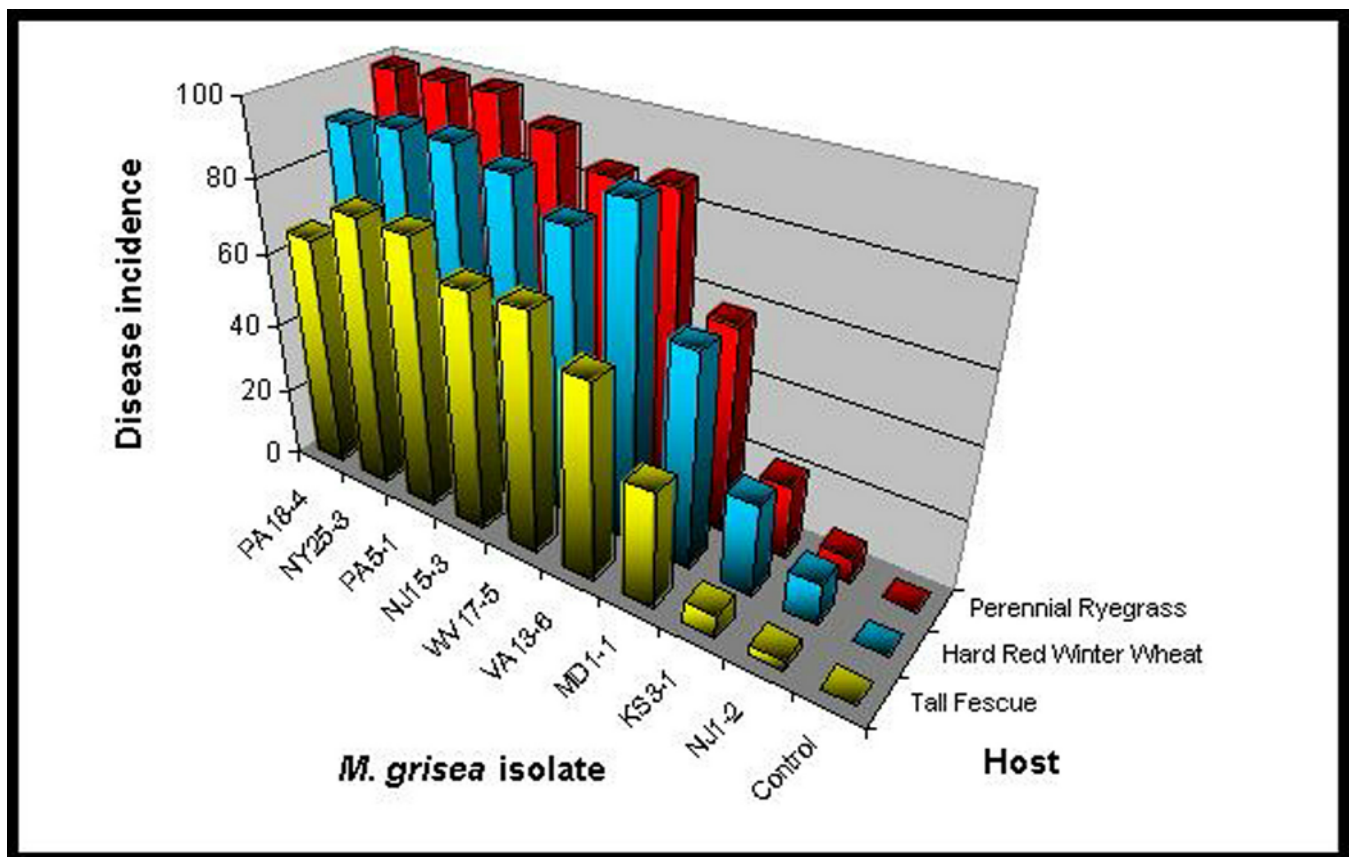
In 1991, Landschoot and Hoyland (22) reported gray leaf spot on perennial ryegrass turf in golf course fairways in Pennsylvania. The epidemic was confined to the southeastern region of Pennsylvania where extensive damage was reported. Since this first report, outbreaks of gray leaf

spot have occurred sporadically, resulting in serious loss of turf in 1995, 1998, and 2000 in the mid-Atlantic region (10,43). The disease has recently been reported from the Midwest (19,30,64), New England (32), and the western United States (53).

### Pathogen diversity and biology

Genetic diversity among perennial ryegrass isolates collected from various regions of the U.S. using different transposons as DNA probes in RFLP analysis indicated the presence of three lineages (Fig. 2) (61). Comparison of DNA fingerprints obtained with the probe Pot2, showed a close relationship between isolates of *M. grisea* from perennial ryegrass and those from wheat and triticale. Furthermore, the perennial ryegrass isolates had the same sequence in the rDNA internal transcribed spacer ITS region as that of the wheat and triticale isolates (61).

A close relationship between an isolate from perennial ryegrass and wheat has also been



**Figure 3.** Incidence and severity of gray leaf spot or blast on tall fescue, hard red winter wheat, and perennial ryegrass inoculated with isolates of *Magnaporthe grisea* from perennial ryegrass

reported by Farman (15). However, the study indicated that vast majority of the perennial ryegrass isolates tested were distinct from wheat blast isolates based on fingerprint obtained with Pot2 and MGR583. Additionally, the perennial ryegrass isolates were closely related to isolates from tall fescue (*Festuca arundinacea* L.) based on fingerprints obtained with these probes (15). *Magnaporthe grisea* isolates from perennial ryegrass were genetically distinct from the isolates from rice, crabgrass (*Digitaria spp.*) and *Setaria spp.* This was evidenced by the uneven distribution of the MAGGY retrotransposon in the perennial ryegrass isolates, where some isolates contained 6-8 or 10-30 copies of the element and other isolates completely lacked the element (15).

Isolates of *M. grisea* from perennial ryegrass and wheat have been shown to cross-infect each other's hosts. Pathogenicity tests have indicated that isolates of *M. grisea* from perennial ryegrass are virulent on wheat and tall fescue (Fig. 3). The isolates also caused gray leaf spot symptoms on triticale, but not on rice. Isolates from wheat and triticale were also virulent on perennial ryegrass indicating that these cereal grain crops have the potential to serve as an additional source of inoculum for the spread of gray leaf spot.

The perfect stage of *P. grisea* was first described in 1971 in crosses between isolates from cereals and wild grasses (20). Since then,

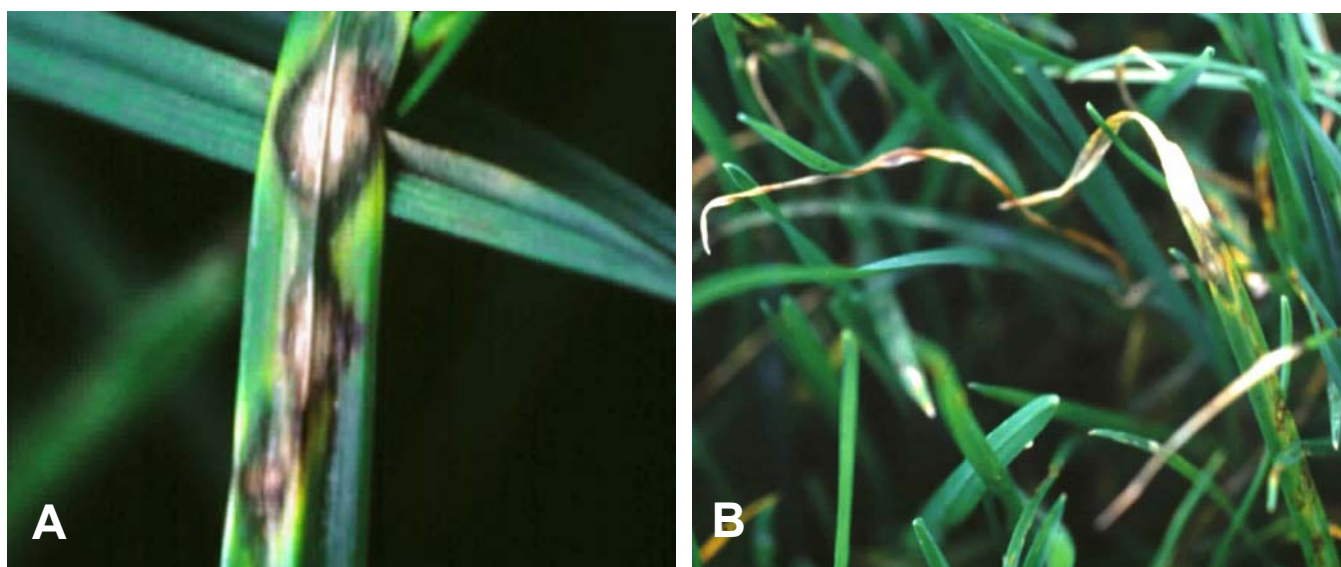
efforts have been made to produce perithecia under controlled conditions using hermaphroditic tester strains to study the genetic relationships among isolates. In a recent study, 73 isolates of perennial ryegrass were paired with fertile hermaphroditic tester strains from finger millet, rice, and wheat in order to determine the mating type distribution, fertility and the degree of sexual compatibility (59).

All isolates from perennial ryegrass produced perithecia only when crossed with MAT1-1 tester strains, and belonged to one mating type (MAT1-2), indicating that sexual recombination does not occur among these isolates. The perennial ryegrass that formed perithecia were male-fertile (female sterile) suggesting that the capacity for expressing the female characteristic may have been lost.

Additionally, none of the perennial ryegrass isolates were capable of forming asci or ascospores, regardless of their developmental stage. The low levels of fertility in the *M. grisea* isolates of perennial ryegrass along with the relatively simple population structure suggests the clonality of the pathogen, and also indicates an exclusive asexual reproduction.

### Symptoms

Gray leaf spot develops on the leaf blades of perennial ryegrass as small water-soaked



**Figure 4.** Gray leaf spot symptoms on perennial ryegrass: A, a characteristic necrotic lesion, B, and flagging of a leaf blade

lesions that subsequently turn into dark-colored, 1-3 mm diameter necrotic spots. The spots expand rapidly and become gray, grayish-brown or light brown, circular to oblong lesions with purple to dark brown borders that often are surrounded by a yellow halo (Fig. 4A). The necrotic lesions coalesce, become irregular in shape and cause partial blighting (tip blighting) or complete blighting of the leaves. Blighted leaf blades may also exhibit twisting or flagging (Fig. 4B). Complete necrosis of the leaves results in the death of entire plant.

There is no evidence of infection of crown tissue by the pathogen. Blighted leaf blades may appear grayish-white to tan and may have dusty or velvety texture when conidia are produced profusely. Aerial mycelium is usually not evident on necrotic leaves under humid conditions.

Diseased turfgrass stands develop an off-color, diffuse blighted or wilted appearance. This is followed by the development of sunken or pocketed areas or irregularly-shaped large patches. When severe the entire ryegrass stand may be killed leaving annual bluegrass and other grassy weeds in the fairways. The disease may be distributed along low-lying or drainage areas where high relative humidity and prolonged leaf wetness periods occur in the turf canopy (Fig. 5).

Gray leaf spot is usually confirmed by observing the hyaline, pyriform, septate (1-3 septa) conidia on diseased leaf tissue (14) (Fig. 6).



**Figure 5.** Gray leaf spot development in drainage areas where high relative humidity and prolonged leaf wetness periods occur

One of the major challenges in using this method is that the conidia of *M. grisea* are not always present on the infected leaf tissue during microscopic examination. Symptomatic leaves may require incubation in a moist chamber for up to 48 hours to induce sporulation.

Recently, a rapid immuno-recognition assay using a monoclonal antibody was developed for diagnosis of gray leaf spot (54). This method of detection has a great potential for practical use as a diagnostic kit in processing turf samples. In this assay, the monoclonal antibody did not react with antigens from *Rhizoctonia solani* (brown patch), *Bipolaris sorokiniana* (Bipolaris leaf spot), or *Pythium aphanidermatum* (Pythium blight). These pathogens are prevalent in perennial ryegrass during the periods of gray leaf spot epidemic development and the symptoms they cause often resemble those of gray leaf spot (Fig. 7).

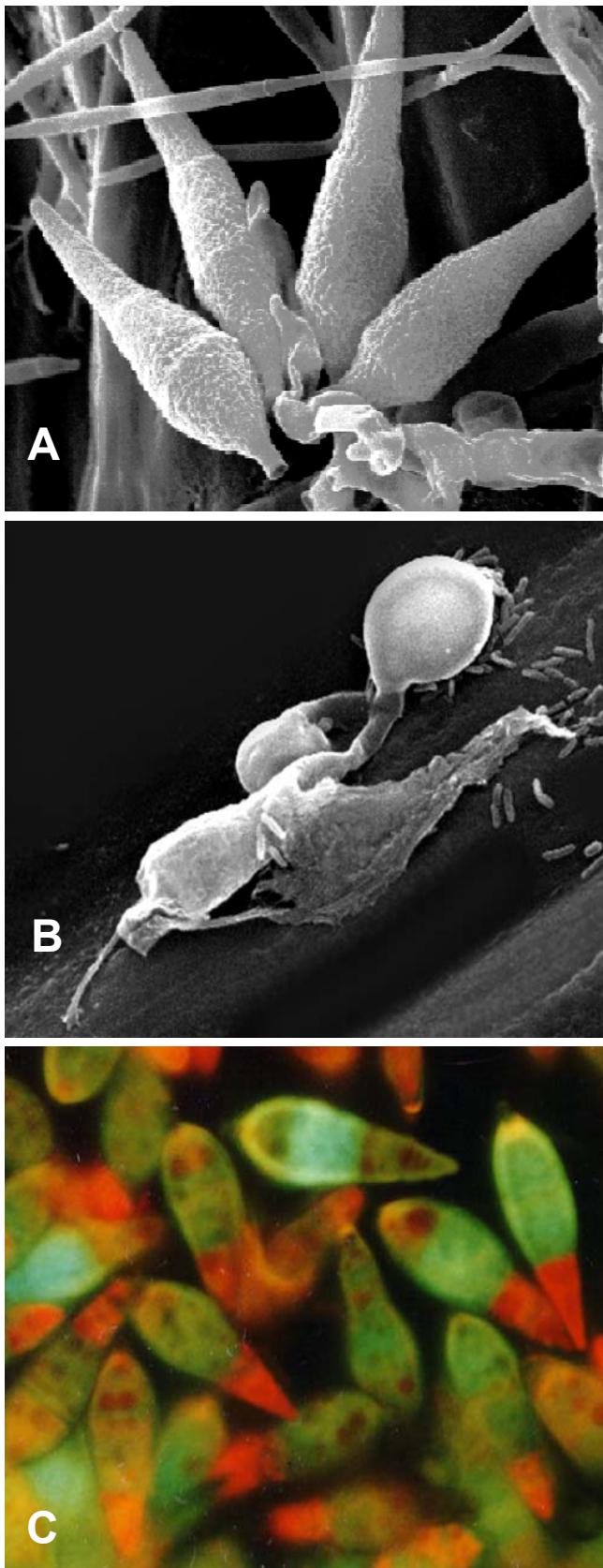
The monoclonal antibody was effective in detecting *M. grisea* in symptomatic perennial ryegrass leaves, but not during the incubation period. Although significant advances have been made in PCR-based procedures for diagnosis of a number of turfgrass pathogens in recent years, it is not yet available for diagnosing gray leaf spot.

### Disease Development

*Magnaporthe grisea* overwinters as dormant mycelium in dead leaves. Harmon and Latin (18) found that survival of *M. grisea* was greatly reduced during the winter, but they successfully induced sporulation of the fungus from infected plant debris in the spring. Conidia produced from the leaf debris apparently serve as the primary inoculum for leaf infections early in the growing season, although details of this early infection process need to be determined.

It is also possible that at least some infection foci are established via long distance dispersal of conidia. Based on field observations, we hypothesize that gray leaf spot develops at visually undetectable levels in early- to mid-summer. Conidia produced on infected leaves during this period eventually trigger a series of secondary infections that contribute to the build-up of inocu-





**Figure 6.** Cryo-scanning electron micrograph of: A, conidia, B, a germinating conidium with appressorium, and C, fluorescent micrograph of conidia of *Pyricularia grisea* causing gray leaf spot of perennial ryegrass turf

lum during the late summer periods (Fig. 8).

Gray leaf spot is often observed first in turf in golf course rough maintained at higher mowing heights. These areas may be partially shaded and have extended leaf wetness periods and high humidity that are more conducive for infection. The disease may be detected in roughs several days before extensive damage of turf in fairways becomes evident.

Dispersal of inoculum is by wind, wind-blown rain, water-splash from sprinkler irrigation, movement by ground maintenance equipment, and other golfing activities. Dispersal of conidia by mowers, spray rigs, spreaders, core aerifiers, and golf carts is important in the spread of the disease in golf courses. Although transmission of *M. grisea* by seeds is well known in rice blast disease, this has not been documented in perennial ryegrass.

Gray leaf spot normally develops from early August to mid-October. Environmental conditions prevailing during this late summer period and availability of inoculum are major determinants in the development of gray leaf spot epidemics. Efforts to quantitatively describe the relationships between environmental factors and gray leaf spot development were first studied on Italian ryegrass in 1972 (27) and continued on tall fescue (41) and perennial ryegrass (22). These studies showed that temperatures between 26-29 C (79-84 F) were optimal for disease development.

A recent study on effects of environmental factors on development of gray leaf spot on perennial ryegrass showed similar temperature effect on disease development (44). The study showed that the effect of temperature on gray leaf spot incidence and severity is cubic, indicating that disease incidence and severity increase with increases in temperature from 20 to 28 C (68 to 82 F) and decrease with increase in temperature above 28 C (82 F).

Leaf wetness duration is also important in the development of gray leaf spot (41,44). Uddin et al (44) reported that that disease incidence and severity increased with increased leaf wetness duration at all temperatures (Fig 9). A shorter leaf wetness duration was required for disease devel-



opment under warmer temperatures.

In addition to leaf wetness duration, relative humidity is also an important environmental component influencing gray leaf spot development. Although expansion of necrotic lesions is rapid under prolonged leaf wetness periods, conidia are not produced when excessive free moisture is present on the leaf tissue. Removal of free moisture from the infected leaf blades under high humidity is necessary for production of conidia. Therefore, warm day and night temperatures, subsequent wetting and drying of leaf blades, and high humidity regimes are major factors in development of gray leaf spot epidemics and perpetuation of the disease. Further studies on epidemiology, particularly the role of relative humidity in gray leaf spot epidemic development and testing of the model under various turfgrass cultural management practices are warranted.

In turfgrass management, the assessment of damage caused by gray leaf spot is exclusively based on disease severity measured by the percent area of dead turf in fairways. Such an assessment may be impractical when the disease appears as off-color, diffused blight at its early stage of development. At this stage when the disease is initially detected, incidence data (% leaf blades symptomatic) can be obtained and the severity estimated; thus a disease threshold for a fungicide spray program can be established. A recent study has shown that gray leaf spot severity can be estimated by incidence ( $Y = 0.25 - 0.002x + 0.0009x^2$ ;  $r^2 = 0.90$ , where  $Y$  = disease severity, and  $x$  = disease incidence) (44).

### Disease Management Strategies

Cultural management practices often do not provide adequate control of gray leaf spot due to rapid development of the disease and high susceptibility of currently available cultivars. An integrated approach that entails various cultural management practices and a sound fungicide program provide effective control of gray leaf spot.

#### Fungicidal Control

Among currently labeled fungicides, the most effective materials for gray leaf spot control



**Figure 7.** Gray leaf spot symptoms resemble those of brown patch and Pythium blight.

are azoxystrobin, trifloxystrobin, and thiophanate methyl (Fig. 10). Azoxystrobin is labeled for gray leaf spot as Heritage 50WG at the rates of 0.61-1.22 kg of formulated product/ha (0.2-0.4 oz/1000 ft<sup>2</sup>) at 14 to 28-day intervals. While the 0.61 kg/ha (0.2 oz/1000 ft<sup>2</sup>) rate of Heritage 50WG has proven very effective in some tests (11, 36, 37, 38, 45,50,72), biweekly applications of the 0.61 kg product/ha (0.2 oz/1000 ft<sup>2</sup>) rate sometimes have resulted in a small but significant amounts of foliar blighting (12, 39, 56, 69).

In contrast, application of Heritage 50WG at the 1.22 kg/ha (0.4 oz/1000 ft<sup>2</sup>) rate typically has provided excellent disease control for at least three weeks under high disease pressure (45, 67, 69, 70, 71). The performance of trifloxystrobin (Compass 50WG) in field tests typically has been very satisfactory. In some studies, there was no statistical difference between Compass used at labeled rates at two-week intervals and the top-performing treatment in the test (12, 38, 45, 63, 70). However in several tests, use of the compound according to label directions provided slightly lower disease control than the top treatment in the test (56,65). The product has not always provided acceptable disease control when used at labeled intervals exceeding two weeks, even at the highest labeled rate (65).

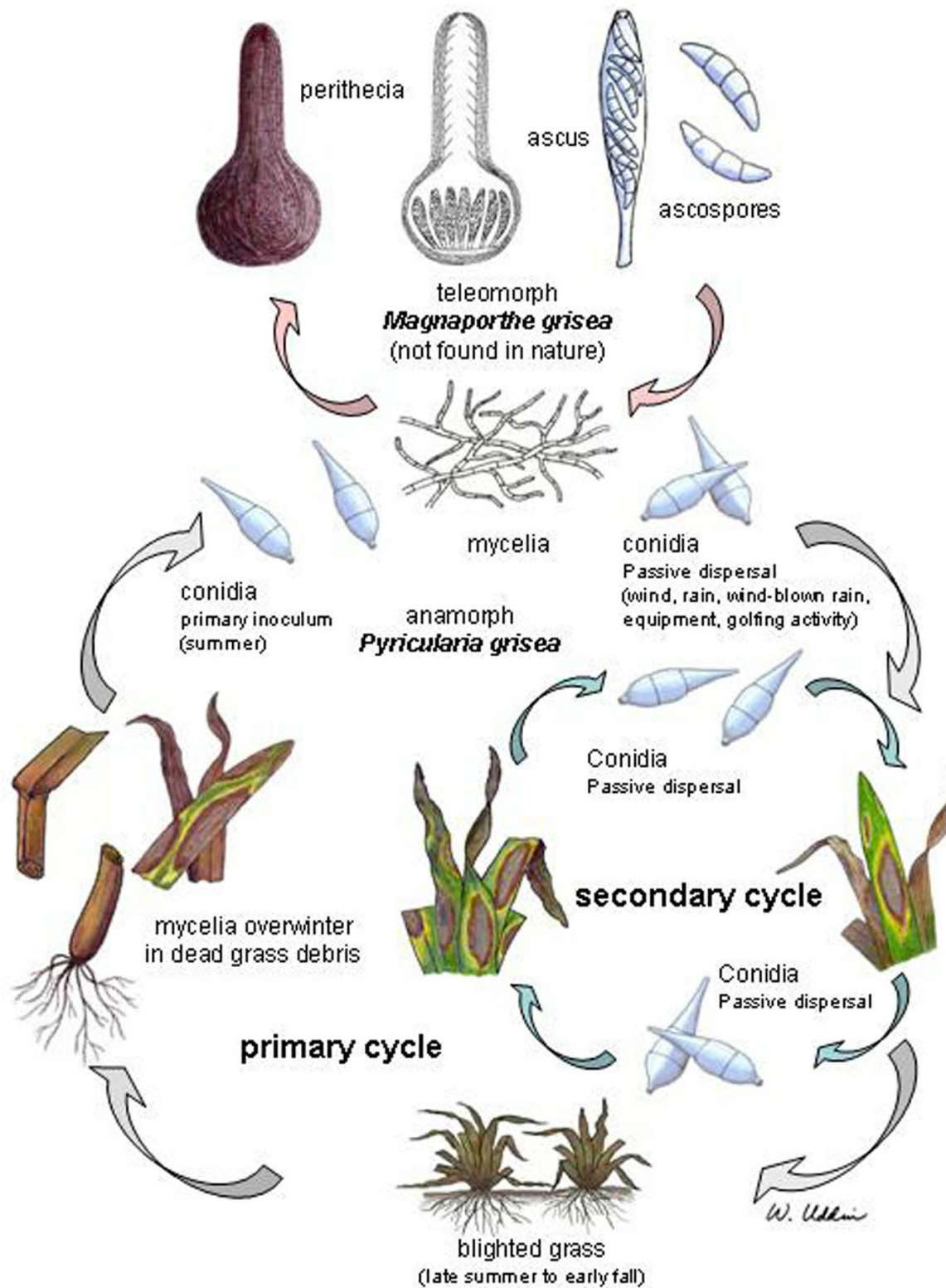


Figure 8. Life cycle of *Pyricularia grisea*, causal organism of gray leaf spot

Thiophanate methyl (Clearys 3336 50WP) and similar products typically have provided excellent control under high disease pressure when used at a minimum of 9.15 kg ai/ha (3 oz ai/1000 ft<sup>2</sup>) at 14-day intervals (37, 50, 56, 70). Rates as low as 6.1 kg ai/ha (2 oz ai/1000 ft<sup>2</sup>) have been effective under low to moderate disease pressure (38, 39, 63). In one test, thiophanate methyl applied biweekly at 9.15 kg ai/ha (3 oz ai/1000 ft<sup>2</sup>) provided excellent control for most of the season, but diminished somewhat at the end of the epidemic (11), suggesting that under the highest disease pressure, the 12.2 kg ai/ha (4 oz ai/1000 ft<sup>2</sup>) rate may be necessary.

Formulations of two demethylation inhibitor (DMI) fungicides, propiconazole and triadimefon, are labeled for gray leaf spot. These DMI fungicides, when used alone following label directions, have usually provided poor control under high disease pressure (36, 37, 38, 45, 56, 68, 69, 70, 73). Chlorothalonil and mancozeb, both contact fungicides that act as non-specific enzyme inhibitors, are also labeled for this disease. Although some studies show have shown good results with these materials (12, 38, 39, 48, 50, 56, 72), chlorothalonil (11, 36, 37, 65, 69, 70) and mancozeb (56, 68, 73) have not consistently provided acceptable control under high disease pressure. Tank-mixes of a DMI fungicide and chlorothalonil at labeled rates can often provide excellent control (11, 12, 38, 48, 67, 70), but control is sometimes not complete under high disease pressure (65, 68).

Timely use of fungicides can help prevent the disease epidemic from reaching its logarithmic phase. Although infections of *M. grisea* can be found as much as 4-6 weeks in advance of the logarithmic phase, rapid increase in disease development must be prevented (66, 71). In the absence of site-specific information on disease development, some turf managers will initiate a spray program a week or two before the time of year when epidemics historically have begun in the area; others will begin spraying when the disease is first reported in the region. Both approaches carry risks. The frequency and longevity of a spray program often depends on a combination of the past

history of the disease at the site and in the region, and the weather conditions favorable to disease development.

In the Midwest and Northeast, the window where fungicide protection is needed is usually from early August to early September and often beyond. In seasons with low disease pressure, no fungicide protection may be needed beyond early September (66), whereas under high disease pressure, fungicides are needed into October (71).

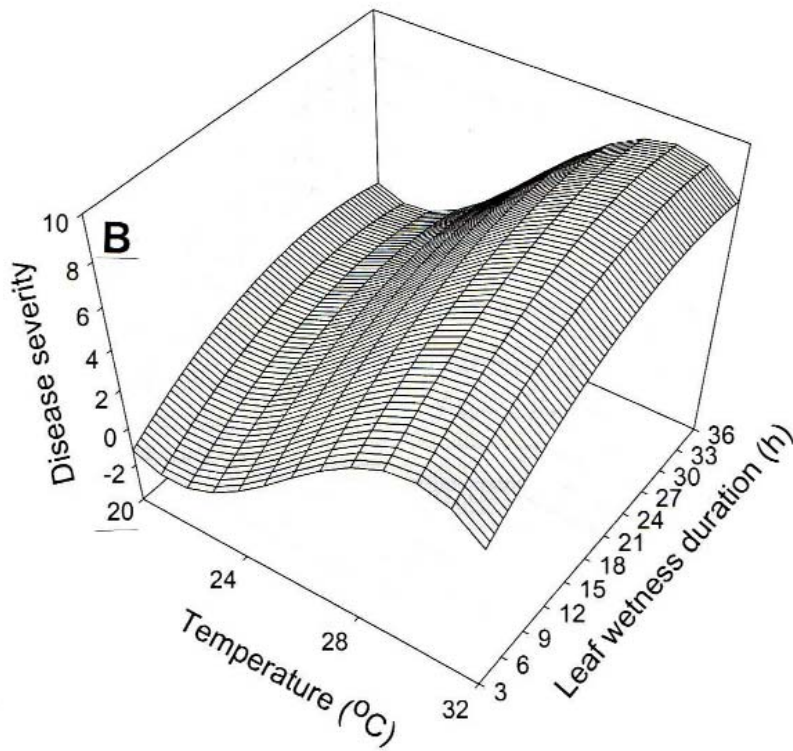
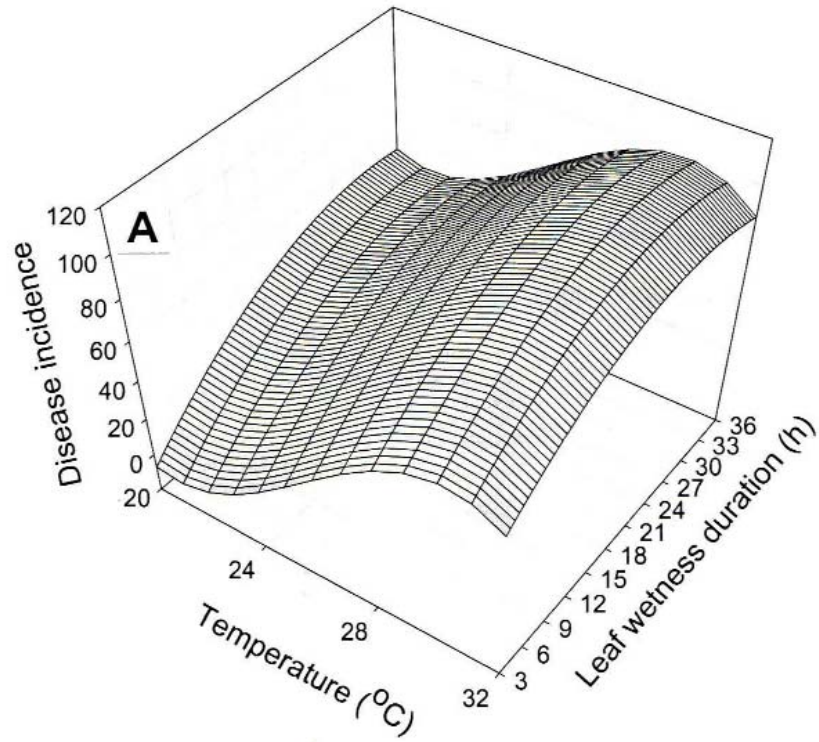
### Fungicide Resistance

The three most effective active ingredients against gray leaf spot belong to two fungicide groups with a high risk for selection of fungicide-resistant subpopulations. Azoxystrobin and trifloxystrobin are strobilurins in a relatively new fungicide class, the Q<sub>o</sub>I fungicides, to which several pathogens have developed resistance (3); and thiophanate methyl is a benzimidazole fungicide, a group in which highly resistant pathogen subpopulations have been selected relatively frequently (34).

To date, *M. grisea* isolates from perennial ryegrass with resistance to Q<sub>o</sub>I fungicides have been found in a total of nine locations in five states (Kentucky, Illinois, Indiana, Maryland, and Virginia) since azoxystrobin was first commercially available for gray leaf spot control in 1998. In cases where we have access to records, the disease outbreaks occurred after Q<sub>o</sub>I fungicides had been applied according to label directions. Resistant isolates have exhibited significantly reduced sensitivity to azoxystrobin *in vitro* as compared to baseline isolates (64, Vincelli and Dixon, unpublished), and they have one or the other of the target site mutations in the mitochondrial cytochrome b gene associated with resistance to Q<sub>o</sub>I fungicides (3, 21).

Although there appears to be a significant resistance risk to Q<sub>o</sub>I fungicides in *M. grisea* isolates from perennial ryegrass, these materials should continue to have a place in control programs for gray leaf spot. Rapid development of a resistant subpopulation in the field does not necessarily preclude the effective use of the fungicide





**Figure 9.** A surface response for disease incidence and severity derived from the polynomial model,  $Y_{is} = b_0 + b_1T + b_2T^2 + b_3T^3 + b_4W + b_5W^2 + b_6TW$ , where  $Y_{is}$  = disease incidence or disease severity,  $T$  = temperature, and  $W$  = leaf wetness duration in hours

in other locations for a number of years. For example, resistance in strains of *Podosphaera xanthii* to benomyl and to DMI fungicides occurred one and two years after commercial introduction, respectively, and yet these fungicides remained important disease control tools in other areas for years until resistant strains became widespread (26).

The Q<sub>o</sub>I fungicides have been an important component of a gray leaf spot management program since 1998, when azoxystrobin received a federal registration for the disease. Avoiding the use of Q<sub>o</sub>I fungicides in the many locations with no evidence of control failure would exert additional selection pressure on *M. grisea* towards resistance to the few remaining effective fungicides (e.g. thiophanate-methyl and DMI fungicides) (62). The potential for rather rapid selec-

tion for resistance does, however, suggest that users of Q<sub>o</sub>I fungicides for gray leaf spot control should aggressively use strategies for reducing the risk of resistance.

### Biological Control

Significant efforts have been made in development of biological control that can be incorporated into a broader integrated management strategy for gray leaf spot (52). A recent study has shown that *Pseudomonas aeruginosa* isolated from spent mushroom substrate is highly antagonistic to the gray leaf spot pathogen (60). In controlled-environment chamber studies, bacterial isolates provided a level of control that was comparable to that of propiconazole, but significantly lower than that of azoxystrobin. In field experiments, *P. aeruginosa* provided significant



**Figure 10.** . Efficacy of various fungicides for control of gray leaf spot. Azoxystrobin, trifloxystrobin, and thiophanate methyl provided excellent control (plots in the background), and chlorothalonil provided moderate control (plot with small patches in the left). Flutolanil, and propiconazole provided poor control (plots in the right) which were not different from the non-treated control plot (foreground)



suppression of gray leaf spot in perennial ryegrass turf with the efficacy up to 14 days when applied as preventive application (74).

Another bacterium, *Bacillus lentimorbus*, isolated from the rhizosphere of perennial ryegrass has also been shown to be suppressive to *M. grisea* (58). In dual plate assays, the antagonist significantly inhibited mycelial growth of *M. grisea* isolates from various hosts including perennial ryegrass, tall fescue, and rice. In controlled environment chamber experiments, the bacterium significantly reduced disease severity and incidence with the efficacy up to 20 days when applied as preventive application. In addition to bacteria, various pathogenic and non-pathogenic isolates of *R. solani* have been shown to be suppressive to gray leaf spot (46). Further studies addressing the safety and practical use of the bio-control agents are warranted.

#### Turfgrass height and grass clipping management

Results from experiments on the effects of mowing height on gray leaf spot severity have been contradictory. A study conducted in the northeastern region of U.S. indicated gray leaf spot was more severe at a mowing height of 8.9 cm (3.5 inches) compared to 1.27 cm (0.5 inches) (55). In Kentucky, Williams et al (74) found no differences in disease severity on perennial ryegrass mowed at 1.9 cm (0.75 inches) and 6.4 cm (2.5 inches). This discrepancy may have been due to various cultural and environmental factors influencing disease development.

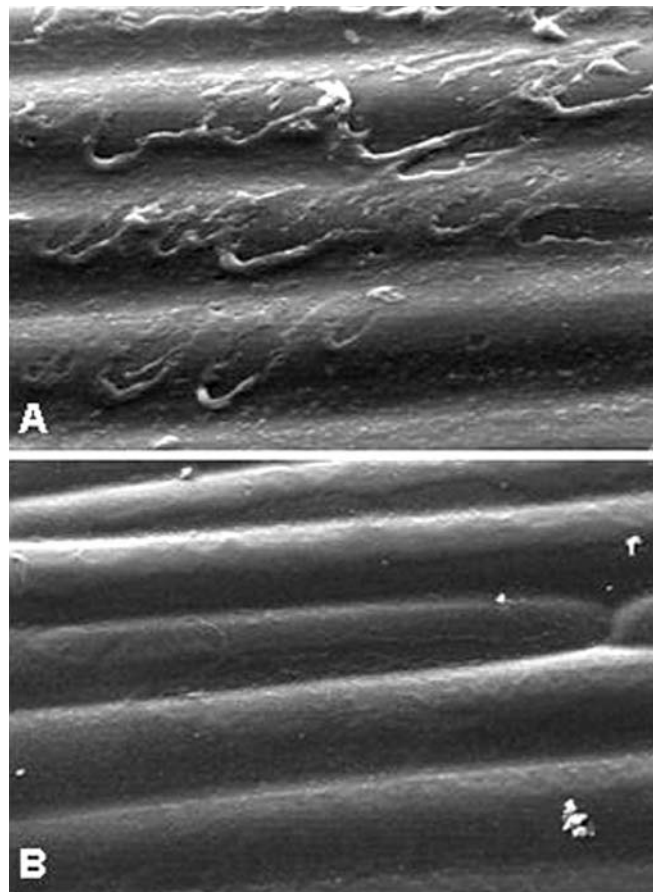
Turf managers in the northeastern and mid-Atlantic states are generally advised to avoid raising mowing heights, particularly during the periods of gray leaf spot epidemics. Grass clipping management is also important, as the removal of clippings can reduce disease incidence substantially under low disease intensity; however, under high disease intensity the effect of clipping removal is not significant (7, 55). Although clipping removal effectively reduces disease intensity under low to moderate disease intensity, collecting the clippings from large fairways and disposing of them is impractical in the operation of most golf courses.

#### Fertility

Increasing the amount nitrogen increases gray leaf spot severity (51, 55, 74). Source of nitrogen also influences gray leaf spot development. A recent study has shown that gray leaf spot severity was lower when controlled-release forms of nitrogen such as isobutylidene diurea (IBDU) and sewage sludge-based Milorganite were applied compared to quick-release forms such as ammonium nitrate and urea (51).

#### Herbicides

The herbicide ethofumesate (Prograss) is widely used to control annual bluegrass in perennial ryegrass fairways. Its use in the spring has been associated with increased severity of gray leaf spot (47). These effects were not evident when the herbicide was applied during fall. The mechanism for the increase in severity of gray leaf



**Figure 11.** Cryo-scanning electron micrographs of perennial ryegrass leaf blades showing the effect of ethofumesate (Prograss) on epicuticular wax deposition: (A) four weeks after treatment with ethofumesate (B) control (distilled water).



spot by ethofumesate is unclear. The herbicide interferes with fatty acid biosynthesis in plants (13) and causes aggregation of the epicuticular wax on leaves (23) (Fig. 12). More efficient penetration of host plant surface at the thinning areas of leaf tissue by *M. grisea* may have resulted in higher gray leaf spot severity. A more rigorous fungicide spray program for gray leaf spot may be required in areas where ethofumesate is applied in spring for annual bluegrass seedhead suppression (47).

#### Breeding for resistance

Resistant cultivars of perennial ryegrass to gray leaf spot pathogen are not currently available. Polygenic resistance appears to be present in perennial ryegrass (9). A number of field studies to identify novel sources of resistance in a worldwide collection of perennial ryegrass cultivars and lines are currently underway. Thus far, some progress in identifying resistant germplasm has been made and several improved lines and cultivars have been identified (5,17).

Research to identify patterns of inheritance and DNA markers associated with resistance in progenies from cultivars, experimental selections, and single plot progenies of perennial ryegrass are in progress. Recently, microsatellite DNA markers in perennial ryegrass progenies associated with moderate to good resistance to *M. grisea* have been identified (4). Breeding for gray leaf spot resistance in perennial ryegrass still remains a long-term goal in the turfgrass industry.

#### **Current Status and Future Outlook**

The impact of gray leaf spot on the management of perennial ryegrass in golf courses has been profound. Additionally, the disease is an emerging problem on ryegrass turf in residential lawns, and sports and recreational turf. Although the economic loss due to damages in residential and sports turf has not been well documented, the impact of gray leaf spot in these turf applications appears to be significant.

As gray leaf spot emerges as a serious disease of perennial ryegrass turf, significant progress in research on various aspects of the dis-

ease such as biology of the pathogen, and epidemiology and management of the disease has been made in recent years. The genetic relationship among the isolates of *M. grisea* from perennial ryegrass and other grass hosts has provided the basic understanding of the biology of the pathogen (15,61).

Development of a weather-based model for predicting gray leaf spot epidemic has been a focus of our research, and currently a temperature and leaf wetness duration based model is available (44). Efforts to quantitatively describe the relationship between the relative humidity and gray leaf spot development are currently underway. A major breakthrough in research on gray leaf spot management is the development of effective fungicide programs for the disease. While intensive use of fungicides is not a desirable long-term disease control strategy, spray programs can be used to prevent epidemics until more sustainable management options are available.

While significant advances have been made in understanding gray leaf spot of perennial ryegrass turf, some major challenges still exist. One of the most serious challenges is the identification of resistant germplasms and development of resistant cultivars. Additionally, very little is known about the life cycle of the gray leaf spot pathogen. Further understanding of the life cycle and its significance to the epidemic development in ryegrass fairways in late summer periods will require major efforts in gray leaf spot research. Although there may be conclusive evidence that the teleomorph of the rice blast fungus is *M. oryzae* as suggested by a recent study (8), genetic relationships between the pathogens of rice blast and gray leaf spot disease of perennial ryegrass needs to be examined more closely.

Phylogenetic analysis of a large pool of isolates of *P. grisea* from perennial ryegrass from a diverse population may be required to reach a reasonable conclusion that the teleomorph of gray leaf spot pathogen is indeed *M. oryzae*. Additionally, determination of the population structure of the gray leaf spot pathogen that encompasses wider geographic regions of the U.S. will require development of a database on *M.*

*grisea* isolates to support such an undertaking.

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