Genetic Structure of *Magnaporthe grisea* Populations Associated with St. Augustinegrass and Tall Fescue in Georgia

L. P. Tredway, K. L. Stevenson, and L. L. Burpee

First and second authors: Department of Plant Pathology, University of Georgia, Athens 30602; and third author: Department of Plant Pathology, Georgia Station, University of Georgia, Griffin 30223.

Current address of L. P. Tredway: Department of Plant Pathology, North Carolina State University, Raleigh 27695.

Accepted for publication 23 December 2004.

ABSTRACT

Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2005. Genetic structure of *Magnaporthe grisea* populations associated with St. Augustinegrass and tall fescue in Georgia. *Phytopathology* 95:463-471.

Amplified fragment length polymorphisms (AFLPs) were used to estimate phylogenetic relationships within *Magnaporthe grisea* and determine the genetic structure of *M. grisea* populations associated with tall fescue and St. Augustinegrass in Georgia. Sixteen clonal lineages were identified in a sample population of 948 isolates. Five lineages were isolated from tall fescue (E, G1, G2, G4, and H), with lineage G4 comprising 90% of the population. Isolates from tall fescue were closely related to those from perennial ryegrass, weeping lovegrass, and wheat. Two *M. grisea* lineages were isolated from St. Augustinegrass (C and K),

with lineage C comprising 99.8% of the population. Populations from crabgrass were dominated (98%) by lineage K, but also contained a single lineage C isolate. Haplotype diversity indices ranged from 0.00 to 0.29 in tall fescue populations and from 0.00 to 0.04 in St. Augustinegrass populations. Selection due to host species was the primary factor determining population structure according to analysis of molecular variance; host cultivar and geographical region had no significant effect. The host range of *M. grisea* lineages from turfgrasses was determined in growth chamber experiments and supports the prominent role of host species in determining the genetic structure of *M. grisea* populations from turfgrasses in Georgia.

Additional keywords: gray leaf spot, *Pyricularia grisea*.

Gray leaf spot, caused by *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa (anamorph *Pyricularia grisea* (Cooke) Sacc.), is an emerging disease problem in the turfgrass industry. Before 1971, gray leaf spot was confined to St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), a warm-season grass grown in subtropical and tropical climates (20). In 1971, a severe epidemic occurred on annual ryegrass (*Lolium multiflorum* Lam.) pastures in Mississippi and Louisiana (1,3). *M. grisea* was first reported as a pathogen of perennial ryegrass (*L. perenne* L.) in 1991, when a localized epidemic occurred on golf course fairways in Pennsylvania (16). Since then, gray leaf spot of perennial ryegrass has been observed throughout the United States wherever this species is cultivated (4,11,23,27,30). Gray leaf spot of tall fescue (*Festuca arundinacea*) was first reported in the United States in 1996, when a severe epidemic occurred in North Carolina (9). Turfgrass breeders have recently identified sources of resistance to *M. grisea* in selections of perennial ryegrass (12) and tall fescue (9) and are attempting to transfer this resistance to commercial cultivars.

M. grisea also causes blast disease of rice (*Oryza sativa* L.), which is one of the most important plant diseases worldwide (21). Management of rice blast with pathogen-resistant cultivars has not been a successful long-term strategy because resistant cultivars lose their effectiveness after 2 to 3 years of widespread use (21). Recently, studies on the population structure and genetics of *M. grisea* have led to the development of novel methods for developing rice cultivars with durable blast resistance (33). A similar understanding of the genetic structure of *M. grisea* populations

associated with turfgrasses may enable turfgrass breeders to develop varieties with durable resistance to this pathogen.

Little is known about the origin and structure of *M. grisea* populations associated with turfgrass hosts. The strains of *M. grisea* infecting annual ryegrass, tall fescue, and perennial ryegrass may have been introduced to the United States from abroad or generated by mutation of endemic strains. Viji et al. (29) and Farman (7) reported that *M. grisea* isolates from perennial ryegrass are genetically similar to, but distinct from, isolates from wheat collected in Brazil according to rDNA sequences and transposon-detected restriction fragment length polymorphisms (RFLPs).

Evidence collected to date indicates that *M. grisea* populations associated with turfgrasses exhibit a clonal structure and that sexual recombination is rare. Viji et al. (29) identified three clonal lineages among 194 *M. grisea* isolates from perennial ryegrass in 33 locations in the United States. Mating studies in vitro demonstrated that 56% of isolates from perennial ryegrass were male fertile, but no female fertile isolates were identified (28). In a tall fescue population from Georgia, 54% of *M. grisea* isolates from tall fescue were male fertile, but no isolates were female fertile (25). A single isolate from St. Augustinegrass was male fertile, whereas all others were completely sterile (25).

RFLP using cloned transposable elements as probes is the most common method for population analysis in *M. grisea* (5). These transposable elements are present in low copy numbers in the genome of *M. grisea* isolates from turfgrasses (5,7,29) and are consequentially of limited value for analysis of turfgrass populations. Amplified fragment length polymorphisms (AFLP) may be useful for analysis of population structure and genetics in plant-pathogenic fungi (2,19,31). With the AFLP technique, a potentially unlimited number of molecular markers can be generated using multiple polymerase chain reaction (PCR) primer combinations or sets of restriction enzymes (31). AFLPs meet the criteria for selection of molecular markers for population genetics (2),

Corresponding author: L. P. Tredway; E-mail address: lane_tredway@ncsu.edu

DOI: 10.1094/PHYTO-95-0463

© 2005 The American Phytopathological Society

and may represent a viable alternative to transposon-detected RFLPs for analysis of *M. grisea* populations.

In Georgia, St. Augustinegrass and tall fescue are the primary turfgrass hosts of *M. grisea*. St. Augustinegrass is well-adapted to the coastal plain region of the state, whereas tall fescue is most well-adapted to the piedmont, and ridge and valley geological zones. A wide “transition zone” surrounds the border between the coastal plain and piedmont, where both tall fescue and St. Augustinegrass are grown but each is marginally adapted. The objectives of this study were to describe the genetic structure of *M. grisea* populations associated with turfgrass hosts in Georgia, determine which evolutionary forces are important in shaping the observed population structure, estimate the genetic relationships among *M. grisea* isolates from diverse hosts using AFLP markers, and determine the host range of *M. grisea* isolates from turfgrasses and other hosts.

MATERIALS AND METHODS

Sampling of *M. grisea* populations. Isolates of *M. grisea* were collected from St. Augustinegrass and tall fescue turf stands in Georgia in 1999 and 2000 (Fig. 1). Sampling locations were selected to allow the separation of factors that may influence

Fig. 1. Location of *Magnaporthe grisea* populations sampled in 1999 and 2000 for analysis of population structure. Table 1 provides specific information on each population.

population structure, such as geographic region (South Georgia, Middle Georgia, and North Georgia), host species (tall fescue and St. Augustinegrass), and host cultivar (‘Mercedes’, ‘Palmetto’, ‘Rebel III’, ‘Confederate’, ‘Bonzai’, and ‘Raleigh’) (Table 1). Populations 1 through 6 were from sod production fields, and populations 7 and 8 were from a research facility with stands of tall fescue and St. Augustinegrass separated by approximately 100 m. At each location, symptomatic leaf blades were collected on 3-m centers in a 10 × 10 grid to yield 100 collection points from a 729-m² area. The leaf samples were allowed to dry overnight in paper bags at room temperature (23 to 25°C) and then stored at 4°C for further use. Monoconidial isolates were obtained and prepared for long-term storage as described by Tredway et al. (25).

Isolates of *M. grisea* from several other hosts and geographic regions (Table 2) were obtained for phylogenetic comparisons to turfgrass isolates. Isolates were provided by the French Agricultural Research Center for International Development (CIRAD, Paris, France), J. C. Correll (University of Arkansas), M. L. Farman (University of Kentucky), and B. B. Clarke (Rutgers University, NJ). Crabgrass (*Digitaria sanguinalis*), pearl millet (*Pennisetum glaucum*), and weeping lovegrass (*Eragrostis curvula*) leaves exhibiting gray leaf spot symptoms were collected in Georgia, and monoconidial isolates were obtained as described by Tredway et al. (25).

The mating type of each isolate in this study was previously determined using a PCR-based assay as reported by Tredway et al. (25). Mating-type data is included in the current manuscript for comparison to phylogenetic relationships revealed by AFLP markers.

AFLP analysis. Each of the 948 isolates of *M. grisea* were grown for 7 days at room temperature (23 to 25°C) in 2 ml of potato dextrose broth in glass culture tubes (16 by 100 mm). The mycelial suspension was transferred to a 1.5-ml microcentrifuge tube, harvested by centrifugation for 5 min at 13,200 rpm, and ground with a plastic pestle, and genomic DNA was extracted with the Easy-DNA Kit (Invitrogen Corp., Carlsbad, CA). The DNA samples were treated with RNase (40 µg/µl) and incubated at 37°C for 30 min. Samples were analyzed for purity by spectrophotometry and standardized to a concentration of 25 ng ml⁻¹. Samples with an A₂₆₀:A₂₈₀ ratio of less than 1.8 were rejected to ensure maximum restriction enzyme activity. For rejected samples, the entire DNA extraction procedure was repeated until a genomic DNA sample of acceptable quality was obtained for that isolate.

AFLP reactions were conducted with AFLP Analysis System II (Life Technologies, Inc., Gaithersburg, MD). Template DNA (125 ng) was digested (1.25 units of *EcoRI*, 1.25 units of *MseI*, and 2.5 µl of 5× reaction buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, and 250 mM K-acetate]) at 37°C for 2 h followed by heat deactivation of the enzymes at 70°C for 10 min. Double-stranded *EcoRI* and *MseI* adapters were ligated with 0.5 units of T4 DNA ligase and 12 µl of adapter/ligation solution (*EcoRI*/*MseI* adapters, 0.4 mM ATP, 10 mM Tris-HCl [pH 7.5],

TABLE 1. *Magnaporthe grisea* populations from Georgia sampled in 1999 and 2000 for analysis of population structure

Population	County	Host species	Cultivar	1999		2000	
				Date	N ^a	Date(s)	N
1	Lanier	St. Augustinegrass	Mercedes	7/13/1999	65	7/18/2000	78
2	Webster	St. Augustinegrass	Palmetto	7/14/1999	55	7/17/2000	79
3	Taylor	St. Augustinegrass	Palmetto	7/29/1999	43	7/31/2000	64
4	Laurens	Tall fescue	Rebel III	8/16/1999	44	8/14/00, 8/24/00	85
5	Cherokee	Tall fescue	Rebel III	9/1/1999	61	9/14/2000	87
6	Floyd	Tall fescue	Confederate	9/1/1999	66	9/14/2000	70
7	Paulding	Tall fescue	Bonzai	8/13/1999	10	9/7/2000	68
8	Paulding	St. Augustinegrass	Raleigh	8/13/1999	16	8/9/2000	85

^a Number of isolates.

10 mM Mg-acetate, and 50 mM K-acetate) at 20°C for 2 h and then diluted 10×. Pre-amplification reactions were performed with primers complementary to the adapter sequences with no selective nucleotides. Diluted ligation reaction (2 µl) was added to 2 µl of 10× PCR buffer (100 mM Tris-HCl [pH 8.3] and 500 mM KCl), 1.5 mM MgCl₂, 1 unit of *Taq* DNA polymerase, 50 ng of *Eco*RI primer, 50 ng of *Mse*I primer, 0.2 mM each dNTPs, and water to a final volume of 20 µl. Thermal cycling conditions included an initial denaturation step at 94°C for 1 min followed by 30 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 1 min. The pre-amplification products were diluted 20× for use in selective amplification reactions.

Selective amplification reactions were performed with *Eco*RI and *Mse*I primers that included two selective nucleotides (Applied Biosystems Inc., Foster City, CA). A single *Mse*I primer (M-CA) was used in combination with three *Eco*RI primers (E-AA, E-TA, and E-AC) to yield three primer pair combinations. Each *Eco*RI primer included a 5' fluorescent label (FAM, HEX, or NED) for detection of PCR products by automated sequencing equipment. Selective PCRs (4-µl volume) contained 1 µl of diluted pre-amplification product, 1.5 ng of *Eco*RI primer, 9 ng of *Mse*I primer, 0.15 unit of *Taq* DNA polymerase, 0.4 µl of 10× PCR buffer (100 mM Tris-HCl [pH 8.3] and 500 mM KCl), 1.5 mM MgCl₂, and 0.2 mM each dNTPs. Thermal cycling conditions included an initial cycle at 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min, followed by a touchdown phase where the annealing temperature was lowered 0.7°C each cycle for 12 cycles, and finally 23 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min.

For each *M. grisea* isolate, 1.5 µl of selective amplification product from each of the three primer combinations was combined with 0.85 µl of formamide, 0.30 µl of GS-500 ROX internal size standard (Applied Biosystems), and 0.35 µl of loading dye (Applied Biosystems). The mixture was denatured at 95°C for 5 min, and then separated in a 5% polyacrylamide gel on a Perkin-Elmer ABI Prism 377 DNA sequencer (Applied Biosystems). The molecular weight of amplified restriction fragments was determined in Genescan version 3.1 (Applied Biosystems) by

comparison to the GS-500 ROX internal size standard. Restriction fragments were scored in Genotyper version 2.5 (Applied Biosystems) and recorded as present or absent for each taxa. Each marker was labeled using its molecular weight in base pairs and the *Eco*RI primer that amplified the marker. For example, the designation 281AA denotes a 281-bp marker amplified by primer E-AA. Pre-amplification and selective amplification was performed twice for each isolate from the same restriction/ligation reaction. Restriction fragments that were not amplified in both reactions were excluded from further analysis.

Phylogenetic relationships among *M. grisea* isolates from diverse hosts. The Windist program (32) was used to calculate Dice similarity coefficients (D_s) among all pairs of isolates using the formula $D_s = 2N_{xy}/(N_x + N_y)$, where N_x is the number of markers amplified for isolate X , N_y is the number of markers amplified for isolate Y , and N_{xy} is the number of markers shared by isolates X and Y . Phylogenetic trees were constructed from D_s using the unweighted pair-group method, arithmetic mean (UPGMA) algorithm in MEGA (14). Bootstrap values (8) were determined in Winboot (32) based on 1,000 random samplings of the data set.

Genetic structure of *M. grisea* populations from turfgrass hosts in Georgia. The haplotype diversity index (HDI) in each *M. grisea* population from tall fescue and St. Augustinegrass was determined using the Shannon information statistic, calculated using the formula $h_0 = -\sum p_i \ln p_i$, where p_i is equal to the frequency of the i th multilocus genotype (10). HDI was normalized based on sample size using the formula $H_0 = h_0 \ln k^{-1}$, where k is the number of loci or isolates (10).

Analysis of molecular variance (AMOVA) procedure (6) was used to compare groups of *M. grisea* populations differing in host species, host cultivar, or geographic region and test the significance of these characteristics as selective forces. AMOVA calculations were conducted in Arlequin version 2.0 (22). From user-defined groups of populations, the AMOVA procedure partitions variance into the following covariance components: among groups (V_a), among populations within groups (V_b), and within populations (V_c). The proportion of variation explained by each covariance component was determined by dividing by the total covari-

TABLE 2. Origin and mating type of *Magnaporthe grisea* isolates belonging to lineages identified with amplified fragment length polymorphism (AFLP) markers

AFLP lineage	N ^a	Host	Mating type	Origin
A	9	Rice (<i>Oryza sativa</i>)	Mat1-1, Mat1-2	Arkansas, Cote d'Ivoire, France, French Guiana, India
B	1	Giant foxtail (<i>Setaria faberii</i>)	Mat1-1	Kentucky
B	1	Foxtail millet (<i>Setaria italica</i>)	Mat1-1	Kentucky
B	8	Yellow foxtail (<i>Setaria viridis</i>)	Mat1-1, Mat1-2	Kentucky
C	410	St. Augustinegrass (<i>Stenotaphrum secundatum</i>)	Mat1-1	Georgia, Florida
C	1	Large crabgrass (<i>Digitaria sanguinalis</i>)	Mat1-1	Georgia
D	5	Finger millet (<i>Eleusine coracana</i>)	Mat1-1, Mat1-2	Cote d'Ivoire, Japan, Philippines, Rwanda
E	6	Weeping lovegrass (<i>Eragrostis curvula</i>)	Mat1-2	Georgia
E	16	Tall fescue (<i>Festuca arundinacea</i>)	Mat1-2	Georgia
F	2	Wheat (<i>Triticum aestivum</i>)	Mat1-1	Brazil
G1	10	Tall fescue (<i>Festuca arundinacea</i>)	Mat1-1	Georgia
G2	14	Tall fescue (<i>Festuca arundinacea</i>)	Mat1-2	Georgia
G3	1	Perennial ryegrass (<i>Lolium perenne</i>)	Mat1-2	New Jersey
G4	387	Tall fescue (<i>Festuca arundinacea</i>)	Mat1-2	Georgia
G4	10	Perennial ryegrass (<i>Lolium perenne</i>)	Mat1-2	Kentucky, Maryland, New Jersey, Pennsylvania, Virginia
H	2	Tall fescue (<i>Festuca arundinacea</i>)	Mat1-2	Georgia
I	1	Ginger (<i>Zingiber</i> sp.)	Mat1-1	Reunion Island
J	2	Pearl millet (<i>Pennisetum glaucum</i>)	Nd ^b	Georgia
K	58	Large crabgrass (<i>Digitaria sanguinalis</i>)	Mat1-1, Mat1-2	Georgia, Kentucky
K	1	St. Augustinegrass (<i>Stenotaphrum secundatum</i>)	Mat1-2	Georgia
K	1	<i>Pennisetum</i> sp.	Mat1-1	Philippines
L	1	Sedge (<i>Cyperus</i> sp.)	Nd	Philippines
M	1	Sedge (<i>Cyperus</i> sp.)	Nd	Philippines

^a Number of isolates.

^b Mating type not determined.

ance (V_T). Nonparametric permutation of the data set was used to determine probability values and test the significance of each covariance component (6).

Host range of selected *M. grisea* isolates. The host range of *M. grisea* isolates representing each lineage from tall fescue, St. Augustinegrass, wheat, and weeping lovegrass was determined by artificial inoculation of six turfgrass species: tall fescue (cv. Rebel III), perennial ryegrass (cv. Manhattan III), annual ryegrass (cv. Gulf), Kentucky bluegrass (*Poa pratensis* cv. Merit), crabgrass (*D. sanguinalis*, Valley Seed Service, Fresno, CA), and St. Augustinegrass (cv. Palmetto); and two non-turfgrass species: wheat (*Triticum aestivum* cv. Roberts) and weeping lovegrass (cv. Ermelo). Each grass species was seeded at standard field rates in 946.4-cm³ styrofoam cups (Dart Container Corp., Mason, MI) containing calcined clay (Turface Allsport, Profile Products LLC, Buffalo Grove, IL), except St. Augustinegrass, which was propagated vegetatively. Seeded pots were covered with paper and misted four times per day to ensure rapid germination. After establishment, the grasses were irrigated to saturation twice per day and fertilized weekly with 1.3 g of N per m² with Peters 20-10-20 (Scotts-Sierra Horticultural Products Co., Marysville, OH). Turfgrass species were cut to a height of 7 cm weekly, with the exception of wheat and weeping lovegrass, which were not cut. Tall fescue, perennial ryegrass, annual ryegrass, Kentucky bluegrass, crabgrass, and St. Augustinegrass were inoculated with an isolate of *M. grisea* 10 to 12 weeks after seeding, whereas wheat and weeping lovegrass were inoculated 6 weeks after seeding.

Preparation of conidial suspensions, inoculation, and incubation were conducted as previously described (26). Selected *M. grisea* isolates from lineages C, E, F, G1, G2, G4, H, and K were transferred to 90-mm petri dishes containing 1.5% water agar and overlaid with 15 to 20 sterilized alfalfa stem sections (6 cm long). The cultures were placed at room temperature (23 to 25°C) with continuous fluorescent illumination. After 3 weeks, conidia were harvested from the alfalfa stem sections by vortexing for 10 s in 20 ml of H₂O and then filtering through a single layer of cheese-cloth. Conidial suspensions were adjusted to a concentration of 2×10^5 conidia ml⁻¹ with a hemacytometer.

A split-plot, randomized complete block experimental design was used in this experiment, with host species representing the subplots and *M. grisea* isolates applied to main plots. Prior to inoculation, one pot of each host species was randomly arranged in a plastic container (41.2 × 28.7 × 25.3 cm) that had a single 6.35-mm-diameter hole at a height of 2.54 cm to permit water drainage. The grass in each container was inoculated with 30 ml of conidial suspension from a single isolate applied with an air-brush (Model 350, Badger Air-Brush Co., Franklin Park, IL) powered with compressed CO₂ at 2.2 kg m⁻². Immediately after inoculation, the plastic containers were covered with the supplied lids and transferred to a growth chamber. Incubation conditions for the first 24 h were 24°C, 100% relative humidity (RH), and no light. Subsequently, the growth chamber was programmed for 12-h days at 30°C and 75% RH and 12-h nights (no light) at 24°C and 100% RH. The lids were removed from the plastic containers for each day cycle. At the beginning of each night cycle, the pots were misted with water and the lids were placed on the containers to maintain continuous and uniform humidity and leaf wetness. An auxillary humidifier (Herrmidifier 500, Trion Inc., Sanford, NC) was used to maintain 100% RH in the growth chamber during the initial incubation and during each night cycle.

Inoculated pots were observed for symptoms induced by *M. grisea* 7 days after inoculation, and the host reaction was rated as either pathogenic (+, presence of disease symptoms) or non-pathogenic (–, absence of disease symptoms). Each isolate × host combination was repeated in two independent experiments, with three replications per experiment. Noninoculated control plants of each species were included in each experiment.

RESULTS

Phylogenetic relationships among *M. grisea* isolates from diverse hosts. A total of 193 unique markers were amplified using three AFLP primer combinations on 948 *M. grisea* isolates. The number of markers amplified in an individual isolate ranged from 31 to 53, with an average of 44.5 markers per individual. Using the 193 AFLP markers, a total of 71 unique AFLP haplotypes were identified in the sample population. A single representative of each haplotype was selected for calculation of genetic distance and phylogenetic analysis (Fig. 2). The UPGMA tree revealed the presence of 13 AFLP lineages, designated A to M, which were distinguished at the 95% similarity level. Significant bootstrap support values, ranging from 84 to 100, were observed for all lineages except I, L, and M. Similarities among groups of lineages were evident in the phylogram. Lineages A to H formed one such group, joined by a single node at the 83% similarity level. Within the lineage A to H group, two smaller groups of lineages were also observed. Lineages A to D are joined by a single node at the 91% similarity level, whereas lineages E to G are joined by a single node at the 89% similarity level.

Lineage G contained 422 out of 440 (96%) of the isolates from tall fescue and perennial ryegrass (Table 2). This lineage can be further divided into four sublineages, designated G1 to G4, at the 98% similarity level. The identity of these sublineages is supported by the consistent presence or absence of certain markers within each group. Compared with sublineages G2 to G4, sublineage G1 is characterized by absence of markers of 121AA and 202AA and presence of markers of 380AA, 456AA, and 205TA. Sublineage G2 is characterized by presence of markers 154AA, 274AA, and 274TA and absence of marker 272TA. Sublineage G3 is unique in the presence of markers 87AA, 308AA, 353AA, and 157AC. Mating-type data also support the identity of these lineages. All isolates in sublineage G1 are *Mat1-1*, whereas sublineages G2 to G4 contain only *Mat1-2* isolates (Table 2).

There is some evidence of additional structure within lineages D and K. However, there was a relatively small number of isolates representing these lineages (5 and 60, respectively). Lineage K was also a heterogeneous group, containing 29 unique haplotypes among 60 total isolates. Therefore, additional isolates must be analyzed to determine if there is additional phylogenetic structure within these groups.

Each AFLP lineage identified in the collection was associated with a single host or small number of hosts (Table 2). Eleven lineages contained isolates from a single host species, and lineage B contained isolates from three *Setaria* species. Four lineages contained isolates from multiple host genera: lineage C contained isolates from St. Augustinegrass and large crabgrass; lineage E contained isolates from weeping lovegrass and tall fescue; lineage G4 contained isolates from tall fescue and perennial ryegrass; and lineage K contained isolates from crabgrass, St. Augustinegrass, and *Pennisetum* sp.

Genetic structure of *M. grisea* populations from turfgrass hosts in Georgia. In 1999, four of the *M. grisea* populations from turfgrasses were composed of a single AFLP haplotype, and the other four populations were composed of two AFLP lineages and between two and four haplotypes (Table 3). The HDI for Georgia turfgrass populations of *M. grisea* ranged from 0.00 to 0.18. For St. Augustinegrass, genetic variation was only detected in the Taylor population in 1999. For tall fescue, the highest HDI in 1999 was observed in the Cherokee population.

Results similar to those from 1999 were obtained in 2000, but some differences were noted (Table 3). The Lanier, Webster, Laurens, Floyd, and Paulding St. Augustinegrass populations were comprised of the same number of lineages in 1999 and 2000. One additional lineage was found in the Paulding tall fescue population, three additional lineages were found in the Cherokee population, and only one lineage was found in the Taylor population in

2000. The Cherokee population in 2000 was composed of five AFLP lineages and 10 distinct haplotypes, resulting in the highest HDI (0.29) observed in this study.

All populations of *M. grisea* sampled from St. Augustinegrass and tall fescue were dominated by single AFLP lineages (Table

4). All isolates from St. Augustinegrass belonged to lineage C, with the exception of a single isolate (455-2) from the Taylor population in 1999, which was placed in lineage K. Isolate 455-2 was identical to a haplotype commonly isolated from large crabgrass in Georgia. No lineage K isolates were obtained from St.

Fig. 2. Unweighted pair-group method, arithmetic mean phylogram produced from amplified fragment length polymorphism (AFLP) fingerprint patterns observed in *Magnaporthe grisea* isolates. Scale bar indicates the horizontal distance corresponding to genetic similarity as measured by the Dice similarity coefficient. Bootstrap values greater than 70 are indicated adjacent to the nodes and are based on 1,000 replications. Clonal lineages with significant bootstrap support are indicated.

Augustinegrass in 2000. However, one isolate (1203-2) from large crabgrass at the Webster location in 2000 belonged to lineage C and was identical to the common haplotype from St. Augustinegrass.

Each tall fescue population sampled was dominated by lineage G4, but most tall fescue populations also contained other lineages (Table 4). Lineage G1 was found in the Laurens and Floyd populations in 1999, but only in the Cherokee population in 2000. Lineage G2 was present in all populations from tall fescue in 2000, but not in 1999. Lineages E and H were found in the Cherokee population, but not in the other *M. grisea* populations from tall fescue.

When groups of populations differing in host species were compared, differences among groups accounted for 95.24 and 92.97% of the genetic variation detected by AFLP markers in

1999 and 2000, respectively (Table 5). Host species was a significant selective force in 1999 and 2000 ($P < 0.0001$), but significant genetic variation was also detected within host species and within populations in 1999 and 2000 ($P < 0.0001$), indicating that other factors also influence population structure. When groups of populations differing in geographic region or cultivar were compared, differences within populations accounted for 89.55 to 100% of the genetic variation. Groups of populations differing in geographic region or host cultivar were not significantly different, indicating that selection due to geographic region or host cultivar are not significant forces in determining the genetic structure of these populations.

Host range of selected *M. grisea* isolates. Isolates within AFLP lineages exhibited similar host ranges (Table 6). All *M. grisea* isolates were pathogenic to their host of origin, with the exception

TABLE 3. Measures of diversity in populations of *Magnaporthe grisea* associated with tall fescue and St. Augustinegrass in Georgia

Population	County	Host	Cultivar	1999				2000			
				<i>N</i> ^a	<i>L</i> ^b	<i>H</i> ^c	HDI ^d	<i>N</i>	<i>L</i>	<i>H</i>	HDI
1	Lanier	SA ^e	Mercedes	56	1	1	0.00	36	1	2	0.04
2	Webster	SA	Palmetto	50	1	1	0.00	72	1	1	0.00
3	Taylor	SA	Palmetto	37	2	2	0.03	54	1	2	0.04
4	Laurens	TF ^f	Rebel III	34	2	3	0.08	80	2	3	0.06
5	Cherokee	TF	Rebel III	45	2	4	0.18	76	5	10	0.29
6	Floyd	TF	Confederate	55	2	4	0.11	61	2	2	0.04
7	Paulding	TF	Bonzai	11	1	1	0.00	66	2	2	0.04
8	Paulding	SA	Raleigh	15	1	1	0.00	82	1	1	0.00

^a Number of isolates.

^b Number of lineages identified by amplified fragment length polymorphism (AFLP) markers.

^c Number of haplotypes identified by AFLP markers.

^d Haplotype diversity index determined by Shannon's information statistic formula.

^e St. Augustinegrass (*Stenotaphrum secundatum*).

^f Tall fescue (*Festuca arundinacea*).

TABLE 4. Frequency of amplified fragment length polymorphism lineages in populations of *Magnaporthe grisea* associated with tall fescue and St. Augustinegrass in Georgia

Population	County	Host	Cultivar	1999							2000						
				C	E	G1	G2	G4	H	K	C	E	G1	G2	G4	H	K
1	Lanier	SA ^a	Mercedes	1.00	...	...	...	...	...	...	1.00	...	...	...	...	...	...
2	Webster	SA	Palmetto	1.00	...	...	...	...	...	...	1.00	...	...	...	...	...	...
3	Taylor	SA	Palmetto	0.97	...	...	...	...	...	0.03	1.00	...	...	...	...	...	...
4	Laurens	TF ^b	Rebel III	...	...	0.06	...	0.94	...	...	...	...	...	0.05	0.95	...	...
5	Cherokee	TF	Rebel III	...	0.04	...	...	0.96	...	...	...	0.18	0.07	0.07	0.65	0.03	...
6	Floyd	TF	Confederate	...	...	0.04	...	0.96	...	...	...	...	...	0.03	0.97	...	...
7	Paulding	TF	Bonzai	...	...	...	...	1.00	...	...	...	...	...	0.05	0.95	...	...
8	Paulding	SA	Raleigh	1.00	...	...	...	...	...	...	1.00	...	...	...	...	...	...

^a St. Augustinegrass (*Stenotaphrum secundatum*).

^b Tall fescue (*Festuca arundinacea*).

TABLE 5. Summary statistics from analysis of molecular variance (AMOVA) used to test the significance of potential selection factors in Georgia turfgrass populations of *Magnaporthe grisea*

Selection factor	Groups ^a	1999						2000					
		Among groups		Within groups		Within populations		Among groups		Within groups		Within populations	
		<i>V_a/V_T</i> (%) ^b	<i>P</i> value ^c	<i>V_b/V_T</i> (%)	<i>P</i> value	<i>V_c/V_T</i> (%)	<i>P</i> value	<i>V_a/V_T</i> (%)	<i>P</i> value	<i>V_b/V_T</i> (%)	<i>P</i> value	<i>V_c/V_T</i> (%)	<i>P</i> value
Host	(1,2,3,8) vs. (7,4,5,6)	95.24	<0.0001	0.07	<0.0001	4.69	<0.0001	92.97	<0.0001	0.94	<0.0001	6.09	<0.0001
Region-SA ^d	(1,2) vs. (3) vs. (8)	3.09	0.3275	-1.87	1.0000	98.78	0.0899	-0.81	0.1603	2.65	<0.0001	98.15	0.0215
Region-TF ^e	(4) vs. (5,6,7)	0.54	0.2405	2.34	0.1105	97.12	0.0264	-9.66	0.2649	19.31	<0.0001	90.36	<0.0001
Cultivar-SA	(1) vs. (2,3) vs. (8)	-4.05	0.6716	3.45	<0.0001	100.6	0.0919	-0.79	0.0782	2.59	<0.0001	98.2	0.0147
Cultivar-TF	(4,5) vs. (6) vs. (7)	-7.72	0.8407	8.79	0.0059	98.92	0.0332	-17.13	0.8260	27.59	<0.0001	89.55	<0.0001

^a Groups of populations compared with AMOVA to test significance of selection factors. Population numbers are from Table 3.1.

^b Percentage of genetic variation (*V_T*) partitioned into components (among groups [*V_a*], within groups [*V_b*], and within populations [*V_c*]) by AMOVA.

^c *P* values for covariance components obtained by comparison to random data sets created by nonparametric permutations.

^d St. Augustinegrass.

^e Tall fescue.

of isolates 1203-2 (lineage C) and 455-2 (lineage K). All isolates from lineages E, F, G1, G2, G4, and H were pathogenic to annual ryegrass, perennial ryegrass, Kentucky bluegrass, and tall fescue. Isolates from lineages E, F, G1, G2, and H were also pathogenic to wheat and weeping lovegrass, inducing symptoms similar to those observed in the field (Fig. 3A). Isolates from lineage G4 were pathogenic to weeping lovegrass, but isolates within this lineage varied in their pathogenicity to wheat. Isolates 1207-59, 1212-71, and 1213-77 (lineage G4) were weakly virulent to wheat, inducing lesions that were limited in their expansion (Fig. 3B). Isolates 783 and 1210-57 were nonpathogenic and induced a hypersensitive response (HR) (Fig. 3C). All isolates from lineages E, F, G1, G2, G4, and H were nonpathogenic to crabgrass, but several were pathogenic to St. Augustinegrass (Table 6; Fig. 4). All isolates from lineage C were pathogenic to St. Augustinegrass and annual ryegrass. Isolates from lineage K were pathogenic to crabgrass and annual ryegrass, and one isolate (1100-4) was pathogenic to weeping lovegrass.

DISCUSSION

Molecular diversity was low in populations of *M. grisea* from turfgrasses in Georgia compared with previous reports of molecular diversity in populations associated with rice. The HDI in turfgrass populations ranged from 0.00 to 0.29 in the current study compared with 0.89 to 0.92 observed in populations from rice in the Indian Himalayas (13). Haplotype diversity was generally lower in *M. grisea* populations from St. Augustinegrass than in populations from tall fescue. This result was unexpected, because *M. grisea* has been associated with St. Augustinegrass in the United States since the 1950s but has only been associated with tall fescue since 1996. Differences in host diversity may be responsible for the increased pathogen diversity in populations from tall fescue. Tall fescue is an outbreeding species, and culti-

vars of this grass are composed of a mixture of genotypes. On the other hand, St. Augustinegrass is propagated vegetatively and cultivars are composed of a single clone. The increased genetic diversity in tall fescue cultivars may favor increased genetic diversity in the pathogen population.

Fig. 3. Symptoms induced on wheat by *Magnaporthe grisea* isolates: **A**, typical wheat blast symptoms induced by isolate BR32 (lineage F); **B**, weakly virulent response induced by isolate 1212-71 (lineage G4); and **C**, hypersensitive response induced by isolate 1210-57 (lineage G4).

TABLE 6. Partial host range of *Magnaporthe grisea* isolates belonging to clonal lineages associated with large crabgrass, St. Augustinegrass, weeping lovegrass, tall fescue, and wheat

Lineage	Host origin	Isolate	Inoculated host ^a							
			Annual ryegrass	Perennial ryegrass	Kentucky bluegrass	Tall fescue	Wheat	Weeping lovegrass	Large crabgrass	St. Augustinegrass
C	Large crabgrass	1203-2	--/+--	---/---	---/---	---/---	---/---	--+/---	---/---	+++/-++
C	St. Augustinegrass	1173	+++/-++	---/---	---/---	---/---	---/---	---/---	---/---	+++/-++
C	St. Augustinegrass	1345	++/-++	---/---	---/---	---/---	---/---	---/---	---/---	+++/-++
C	St. Augustinegrass	1204-50	++/-++	---/---	---/---	---/---	---/---	---/---	---/---	+++/-++
E	Weeping lovegrass	1208-2	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	---/---
E	Weeping lovegrass	1208-4	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	---/---
E	Tall fescue	1213-50	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	---/---
E	Tall fescue	1213-58	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	---/---
F	Wheat	BR32	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	---/---
G1	Tall fescue	1213-22	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	---/---
G1	Tall fescue	1213-71	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G1	Tall fescue	1213-8	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G1	Tall fescue	533-78	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G2	Tall fescue	1207-50	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G2	Tall fescue	1210-17	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G2	Tall fescue	1212-62	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G2	Tall fescue	1213-26	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G4	Tall fescue	783	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G4	Tall fescue	1207-59	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G4	Tall fescue	1210-57	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G4	Tall fescue	1212-71	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
G4	Tall fescue	1213-77	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
H	Tall fescue	1213-37	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
H	Tall fescue	1213-74	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	---/---	+++/-++
K	Large crabgrass	1100-20	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++
K	Large crabgrass	1100-4	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++
K	Large crabgrass	1206-6	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++
K	St. Augustinegrass	455-2	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++	+++/-++

^a Reaction observed for each host × isolate combination. + indicates a pathogenic response, whereas - indicates a nonpathogenic response. Each data point represents a single replication. Sets of data points separated by / represent independent experiments.

Phylogenetic analysis using AFLP markers in this study produced groupings of *M. grisea* isolates that are similar to those previously identified with internal transcribed spacer (ITS) sequences (15) and transposon-detected RFLPs (5,15). Lineages A to H in this study correspond to groups r1 to r7 described by Kusaba et al. (15) and Eto et al. (5). The phylogeny created from AFLP markers, however, provided greater resolution within the species than ITS sequences or RFLPs. Thirteen distinct lineages were identified by AFLP markers, nine of which had significant bootstrap support. Within lineage G, four sublineages were identified based on the consistent presence or absence of certain markers.

M. grisea isolates from tall fescue in Georgia are closely related to those from weeping lovegrass, wheat, and perennial ryegrass according to the AFLP phylogeny. Lineage G4 contained 387 of 429 (90%) tall fescue isolates and 10 of 11 (91%) perennial ryegrass isolates. All isolates from weeping lovegrass were placed within lineage E, along with 16 isolates from tall fescue. Isolates from wheat were distinct from all others and belonged to lineage F. Based on ITS sequences and RFLPs, Viji et al. (29) demonstrated that isolates from perennial ryegrass were highly similar to those from wheat. AFLP markers in this study support this close relationship but differentiate isolates from perennial ryegrass and wheat into distinct lineages. Our study included perennial ryegrass isolates that were analyzed by Viji et al. (29), but the same wheat isolates were not analyzed.

Of 411 isolates from St. Augustinegrass analyzed in this study, 410 (99.8%) were placed in lineage C and one (0.2%) was placed in lineage K. Conversely, 58 (98%) isolates from large crabgrass (*D. sanguinalis*) belonged to lineage K, whereas 1 of 59 (2%) was placed in lineage C. These results indicate that cross-infection may occur at low frequencies among St. Augustinegrass and large crabgrass, which is a common weed in turfgrass stands. Such cross-infection could create the potential for sexual recombination

in populations associated with St. Augustinegrass, since lineage C contains *Mat1*-1 only, whereas lineage K contains both mating types. We were unable to reproduce this cross-infection in growth chamber experiments. The observed cross-infections in field populations may represent opportunistic infection of wounds or damaged tissue rather than true pathogenic relationships.

Host species is the dominant force influencing the genetic structure of *M. grisea* populations in turfgrasses based on comparison of populations using AMOVA. All populations of *M. grisea* sampled from tall fescue and St. Augustinegrass were dominated by a single AFLP lineage, but the dominant lineage differed among hosts. There were no lineages in common to populations from St. Augustinegrass and tall fescue; therefore, cross-infection among these hosts does not occur at detectable levels in nature. Although host species was the dominant selection force, AMOVA detected additional genetic variation within and between populations that could not be explained by differences in host species. Host cultivar and geographical region did not account for this genetic variation according to AMOVA. Other selection forces, gene flow, genetic drift, mutation, genetic recombination, or founder effects may be responsible for the differences observed among populations and among growing seasons. Further research is required to investigate the potential role of these evolutionary forces.

The distribution of mating types among AFLP lineages provides insight to the occurrence of sexual recombination in turfgrass populations of *M. grisea*. Even though both mating types occur in tall fescue populations (25), all AFLP lineages from tall fescue were comprised of a single mating type. Lineage C, which comprised 99.8% of St. Augustinegrass populations, contained only *Mat1*-1. The single lineage K isolate from St. Augustinegrass was *Mat1*-2. The absence of lineages that contain both mating types provides additional support for the lack of sexual recombination in turfgrass populations of *M. grisea*.

This report represents the most comprehensive host range study of *M. grisea* isolates from turfgrasses conducted to date. Isolates belonging to lineages E, F, G1, G2, and H were pathogenic to annual ryegrass, perennial ryegrass, Kentucky bluegrass, tall fescue, wheat, and weeping lovegrass but were nonpathogenic to large crabgrass. Isolates from lineage G4 exhibited a similar host range, but were either weakly virulent or induced an HR on wheat cv. Roberts (Fig. 3B and C). The observation of HR on wheat indicates that lineage G4 may correspond to a distinct pathogenic race of *M. grisea*. Additional research is needed to determine the relationship between lineage and race in turfgrass populations because of its important implications to management of *M. grisea* with resistant cultivars.

The majority of *M. grisea* isolates in field populations from tall fescue and perennial ryegrass belonged to lineage G4. Therefore, it is likely that the *M. grisea* populations associated with these hosts originated from a common source. Wheat and weeping lovegrass represent potential sources of these populations based on the genetic similarities of the lineages and their cross-pathogenicity among hosts. Gray leaf spot, or blast, is not known to occur on wheat in the United States, but is a common disease of weeping lovegrass, which is used extensively as an ornamental grass surrounding golf courses and other turf areas. Weeping lovegrass was introduced to the United States from Africa in 1927 (18) and has been established on more than 0.4 million ha since 1985 as part of the Conservation Reserve Program administered by the U.S. Department of Agriculture (24). Although the first appearance of gray leaf spot in weeping lovegrass is not documented, the introduction of this grass and its increased use in recent years may be involved in the recent appearance of gray leaf spot in tall fescue and perennial ryegrass. More intensive sampling of *M. grisea* populations from weeping lovegrass and wheat is needed to determine if these grasses are hosts of lineages G1, G2, G3, G4, or H in nature.

Fig. 4. Symptoms observed on St. Augustinegrass 7 days after inoculation with *Magnaporthe grisea* A, isolate 1213-71 from lineage G1 or B, isolate 1204-50 from lineage C.

Several isolates in lineages E, F, G1, G2, G4, and H induced symptoms on St. Augustinegrass, but this response varied among replications and experiments. In addition, the virulence of these isolates toward St. Augustinegrass was low, inducing small lesions that did not expand beyond 1 mm in length (Fig. 4). St. Augustinegrass does not appear to be an effective host for these lineages, which is consistent with the absence of cross-infection among tall fescue and St. Augustinegrass in field populations. The pathogenicity of *M. grisea* isolates from lineages E, F, G1, G2, G4, and H toward Kentucky bluegrass was unexpected, as this grass species is not known to be a host in nature. However, the virulence of these isolates toward Kentucky bluegrass was also low. Seven days after inoculation, lesions observed on Kentucky bluegrass were 1 to 2 mm in length compared to 5 to 10 mm on ryegrasses or tall fescue (data not shown). In addition, secondary inoculum (conidia) was not produced in visible quantities on Kentucky bluegrass as it was on other hosts (data not shown).

Based on the results of this study, we conclude that selection due to host species is the dominant force determining the genetic structure of *M. grisea* populations associated with turfgrasses in Georgia. Low haplotype diversity and perfect mating type–lineage relationships in turfgrass populations indicate that sexual recombination does not occur with significant frequency. The distinct AFLP lineages identified in this study are therefore proposed to comprise clonal lineages of the pathogen. It is unknown if these clonal lineages correspond to distinct pathogenic races, as is common in rice populations (17). However, the induction of HR by isolates in lineage G4 on wheat cv. Roberts suggests that this may be the case. Further characterization of *M. grisea* populations associated with wheat and weeping lovegrass is needed, because these grasses may harbor additional clonal lineages that are pathogenic to tall fescue and perennial ryegrass. Identification of these lineages is critical to efforts to develop turfgrass cultivars with durable resistance to *M. grisea*.

ACKNOWLEDGMENTS

We thank B. B. Clarke (Rutgers University, New Brunswick, NJ), J. C. Correll (University of Arkansas, Fayetteville), M. L. Farman (University of Kentucky, Lexington), and D. Tharreau (CIRAD, Paris, France) for providing *M. grisea* isolates from their collections.

LITERATURE CITED

- Bain, D. C., Patel, B. N., and Patel, M. V. 1972. Blast of ryegrass in Mississippi. Plant Dis. Rep. 56:210.
- Brown, J. K. M. 1996. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol. 133:183-195.
- Carver, R. B., Rush, M. C., and Lindberg, G. D. 1972. An epiphytotic of ryegrass blast in Louisiana. Plant Dis. Rep. 56:157-159.
- Dernoeden, P. H. 1996. Perennial ryegrass and gray leaf spot. Golf Course Manage. 64:49-52.
- Eto, Y., Ikeda, K., and Mayama, S. 2001. Comparative analyses of the distribution of various transposable elements in *Pyricularia* and their activity during and after the sexual cycle. Mol. Gen. Genet. 264:565-577.
- Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. Genetics 131:479-491.
- Farman, M. L. 2002. *Pyricularia grisea* isolates causing gray leaf spot on perennial ryegrass (*Lolium perenne*) in the United States: Relationship to *P. grisea* isolates from other host plants. Phytopathology 92:245-254.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.
- Fraser, M. L. 1996. Susceptibility of tall fescues to gray leaf spot, 1995. Biol. Cult. Tests 12:130.
- Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1995. Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85:669-676.
- Harmon, P., Kane, R., Ruhl, G., and Latin, R. 2000. First report of gray leaf spot on perennial ryegrass in Indiana. Plant Dis. 84:492.
- Hofmann, N. E., and Hamblin, A. M. 2000. Reaction of perennial ryegrass to gray leaf spot following inoculation in the greenhouse, 1999. Biol. Cult. Tests 16:55.
- Kumar, J., Nelson, R. J., and Zeigler, R. S. 1999. Population structure and dynamics of *Magnaporthe grisea* in the Indian Himalayas. Genetics 152:971-984.
- Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: Molecular Evolutionary Genetics Analysis Version 1.01. The Pennsylvania State University, University Park.
- Kusaba, M., Eto, Y., Don, L. D., Nishimoto, N., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Genetic diversity in *Pyricularia* isolates from various hosts revealed by polymorphisms of nuclear ribosomal DNA and the distribution of the MAGGY retrotransposon. Ann. Phytopathol. Soc. Jpn. 65:588-596.
- Landschoot, P. J., and Hoyland, B. F. 1992. Gray leaf spot of perennial ryegrass turf in Pennsylvania. Plant Dis. 76:1280-1282.
- Levy, M., Correa-Victoria, F. J., Zeigler, R. S., Xu, S., and Hamer, J. E. 1993. Genetic diversity of the rice blast fungus in a disease nursery in Colombia. Phytopathology 83:1427-1433.
- Magness, J. R., Markle, G. M., and Compton, C. C. 1971. Food and feed crops of the United States. Interregional Research Project IR-4, Bull. 828. New Jersey Agric. Exp. Stn., New Brunswick, NJ.
- Majer, D., Mithen, R., Lewis, B. G., Vos, P., and Oliver, R. P. 1996. The use of AFLP fingerprinting for the detection of genetic variation in fungi. Mycol. Res. 100:1107-1111.
- Malca, I., and Owen, J. H. 1957. The gray leaf spot disease of St. Augustinegrass. Plant Dis. Rep. 41:871-875.
- Ou, S. H. 1980. A look at worldwide rice blast control. Plant Dis. 64:439-445.
- Schneider, S., Roessli, D., and Excoffier, L. 2000. Arlequin: A Software for Population Genetics Data Analysis, Version 2.0. University of Geneva, Geneva, Switzerland.
- Schumann, G. L., and Jackson, N. 1999. First report of gray leaf spot (*Pyricularia grisea*) on perennial ryegrass (*Lolium perenne*) in New England. Plant Dis. 83:1073.
- Torell, L. A., Kirksey, R. E., Donart, G. B., and Libbin, J. D. 2000. Economics of grazing weeping lovegrass with stockers in the Southern Great Plains. J. Range Manage. 53:268-276.
- Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2003. Mating type distribution and fertility status in *Magnaporthe grisea* populations associated with turfgrass hosts in Georgia. Plant Dis. 87:435-441.
- Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2003. Components of resistance to *Magnaporthe grisea* in 'Coyote' and 'Coronado' tall fescue. Plant Dis. 87:906-912.
- Uddin, W., Soika, M. D., Moorman, F. E., and Viji, G. 1999. A serious outbreak of blast disease (gray leaf spot) of perennial ryegrass in golf course fairways in Pennsylvania. Plant Dis. 83:783.
- Viji, G., and Uddin, W. 2001. Mating behavior of *Magnaporthe grisea* isolates causing gray leaf spot (blast) of perennial ryegrass turf. (Abstr.) Phytopathology 91(suppl.):S92.
- Viji, G., Wu, B., Kang, S., Uddin, W., and Huff, D. R. 2001. *Pyricularia grisea* causing gray leaf spot of perennial ryegrass turf: Population structure and host specificity. Plant Dis. 85:817-826.
- Vincelli, P. 1999. Gray leaf spot: An emerging disease of perennial ryegrass. Turfgrass Trends 7:1-8.
- Vos, P., Hogers, R., Bleeker, M., Reijmans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kulper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.
- Yap, I. V., and Nelson, R. J. 1996. Winboot: A program for performing bootstrap analysis of binary data to determine confidence limits of UPGMA-based dendrograms. Int. Rice Res. Inst. Dis. Paper Ser. 14. Manila, Philippines.
- Zeigler, R. S., Tohme, J., Nelson, R., Levy, M., and Correa-Victoria, F. J. 1994. Lineage exclusion: A proposal for linking blast population analysis to resistance breeding. Pages 267-292 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong, and P. S. Teng, eds. CAB International, Oxon, UK.