Gray Leaf Spot of Perennial Ryegrass Turf in Pennsylvania

P. J. LANDSCHOOT, Assistant Professor, and B. F. HOYLAND, Research Technician, Department of Agronomy, The Pennsylvania State University, University Park 16802

ABSTRACT

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A severe foliar disease of unknown etiology occurred on perennial ryegrass (*Lolium perenne*) turf during the summer of 1991 in southeastern Pennsylvania. Symptoms began as small brown lesions and rapidly progressed to a foliar blight on both seedling and mature perennial ryegrass. Isolation from diseased leaf tissue yielded a high percentage of *Pyricularia grisea* colonies. Inoculation of 4- and 20-wk-old plants resulted in symptoms very similar to naturally infected perennial ryegrass. Plants 4 wk old were more severely diseased than 20-wk-old plants, and necrosis of leaf tissue was more extensive at 29 C than at 22 C. *P. grisea* was consistently isolated from inoculated plants. Given the results of this study, it was concluded that *P. grisea* was the primary cause of the foliar blight that occurred in Pennsylvania. Gray leaf spot was chosen as the name of this disease.

Blast and leaf spot diseases caused by *Pyricularia grisea* (Cooke) Sacc. (perfect stage *Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udagawa) occur on a number of grass species worldwide (1,13). Blast of annual ryegrass (*Lolium multiflorum* Lam.) forage was first reported in Louisiana and Mississippi in 1971 (2,3). Carver et al (3) noted that several thousands of acres of annual ryegrass were affected by the disease. Gray leaf spot is a common and severe disease of St. Augustine grass (*Stenotaphrum secundatum* (Walter) Kuntze) turf in the southern United States (5). Blast and leaf spot diseases are more severe during warm (25–30 C) and humid weather, under high rates of nitrogen fertilizer, and in newly sprigged or seedling grasses (5,7,12).

Infection of perennial ryegrass (*L. perenne* L.) by *P. grisea* has been achieved through artificial means (8,15), and Shurtleff et al (11) listed perennial ryegrass as a host of *P. grisea*. However, a search of literature pertaining to *P. grisea* yielded no detailed reports citing this fungus as the causal agent of a foliar disease of perennial ryegrass turf.

A severe foliar disease exhibiting symptoms similar to gray leaf spot of St. Augustine grass and blast of annual ryegrass (2,6) was observed on perennial ryegrass fairways in southeastern Pennsylvania during the last week of August and the first week of September, 1991. Unseasonably warm temperatures and high relative humidity occurred during this period and coincided with perennial ryegrass-overseeding operations on many area golf courses.

Initial disease symptoms on leaves included small, oval, brown lesions or spots (1–3 mm long) with darker brown borders. On mature, tillered perennial ryegrass, a zone of chlorotic tissue developed around spots, eventually enveloping most or all of the leaf. In some cases leaves turned tan and appeared blighted. In most cases, crowns were not damaged. Infected seedlings appeared flaccid and water-soaked and were blue-gray. Many affected plants collapsed within 4 or 5 days after symptoms first appeared. Some seedlings survived but suffered extensive leaf necrosis. Within 1 wk of initial symptom development, irregular patches, several meters in diameter, of chlorotic or blighted turf were evident on golf course fairways.

The purpose of this study was to determine the cause of the foliar disease of perennial ryegrass turf in Pennsylvania and examine the influence of temperature and plant age on disease severity.

MATERIALS AND METHODS

Isolation. Samples of diseased perennial ryegrass were collected from two golf courses in southeastern Pennsylvania: Doylestown Country Club in Doylestown, and Springhaven Country Club in Wallingford. Leaf blades with lesions were cut into 5-mm segments, immersed in 0.5% sodium hypochlorite for 3–5 min, then rinsed in sterile distilled water, blotted, and placed on half-strength potato-dextrose agar (PDA). Plates were incubated in total darkness at 25 C. Within 4–5 days, white to light gray colonies, as well as other colonies, grew out of the leaf segments. All colonies were transferred to fresh half-strength PDA and stored at 20 C.

Growth rates. Four isolates of *P. grisea* were selected for determination of temperature effects on radial colony growth. Two isolates were from the Doylestown site (DOY-2 and DOY-3) and two were from Springhaven Country Club (SPR-2 and SPR-3). All isolates were maintained on half-strength PDA at 25 C in darkness for 6 days prior to transferring. Agar plugs (5 mm in diameter) were cut from actively growing margins of each colony and transferred to the center of plastic petri dishes, 90 mm in diameter, containing approximately 20 ml of half-strength PDA. Three replicate plates were used for each isolate, and plates were incubated at 9, 14, 19, 24, 29, 34, and 39 C in darkness. Growth rates were determined by measuring radial growth every 24 hr for 6 days, or until the colony margin reached the edge of the plate. Two measurements of colony radius were taken at right angles to each other, and the values were averaged. Growth measurements used for temperature comparisons were from the 24-hr period showing maximum growth for each isolate. The experiment was a completely randomized design, and the test was repeated once. Data from each test were pooled.

Pathogenicity studies. One single-conidium isolate of *P. grisea* (DOY-3) was selected for pathogenicity studies. Selection was based on stability of growth and ability to produce abundant conidia in culture. Inoculum was produced by placing several plugs of colonized half-strength PDA on plates of rabbit food agar (25 g of Big Red rabbit food [Agway Inc., Syracuse, NY] per liter of sterile distilled water) and allowing plates to incubate under constant fluorescent light (21 μE m^{-2} s^{-1}) at 25 C for 2–3 wk. Rabbit food agar yielded the highest number of conidia when compared with half-strength PDA, potato-sucrose agar, and V8 juice agar. Conidia were harvested by flooding each plate with 3 ml of sterile distilled water and dislodging conidia with a glass rod. The resulting suspension was filtered through four layers of cheesecloth to remove large pieces of mycelium. The final conidia suspension was adjusted to a concentration of 7×10^3 conidia per milliliter with additions of sterile distilled water.

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The perennial ryegrass cultivar Pennfine was seeded into pots, 12 cm in diameter, at a rate of 1.5 g of seed per pot. The growing medium was calcined clay (Terra-Green coarse grade, Oil-Dri Corp. of America, Chicago, IL) (N at 7 mg/kg, P at 20 mg/kg, and K at 842 mg/kg, pH 5.4). Plants were placed on a greenhouse bench under natural light (no supplemental lighting) and irrigated twice per day and fertilized biweekly with a 20-10-20 fertilizer at approximately 0.5 g per 100 ml of water per pot. The turf was cut once per week and maintained at a height of 3.5 cm. One group of plants was allowed to grow for 20 wk before inoculation, and the other group consisted of 4-wk-old plants.

Both groups of plants were inoculated at the same time by spraying the conidial suspension through an atomizer until runoff. One drop of Tween 20 (monolaurate polyoxyethylenesorbatin) was added to the spore suspension to aid in wetting the leaf surface. Control plants were sprayed with sterile distilled water and Tween 20. Inoculated and control plants were covered with glass jars for 48 hr at 22 ± 3 C to maintain a humid environment. Upon removal of the jars, 4- and 20-wk-old plants were placed in growth chambers at 22 ± 3 C and 29 ± 3 C with a 12-hr photoperiod (329 μE m^{-2} s^{-1}) and 50–70% relative humidity. Pots were arranged in a completely randomized design with three replications per treatment. The experiment was repeated once.

The criterion for evaluating pathogenicity included measuring the length of individual lesions on each of 10 leaves per pot. Measurements were made with a plastic ruler. Measurements of lesions were collected on three separate dates for each of two tests at 10, 14, and 18 days after inoculation. No measurements were taken on control plants, since no foliar lesions occurred. The experiment was analyzed as a split plot design with temperature serving as whole plots, age as subplots, and the two sequential experiments as blocks.

RESULTS AND DISCUSSION

Isolation and identification. Several different fungi were isolated from diseased perennial ryegrass from southeastern Pennsylvania. Of the 75 isolates obtained, the majority (72%) were identified as *P. grisea*. Identification of *P. grisea* was based on features of the anamorphic state and conformed to descriptions in Asuyama (1), Ellis (4), and Ou (9). Conidia were pyriform or obclavate and three-celled and ranged from 17.5 to 29.0 μm in length and 6.3 to 10.0 μm in width (Fig. 1). Most colonies were white or light gray and produced abundant aerial mycelium. A few colonies were appressed to the medium surface.

The remainder of the fungi included species in the genera *Curvularia* (11%), *Leptosphaerulina* (8%), *Colletotrichum* (7%), *Pythium* (1%), and *Rhizoctonia* (1%). Of these fungi, only *P. grisea* and *Curvularia* spp. were isolated from both the Doylestown and Wallingford sites. Because of the high percentage of *P. grisea* isolated from diseased leaf tissue, the decision was made to determine whether a causal relationship existed between this fungus and the foliar disease of perennial ryegrass.

Radial growth rates. Radial growth rates of *P. grisea* were very similar among the four isolates, with optimum growth of all isolates occurring at 29 C (Fig. 2). No detectable growth was evident during the experiment at 9 and 39 C. These results are similar to studies cited by Ou (9), in which optimum growth occurred at 28 C, but no mycelial growth occurred below 8 C or above 37 C.

Pathogenicity studies. Although the inoculum concentration of 7×10^3 conidia per milliliter was considerably lower than those used in previous studies with annual ryegrass (7,14), it was sufficient to produce one or two lesions per plant. Symptoms of plants inoculated with *P. grisea* were very similar to those that occurred in the field (Fig. 3). On 4-wk-old inoculated plants, initial symptoms appeared as small brown lesions, which rapidly expanded to form spots with dark borders (Fig. 4). Within 10–18 days, many leaves were blighted, becoming blue-gray and then tan. After 18 days at 29 C, necrosis was extensive above and below spots (Fig. 5). On 20-wk-old plants, chlorosis and necrosis were evident around individual leaf spots. *P. grisea* was consistently isolated from both 4-wk-old and 20-wk-old plants showing disease symptoms.

The main effect of plant age was highly significant on all three rating dates and

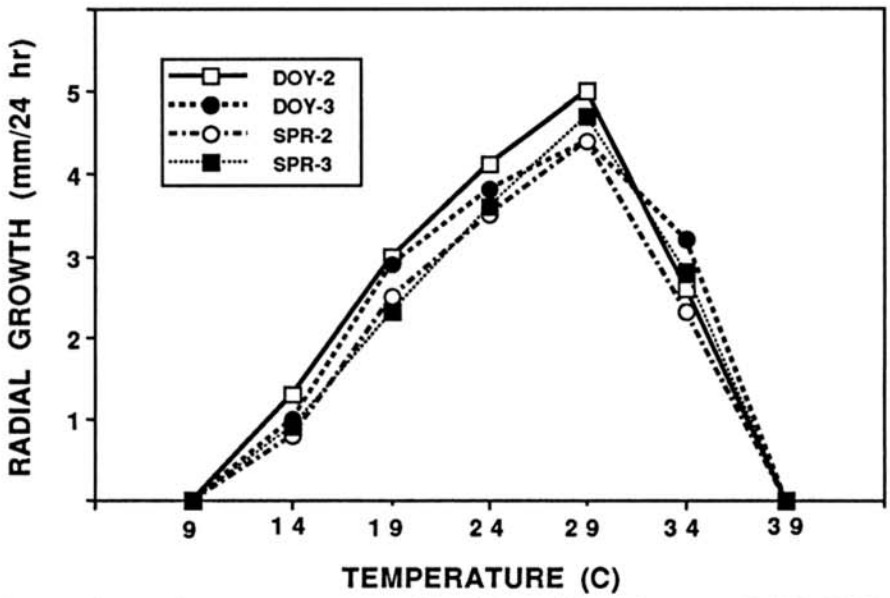

Fig. 2. Influence of temperature on growth of four isolates of *Pyricularia grisea* (DOY-2, DOY-3, SPR-2, and SPR-3) on half-strength potato-dextrose agar.

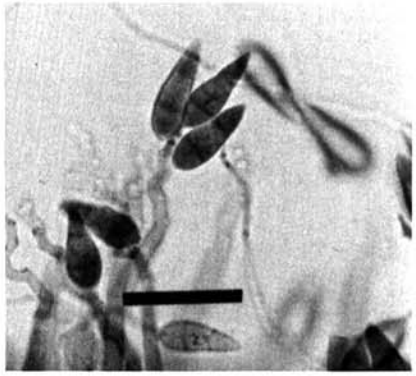

Fig. 1. Conidia of *Pyricularia grisea*. Scale bar = 30 μm.

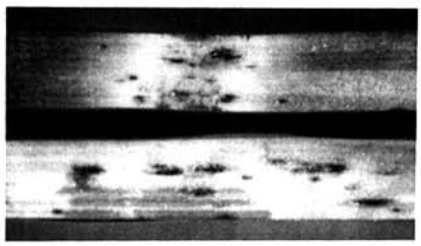

Fig. 3. Lesions and chlorosis on tillered perennial ryegrass leaves from a golf course fairway in Doylestown, Pennsylvania.

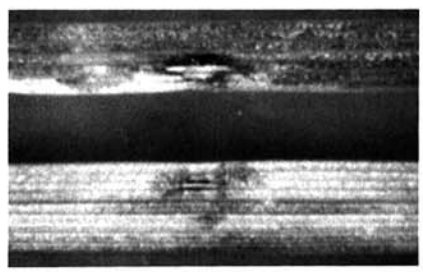

Fig. 4. Early stages of disease symptoms on 4-wk-old perennial ryegrass inoculated with *Pyricularia grisea*.

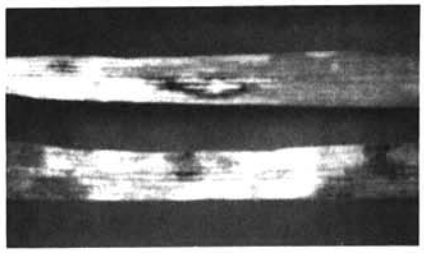

Fig. 5. Necrosis on leaf tissue of 4-wk-old perennial ryegrass inoculated with *Pyricularia grisea*.

when measurements were averaged over the three rating dates (Table 1). Leaf lesion measurements indicate that younger (4-wk-old) perennial ryegrass was more severely affected by *P. grisea* than older (20-wk-old) turf (Table 2). This appears to confirm field observations in which newly seeded turf was more extensively damaged than older, tillered plants. Moss and Trevathan (7) reported that annual ryegrass was susceptible to *P. grisea* in all stages of development, but that 4- to 5-wk-old plants were most susceptible. The authors noted that fewer lesions developed on plants older than 5 wk than on younger plants. The main effect of temperature was significant with respect to extent of leaf lesions on two of three rating dates and when leaf lesion measurements were averaged over the three dates (Table 1). Leaf lesions were greater at 29 C than at 22 C on both 4- and 20-wk-old perennial ryegrass (Table 2). There was a significant temperature–age interaction 10 days after inoculation but not at 14 or 18 days. The interaction indicated that differences between 4- and 20-wk-old plants were greater at 29 C than at 22 C with respect to foliar lesions. Therefore, it is possible that the unseasonably warm and humid conditions that existed in southeastern Pennsylvania at the time this disease occurred played an important role in disease development.

It is unknown if resident populations of *P. grisea* were responsible for the foliar blight disease occurrence in southeastern Pennsylvania. Bain et al (2) speculated that time and location of the first large-scale epidemic of annual ryegrass blast may have been due to spores of *P. grisea* transported from the Caribbean or Southern Louisiana into Mississippi by Hurricane Edith in 1971. It is interesting to note that a tropical storm passed near the southeastern portion of Pennsylvania immediately prior to the occurrence of this disease in 1991. It is also possible that the pathogen could be seedborne.

Given the high percentage of isolates of *P. grisea* from diseased perennial ryegrass, the production of symptoms on inoculated plants resembling those from the field, and the reisolation of *P. grisea* from inoculated plants, it is concluded that this fungus was the primary cause of a foliar blight disease of perennial ryegrass in Pennsylvania. Gray leaf spot is the name proposed for this disease. The epithet *leaf spot* was chosen rather than *blast*, since blast denotes symptoms characterized by shedding of unopened buds or failure to produce fruit or seed (10).

Table 1. Analysis of variance for foliar lesions on perennial ryegrass plants inoculated with *Pyricularia grisea*

		Mean squares[a]			
Source	df	10 days	14 days	18 days	Mean
Experiment (E)	1	0.70	0.18	5.13	0.38
Temperature (T)	1	40.30*[b]	61.12	3,569.72*	610.04**
T × E	1	0.07	2.10	8.28	0.14
Age (A)	1	284.97***	637.57***	1,271.67***	674.16***
T × A	1	9.50**	4.25	21.09	10.67*
Error	18	19.78	19.59	217.72	1.69
Corrected total	23	...	...	...	...

[a] Mean squares of foliar lesions on perennial ryegrass 10, 14, and 18 days after inoculation, and the mean of the three ratings.

[b] * = Significant at the 0.05 probability level, ** = significant at the 0.01 probability level, and *** = significant at the 0.001 probability level.

Table 2. Influence of *Pyricularia grisea* on the length of foliar lesions at two temperatures on 4- and 20-wk-old perennial ryegrass

	Length of foliar lesions (mm)							
	22 C				29 C			
Age of plant	10 days[a]	14 days	18 days	Mean[b]	10 days	14 days	18 days	Mean
4 wk	10.7[c]	14.8	25.4	17.0	14.6	18.8	51.7	28.4
20 wk	5.1	5.3	12.8	7.7	6.4	7.7	35.3	16.5

[a] Days after inoculation.

[b] Length of foliar lesions averaged over three rating dates.

[c] Mean foliar lesion length from two experiments.

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