

Pathogenicity on Ryegrass and Cultural Variability of Mississippi Isolates of *Pyricularia grisea*

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ABSTRACT

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The occurrence of *Pyricularia grisea* on hosts common to Mississippi was assessed to elucidate relationships among isolates and to determine their relative pathogenicity to Italian ryegrass (*Lolium multiflorum*). Isolates of *P. grisea* pathogenic to ryegrass were obtained from symptomatic leaf tissue of ryegrass, crabgrass (*Digitaria sanguinalis*), St. Augustinegrass (*Stenotaphrum secundatum*), spurge (*Euphorbia preslii*), and smartweed (*Polygonum pensylvanicum*) and from asymptomatic soybean (*Glycine max*) pod tissue. Colony morphology and growth rate varied with isolate and growth medium. The ryegrass cultivar Gulf was consistently more susceptible to the different isolates of *P. grisea* than was Magnolia.

Blast of ryegrass (*Lolium multiflorum* Lam.), caused by *Pyricularia grisea* (Cke.) Sacc., was first reported in 1972 in Louisiana and Mississippi (3,4). Heavy losses in forage for winter grazing have been sustained, especially in central and southwestern Mississippi (3) and southeastern Louisiana (4). The *Pyricularia* sp. found on lesions and dead portions of ryegrass leaves (4) was later identified as *P. grisea* (2). It has been documented that *P. grisea* and *P. oryzae* Cavara are not readily distinguishable (5,6) and that the latter exhibits physiologic specialization (1).

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Bain emphasized that *P. grisea* also occurs on wild grasses and other hosts that could act as reservoirs of inoculum for infection of ryegrass (2). This study was undertaken to assess the occurrence of *P. grisea* on hosts commonly growing in Mississippi, to understand more about the relationship between these isolates, and to determine their relative pathogenicity to ryegrass.

MATERIALS AND METHODS

Pyricularia isolates. Isolates of the pathogen were obtained from various sites throughout Mississippi from the following hosts: Italian ryegrass, crabgrass (*Digitaria sanguinalis* (L.) Scop.), soybean (*Glycine max* (L.) Merr.), spurge (*Euphorbia preslii* Guss.), smartweed (*Polygonum pensylvanicum* L.), and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze). Sections of tissue from symptomatic or asymptomatic hosts were surface sterilized with 95% ethanol for 5 sec and with 1% sodium hypochlorite (20% Clorox) for 60 sec,

then rinsed in sterile water and plated on Difco potato-dextrose agar (PDA). The tissue sections were incubated in darkness at 15 C for 4 days, after which *P. grisea* isolates growing from them were transferred to fresh PDA.

Cultural comparisons. Isolates of *P. grisea* were grown on PDA, V-8 juice agar, and oatmeal agar (OMA) in standard petri plates. Point inoculations with agar plugs were made at the extreme edge of petri plates, and cultures were incubated in the laboratory in alternate periods of 8 hr of daylight and 16 hr of darkness at ambient temperatures (~25 C). Growth of isolates was determined by measuring from the agar plug to the leading edge of mycelial growth at 2-day intervals on three plates of each isolate on a particular medium. Measurements were continued until the fastest growing isolate had reached the opposite edge of the plate on any medium. This experiment was repeated three times.

Pathogenicity studies. Sporulating isolates from the three media used for cultural comparisons were streaked onto OMA and maintained as previously described for 10 days. Conidia were harvested by flooding 20 cultures of each isolate with sterile, distilled water; dislodging conidia and mycelium from the agar surface with a flamed loop; and filtering the conidial-mycelial suspensions through cheesecloth. Conidial suspensions of each isolate were adjusted with sterile, distilled water to a final concentration of 2×10^5 conidia per milliliter.

For inoculation tests, plants of two ryegrass cultivars, Gulf and Magnolia,

were grown from seed in a mixture of sand, soil, and vermiculite (2:2:1, v/v/v) and 85 g of 14:14:14 (N-P₂O₅-K₂O) fertilizer per 1.18 cm³ of soil mixture in plastic flats containing 12 peat pots. Plants were thinned after emergence to five per pot for a total of 60 plants per flat. All plants were inoculated at 4 wk of age.

Each treatment consisted of spraying all plants in a flat with a conidial suspension from a single isolate of *P. grisea* until runoff; three replicate flats of each cultivar were sprayed. Flats were immediately placed in a mist chamber in the greenhouse (~25 C) for 72 hr. Upon removal from the mist chamber, plants were maintained with daily watering in the greenhouse under natural conditions of light and a mean temperature of 25 C. Seventy-two hours after misting, all plants were examined for symptom development. A disease rating was recorded for the number of plants with symptoms as a percentage of total plants inoculated.

RESULTS

Pyricularia isolates. Cultural studies of tissue samples confirmed the presence of 13 isolates of *P. grisea* on several hosts. Six isolates (1-6) were cultured from symptomatic leaf tissue of crabgrass. Single isolates were obtained from asymptomatic soybean pod tissue (isolate 7) and from lesions on leaf tissue of spurge (isolate 8), smartweed (isolate 9), and Italian ryegrass (isolate 10). Three isolates (11-13) from St. Augustinegrass were cultured from symptomatic leaf tissue. Isolate 14 was obtained from the American Type Culture Collection, ATCC 15022 from crabgrass, and was included only in pathogenicity studies.

Cultural comparisons. Colony morphology was dependent upon both isolate and growth medium (Fig. 1). Growth rate also varied among isolates grown on the same medium and for each isolate on the three different media used (Table 1).

Pathogenicity studies. Pathogenic isolates incited small, water-soaked spots on ryegrass leaves. Four days after inoculation, leaf lesions began to assume a round to oval conformation with a gray center. Symptom development was most obvious 5 days after plants were inoculated. Lesions were concentrated on the adaxial leaf surface. Lesions often coalesced, extending the width of leaf blades and killing the leaf from that point to the leaf tip. Dieback of leaves was also observed in association with older lesions that were surrounded by a chlorotic border. Chlorosis tended to expand from the area of the original lesion toward the leaf tip.

All isolates, with the exception of isolate 4 from crabgrass, incited visible symptoms on one or both inoculated cultivars (Fig. 2). Isolate 10 from ryegrass was the most virulent one tested, followed by isolate 5 from crabgrass, isolates 11-13

from St. Augustinegrass, and isolate 9 from smartweed. All isolates except 4 and 7 were more virulent to the ryegrass cultivar Gulf than to Magnolia.

DISCUSSION

The original postulation by Bain (2) that wild hosts serve as reservoirs of inoculum of *P. grisea* for infection of

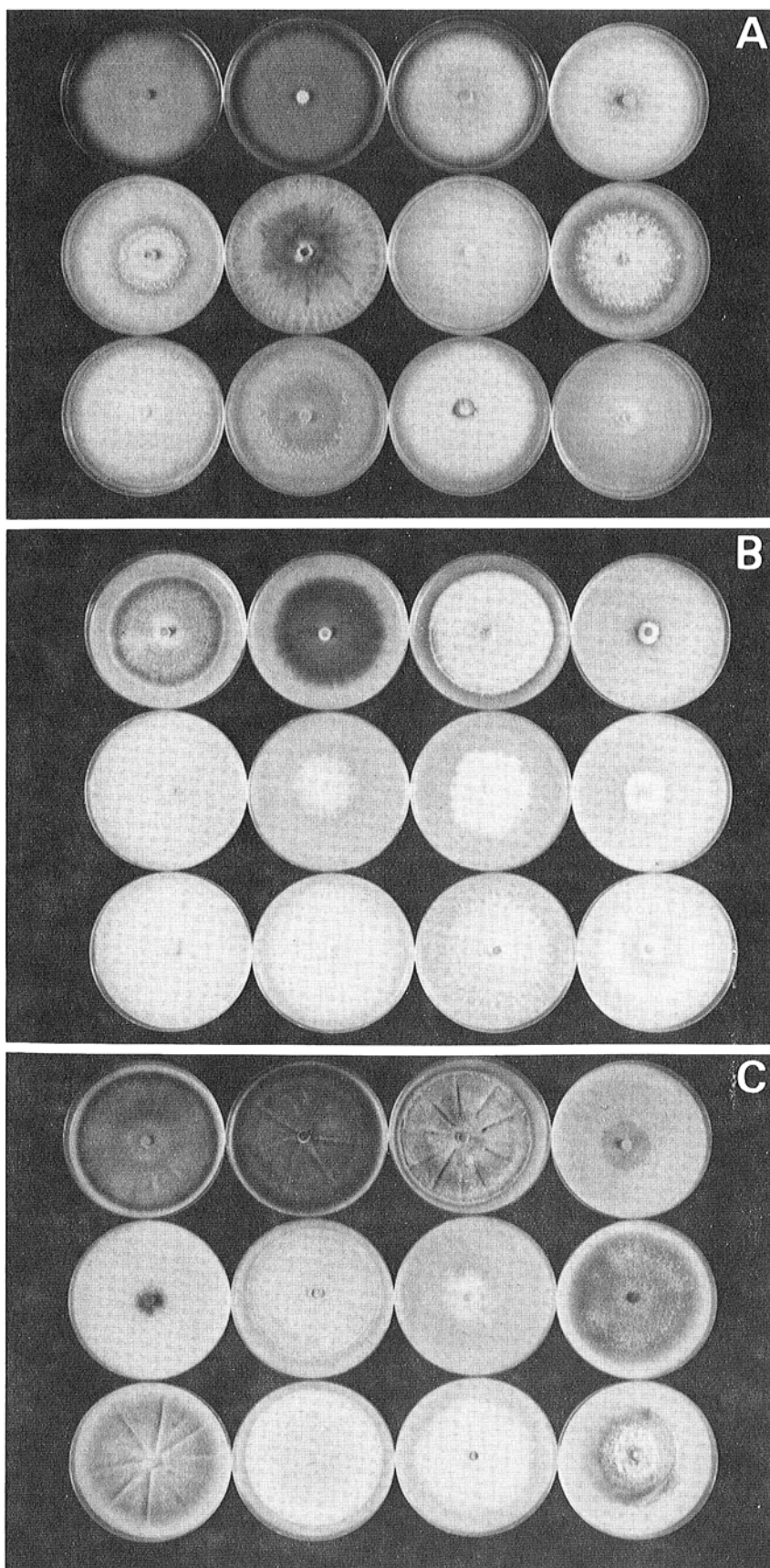


Fig. 1. Colony morphology of *Pyricularia grisea* isolates grown on (A) potato-dextrose agar, (B) V-8 juice agar, and (C) oatmeal agar. Beginning with the top row and reading left to right, the isolates are numbered 1, 3, 4, 5, 7, 8, 9, 6, 10, 11, 12, and 13.

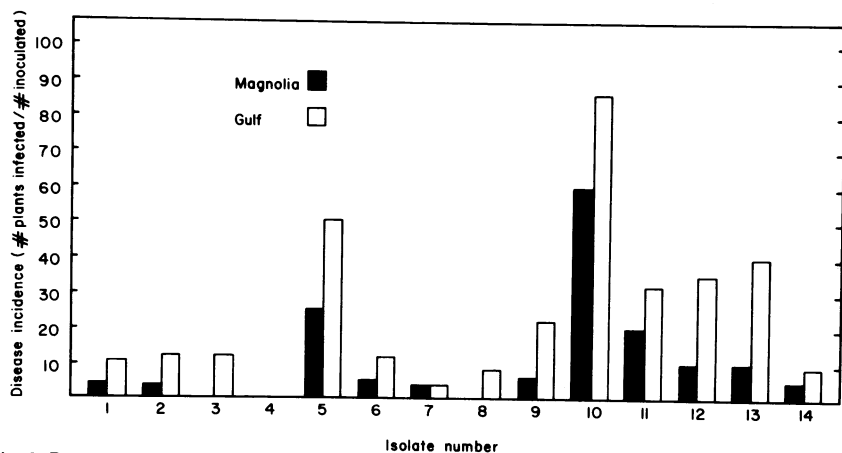


Fig. 2. Response of ryegrass cultivars Magnolia and Gulf to isolates of *Pyricularia grisea*. Source hosts for isolates include crabgrass (isolates 1–6), soybean (7), spurge (8), smartweed (9), Italian ryegrass (10), St. Augustinegrass (11–13), and ATCC 15022 from crabgrass (14). For isolate 3, data are missing for Magnolia; for isolate 4, disease incidence for both cultivars was zero; for isolate 8, disease incidence for Magnolia was zero.

Table 1. Relative growth rate of *Pyricularia grisea* isolates on potato-dextrose agar (PDA), V-8 juice agar (V8), and oatmeal agar (OMA)

Isolate	Host	Growth rate ^a		
		PDA	V8	OMA
1	<i>Digitaria sanguinalis</i>	3.7	2.5	3.5
2	<i>D. sanguinalis</i>	3.9	3.5	3.9
3	<i>D. sanguinalis</i>	4.1	4.0	3.8
4	<i>D. sanguinalis</i>	2.7	3.4	3.3
5	<i>D. sanguinalis</i>	3.5	3.8	3.8
6	<i>D. sanguinalis</i>	3.0	3.8	3.6
7	<i>Glycine max</i>	4.0	3.7	3.7
8	<i>Euphorbia preslii</i>	2.8	3.5	3.5
9	<i>Polygonum pensylvanicum</i>	2.9	2.4	2.3
10	<i>Lolium multiflorum</i>	4.1	3.9	3.5
11	<i>Stenotaphrum secundatum</i>	3.2	3.8	3.4
12	<i>S. secundatum</i>	4.0	3.6	3.9
13	<i>S. secundatum</i>	2.7	3.2	2.9

^aNumbers represent the slope of the growth curve based on measurements made at 2-day intervals over 16 days of at least three cultures of each isolate on each medium. Differences were not significant ($P=0.05$). The experiment was repeated three times; test results were similar and have been averaged for presentation.

ryegrass was confirmed. His inclusion of only gramineous hosts seems to be too restrictive in view of isolates from soybean, spurge, and smartweed that were pathogenic to ryegrass. Isolation from asymptomatic, surface-sterilized soybean pod tissue indicated that the organism may be capable of existing either intercellularly or intracellularly in this host without inciting symptoms.

Such evidence could have important implications in Mississippi where soybean and ryegrass are commonly grown in a double-cropping system.

Virulent isolates were generally gray to white in coloration, whereas black isolates were weakly virulent. Mycelial production varied from the abundant white, aerial mycelium of isolates 11–13 to the very sparse, appressed growth of

isolate 5.

The most virulent isolate, 10, had the most rapid growth rate of all isolates on PDA and the second most rapid growth rate on V-8 juice agar, but it did not grow as rapidly as eight other isolates on OMA. Isolate 12 grew faster than other isolates on OMA and was one of the more virulent isolates. Isolate 5, the second most virulent one, produced moderate to fast growth on all media tested. However, no definite relationship between colony growth rate and virulence was determined.

Although disease varied with the isolate tested, symptom expression and disease development were the same regardless of inoculum source. Chlorotic areas around lesions and chlorosis from lesions to leaf tips were interpreted as possible evidence of a diffusible toxin produced by the causal organism or in association with the host. Concentration of lesions on adaxial leaf surfaces may indicate accumulation of conidia as a result of the inoculation technique or may be a function of runoff during the 72-hr misting period.

Gulf and Magnolia ryegrass share a common genetic background, but Magnolia was consistently more resistant to isolates of *P. grisea* than was Gulf. Thus, a degree of resistance to *P. grisea* may be available in existing ryegrass cultivars. Such material, along with other sources of genes for resistance to *P. grisea*, probably represents the most practical measure of minimizing economic losses from this pathogen on ryegrass.

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