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Genetic Structure of *Magnaporthe grisea* Populations Associated with Turfgrass Hosts in Georgia.
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Magnaporthe grisea, causal agent of gray leaf spot, is a pathogen of increasing importance in the turfgrass industry, yet little is known about the genetic structure of populations from turfgrasses. Populations of *M. grisea* from St. Augustinegrass and tall fescue in Georgia were sampled in 1999 and 2000. Populations were selected to allow separation of factors that may influence population structure, such as host species, host cultivar, and geographic region. A PCR-based assay was developed for mating type determination in *M. grisea*. Populations from St. Augustinegrass and tall fescue were dominated by *Mat*1-1 and *Mat*1-2, respectively, but the opposite mating type was also obtained from each host. No female fertile isolates were identified in crosses with fertile tester strains in vitro. Although both mating types exist in populations of *M. grisea* from turfgrass, the potential for sexual reproduction appears to be low due to the absence of female fertile isolates. According to AFLP markers, each *M. grisea* population was dominated by a single lineage, with other lineages present in low frequencies. Host species significantly influenced population structure, but differences were observed among populations which could not be explained by host species, host cultivar, or geographic region. *M. grisea* populations from tall fescue and perennial ryegrass appear to have been established from a common source, but no alternative host for the dominant lineage from these hosts was identified. Weeping lovegrass was an alternative host for lineage E from tall fescue, and isolates from wheat were also closely related, but distinct. In growth chamber experiments, isolates from lineage G4 induced a hypersensitive response on wheat cultivar Roberts, but other *M. grisea* lineages from tall fescue induced typical blast symptoms. The clonal lineages associated with tall fescue in Georgia may correspond to distinct pathogenic races of *M. grisea*. Several

components were responsible for the *M. grisea* resistance of ‘Coyote’ and ‘Coronado’ tall fescue, and the resistance was equally effective against all lineages associated with tall fescue in Georgia. These cultivars represent sources of partial resistance to *M. grisea* and will be a valuable component of integrated programs for management of gray leaf spot.

INDEX WORDS: Gray leaf spot, *Pyricularia grisea*, Population genetics, Mating type, Amplified Fragment Length Polymorphisms

GENETIC STRUCTURE OF *MAGNAPORTHE GRISEA* POPULATIONS ASSOCIATED WITH TURFGRASS HOSTS IN GEORGIA

by

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INTRODUCTION AND LITERATURE REVIEW

Introduction

Magnaporthe grisea (Hebert) Yaegashi & Udagawa (anamorph *Pyricularia grisea* (Cooke) Sacc.) is a pathogen of grasses belonging to 22 genera, including several of global economic importance, such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and finger millet (*Eleusine coracana* (L.) Gaertn.) (26). The plant organs infected by *M. grisea* vary among host species, but include all aboveground tissues. Pathogen activity results in various symptoms, including leaf spots, foliar blight, or blast of the inflorescence. *Magnaporthe grisea* is believed to be the most important plant pathogen worldwide, primarily due to its role in causing blast disease of rice (91), the main food source for about half of the world's population (46).

Gray leaf spot, also caused by *M. grisea*, is an emerging disease problem in the turfgrass industry. Before 1971, gray leaf spot was limited to St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), a warm-season grass grown in subtropical and tropical climates (70). Since 1971, the host range of *M. grisea* has expanded to include three major cool-season grasses: annual ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*Lolium perenne* L.), and tall fescue (*Festuca arundinacea* Schreb.).

Several fungicides are available for gray leaf spot management, but must be applied on a preventative basis in order to provide adequate control (127). Many turfgrass managers are unable to justify the expense of preventative fungicide applications when occurrence of a severe epidemic is uncertain. For this reason, disease resistant cultivars would be a valuable tool for gray leaf spot management. Sources of resistance to *M. grisea* have been identified, and turfgrass breeders are working to move this resistance into commercial cultivars (7,27,40,42,43,113).

Long-term management of rice blast with disease resistant cultivars has not been successful because resistant cultivars typically lose their effectiveness, or “break down”, after 2 to 3 years of widespread use (92). Recent studies of *M. grisea* population structure have spurred the development of strategies for breeding rice cultivars with durable blast resistance (144).

The purpose of this research is to analyze the genetic structure of *M. grisea* populations associated with turfgrass hosts in Georgia. This information will be directly useful to turfgrass breeders in their efforts to develop turfgrass cultivars with durable resistance to *M. grisea*.

Gray Leaf Spot of Turfgrasses

Magnaporthe grisea was first reported as a turfgrass pathogen in 1954 by Malca and Owen (70), who demonstrated its pathogenicity to St. Augustinegrass, a warm-season grass used for landscape turf in subtropical and tropical climates. Malca and Owen coined the name “gray leaf spot” to describe the distinct foliar lesions, which turn gray with profuse sporulation by the pathogen (70). Gray leaf spot is now a chronic problem in St. Augustinegrass turf throughout the southeastern United States.

In 1971, *M. grisea* was first recognized as a pathogen of a cool-season turfgrass, when a severe epidemic occurred on annual ryegrass pastures in Mississippi and Louisiana (4,10). The name “ryegrass blast” was coined for this disease because of the role of *M. grisea* in causing blast of rice. Localized epidemics of ryegrass blast continue to occur annually in annual ryegrass pastures (114).

Magnaporthe grisea was first reported as pathogen of perennial ryegrass in 1991, when a localized epidemic occurred on golf course fairways in Pennsylvania (59). The authors suggested that “gray leaf spot” provided a more accurate description of the

symptoms induced by *M. grisea* in turfgrasses, since “blast” describes symptoms on the inflorescence (98), which is removed from turfgrasses by mowing or grazing. In 1995, a widespread gray leaf spot epidemic occurred on perennial ryegrass in the mid-Atlantic United States, causing severe damage to many golf courses and landscapes (22). Since then, gray leaf spot of perennial ryegrass has been observed throughout the United States wherever this species is cultivated (22,33,104,107,117,126).

The history of gray leaf spot of tall fescue is not well documented. The symptoms induced by *M. grisea* on tall fescue often appear very similar to those induced by *Rhizoctonia solani* Kühn, another important foliar pathogen. Confusion between these two diseases may have contributed to the poor documentation of gray leaf spot. Gray leaf spot was not mentioned in a review of tall fescue diseases in Georgia by Luttrell in 1951 (67). In a 1977 study of mating behavior in *M. grisea*, Yaegashi utilized a strain isolated in Japan from meadow fescue (*Festuca eliator* L.), which is closely related to tall fescue (135). Gray leaf spot of tall fescue was first reported in the United States in 1996, when a severe epidemic occurred in North Carolina (27).

Turfgrass species vary widely in their susceptibility to *M. grisea*. Although gray leaf spot is a chronic problem in St. Augustinegrass, severe damage is rarely incurred by this host. In contrast, the cool-season grasses are highly susceptible to *M. grisea* and sustain severe foliar blighting in many cases. Cultivars of St. Augustinegrass with improved resistance to *M. grisea* are currently available (43). Turfgrass breeders have recently identified sources of resistance to *M. grisea* in selections of annual ryegrass (113), perennial ryegrass (7,40,42), and tall fescue (27), and are working to transfer this resistance into commercial cultivars.

In Georgia, gray leaf spot is primarily a problem in St. Augustinegrass and tall fescue, which are important lawn and landscape grasses in the state. St. Augustinegrass is

well-adapted to the coastal plain region of the state, whereas tall fescue is best 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. Perennial ryegrass is used on a limited basis in Georgia as a winter over-seeding crop, but gray leaf spot of perennial ryegrass has not been reported as a significant problem in Georgia.

Biology of *Magnaporthe grisea*

Magnaporthe grisea is a filamentous fungus belonging to Order Diaporthales of Phylum Ascomycota (53). The sexual stage of *M. grisea* has only been observed in vitro. In nature, the fungus exists in its anamorphic state, *Pyricularia grisea*, which is classified in the Deuteromycota (Order Moniliales, Family Moniliaceae), an informal group created for classification of asexual fungi. The fungus reproduces through growth of somatic hyphae and production of pyriform, two-septate conidia in clusters of three to five on the end of long, unbranched conidiophores.

Magnaporthe grisea is known to infect hosts belonging to 22 genera, including many of economic importance, such as rice, wheat, and finger millet (26). The plant organs infected by *M. grisea* vary among host species, but include all aboveground tissues. Infection results in various symptoms, including leaf spots, foliar blight, or blast of the inflorescence. Generally, conidia are produced during warm (optimum 25ºC to 28ºC; minimum 9ºC; maximum 35ºC), humid (minimum 89%) conditions and dspersed to potential hosts by wind, water, or other means (108). Once deposited, the conidia attach to hydrophobic surfaces by an apical droplet of spore tip mucilage (44). If free moisture is available on the leaf surface, the conidia germinate, and an appressorium is produced on the end of the resulting germ tube (108). An infection peg arises from the

appressorium that penetrates the epidermis through a combination of enzymatic degradation and mechanical force (52). The infection process, from germination to invasion of epidermal cells, may occur within 6 hours at the optimal temperature of 24ºC (108), but most researchers report that a minimum of 12 hours continuous leaf wetness following inoculation is required for optimal disease development (2,70,84,116). Incubation period, the amount of time between inoculation and symptom development, ranges from several hours to several days depending on the host species and pathogen isolate (105,108). After a latent period of five to seven days, conidia are produced on the surface of infected tissue to complete the disease cycle (105,108). During extended periods of warm, humid weather, epidemics of diseases caused by *M. grisea* progress rapidly through repeated cycles of infection and conidiation (108).

M. grisea is a heterothallic ascomycete, requiring the interaction of two mating types, *Mat*1-1 and *Mat*1-2, in order to reproduce sexually (141). Sexual reproduction occurs through the fusion of haploid nuclei from strains of opposite mating type to form a diploid zygote. The zygote then undergoes meiosis to produce haploid recombinant progeny, which are released from a flask-shaped perithecium as asci containing 8 ascospores.

The genetics of the mating system have been well characterized in the ascomycetes *Neurospora crassa* (80), *Cochliobolus heterostrophus* (115), and *M. grisea* (141). The mating system in ascomycetes is controlled by one locus that exists in two different forms (*Mat*1-1 and *Mat*1-2). The two forms found at the mating type locus are referred to as “idiomorphs” instead of “alleles” because they do not share significant sequence homology, which is implied by the term “allele” (79). The *Mat*1-1 and *Mat*1-2 idiomorphs encode proteins with DNA-binding motifs and may determine mating type by controlling expression of genes for pheromones and pheromone receptors (5,6,106).

Kang et al. (51) isolated and cloned the mating type idiomorphs, *Mat*1-1 and *Mat*1-2, from *M. grisea* using genomic subtraction. This technique was necessary because the *M. grisea* idiomorphs could not be isolated based on homology with those from *N. crassa* or *C. heterostrophus*. The *Mat*1-1 and *Mat*1-2 idiomorphs were approximately 2.5 kbp and 3.5 kbp in length, respectively, which are significantly different from those in *N. crassa* (3.2 and 5.3 kbp) and *C. heterostrophus* (1.1 and 1.0 kbp). Although the function of mating type idiomorphs is conserved within Phylum Ascomycota, little sequence homology is observed between Orders (15).

For a given pair of isolates of opposite mating type, additional criteria must be met for mating and sexual reproduction to occur. At least one of the strains must be female fertile and the other must be male fertile. Isolates of *M. grisea* may be hermaphroditic (male and female fertile), female fertile (male sterile), male fertile (female sterile), or completely sterile (141). Additional compatibility factors independent of fertility are also present in *M. grisea*, but are not well understood (13,21,56,64,90). As a result, the apparent fertility of a cross depends on the interaction between the parental genotypes, rather than the fertility status of the individual isolates alone (13).

Another means of genetic recombination in ascomycetes is the parasexual cycle. This process involves anastomosis of compatible hyphae to form heterokaryons, fusion of nuclei to yield heterozygous diploids, and random assortment of chromosomes to yield recombinant haploid progeny (100). In rare cases, random associations between homologous chromosomes may result in mitotic crossing over and creation of recombinant chromosomes. Parasexual recombination has been identified in *M. grisea* through complementation of auxotrophic mutants in vitro, followed by isolation of haploids with recombinant phenotypes (19,29). Using molecular markers, Zeigler et al. (143) demonstrated that parasex in vitro results in the production of recombinant genotypes,

and also discovered recombinant genotypes in the field that may have been produced through parasexual recombination.

Population Genetics and Population Structure

Population structure is defined as a non-random distribution of individuals in populations (34). Studies of population structure describe the distribution of individuals in populations, but the evolutionary forces responsible for the observed structure are not determined. Population genetics is the study of evolutionary forces, including selection, mutation, recombination, gene flow, and genetic drift, that determine population structure and drive evolutionary changes in populations over time (34,81). Using the tools of population genetics, it is possible to measure the relative impact of evolutionary forces on populations and to predict future changes in population structure.

The population genetics of several important plant pathogens have been studied in depth, including *Phytophthora infestans* (31), *Cryphonectria parasitica* (83), *Septoria tritici* (76), and *M. grisea* (17,65,141,142). These studies have yielded valuable information that is directly applicable to the development of disease management strategies. For example, analysis of *P. infestans* populations has allowed researchers to identify the center of origin for this pathogen and identify populations where sexual recombination may lead to the development of new virulence phenotypes (31). Studies of the population genetics of *Septoria tritici*, the causal agent of wheat leaf blotch, have revealed that ascospores are the source of primary inoculum for this disease and facilitated the identification of optimal cultivar mixtures for disease management (76). Finally, analysis of *M. grisea* populations associated with rice has led to the development of new strategies for managing the disease (145) and production of durably resistant cultivars (110,144).

Population structure is determined by the relative contributions of evolutionary forces that reduce genetic variation (selection and genetic drift) and those that increase genetic variation (mutation, gene flow, and recombination). In agricultural systems, selection is the dominant force driving the evolution of plant pathogens (63). Disease control practices, such as resistant cultivars and fungicide applications, inhibit the growth and reproduction of plant pathogens and therefore place tremendous selection pressure on pathogen populations (63,82). Selection pressure in the form of disease control practices reduces the size of pathogen populations, resulting in reduced genetic variation through a process called genetic drift (63). How the pathogen population responds to selection and genetic drift is determined by the frequency of mutation (63), genetic recombination (81), and gene flow (75). These forces determine the amount of genetic variation present in the population prior to selection and the pathogen's capacity to generate new genetic variation following selection. As a result, these forces determine the potential for a pathogen population to develop resistance to a fungicide or overcome cultivar resistance (82).

Using knowledge of pathogen population genetics, it may be possible to develop disease management strategies that are not subject to failure due to pathogen evolution (111). For plant pathogens that are strictly clonal in nature, disease management practices must be directed toward the clonal lineages or individual genotypes present in populations (143). In this situation, gene pyramiding or fungicide tank-mixtures may be most effective as long-term control practices (144). However, for pathogens that undergo frequent genetic recombination, clonal lineages and genotypes are transient, and the targets of control must be the entire population and all future variants that may arise through recombination (111). In this situation, cultivar mixtures, multilines, dynamic multilines, or fungicide rotations may be the most durable management strategies (85).

Mating Type Distribution and Fertility Status in *Magnaporthe grisea* Populations

Sexual recombination is an important source of genotypic variation in fungal populations and is a primary determinant of population structure (81). In a heterothallic fungus, such as *M. grisea*, both mating types must be present for sexual reproduction to occur (141). While the absence of one mating type in a population precludes sexual recombination, other barriers also exist, including female, male, or complete sterility (141) and other poorly understood compatibility factors (13,21,56,64,90).

Populations of *M. grisea* associated with several hosts have been analyzed for mating type distribution and fertility status. Both mating types co-exist in the majority of *M. grisea* populations analyzed, although one mating type is typically dominant in a population (21,35,47,48,53,57,77,89,97,119,122,138). The mating type that is dominant in a population is influenced by host species, host cultivar, and geographical location (35,57,89,122,138). The forces responsible for dominance of a single mating type in fungal populations are unknown. Because both mating types are present in many populations, it does not appear that populations associated with a particular host are fixed for a single mating type. Isolates of a certain mating type may have a selective advantage in survival or virulence in certain environments (141).

The majority of *M. grisea* isolates from rice are male-fertile or completely sterile (24,35,47,53,77,89,97,119,122,138). However, a significant number of hermaphroditic isolates were identified in rice populations in the Central Himalayas (57) and India (21). The frequency of hermaphroditic isolates is generally higher in populations associated with grasses other than rice (47,48,119,122,135,137). In fact, the first report of sexual reproduction in *M. grisea* originated from a cross between isolates collected in the southeastern United States from large crabgrass (*Digitaria sanguinalis* (L.) Scop.), a

common weed in turfgrass swards (39). As discussed by Leslie and Klein (62), the presence of hermaphroditic isolates in fungal populations may be evidence of a recent sexual recombination event.

Little is known about the distribution of mating types and fertility in turfgrass populations of *M. grisea*. A single isolate from meadow fescue analyzed by Yaegashi (135) was sterile and its mating type was undetermined. Among seven isolates from St. Augustinegrass, two were male-fertile in controlled crosses and were mating type *Mat*1-1 (135). Viji et al. (125) found only *Mat*1-2 isolates in 194 isolates from 33 perennial ryegrass populations. Among 126 perennial ryegrass isolates from 37 locations, 71 were male fertile in crosses with tester strains, but the perithecia were barren (124). Given the fact that hermaphroditic isolates are relatively common in hosts other than rice, and that sexual reproduction was first observed among *M. grisea* isolates from the southeastern United States, a study of mating type distribution and fertility status is warranted to determine the potential for sexual reproduction in Georgia turfgrass populations of *M. grisea*.

The mating type of unknown isolates may be determined by one of two methods: in vitro crosses with hermaphroditic tester strains (47) or Southern hybridization of genomic DNA with cloned mating type idiomorphs (18). Isolates that do not mate with tester strains in vitro are assumed to be sterile. However, because of poorly understood incompatibility factors, the apparent fertility of an unknown isolate depends on the tester strains utilized (13,21,56,64,90). Therefore, results from in vitro crosses give little insight to the potential for sexual reproduction in natural populations. Using a molecular assay, it may be possible to determine mating type independent of fertility and incompatibility factors. Then, isolates of opposite mating type from the same population can be

crossed, giving a more accurate assessment of the potential for sexual reproduction in that population.

The idiomorphic nature of the mating type locus in ascomycetes (30) facilitates the development of PCR-based assays for mating type determination (129). Xu et al. (134) developed a PCR-based assay for mating type determination in *M. grisea*; however, two disadvantages have prevented this method from gaining widespread use. First, the PCR primers are not specific to the mating type locus, resulting in amplification of multiple bands in some cases (unpublished results; ML. Farman, personal communication). Second, the expected size of PCR products from each idiomorph is nearly identical (372 bp for *Mat*1-1; 376 bp for *Mat*1-2). This prevents the use of multiplexed PCR reactions, reducing efficiency and creating control problems when screening isolates. Development of a new PCR-based mating type assay with multiplexed reactions will greatly facilitate future studies of mating type distribution in *M. grisea*.

Population Genetics of *Magnaporthe grisea*

Although many researchers have conducted studies of *M. grisea* population structure (14,16,17,18,23,24,57,65,66,102,118,125,132,133,142), only two studies used molecular data to conduct population genetic analyses (14,57).

Kumar et al. (57) studied the population genetics of *M. grisea* associated with rice in the Himalayas region of India, which is one of the proposed centers of diversity for rice (12). In this study, two statistical approaches were used to test for evidence of genetic recombination. The two-locus gametic disequilibrium method uses Fisher's exact test to detect pairs of markers that are associated non-randomly. Non-random associations among molecular markers is interpreted as evidence that genetic recombination does not occur in the population (81). Another statistical approach, called the Index of Association

(I_a), estimates the degree of non-random associations among all markers and tests for significant departures from random mating (74,81). Kumar et al. (57) reported that 97.2%, 96.3%, and 98.8% of marker pairs were randomly associated in populations sampled in 1992, 1993, and 1994, respectively. When data from all three seasons were combined, 93.4% of the marker pairs were randomly associated. In all three seasons and the combined data set, the null hypothesis of random mating was not rejected by the I_a method. The authors concluded that genetic recombination had a significant impact on population structure in the Indian Himalayas. Approximately equal frequencies of *Mat*1-1 and *Mat*1-2 isolates in the population, and a significant number of male fertile and hermaphroditic strains, provided additional support for the occurrence of genetic recombination in that region.

In a study of two *M. grisea* populations associated with rice in the Philippines, Chen et al. (14) reported that 60% of marker pairs were randomly associated, but I_a was not reported. While the percentage of randomly associated marker pairs is considerably lower than that reported from India (57), genetic recombination appears to play a significant role in shaping *M. grisea* population structure in the Philippines.

Because of the low frequency or complete absence of female fertile isolates in most populations from rice, many researchers assume that *M. grisea* is clonal and focus their studies on identification of clonal lineages in populations of this pathogen. Interest in clonal lineages was spurred by Levy et al. (66), who reported a close correspondence between clonal lineage and pathogenic races in *M. grisea*. Of the eight lineages identified in *M. grisea* populations from rice in the United States, six contained a single race, whereas two lineages each contained two races with similar virulence profiles. These results indicated that race is a stable characteristic in *M. grisea* that is not subject to frequent mutation or recombination, as was previously thought (92). The authors also

suggested that DNA fingerprinting could be used to identify races of *M. grisea* with a high degree of confidence, and thus would assist rice breeders in their efforts to develop cultivars with resistance to all *M. grisea* races that may be encountered in the field.

Since the report by Levy et al., researchers have identified similar relationships between clonal lineage and pathogenic race in rice populations from Arkansas (18,132,133), Colombia (16,17,65), the Philippines (142), and Europe (102). In contrast, no apparent relationship among clonal lineage and race could be identified in *M. grisea* populations from rice in Japan (23) or Vietnam (24). In populations where relationships between lineage and race were detected, the relationships were typically not as simple as those originally reported by Levy et al. (66). Many clonal lineages contained multiple pathogenic races; however, the majority of races were limited to a single clonal lineage (16-18,65,102,132,133,142). For example, among 39 races identified in Colombia, 33 were limited to one of the six clonal lineages (65). In the Philippines, 65 of the 71 races identified were restricted to a single clonal lineage (142). Finally, 41 distinct races were identified in European populations of *M. grisea*, all of which were limited to one of the five clonal lineages identified by MGR586 markers (102).

Although multiple races are present in most clonal lineages of *M. grisea*, the races within each lineage form a network of closely related races with single differences in virulence profile. Correa-Victoria et al. (16,17) expanded on the work of Levy et al. (65) in Colombia to further explore the relationship between clonal lineage and race. Among 131 isolates collected from 15 rice cultivars, 45 pathogenic races were detected, which belonged to nine clonal lineages containing groups of races with closely related virulence profiles. Artificial inoculation of rice lines with single, known resistance genes revealed that virulence to all known resistance genes was present in the population. However, despite the complexity of virulence profiles in each clonal lineage, single resistance

genes were identified which were effective against all races in a particular lineage. The authors proposed that changes in virulence profiles are not random. Instead, isolates within a lineage have a limited virulence spectrum due to fitness costs associated with mutation of avirulence genes, many of which are involved in pathogenicity (16,17). A fitness cost associated with mutation of avirulence genes was also proposed by Mekwatanakarn et al. (78), who found that the dominant races in Thailand populations of *M. grisea* had simple virulence profiles.

Based on the work of Correa-Victoria et al., Zeigler et al. (144) proposed a new method for *M. grisea* resistance breeding called “lineage exclusion”. This method is based on the hypothesis that clonal lineages of *M. grisea* have limited virulence spectra. The lineage exclusion method differs from traditional breeding practices in the criteria used to select resistance genes. In the lineage exclusion method, resistance genes that exclude entire lineages of the pathogen are identified. Multiple genes, which together exclude all lineages of the pathogen present in the region of interest, are then integrated into a single cultivar. Cultivars produced using the lineage exclusion method are expected to be durable because of the restricted virulence spectrum of *M. grisea* lineages. The effectiveness of lineage exclusion depends on a lack of genetic recombination in *M. grisea* populations (144). Therefore, this method may not be effective in locations where evidence of genetic recombination has been detected, such as the Philippines and Himalayas, emphasizing the practical significance of population genetic studies in plant disease management.

Unlike work on rice, studies of *M. grisea* population structure in turfgrasses are limited. Viji et al. (125) used MGR586 and Pot2 markers to characterize 194 *M. grisea* isolates collected from perennial ryegrass in 33 locations across 4 states. Three clonal lineages were identified in the sample population. According to Analysis of Molecular

Variance (AMOVA), a significant amount of genetic divergence was detected among all states, except for Maryland and Pennsylvania, which were not significantly different. However, the sampling strategy employed by Viji et al. (125), with less than 6 isolates per population on average, is generally considered to be inadequate for estimating population structure (9). Larger sample sizes (>50 isolates) from each population will be required to characterize the structure of *M. grisea* populations associated with perennial ryegrass.

Selection of Molecular Markers for Population Genetic Studies

Brown (9), McDonald and McDermott (75), and Milgroom and Fry (82) discussed the criteria that are most important for selection of molecular marker techniques for population genetic studies. First, the technique must permit unambiguous and reliable identification of genotypes. Nonhomologous markers must be easily differentiated, and the assumption that co-migrating fragments are homologous must be valid. Second, the molecular markers must be independent. The results obtained from different markers should not be directly correlated. For example, markers that map to the same genetic locus are dependent markers. Third, the molecular markers must be selectively neutral so that unbiased estimates of population structure can be obtained. If genes for virulence or fungicide resistance are used for population genetics, the effect of selection on allele frequency may obscure the importance of mutation, gene flow, and recombination (75). Fourth, the molecular markers must exhibit a constant mutation rate in the species being studied (87). In populations of clonal pathogens, such as *M. grisea*, identification of clonal lineages through phylogenetic analysis is an important aspect of population genetics (63). Molecular markers with mutation rates that vary among subgroups in the population may yield phylogenetic trees that do not reflect true evolutionary relationships (87).

To date, all studies of population genetics in *M. grisea* have employed transposable elements as molecular markers. Transposable elements, or transposons, are segments of DNA that possess the ability to move spontaneously and re-insert themselves into new locations in the genome (20,54). Based on their mechanism of movement, transposons can be divided into two classes: Class I transposons, also called retrotransposons, move via an RNA intermediate, whereas Class II transposons move via a DNA intermediate (25). Within each class, many different transposons have diverged at the DNA sequence level and proliferated in the genome of prokaryotic and eukaryotic individuals (20).

Several transposons have been identified in the *M. grisea* genome, including MGR586, Pot2, Grasshopper, MAGGY, Fosbury, MGR583, MGSR1, and Mg-SINE (25). Some of these transposons have proliferated in the *M. grisea* genome and are present in high numbers (50 to 100 copies) in some individuals. These high-copy-number transposons are useful as molecular probes to detect Restriction Fragment Length Polymorphisms, or RFLPs (32). All studies of population genetics in *M. grisea* to date have employed these Transposon-Detected Restriction Fragment Length Polymorphisms (TD-RFLPs).

There are several drawbacks to the use of TD-RFLPs for population genetic analysis in *M. grisea*, which underscores the need for new techniques. These drawbacks include:

1. All transposons characterized in *M. grisea* to date have proliferated only in certain lineages within the species (25). For example, the transposon MGR586 is present in high copy number in isolates pathogenic to rice (50 to 60 copies), but in lower copy number (10 to 25 copies) in isolates from all other hosts (8). The transposon MAGGY is also found in high copy number (50 to 100 copies) in rice isolates, but is in lower copy number (1 to 30

copies) or absent in the genome of isolates from other hosts. The disparity in copy number indicates that the mutation rate of transposons is not constant in *M. grisea* and brings their utility for phylogenetic analysis into question (87).

2. Transposable elements are distributed throughout the *M. grisea* genome (99), but their location is not random. Nitta et al. (88) found that MAGGY, MGR586, Pot2, and Mg-SINE were located adjacent to one another in the *M. grisea* genome more frequently than expected by chance. Preferential integration of transposons into specific regions of the genome has also been documented in maize (103) and may be associated with "hot spots" for meiotic crossing-over. The linkage of transposons in the *M. grisea* genome violates the requirement that molecular markers be independent for use in population genetics (9).
3. High-copy-number transposons have not yet been identified in *M. grisea* isolates from several hosts, including annual ryegrass, perennial ryegrass, tall fescue, and St. Augustinegrass. Viji et al. (125) analyzed *M. grisea* populations from perennial ryegrass, and found MGR586 and Pot2 markers in copy numbers of 25 to 35 and 15 to 25, respectively. This quantity of markers is generally considered to be insufficient for population genetic analyses (63).
4. Population genetic studies using TD-RFLPs are limited by the number of transposon copies present in the genome. If the resolution provided by transposons is inadequate for statistical analyses, no means are available to collect additional data. The use of additional restriction enzymes or other transposable elements may yield markers that are not independent of the previous data.

A relatively new technique, called Amplified Fragment Length Polymorphisms (AFLPs), may be useful for population genetic studies (9). The AFLP procedure was developed and patented by Keygene N.V. (140). This technique was specifically designed to overcome some of the major drawbacks of previous molecular techniques such as RFLP and Random Amplified Polymorphic DNA (RAPD) (128).

In the AFLP procedure, template DNA is cut with two restriction enzymes, a “rare cutter” with a recognition sequence of 6 base pairs (usually *Eco*RI) and a “frequent cutter” with a recognition sequence of 4 base pairs (usually *Mse*I). Double stranded adapters are then ligated to the “sticky ends” of the restriction fragments. PCR amplification of digested, ligated template DNA is typically conducted in two steps. The first, or preamplification, step is performed with primers complimentary to the adapter sequences. The final, or selective, amplification step utilizes primers with “selective nucleotides” that extend into the restriction fragments. The reaction products are separated on a polyacrylamide sequencing gel. Polymorphisms are visualized through radioactive or fluorescent labeling of the primer complimentary to the rare cutter adapter.

The molecular processes that lead to polymorphisms in AFLP markers are very similar to those for RFLP (69). Polymorphisms between individuals primarily arise in four ways: (1) point mutations in the template DNA that create or eliminate restriction sites; (2) substitutions, insertions, or deletions in the bases adjacent to restriction sites that change the selective primer that anneals to that restriction fragment; (3) large insertions or deletions that create or eliminate restriction fragments; and (4) insertions or deletions that change the length of restriction fragments.

As mentioned above, the AFLP technique was designed to overcome some of the major disadvantages of current techniques used for generating molecular markers. The primary advantages of AFLP markers are:

1. The AFLP technique may be applied to any organism with no prior genetic information other than an estimate of genome size (128).
2. Polymorphisms are plentiful and arise through known molecular processes (69).
3. AFLP markers are randomly distributed throughout the genome (96) and therefore may be treated as independent molecular markers (9).
4. A large number of independent loci (up to 100) can be assayed with one AFLP reaction. Through the use of multiple primer combinations and sets of restriction enzymes, an unlimited number of independent molecular markers can be generated using this technique (128).
5. The restriction fragments amplified by AFLP are in a narrow size range and can be separated on a polyacrylamide gel for maximum resolution (128). Higher resolution increases the probability that co-migrating fragments are homologous and improves the reliability of genotype identification (9).
6. Stringent annealing temperatures during PCR produce reliable and repeatable results (128). Among nine laboratories in Europe, Jones et al. (50) reported that AFLP markers were highly reproducible, whereas large amounts of variation in RAPD markers were observed among locations.
7. Small amounts of template DNA (approximately 125 ng) are required for a potentially unlimited number of AFLP reactions (128).

The primary advantage of the AFLP technique over TD-RFLPs is the means by which the molecular markers are detected. TD-RFLPs are detected by probing the genome with radioactively labeled transposable elements, which are present in limited copy numbers in specific regions of the genome (88). AFLPs are detected by PCR amplification of a specific subset of restriction fragments that only share an identical DNA sequence immediately adjacent to the restriction sites (128). Because variation in AFLP

and TD-RFLP markers are generated by similar mechanisms (69), AFLP fingerprinting represents an appropriate alternative to TD-RFLP for generating independent molecular markers for use in population genetic analyses (9). Although there are no published reports of their use in population genetics, AFLP markers are being used successfully at Rutgers University to study the population genetics of *Monilinia oxycocci*, the causal agent of cottonball of cranberry (95).

Non-Host Resistance to *Magnaporthe grisea*

Although *M. grisea* as a species is pathogenic to grasses belonging to 22 genera (26), individual isolates have a narrow host range consisting of one to several genera (52). As discussed by Zeigler et al. (141), rigorous studies of host range in *M. grisea* have been lacking. In addition, there is considerable disagreement among the small host range studies that have been reported (56,58,61,70,86,112,118-120,125,136). For example, Igarashi et al. (45) and Viji et al. (125) reported that *M. grisea* isolates from wheat were highly virulent on rice, whereas Urashima et al. (119) found that wheat isolates were non-pathogenic on rice. The incongruity observed among host range studies may be due in large part to a lack of standardized inoculation methods for *M. grisea* (60).

Although host range studies have not provided clear evidence, field and laboratory data have conclusively demonstrated that *M. grisea* isolates have a limited host range. Analyses of *M. grisea* populations with molecular markers have consistently shown that cross-infection among hosts does not occur in nature with detectable frequency (8,118,123). In some cases, single genes encoding host specificity have been identified in *M. grisea*. Yaegashi (136) analyzed the progeny of a cross between *M. grisea* isolates from finger millet and weeping lovegrass (*Eragrostis curvular*) and determined that

pathogenicity to these hosts was controlled by single, separate genes. Valent and Chumley (120) used crosses between isolates from goosegrass (*Eleusine indica*) and weeping lovegrass to determine that pathogenicity to goosegrass was also controlled by a single gene. In a cross between isolates from wheat and finger millet, Murakami et al. (86) identified two genes, designated Pwt1 and Pwt2, that controlled pathogenicity to wheat.

Sweigard et al. (109) isolated and cloned the *PWL2* gene, which controls specificity to weeping lovegrass, and demonstrated the functionality of the allele that confers non-pathogenicity. When this allele was expressed in an *M. grisea* isolate from weeping lovegrass, pathogenicity to weeping lovegrass was lost. This allele encodes a protein with a putative secretion signal sequence, indicating that the product is excreted from the cell. On the outside of the cell, this protein may act as an elicitor of a defense response in hosts with the corresponding receptor. The authors speculated that the gene encoding the host receptor could be cloned from weeping lovegrass and transformed into rice cultivars, possibly conferring resistance to *M. grisea* (109).

Heath et al. (37,38) utilized microscopy to examine the *M. grisea* infection process and discover mechanisms that determine host specificity. Several processes were observed in incompatible host-pathogen combinations. In some cases, infection hyphae failed to develop from appressoria or failed to penetrate the epidermis. When infection hyphae formed and penetrated the epidermis, they did not develop into secondary hyphae or were confined to a single host epidermal cell by an active host response. In their analysis of host specificity to wheat, Murakami et al. (86) found that the Pwt1s allele elicited a hypersensitive response, whereas the Pwt2s allele induced papilla formation.

Several host range studies have included turfgrass isolates of *M. grisea*, but few conclusions can be drawn from these experiments due to their limited scope. Malca and

Owen (70) reported that cross-infection occurred between crabgrass and St. Augustinegrass, but noted that virulence was significantly reduced on the host of non-origin. Latterell et al. (61) reported that *M. grisea* isolates from St. Augustinegrass induced pinpoint lesions on some rice varieties, but isolates from rice induced typical gray leaf spot symptoms on St. Augustinegrass. In their initial description of ryegrass blast, Bain et al. (4) reported that *M. grisea* isolates from annual ryegrass induced symptoms on oats (*Avena sativa* L.), wheat, rice, pearl millet (*Pennisetum glaucum* (L.) R. Br.), and corn (*Zea mays* L.). Later, Trevathan (112) found that isolates from crabgrass, soybean (*Glycine max* (L.) Merrill), spurge (*Chamaesyce* sp.), smartweed (*Polygonum* sp.), and St. Augustinegrass were pathogenic to annual ryegrass. Urashima et al. (119) found that *M. grisea* isolates from wheat and rice were pathogenic to Kentucky bluegrass (*Poa pratensis* L.), annual ryegrass, perennial ryegrass, tall fescue, and creeping red fescue (*Festuca rubra* L.). Finally, Viji et al. (125) reported that *M. grisea* isolates from wheat and perennial ryegrass induced symptoms on perennial ryegrass, tall fescue, and wheat, whereas isolates from rice were pathogenic only to wheat and rice.

Host Resistance to *Magnaporthe grisea*

In general, attempts to manage rice blast with resistant cultivars have been unsuccessful. Rice breeders have produced many cultivars with complete resistance to *M. grisea*, but the resistance has been short-lived in most cases (91). Resistant cultivars typically lose their effectiveness after two to three years of widespread use, a phenomenon known as “resistance breakdown” (55). Four possible explanations for the short-lived nature of *M. grisea* resistance have been proposed:

1. **Frequent mutation of avirulence genes yields an unlimited number of new races.** Ou et al. (93) found 204 distinct pathogenic races among 628 subcultures derived from a monoconidial isolate of *M. grisea*, and speculated that mutations in nature frequently gave rise to new races. In contrast, Latterell and Rossi (60) reported that race was a stable characteristic in *M. grisea* isolates over 20-30 years in culture, and suggested that inappropriate inoculation methods, differential lines, and methods of disease evaluation were responsible for the frequent appearance of new races reported by Ou et al. (93). In light of the stable relationship between clonal lineage and race in natural populations (65,66,142), it is generally accepted that race is a stable characteristic in *M. grisea* and that frequent mutations are not responsible for resistance breakdown.
2. **Frequent recombination in *M. grisea* populations result in production of new races.** Despite evidence of genetic recombination in the Philippines (142) and Himalayas (57), the stability of clonal lineages over extended periods of time (18) and close relationship between lineage and virulence profile (8,16,65,66,142) indicate that recombination does not occur with significant frequency in most populations. Despite an extensive search for the teleomorph over 10 years in China (36), the sexual stage of *M. grisea* has never been observed in nature.
3. **High rates of migration lead to re-establishment of the pathogen population with foreign races of *M. grisea*.** Gene flow, or migration of individuals among populations, appears to occur frequently at the continental level in *M. grisea*. No evidence of population subdivision was observed in Japan (23), Europe (102), or India (57). In contrast, no evidence of

intercontinental migration has been observed in analyses of population structure. Isolates of *M. grisea* collected from rice in Europe were less than 50% similar to all isolates from North America and Asia (102). Climatic conditions appear to play an important role in limiting successful migration among locations (24).

4. **Inadequate sampling techniques during resistance screening fail to detect compatible races present in pathogen populations at low frequencies. Resistance breakdown occurs when compatible races become dominant in the population through selection.** Although most *M. grisea* populations are composed of several clonal lineages, a single lineage is typically dominant in a population. For example, lineage Lf1 comprised 97% of the *M. grisea* population from rice in Japan, with lineage Lf2 comprising the remaining 3% (23). Populations of *M. grisea* from rice in Arkansas are dominated by either lineage A or B, depending on the cultivar from which isolates are obtained, with lineage C and D comprising 24% and 13% of the population, respectively (133). It is unlikely that small plot breeding trials provide adequate exposure of resistant germplasm to infrequent races in *M. grisea* populations (65). It is now generally accepted that breakdown of resistance to *M. grisea* in rice is due to the buildup of races already present in the population through selection (144).

Although inadequate sampling techniques are involved, the primary cause of instability in *M. grisea* resistance is the exclusive use of genes conferring complete resistance (73). Complete resistance, also called race-specific resistance or qualitative resistance, renders a plant immune to infection by a pathogen. Complete resistance is desirable to rice breeders because it is effective under severe disease pressure and is

simply inherited because it is controlled by single genes (68,71,73,131). However, individual genes for complete resistance are only effective against some, but not all, races of a pathogen, and are therefore susceptible to resistance breakdown through selection or introduction of compatible races (55).

In contrast, genes conferring partial resistance are not subject to resistance breakdown because they are equally effective against all known races of a pathogen (73). Partial resistance, also known as quantitative or rate-reducing resistance, reduces the growth and reproduction of a pathogen, thus slowing the rate of epidemic development and reducing the negative effects of disease on growth, yield, or quality (1,3,11,49,72,73,94,101,130,131,139).

The combination of partial resistance with genes for complete resistance may significantly reduce the devastating results of a breakdown in *M. grisea* resistance. However, two problems have limited the use of partial resistance in rice breeding. First, it is very difficult to detect partial resistance in breeding lines in the presence of genes for complete resistance because of epistasis (101). As a result, during intensive selection for complete resistance over many years, rice breeding lines have lost genes for partial resistance, a phenomenon described by Vanderplank as the “Vertifolia effect” (121). Second, the genetic basis for partial resistance is poorly understood, making the transfer of this characteristic into commercial cultivars difficult (131). Molecular mapping of genes involved in partial resistance may allow breeders to use marker-assisted selection to transfer partial resistance into desirable genetic backgrounds (131).

Recently, turfgrass breeders have identified sources of resistance to *M. grisea* in tall fescue (27,28) and perennial ryegrass (7) germplasm. In both species, the resistance appears to be under polygenic control (7,42), indicating that the resistance is partial. However, both research groups also noted evidence of major genes in the segregating

populations, suggesting that complete resistance may also be involved (7,42). Research is required to further characterize these sources of resistance so that they can be integrated efficiently into commercial cultivars. Component analysis may be used to determine if the resistance in this germplasm is partial or complete, and may also assist in the breeding process by defining which parameters should be measured in breeding populations (101). In addition, the resistant germplasm should be challenged with diverse *M. grisea* isolates in an effort to identify compatible races. A knowledge of *M. grisea* population structure will aid in the design of experiments to further characterize these resistance sources and determine how they can be used in the most effective and sustainable manner.

Research Objectives

Pathogen population structure and genetics has direct application to the development of disease management strategies (82). This information is especially useful to plant breeders in the development of cultivars with durable pathogen resistance (144). Turfgrass breeders are currently working to develop tall fescue and perennial ryegrass cultivars with resistance to *M. grisea*, the causal agent of gray leaf spot (7,27,28,40,41). Because little is known about *M. grisea* populations associated with turfgrasses, this research was initiated with the following objectives:

1. Develop an improved PCR-based assay for mating type determination in *M. grisea*.
2. Estimate the potential for sexual recombination in populations of *M. grisea* associated with St. Augustinegrass and tall fescue in Georgia through analysis of mating type distribution and fertility status.

3. Utilize AFLP markers to compare and contrast the genetic structure of *M. grisea* populations from different hosts, cultivars, and geographical regions in Georgia, and estimate the importance of evolutionary forces in shaping the observed population structure.
4. Measure the components of resistance to *M. grisea* in Coyote and Coronado tall fescue, and determine if clonal lineages identified with AFLP markers correspond to distinct pathogenic races of *M. grisea*.

Literature Cited

1. Ahn, S. W., and Ou, S. H. 1982. Quantitative resistance of rice to blast disease. Phytopathology 72:279-282.
2. Anderson, A. L., Henry, B. W., and Tullis, E. C. 1947. Factors affecting infectivity, spread, and persistence of *Piricularia oryzae* Cav. Phytopathology 37:94-110.
3. Ashizawa, T., Zenbayashi, K., and Koizumi, S. 1999. Effect of partial resistance on rice blast control with cultivar mixtures. Phytopathology 89:S3.
4. Bain, D. C., Patel, B. N., and Patel, M. V. 1972. Blast of ryegrass in Mississippi. Plant Dis. Rep. 56:210.
5. Bistis, G. N. 1981. Chemotrophic interaction between trichogynes and conidia of opposite mating type in *Neurospora crassa*. Mycologia 73:959-975.
6. Bistis, G. N. 1983. Evidence for diffusible, mating-type-specific trichogyne attractants in *Neurospora crassa*. Exp. Mycol. 7:292-295.
7. Bonos, S.A. and Meyer, W.A. 2001. Gray leaf spot resistance in perennial ryegrass selections from Europe. Agronomy Abstracts 93:249.
8. Borromeo, E. S., Nelson, R. J., Bonman, J. M., and Leung, H. 1993. Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. Phytopathology 83:393-399.
9. Brown, J. K. M. 1996. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytologist 133:183-195.
10. Carver, R. B., Rush, M. C., and Lindberg, G. D. 1972. An epiphytotic of ryegrass blast in Louisiana. Plant Dis. Rep. 56:157-159.

11. Castani, J., MacKenzie, D. R., and Nelson, R. R. 1989. Components analysis of race non-specific resistance to blast disease of rice caused by *Pyricularia oryzae*. J. Phytopath. 127:89-99.
12. Chang, T. T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25:425-441.
13. Chao, C. T., and Ellingboe, A. H. 1991. Selection for mating competence in *Magnaporthe grisea* pathogenic to rice. Can. J. Bot. 69:2130-2134.
14. Chen, D., Ziegler, R. S., Leung, H., and Nelson, R. J. 1995. Population structure of *Pyricularia grisea* at two screening sites in the Philippines. Phytopathology 85:1011-1020.
15. Cisar, C. R., TeBeest, D. O., and Spiegel, F. W. 1994. Sequence similarity of mating type idiomorphs: A method which detects similarity among the Sordariaceae fails to detect similar sequences in other filamentous ascomycetes. Mycologia 86:541-546.
16. Correa-Victoria, F. J., and Zeigler, R. S. 1993. Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" breeding site in eastern Colombia. Plant Dis. 77:1029-1035.
17. Correa-Victoria, F. J., Zeigler, R. S., and Levy, M. 1994. Virulence characteristics of genetic families of *Pyricularia grisea* in Colombia. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, U.K.
18. Correll, J. C., Harp, T. L., Guerber, J. C., Zeigler, R. S., Liu, B., Cartwright, R. D., and Lee, F. N. 2000. Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. Phytopathology 90:1396-1404.
19. Crawford, M. S., Chumley, F. G., Weaver, C. G., and Valent, B. 1986. Characterization of the heterokaryotic and vegetative diploid phases of *Magnaporthe grisea*. Genetics 114:1111-1129.
20. Daboussi, M. J. 1997. Fungal transposable elements and genome evolution. Genetica 100:253-260.
21. Dayakar, B. V., Narayanan, N. N., and Gnanamanickam, S. S. 2000. Cross-compatibility and distribution of mating type alleles of the rice blast fungus *Magnaporthe grisea* in India. Plant Dis. 84:700-704.

22. Dernoeden, P. H. 1996. Perennial ryegrass and gray leaf spot. Golf Course Manage. 64:49-52.
23. Don, L. D., Kusaba, M., Urashima, A. S., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Population structure of the rice blast fungus in Japan examined by DNA fingerprinting. Ann. Phytopath. Soc. Japan 65:15-24.
24. Don, L. D., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Population structure of the rice blast pathogen in Vietnam. Ann. Phytopath. Soc. Japan 65:475-479.
25. 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.
26. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. The American Phytopathological Society, St. Paul, MN.
27. Fraser, M. L. 1996. Susceptibility of tall fescues to gray leaf spot, 1995. Biological and Cultural Tests 12:130.
28. Fraser, M. L. 1997. Tall fescues vary in leaf spot susceptibility. Golf Course Manage. 65:64-66.
29. Genovesi, A. D., and Magill, C. W. 1976. Heterokaryosis and parasexuality in *Pyricularia oryzae* Cavara. Can. J. Microbiol. 22:531-536.
30. Glass, N. L., and Kuldau, G. A. 1992. Mating type and vegetative incompatibility in filamentous ascomycetes. Ann. Rev. Phytopath. 30:201-224.
31. Goodwin, S. B. 1997. The population genetics of *Phytophthora*. Phytopathology 87:462-473.
32. Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. 86:9981-9985.
33. 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.
34. Hartl, D. L., and Clark, A. G. 1997. Principles of Population Genetics. Sinauer Associates, Inc., Sunderland, MA.
35. Hayashi, N., Li, C., Li, J., Iwano, M., Naito, H., Yoshino, R., and Kato, H. 1997. Distribution of fertile *Magnaporthe grisea* fungus pathogenic to rice in Yunnan Province, China. Ann. Phytopath. Soc. Japan 63:316-323.

36. Hayashi, N., Li, C. Y., Li, J., and Naito, H. 1997. In vitro production on rice plants of perithecia of *Magnaporthe grisea* from Yunnan, China. Mycol. Res. 101:1308-1310.
37. Heath, M. C., Valent, B., Howard, R. J., and Chumley, F. G. 1990. Correlations between cytologically detected plant-fungal interactions and pathogenicity of *Magnaporthe grisea* toward weeping lovegrass. Phytopathology 80:1382-1386.
38. Heath, M. C., Valent, B., Howard, R. J., and Chumley, F. G. 1990. Interactions of two strains of *Magnaporthe grisea* with rice, goosegrass, and weeping lovegrass. Can. J. Bot. 68:1627-1637.
39. Hebert, T. T. 1970. The perfect stage of *Pyricularia grisea*. Phytopathology 61:83-87.
40. Hofmann, N. E., and Hamblin, A. M. 2000. Reaction of perennial ryegrass to gray leaf spot following inoculation in the greenhouse, 1999. Biological and Cultural Tests 16:55.
41. Hofmann, N. E., and Hamblin, A. M. 2000. Seeking resistant ryegrasses: gray leaf spot hits all varieties. Golf Course Manage. 68:29-34.
42. Hofmann, N. E., and Hamblin, A. M. 2001. Segregation of gray leaf spot resistance in tall fescue. 2001 Annual Meeting Abstracts [ASA/CSSA/SSSA].
43. Holcomb, G. E. 1995. Reaction of St. Augustinegrass cultivars and selections to gray leaf spot, 1994. Biological and Cultural Tests 11:41.
44. Howard, R. J. 1994. Cell biology of pathogenesis. Pages 3-22 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
45. Igarashi, S. 1990. Update on wheat blast (*Pyricularia oryzae*) in Brazil. Pages 25-33 in: Proc. Int. Conf. Wheat for the Nontraditional Warm Areas. D. A. Saunders, ed. United Nations Development Programme International Maize and Wheat Improvement Center, Mexico.
46. I. R. R. I. 1997. IRRI Rice Facts. International Rice Research Institute, Manila.
47. Itoi, S., Mishima, T., Arase, S., and Nozu, M. 1983. Mating behavior of Japanese isolates of *Pyricularia oryzae*. Phytopathology 73:155-158.
48. Itoi, S., Yamamoto, J., Karino, S., Arase, S., and Kato, H. 1980. Hermaphroditic isolates of *Pyricularia* isolated from ragi, *Eleusine coracana* (L.) Gaertn. Ann. Phytopath. Soc. Japan 46:549-552.

49. Johnson, R. 1984. A critical analysis of durable resistance. Ann. Rev. Phytopath. 22:309-330.

50. Jones, C. J., Edwards, K. J., Castaglione, S., Winfield, M. O., Sala, F., van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Linacero, R., Vazquez, A., and Karp, A. 1997. Reproducibility testing of RAPD, AFLP, and SSR markers in plants by a network of European laboratories. Mol. Breed. 3:381-390.

51. Kang, S., Chumley, F. G., and Valent, B. 1994. Isolation of the mating-type genes of the phytopathogenic fungus *Magnaporthe grisea* using genomic subtraction. Genetics 138:289-196.

52. Kang, S., Mullins, E., Dezwan, T. M., and Orbach, M. J. 2000. Pathogenesis and genome organization of the rice blast fungus. Pages 195-235 in: Fungal Pathology. J. W. Kronstad, eds. Kluwer Academic Publishers, Netherlands.

53. Kato, H., and Yamaguchi, T. 1982. The perfect state of *Pyricularia oryzae* CAV. in culture. Ann. Phytopath. Soc. Japan 48:607-612.

54. Kempken, F., and Kuck, U. 1998. Transposons in filamentous fungi - facts and perspectives. BioEssays 20:652-659.

55. Kiyosawa, S. 1982. Genetics and epidemiological modeling of breakdown of plant disease resistance. Ann. Rev. Phytopath. 20:92-117.

56. Kolmer, J. A., and Ellingboe, A. H. 1988. Genetic relationships between fertility and pathogenicity and virulence to rice in *Magnaporthe grisea*. Can. J. Bot. 66:891-897.

57. 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.

58. Kusaba, M., Don, L. D., Urashima, A. S., Eto, Y., Tosa, Y., Nakayashiki, H., Yamamoto, M., and Mayama, S. 1998. Natural infection of wild grass species with rice blast fungus suggested by DNA fingerprinting. Ann. Phytopath. Soc. Japan 64:125-128.

59. Landschoot, P. J., and Hoyland, B. F. 1992. Gray leaf spot of perennial ryegrass turf in Pennsylvania. Plant Dis. 76:1280-1282.

60. Latterell, F. M., and Rossi, A. E. 1986. Longevity and pathogenic stability of *Pyricularia oryzae*. Phytopathology 76:231-235.

61. Latterell, F. M., Tullis, E. C., and Collier, J. W. 1960. Physiologic races of *Pyricularia oryzae* Cav. Plant Dis. Rep. 44:679-683.

62. Leslie, J. F., and Klein, K. K. 1996. Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics 144:557-567.

63. Leung, H., Nelson, R. J., and Leach, J. E. 1992. Population structure of plant pathogenic fungi and bacteria. Adv. Plant Pathol. 8:157-205.

64. Leung, H., and Williams, P. H. 1985. Genetic analysis of electrophoretic enzyme variants, mating type, and hermaphroditism in *Pyricularia oryzae* Cavara. Can. J. Genet. Cytol. 27:697-704.

65. 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.

66. Levy, M., Romao, J., Marchetti, M. A., and Hamer, J. E. 1991. DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95-102.

67. Luttrell, E. S. 1951. Diseases of tall fescue grass in Georgia. Plant Dis. Rep. 35:83-87.

68. Mackill, D. J., and Bonman, J. M. 1992. Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82:746-749.

69. 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.

70. Malca, I., and Owen, J. H. 1957. The gray leaf spot disease of St. Augustinegrass. Plant Dis. Rep. 41:871-875.

71. Mandel, M. A., Crouch, V. W., Gunawardena, U. P., Harper, T. M., and Orbach, M. J. 1997. Physical mapping of the *Magnaporthe grisea* AVR1-MARA locus reveals the virulent allele contains two deletions. Mol. Plant-Microbe Interact. 10:1102-1105.

72. Marchetti, M. A. 1983. Dilatory resistance to rice blast in USA rice. Phytopathology 73:645-649.

73. Marchetti, M. A. 1994. Race-specific and rate-reducing resistance to rice blast in US rice cultivars. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.

74. Maynard-Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G. 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. 90:4384-4388.
75. McDonald, B. A., and McDermott, J. M. 1993. Population genetics of plant pathogenic fungi. BioScience 43:311-319.
76. McDonald, B. A., Pettway, R. E., Chen, R. S., Boeger, J. M., and Martinez, J. P. 1995. The population genetics of *Septoria tritici* (teleomorph *Mycosphaerella graminicola*). Can. J. Bot. 71:S292-S301.
77. Mekwatanakarn, P., Kositratana, W., Phromraksa, T., and Zeigler, R. S. 1999. Sexually fertile *Magnaporthe grisea* rice pathogens in Thailand. Plant Dis. 83:939-943.
78. Mekwatanakarn, P., Kositratana, W., and Zeigler, R. S. 2000. Pathotype and avirulence gene diversity of *Pyricularia grisea* in Thailand as determined by rice lines near-isogenic for major resistance genes. Plant Dis. 84:60-70.
79. Metzenberg, R. L., and Glass, N. L. 1990. Mating type and mating strategies in *Neurospora*. BioEssays 12:53-58.
80. Metzenberg, R. L., and Randall, T. A. 1995. Mating type in *Neurospora* and closely related ascomycetes: some current problems. Can. J. Bot. 73:S251-S257.
81. Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Ann. Rev. Phytopath. 34:457-477.
82. Milgroom, M. G., and Fry, W. E. 1997. Contributions of population genetics to plant disease epidemiology and management. Adv. Bot. Res. 24:1-30.
83. Milgroom, M. G., Wang, K., Zhou, Y., Lipari, S. E., and Kaneko, S. 1996. Intercontinental population structure of the chestnut blight fungus, *Cryphonectria parastica*. Mycologia 88:179-190.
84. Moss, M. A., and Trevathan, L. E. 1987. Environmental conditions conducive to infection of ryegrass by *Pyricularia grisea*. Phytopathology 77:863-866.
85. Mundt, C. C. 1994. Use of host genetic diversity to control cereal diseases: Implications for rice blast. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
86. Murakami, J., Tosa, Y., and Mayama, S. 2000. Analysis of host species specificity of *Magnaporthe grisea* toward wheat using a genetic cross between isolates from wheat and foxtail millet. Phytopathology 90:1060-1067.

87. Nei, M. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford, UK.

88. Nitta, N., Farman, M. L., and Leong, S. A. 1997. Genome organization of *Magnaporthe grisea*: integration of genetic maps, clustering of transposable elements and identification of genome duplications and rearrangements. Theor. Appl. Genet. 95:20-32.

89. Notteghem, J. L., and Silue, D. 1992. Distribution of the mating type alleles in *Magnaporthe grisea* populations pathogenic on rice. Phytopathology 82:421-424.

90. Orbach, M. J., Chumley, F. G., and Valent, B. 1996. Electrophoretic karyotypes of *Magnaporthe grisea* pathogens of diverse grasses. Mol. Plant-Microbe Interact. 9:261-271.

91. Ou, S. H. 1980. A look at worldwide rice blast control. Plant Dis. 64:439445.

92. Ou, S. H. 1980. Pathogen variability and host resistance in rice blast disease. Ann. Rev. Phytopath. 18:167-187.

93. Ou, S. H., Nuque, F. L., Ebron, T. T., and Awoderu, V. A. 1970. Pathogenic races of *Pyricularia oryzae* derived from monoconidial cultures. Plant Dis. Rep. 54:1045-1049.

94. Ou, S. H., Nuque, F. L., Ebron, T. T., and Awoderu, V. A. 1971. A type of stable resistance to blast disease of rice. Phytopathology 61:703-706.

95. Oudemans, P. V. 2001. personal communication.

96. Powell, W., Thomas, W. T. B., Baird, E., Lawrence, P., Booth, A., Harrower, B., McNicol, J. W., and Waugh, R. 1997. Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms. Heredity 79:48-59.

97. Priyadarisini, V. B., Viji, G., and Gnanamanickam, S. S. 1999. Mating type distribution, fertility and pathogenicity of rice isolates of *Magnaporthe grisea* in four rice growing regions of India. Indian Phytopath. 52:28.

98. Roberts, D. A., and Boothroyd, C. W. 1984. Fundamentals of Plant Pathology. W.H. Freeman, New York.

99. Romao, J., and Hamer, J. E. 1992. Genetic organization of a repeated DNA sequence family in the rice blast fungus. Proc. Natl Acad. Sci. 89:5316-5320.

100. Roper, J. A. 1966. Mechanisms of Inheritance. 3. The parasexual cycle. Pages 589-617 in: The Fungi, an Advanced Treatise. G. C. Ainsworth and A. S. Sussman, ed. Academic Press, New York.

101. Roumen, E. C. 1994. A strategy for accumulating genes for partial resistance to blast disease in rice within a conventional breeding program. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
102. Roumen, E., Levy, M., and Notteghem, J. L. 1997. Characterisation of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. Eur. J. Plant Pathol. 103:363-371.
103. SanMiguel, P., Tikhonov, A., Jin, Y. K., Motchoulskaia, N., Zhakarov, D., Melake-Berhan, A., Springer, P. S., Edwards, K. J., Lee, M., Avramova, Z., and Bennetzen, J. L. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274:765-768.
104. 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.
105. Seebold, K. W., Kucharek, T. A., Datnoff, L. E., Correa-Victoria, F. J., and Marchetti, M. A. 2001. The influence of silicon on components of resistance to blast in susceptible, partially resistant, and resistant cultivars of rice. Phytopathology 91:63-69.
106. Shen, W. C., Bobrowicz, P., and Ebbole, D. J. 1999. Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet. Biol. 27:253-63.
107. Stowell, L. J. 2001. personal communication.
108. Suzuki, H. 1975. Meteorological factors in the epidemiology of rice blast. Ann. Rev. Phytopath. 13:239-256.
109. Sweigard, J. A., Carroll, A. M., Kang, S., Farrall, L., and Chumley, F. G. 1995. Identification, cloning, and characterization of PWL2, a gene for host species specificity in the rice blast fungus. Plant Cell 7:1221-1233.
110. Tapiero, A. L., and Levy, M. 1999. Testing a "lineage-exclusion" strategy for breeding rice resistant to blast disease. Phytopathology 89:S76.
111. Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., Breniere, S. F., Darde, M. L., and Ayalaj, F. 1991. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. Proc. Natl. Acad. Sci. 88:5129-5133.
112. Trevathan, L. E. 1982. Pathogenicity on ryegrass and cultural variability of Mississippi isolates of *Pyricularia grisea*. Plant Dis. 66:592-594.

113. Trevathan, L. E. 1982. Response of ryegrass plant introductions to artificial inoculation with *Pyricularia grisea* under greenhouse conditions. Plant Dis. 66:696-697.

114. Trevathan, L. E., Moss, M. A., and Blasingame, D. 1994. Ryegrass blast. Plant Dis. 78:113-117.

115. Turgeon, B. G., Sharon, A, Wirsel, S., Yamaguchi, K., Christiansen, S. K., and Yoder, O. C. 1995. Structure and function of mating type genes in *Cochliobolus* spp. and asexual fungi. Can. J. Bot. 73:S778-S783.

116. Uddin, W., Burpee, L. L., and Stevenson, K. L. 1999. Influence of temperature, leaf wetness duration, and turfgrass age on development of gray leaf spot (blast) of *Festuca arundinacea*. Pages 136-141 in: 5th International Conference on Plant Protection in the Tropics, Kuala Lumpur, Malaysia.

117. 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.

118. Urashima, A. S., Hashimoto, Y., Don, L. D., Kusaba, M., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Molecular analysis of the wheat blast population in Brazil with a homolog of retrotransposon MGR583. Ann. Phytopathol. Soc. Japan 65:429-436.

119. Urashima, A. S., Igarashi, S., and Kato, H. 1993. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Phytopathology 77:1211-1216.

120. Valent, B., and Chumley, F. G. 1987. Genetic analysis of host species specificity in *Magnaporthe grisea*. Pages 83-93 in: Molecular Strategies for Crop Production. C. J. Arntzen and C. Ryan, eds. Alan R. Liss, Inc., New York.

121. Vanderplank, J. E. 1984. Disease Resistance in Plants. Academic Press, Inc., New York.

122. Viji, G., and Gnanamanickman, S. S. 1998. Mating type distribution and fertility status of *Magnaporthe grisea* populations from various hosts in India. Plant Dis. 82:36-40.

123. Viji, G., Gnanamanickam, S. S., and Levy, M. 2000. DNA polymorphisms of isolates of *Magnaporthe grisea* from India that are pathogenic to finger millet and rice. Mycol. Res. 104:161-167.

124. Viji, G., and Uddin, W. 2001. Mating behavior of *Magnaporthe grisea* isolates causing gray leaf spot (blast) of perennial ryegrass turf. Phytopathology 91:S92.
125. 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.
126. Vincelli, P. 1999. Gray Leaf Spot: An emerging disease of perennial ryegrass. Turfgrass Trends 7:1-8.
127. Vincelli, P. 2000. Fungicidal control of gray leaf spot. Golf Course Manage. 68:54-61.
128. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van der 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.
129. Wallace, M. M., and Covert, S. F. 2000. A molecular mating type assay for *Fusarium circinatum*. Appl. Environ. Microbiol. 66: 5506-5508.
130. Wang, Z., Mackill, D. J., and Bonman, J. M. 1989. Inheritance of partial resistance to blast in indica rice cultivars. Crop Sci. 29:848-853.
131. Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C., and Nelson, R. J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434.
132. Xia, J. Q., Correll, J. C., Lee, F. N., Marchetti, M. A., and Rhoads, D. D. 1993. DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) population in two rice fields in Arkansas. Phytopathology 83:1029-1035.
133. Xia, J. Q., Correll, J. C., and Rhoads, D. D. 2000. Regional population diversity of *Pyricularia grisea* in Arkansas and the influence of host selection. Plant Dis. 84:877-884.
134. Xu, J. R., and Hamer, J. E. 1995. Assessment of *Magnaporthe grisea* mating type by spore PCR. Fungal Genet. Newsl. 42:80.
135. Yaegashi, H. 1977. On the sexuality of blast fungi, *Pyricularia* spp. Ann. Phytopath. Soc. Japan 43:432-439.
136. Yaegashi, H. 1978. Inheritance of pathogenicity in crosses of *Pyricularia* isolates from weeping lovegrass and finger millet. Ann. Phytopath. Soc. Japan 44:626-632.

137. Yaegashi, H., and Nishihara, N. 1976. Production of the perfect stage in *Pyricularia* from cereals and grasses. Ann. Phytopath. Soc. Japan 42:511-515.
138. Yaegashi, H., and Yamada, M. 1986. Pathogenic race and mating type of *Pyricularia oryzae* from Soviet Union, China, Nepal, Thailand, Indonesia and Colombia. Ann. Phytopath. Soc. Japan 52:225-234.
139. Yeh, W. H., and Bonman, J. M. 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathol. 35:319-323.
140. Zeigler, R. S. 1998. Recombination in *Magnaporthe grisea*. Ann. Rev. Phytopath. 36:249-275.
141. Zeigler, R. S., Cuoc, L. X., Scott, R. P., Bernardo, M. A., Chen, D. H., Valent, B., and Nelson, R. J. 1995. The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. Phytopathology 85:443-451.
142. Zeigler, R. S., Scott, R. P., Leung, H., Bordeos, A. A., Kumar, J., and Nelson, R. J. 1997. Evidence of parasexual exchange of DNA in the rice blast fungus challenges its exclusive clonality. Phytopathology 87:284-294.
143. 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.
144. Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J., Yang, S., Hu, L., Leung, H., Mew, T., Teng, P., Wang, Z., and Mundt, C. 2000. Genetic diversity and disease control in rice. Nature 406:718-722.

CHAPTER 2

MATING TYPE DISTRIBUTION AND FERTILITY STATUS IN GEORGIA TURFGRASS POPULATIONS OF *MAGNAPORTHE GRISEA*[1]

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ABSTRACT

Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2002. Mating type distribution and fertility status in Georgia turfgrass populations of *Magnaporthe grisea*. Submitted to Plant Disease March 2002.

Populations of *Magnaporthe grisea* associated with tall fescue and St. Augustinegrass in Georgia were analyzed for mating type distribution and fertility status in 1999 and 2000. A PCR-based mating type assay was developed to facilitate population analysis. The assay was effective for diverse *M. grisea* isolates, but was ineffective for some isolates from *Pennisetum* and *Digitaria* hosts. All *M. grisea* turfgrass populations analyzed were dominated by a single mating type, however, the opposite mating type was found in association with each host. St. Augustinegrass populations were dominated by *Mat*1-1 (97.6% to 100%), whereas tall fescue populations were dominated by *Mat*1-2 (94.3% to 100%). Host cultivar and geographical region did not influence mating type distribution, but populations differed among growing seasons. Fertility status of isolates from selected populations was determined using controlled crosses in vitro. All St. Augustinegrass *Mat*1-1 isolates were sterile in crosses with tester strains, but a single *Mat*1-2 isolate from St. Augustinegrass was male-fertile. In crosses with a *Mat*1-1 hermaphrodite, 47 *Mat*1-2 tall fescue isolates were male-fertile, but 19 produced barren perithecia. Although both mating types exist in Georgia turfgrass populations of *M. grisea*, the potential for sexual reproduction appears to be low due to the absence of female-fertile isolates.

Introduction

Magnaporthe grisea (Hebert) Yaegashi & Udagawa (anamorph *Pyricularia grisea* (Cooke) Sacc.) is a pathogen of grasses belonging to 22 genera (8). Gray leaf spot, caused by *M. grisea*, is a disease of increasing importance in the turfgrass industry. This disease has been a chronic problem in St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) turf since it was first reported in 1957 (24). In 1971, a severe epidemic occurred in annual ryegrass (*Lolium multiflorum* Lam.) pastures in Louisiana and Mississippi (1,2), and localized epidemics continue to occur annually (35). Gray leaf spot of perennial ryegrass (*Lolium perenne* L.) was first detected in southeastern Pennsylvania in 1991 (21), and has since been observed throughout the United States (6,10,33,36,41). Gray leaf spot of tall fescue (*Festuca arundinacea* Schreb.) was first reported in 1996 (9) and occurs annually in the southeastern United States.

Turfgrass breeders have identified sources of resistance to *M. grisea* in annual ryegrass (34), perennial ryegrass (13), St. Augustinegrass (14), and tall fescue (9). The long-term stability of these resistance sources will depend on the mechanisms of resistance (25) and the genetic structure of the *M. grisea* population associated with each host (47). Genetic recombination through sexual reproduction is a primary determinant of pathogen population structure because of its role in generating new virulence profiles (26,28,46).

Magnaporthe grisea is a heterothallic ascomycete, requiring the interaction of two mating types, *Mat*1-1 and *Mat*1-2, in order to undergo sexual reproduction (46). While the absence of one mating type in a population precludes sexual reproduction, other barriers also exist, including female, male, or complete sterility (46) and other poorly understood compatibility factors (3,5,19,23,30).

Both mating types co-exist in the majority of *M. grisea* populations analyzed, although one mating type is typically dominant in a population (5,11,15,16,18,20,27,29,31,37,38,45). The majority of *M. grisea* isolates from rice are male fertile or completely sterile (7,11,15,18,27,29,31,37,38,45), but evidence of genetic recombination has been detected in some rice populations (5,20). The frequency of hermaphroditic isolates in *M. grisea* populations associated with other grasses is generally higher than in rice populations (15,16,37,38,43,44). In fact, the first report of sexual reproduction in *M. grisea* originated from a cross between isolates collected in the Southeastern United States from large crabgrass (*Digitaria sanguinalis* (L.) Scop.), a common weed in turfgrass swards (12). These results justify a study of the potential for sexual reproduction in turfgrass populations of *M. grisea*.

Little is known about the distribution of mating types and fertility status in turfgrass populations. A single isolate from meadow fescue (*Festuca eliator* L.) analyzed by Yaegashi (43) was sterile and its mating type was undetermined. Among seven isolates from St. Augustinegrass, two were male-fertile in controlled crosses and were mating type *Mat*1-1 (43). Viji et al. (40) found only mating type *Mat*1-2 in 194 isolates from 33 perennial ryegrass populations. Among 126 perennial ryegrass isolates from 37 locations, 71 were male-fertile in crosses with hermaphroditic tester strains, but the perithecia were barren (39). No studies of mating type distribution in *M. grisea* turfgrass populations in the Southeastern United States have been reported.

The objective of this research was to determine the potential for sexual reproduction in *M. grisea* turfgrass populations in Georgia. To facilitate mating type determination in *M. grisea*, a PCR-based assay was developed based on sequences of the mating type idiomorphs (17). The distribution of mating types in several tall fescue and St. Augustinegrass populations was determined, and the fertility status of selected

populations was assessed using controlled crosses in vitro with hermaphroditic tester strains and other turfgrass isolates.

Materials and Methods

Populations, collection, and isolation. Isolates of *M. grisea* were collected from St. Augustinegrass and tall fescue turf stands throughout Georgia in 1999 and 2000 (Fig. 2.1). Populations were selected to allow the separation of factors that may influence the distribution of mating types in turfgrass populations, such as geographic region, host species, and host cultivar (Table 2.1). Populations 1 through 6 were from sod production fields, whereas populations 7 and 8 were from a research farm with stands of tall fescue and St. Augustinegrass approximately 100 m apart. 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-m^2 area. The leaf samples were allowed to dry overnight in paper bags at room temperature (23 to 25º C), and then stored at 4º C for further use.

Leaf sections containing individual lesions were excised from dried symptomatic leaves, rinsed in sterile H_2O for 30 s, then surface disinfested in 70% ethanol for 30 s and 0.525% NaOCl for 30 s. The leaf sections were shaken dry and affixed with petroleum jelly to the inside periphery of the lid of a 90-mm petri dish containing 1.5% water agar. The leaf sections were incubated at room temperature (23 to 25º C) under continuous fluorescent illumination to induce sporulation of *M. grisea*. The lid of each plate was rotated 90º daily to facilitate even dispersal of conidia onto the agar surface. Isolates were obtained by transferring a single, germinating conidium to a fresh petri dish containing potato dextrose agar (PDA) amended with 50 mg/liter each of tetracycline, streptomycin, and chloramphenicol. Each culture was overlaid with several sterilized filter paper sections and incubated at 20º C. After 10 to 14 days of incubation, the infested filter paper sections were lifted from the agar surface, placed in small coin

envelopes, allowed to dry overnight at room temperature (23 to 25º C), then stored at –80º C. In addition, each isolate was transferred to a culture tube (16 × 100 mm) containing sterilized rye (*Secale cereale* L.) grains (2.75 g rye grain, 0.08 g $CaCO_3$, 5 ml H_20), incubated at 20º C for 10 to 14 days, then stored at –80º C.

Development of mating type assay. *Magnaporthe grisea* isolates of known and unknown mating type were provided by J.C. Correll (University of Arkansas, Fayetteville, AR), M.L. Farman (University of Kentucky, Lexington, KY), or were isolated from Georgia turfgrass populations (Table 2.2, Table 2.3). Each isolate was grown for 7 days at room temperature (23 to 25º C) in 2 ml potato dextrose broth. The mycelial suspension was transferred to a 1.5-ml microcentrifuge tube, harvested by centrifugation for 5 min at 13,200 rpm, and genomic DNA was extracted using the Easy-DNA Kit (Invitrogen Corp., Carlsbad, CA). Sequences of the mating type idiomorphs *Mat*1-1 and *Mat*1-2 were provided by S. Kang (Pennsylvania State University, University Park, PA). Potential 24-mer PCR primers with a T_m of 60º C were identified with the GCG Wisconsin Package (Genetics Computer Group, Madison, WI). Pairs of PCR primers were selected for each mating type idiomorph based on their product lengths, such that the PCR products could be easily differentiated in an agarose gel. Primers L1 (5'-ATGAGAGCCTCATCAACGGCAACG-3') and L2 (5'-ACAGGATGTAGGCATTCGCAGG AC-3') are complementary to the *Mat*1-1 idiomorph and yield a product of 552 bp. Primers T1 (5'-ACAAGGCAACCATCTTGGACCCTG-3') and T2 (5'-CCAAAACACCGAGTGCCATC AAGC-3') are complementary to the *Mat*1-2 idiomorph and yield a product of 390 bp. PCR reactions were 20 µl in volume and contained 50 ng of template DNA, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase, 200 nM of primers L1, L2, T1, and T2, and 0.2 mM of each dNTP. Thermal cycling conditions involved an initial denaturation step at 94º C for 1 min, followed by 30 cycles of 94º C for 30 s, 60º C for 1 min, and 72º C for 1 min, and a final extension step at

72º C for 1 min. PCR products were separated in a 1% agarose gel at 80 V for 1 h. Southern analysis was used to verify that the observed PCR products were homologous to the mating type idiomorphs. PCR products were transferred to Zeta-Probe Blotting Membrane (BioRad Laboratories, Hercules, CA) and hybridized with *Kpn*I/*Sal*I digested SK43 or *Kpn*I/*Hind*III digested SK44, which were labeled with [α-^{32}P]dCTP using the Decaprime II Kit (Ambion Inc., Austin, TX). Hybridization was conducted at 65º C for 16 h in a buffer containing 7% SDS, 1 mM EDTA, and 0.5 M $NaHPO_4$, followed by two 20-min washes in buffer containing 2X SSC and 0.2% SDS.

Determination of fertility status. One *M. grisea* population from tall fescue (Cherokee County, year 2000) and one from St. Augustinegrass (Lanier County, year 2000) were selected for determination of fertility status using controlled crosses in vitro. Each isolate was crossed with the hermaphroditic rice isolates 2539 (*Mat*1-1) and Guy11 (*Mat*1-2). In addition, each tall fescue isolate was crossed with two *Mat*1-1 isolates from tall fescue (1213-8 and 533-78), whereas each St. Augustinegrass isolate was crossed with a *Mat*1-2 tall fescue isolate (1213-30) and a *Mat*1-2 isolate from St. Augustinegrass (455-2).

Crosses were conducted using a modification of the three-point culture method described by Itoi et al. (15). Plates containing oatmeal agar [40 g single grain oatmeal (Gerber Products Co., Fremont, MI), 15 g Bacto Agar (Becton Dickinson Co, Sparks, MD) per liter H_2O] were divided into eight equal sectors, and plugs of PDA containing mycelium of the unknown and tester isolates were transferred to alternating sectors (Fig. 2.2). Cultures were incubated at 25º C under continuous fluorescent illumination, and monitored weekly for the presence of perithecia. When fully developed perithecia were observed, fecundity was measured by counting the number of perithecia per unit area along the interface between the tester and unknown isolates under a dissecting microscope. Perithecia were then excised from the culture and checked for the

presence of asci and ascospores microscopically. Each cross was conducted in 2 to 3 independent experiments, with 1 to 3 replicates per experiment. Positive control crosses between 2539 and Guy11 were included in each experiment.

Results

Development of mating type assay. PCR products of the expected size were produced from all isolates of known mating type (Fig. 2.3A). For unknown isolates, the PCR products were readily distinguished as corresponding to either *Mat*1-1 or *Mat*1-2 (Table 2.2). Southern analysis of the PCR products by hybridization with the cloned mating type idiomorphs demonstrated that the observed 552-bp fragments were homologous to *Mat*1-1 (Fig. 2.3B), whereas the observed 390-bp fragments were homologous to *Mat*1-2 (Fig. 2.3C). *Magnaporthe grisea* isolates from *Pennisetum glaucum*, *Pennisetum* sp., and some isolates from *Digitaria sanguinalis* consistently yielded no PCR products using the procedure described above (Table 2.3). Gradient PCR was used to determine if lower annealing temperatures would yield PCR products from these isolates. Annealing temperatures of approximately 45°C resulted in optimal amplification (data not shown). When 45ºC annealing temperatures were used, four distinct banding patterns were observed among the *Pennisetum* and *Digitaria* isolates (Fig. 2.4A). Southern analysis was performed as described above to detect homology between the PCR products and the mating type idiomorphs, but no hybridization was detected. When hybridization was conducted at 50ºC, the *Mat*1-1 idiomorph hybridized to bands of approximately 220 bp, 400 bp, and 450 bp from isolates 1206-13 and 1206-17, and to bands of approximately 400 bp and 450 bp from isolates PH55 and DsKY96-1 (Fig. 2.4B). No homologous sequences were detected by the *Mat*1-2 idiomorph in these isolates (Fig. 2.4C). The *Mat*1-2 idiomorph hybridized to bands of 300 bp from isolates 1100-11, 1100-14, 1211-05, and 1211-06 (Fig. 2.4C). The *Mat*1-1 idiomorph also

hybridized to bands of approximately 500 bp and 580 bp in isolates 1211-05 and 1211-06, but did not hybridize to bands from isolates 1100-11 or 1100-14 (Fig. 2.4B).

Mating type distribution in Georgia turfgrass populations. In 1999 and 2000, populations of *M. grisea* from St. Augustinegrass were predominantly *Mat*1-1 (97.6 to 100%), whereas populations from tall fescue were dominated by *Mat*1-2 isolates (94.3 to 100%) (Table 2.4). The opposite mating type was found in low frequency (0 to 5%) in some populations from St. Augustinegrass and tall fescue. In 1999, a single *Mat*1-2 isolate was obtained from St. Augustinegrass in Taylor county, but no *Mat*1-2 isolates were obtained from St. Augustinegrass in 2000. In populations from tall fescue, *Mat*1-1 isolates were obtained only from Laurens and Floyd counties in 1999, but only from Cherokee county in 2000.

Determination of fertility status. In positive control crosses between the hermaphroditic rice isolates 2539 and Guy11, fully developed perithecia containing asci and ascospores were observed approximately 4 weeks after inoculation (Fig. 2.2). In preliminary studies, isolates 1213-30 (tall fescue; *Mat*1-2) and 455-2 (St. Augustinegrass; *Mat*1-2) were male-fertile in crosses with 2539 (rice; *Mat*1-1). Isolates 1213-8 (tall fescue; *Mat*1-1) and 533-78 (tall fescue; *Mat*1-1) were sterile in crosses with Guy11 (rice; *Mat*1-2). Because the fertility of a cross depends on the interaction between both parental genotypes (3), isolates 1213-8 and 533-78 were included in the study even though they were sterile in crosses with rice isolates. Crosses among isolates that have the opportunity to interact in nature are expected to provide a more accurate estimate of the potential for sexual reproduction in natural poplations.

All St. Augustinegrass isolates from the 2000 Lakeland population failed to form perithecia in crosses with 2539, Guy11, 1213-30, and 455-2. All tall fescue isolates from the 2000 Canton population failed to form perithecia in crosses with Guy11, 1213-8, and 533-78, but the reaction of isolates in crosses with isolate 2539 was variable among a

total of seven replicates in three independent experiments. A total of 47 (54%) isolates were male-fertile in crosses with 2539 in at least one replicate, and 28 (32%) of these produced asci and ascospores in at least one replicate (Table 2.5). Fecundity, measured as the number of perithecia produced per unit area, was significantly lower in male-fertile tall fescue isolates than in the hermaphroditic tester isolates. An average of 15 perithecia cm^{-2} was produced in crosses between male fertile tall fescue isolates and isolate 2539, whereas 80 perithecia cm^{-2} were produced in crosses between isolate 2539 and Guy11 (data not shown).

Discussion

The PCR-based assay developed in this study presents several advantages over traditional methods for mating type determination, such as controlled crosses in vitro (15) and Southern hybridization with cloned mating type idiomorphs (4). Due to poorly understood incompatibility factors, the apparent fertility of an unknown isolate is dependent upon the tester strain utilized for in vitro crosses (3,5,19,23,30). Therefore, it is advantageous to determine mating type independent of fertility and compatibility factors using a molecular assay. Compared to Southern analysis, our PCR-based assay is rapid, inexpensive, and does not require the use of radioactive isotopes. The assay, however, is not equally effective for all *M. grisea* isolates, as some isolates from *Digitaria* and *Pennisetum* species consistently yielded no amplification products under standard conditions. It appears that the mating type idiomorphs in these isolates have diverged from those found in other *M. grisea* isolates. It may be necessary to develop a separate PCR assay specifically for these divergent isolates so that their mating types can be determined with confidence.

Each population of *M. grisea* from turfgrasses analyzed in this study was dominated by a single mating type. This is consistent with the majority of *M. grisea* populations

analyzed to date (5,11,15,16,18,20,27,29,31,37,38,45). Populations from St. Augustinegrass were predominantly *Mat*1-1 (97.6%), whereas populations from tall fescue were predominantly *Mat*1-2 (94.3%). The opposite mating type was found in low frequencies (0% to 5.7%) in association with each host, but was not found in the same population in two successive growing seasons. Based on these results, we conclude that selection due to host species influences mating type distribution, whereas host cultivar and geography do not. Other evolutionary forces such as migration, selection, or genetic drift may be responsible for variation among seasons.

The presence of opposite mating types in populations from tall fescue and St. Augustinegrass may indicate a low frequency of cross-infection among these hosts. If this were true, opposite mating types would be expected in greater abundance where tall fescue and St. Augustinegrass are grown in close proximity, but this was not the case in the Paulding County populations. Preliminary AFLP fingerprint data from turfgrass populations indicate that tall fescue and St. Augustinegrass isolates are distinct, but the *Mat*1-2 isolate (455-2) obtained from St. Augustinegrass is closely related to isolates from large crabgrass (Chapter 3). In greenhouse inoculation experiments, Malca and Owen (24) demonstrated cross-infection between crabgrass and St. Augustinegrass, but noted that virulence was significantly reduced on the host of non-origin.

The majority of turfgrass isolates tested for fertility status were completely sterile. The single *Mat*1-2 isolate from St. Augustinegrass was male fertile in crosses with isolate 2539. A total of 47 (54%) tall fescue isolates (all *Mat*1-2) were also male fertile in crosses with 2539, but 19 (22%) produced barren perithecia. Due to the absence of female-fertile isolates in turfgrass populations, no fertile crosses among turfgrass isolates were observed in this study. Although opposite mating types co-exist in some locations, the potential for sexual reproduction in Georgia turfgrass populations of *M. grisea* appears to be low.

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Literature Cited

1. Bain, D. C., Patel, B. N., and Patel, M. V. 1972. Blast of ryegrass in Mississippi. Plant Dis. Rep. 56:210.
2. Carver, R. B., Rush, M. C., and Lindberg, G. D. 1972. An epiphytotic of ryegrass blast in Louisiana. Plant Dis. Rep. 56:157-159.
3. Chao, C. T., and Ellingboe, A. H. 1991. Selection for mating competence in *Magnaporthe grisea* pathogenic to rice. Can. J. Bot. 69:2130-2134.
4. Correll, J. C., Harp, T. L., Guerber, J. C., Zeigler, R. S., Liu, B., Cartwright, R. D., and Lee, F. N. 2000. Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. Phytopathology 90:1396-1404.
5. Dayakar, B. V., Narayanan, N. N., and Gnanamanickam, S. S. 2000. Cross-compatibility and distribution of mating type alleles of the rice blast fungus *Magnaporthe grisea* in India. Plant Dis. 84:700-704.
6. Dernoeden, P. H. 1996. Perennial ryegrass and gray leaf spot. Golf Course Manage. 64:49-52.
7. Don, L. D., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Population structure of the rice blast pathogen in Vietnam. Ann. Phytopath. Soc. Japan 65:475-479.
8. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. The American Phytopathological Society, St. Paul, MN.
9. Fraser, M. L. 1996. Susceptibility of tall fescues to gray leaf spot, 1995. Biological and Cultural Tests 12:130.

10. 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.
11. Hayashi, N., Li, C., Li, J., Iwano, M., Naito, H., Yoshino, R., and Kato, H. 1997. Distribution of fertile *Magnaporthe grisea* fungus pathogenic to rice in Yunnan Province, China. Ann. Phytopath. Soc. Japan 63:316-323.
12. Hebert, T. T. 1970. The perfect stage of *Pyricularia grisea*. Phytopathology 61:83-87.
13. Hofmann, N. E., and Hamblin, A. M. 2000. Reaction of perennial ryegrass to gray leaf spot following inoculation in the greenhouse, 1999. Biological and Cultural Tests 16:55.
14. Holcomb, G. E. 1995. Reaction of St. Augustinegrass cultivars and selections to gray leaf spot, 1994. Biological and Cultural Tests 11:41.
15. Itoi, S., Mishima, T., Arase, S., and Nozu, M. 1983. Mating behavior of Japanese isolates of *Pyricularia oryzae*. Phytopathology 73:155-158.
16. Itoi, S., Yamamoto, J., Karino, S., Arase, S., and Kato, H. 1980. Hermaphroditic isolates of *Pyricularia* isolated from ragi, *Eleusine coracana* (L.) Gaertn. Ann. Phytopath. Soc. Japan 46:549-552.
17. Kang, S., Chumley, F. G., and Valent, B. 1994. Isolation of the mating-type genes of the phytopathogenic fungus *Magnaporthe grisea* using genomic subtraction. Genetics 138:289-196.
18. Kato, H., and Yamaguchi, T. 1982. The perfect state of *Pyricularia oryzae* CAV. in culture. Ann. Phytopath. Soc. Japan 48:607-612.
19. Kolmer, J. A., and Ellingboe, A. H. 1988. Genetic relationships between fertility and pathogenicity and virulence to rice in *Magnaporthe grisea*. Can. J. Bot. 66:891-897.
20. 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.
21. Landschoot, P. J., and Hoyland, B. F. 1992. Gray leaf spot of perennial ryegrass turf in Pennsylvania. Plant Dis. 76:1280-1282.
22. Leung, H., Borromeo, E. S., Bernado, M. A., and Notteghem, J. L. 1988. Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. Phytopathology 78:1227-1233.
23. Leung, H., and Williams, P. H. 1985. Genetic analysis of electrophoretic enzyme variants, mating type, and hermaphroditism in *Pyricularia oryzae* Cavara. Can. J. Genet. Cytol. 27:697-704.

24. Malca, I., and Owen, J. H. 1957. The gray leaf spot disease of St. Augustinegrass. Plant Dis. Rep. 41:871-875.
25. Marchetti, M. A. 1994. Race-specific and rate-reducing resistance to rice blast in US rice cultivars. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
26. McDonald, B. A., and McDermott, J. M. 1993. Population genetics of plant pathogenic fungi. BioScience 43:311-319.
27. Mekwatanakarn, P., Kositratana, W., Phromraksa, T., and Zeigler, R. S. 1999. Sexually fertile *Magnaporthe grisea* rice pathogens in Thailand. Plant Dis. 83:939-943.
28. Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Ann. Rev. Phytopath. 34:457-477.
29. Notteghem, J. L., and Silue, D. 1992. Distribution of the mating type alleles in *Magnaporthe grisea* populations pathogenic on rice. Phytopathology 82:421-424.
30. Orbach, M. J., Chumley, F. G., and Valent, B. 1996. Electrophoretic karyotypes of *Magnaporthe grisea* pathogens of diverse grasses. Mol. Plant-Microbe Interact. 9:261-271.
31. Priyadarisini, V. B., Viji, G., and Gnanamanickam, S. S. 1999. Mating type distribution, fertility and pathogenicity of rice isolates of *Magnaporthe grisea* in four rice growing regions of India. Indian Phytopath. 52:28-34.
32. Roumen, E., Levy, M., and Notteghem, J. L. 1997. Characterisation of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. Eur. J. Plant Pathol. 103:363-371.
33. 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.
34. Trevathan, L. E. 1982. Response of ryegrass plant introductions to artificial inoculation with *Pyricularia grisea* under greenhouse conditions. Plant Dis. 66:696-697.
35. Trevathan, L. E., Moss, M. A., and Blasingame, D. 1994. Ryegrass blast. Plant Dis. 78:113-117.
36. 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.

37. Urashima, A. S., Igarashi, S., and Kato, H. 1993. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Phytopathology 77:1211-1216.
38. Viji, G., and Gnanamanickman, S. S. 1998. Mating type distribution and fertility status of *Magnaporthe grisea* populations from various hosts in India. Plant Dis. 82:36-40.
39. Viji, G., and Uddin, W. 2001. Mating behavior of *Magnaporthe grisea* isolates causing gray leaf spot (blast) of perennial ryegrass turf. Phytopathology 91:S92.
40. 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.
41. Vincelli, P. 1999. Gray Leaf Spot: An emerging disease of perennial ryegrass. Turfgrass Trends 7:1-8.
42. Xia, J. Q., Correll, J. C., Lee, F. N., Marchetti, M. A., and Rhoads, D. D. 1993. DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) population in two rice fields in Arkansas. Phytopathology 83:1029-1035.
43. Yaegashi, H. 1977. On the sexuality of blast fungi, *Pyricularia* spp. Ann. Phytopath. Soc. Japan 43:432-439.
44. Yaegashi, H., and Nishihara, N. 1976. Production of the perfect stage in *Pyricularia* from cereals and grasses. Ann. Phytopath. Soc. Japan 42:511-515.
45. Yaegashi, H., and Yamada, M. 1986. Pathogenic race and mating type of *Pyricularia oryzae* from Soviet Union, China, Nepal, Thailand, Indonesia and Colombia. Ann. Phytopath. Soc. Japan 52:225-234.
46. Zeigler, R. S. 1998. Recombination in *Magnaporthe grisea*. Ann. Rev. Phytopath. 36:249-275.
47. 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.

Table 2.1. *Magnaporthe grisea* populations from Georgia sampled in 1999 and 2000 for analysis of mating type distribution

				1999		2000	
Population	**County**	**Host species**	**Cultivar**	**Date**	**N**	**Date(s)**	**N**
1	Lanier	St. Augustinegrass	Mercedes	7/13/99	65	7/18/00	78
2	Webster	St. Augustinegrass	Palmetto	7/14/99	55	7/17/00	79
3	Taylor	St. Augustinegrass	Palmetto	7/29/99	43	7/31/00	64
4	Laurens	Tall fescue	Rebel III	8/16/99	44	8/14/00, 8/24/00	85
5	Cherokee	Tall fescue	Rebel III	9/1/99	61	9/14/00	87
6	Floyd	Tall fescue	Confederate	9/1/99	66	9/14/00	70
7	Paulding	Tall fescue	Bonzai	8/13/99	10	9/7/00	68
8	Paulding	St. Augustinegrass	Raleigh	8/13/99	16	8/9/00	85

Table 2.2. Diverse collection of *Magnaporthe grisea* isolates successfully characterized for mating type using the PCR-based assay

Lane[v]	Isolate	Host	Origin	Year	Mating type
1	CD156	*Eleusine coracana*	Cote d'Ivoire	1992	*Mat* 1-1
2	JP29	*Eleusine coracana*	Japan	1991	*Mat* 1-1
3	RW18	*Eleusine coracana*	Rwanda	1991	*Mat* 1-1
4	533-78	*Festuca arundinacea*	Georgia, USA	1999	*Mat* 1-1
5	2539	n/a	lab strain	1988[w]	*Mat* 1-1[z]
6	75A1	*Oryza sativa*	Arkansas, USA	1975[x]	*Mat* 1-1[z]
7	FR13	*Oryza sativa*	France	1986[y]	*Mat* 1-1[z]
8	Fr9	*Oryza sativa*	France	1988[y]	*Mat* 1-1[z]
9	IN17	*Oryza sativa*	India	1991	*Mat* 1-1
10	IT2	*Oryza sativa*	Italy	1986[y]	*Mat* 1-1[z]
11	LO1-4	*Oryza sativa*	Arkansas, USA	1991[x]	*Mat* 1-1[z]
12	GF9	*Setaria faberii*	Kentucky, USA	1998	*Mat* 1-1[z]
13	SiKY97-1	*Setaria italica*	Kentucky, USA	unknown	*Mat* 1-1
14	GrF6	*Setaria viridis*	Kentucky, USA	1998	*Mat* 1-1[z]
15	YF2	*Setaria viridis*	Kentucky, USA	1998	*Mat* 1-1[z]
16	Pg470	*Stenotaphrum secudatum*	Georgia, USA	1999	*Mat* 1-1
17	Br32	*Triticum aestivum*	Brazil	1991	*Mat* 1-1
18	Br80	*Triticum aestivum*	Brazil	1991	*Mat* 1-1
19	EP1	unknown	unknown	1991	*Mat* 1-1
20	RN1	*Zingiber* sp.	unknown	1986	*Mat* 1-1
21	531-19	*Digitaria sanguinalis*	Georgia, USA	1999	*Mat* 1-2
22	PH42	*Eleusine coracana*	Philippines	1991	*Mat* 1-2
23	RW12	*Eleusine coracana*	Rwanda	1991	*Mat* 1-2
24	669	*Festuca arundinacea*	Georgia, USA	1999	*Mat* 1-2
25	LpKY97-1C	*Lolium perenne*	Kentucky, USA	1997	*Mat* 1-2[z]
26	GG9	*Lolium perenne*	Kentucky, USA	1997	*Mat* 1-2[z]
27	CR24	*Lolium perenne*	Korea	unknown	*Mat* 1-2
28	Guy11	*Oryza sativa*	French Guyana	1988[w]	*Mat* 1-2[z]
29	LO3-8	*Oryza sativa*	Arkansas, USA	1991[x]	*Mat* 1-2[z]
30	BM1-24	*Oryza sativa*	Arkansas, USA	1991[x]	*Mat* 1-2[z]
31	GrF5	*Setaria viridis*	Kentucky, USA	1998	*Mat* 1-2[z]
32	McC2	*Setaria viridis*	Kentucky, USA	1998	*Mat* 1-2[z]
33	ARC2	*Setaria viridis*	Kentucky, USA	1998	*Mat* 1-2[z]
34	GrF3	*Setaria viridis*	Kentucky, USA	1998	*Mat* 1-2[z]
35	455-2	*Stenotaphrum secundatum*	Georgia, USA	1999	*Mat* 1-2

[v] Lane assignment in agarose gel depicted in Figure 5.

[w] Leung et al., 1988 (22)

[x] Xia et al., 1993 (42)

[y] Roumen et al., 1997 (32)

[z] Mating type of isolates previously known.

Table 2.3. *Magnaporthe grisea* isolates that required reduced annealing temperatures in PCR reactions for successful amplification

Lane[x]	Isolate	Host	Location	Year	Mating type[y]
a	1100-11	*Digitaria sanguinalis*	Georgia, USA	2000	*Mat* 1-2
b	1100-14	*Digitaria sanguinalis*	Georgia, USA	2000	*Mat* 1-2
c	1211-05	*Pennisetum glaucum*	Georgia, USA	2000	nd[z]
d	1211-06	*Pennisetum glaucum*	Georgia, USA	2000	nd[z]
e	1206-13	*Digitaria sanguinalis*	Georgia, USA	2000	*Mat* 1-1
f	1206-17	*Digitaria sanguinalis*	Georgia, USA	2000	*Mat* 1-1
g	PH55	*Pennisetum* sp.	Philippines	1990	*Mat* 1-1
h	DsKY96-1	*Digitaria sanguinalis*	Kentucky, USA	1996	*Mat* 1-1

[x] Lane assignment in agarose gel depicted in Figure 2.4.

[y] Tentative mating type assignment based on Southern analysis.

[z] Mating type could not be determined.

Table 2.4. Distribution of mating types in *Magnaporthe grisea* populations from Georgia associated with St. Augustinegrass and tall fescue in 1999 and 2000

				1999		2000	
Population	**County**	**Host species**	**Cultivar**	***Mat* 1-1**	***Mat* 1-2**	***Mat* 1-1**	***Mat* 1-2**
1	Lanier	St. Augustinegrass	Mercedes	65	0	78	0
2	Webster	St. Augustinegrass	Palmetto	55	0	79	0
3	Taylor	St. Augustinegrass	Palmetto	42	1	64	0
4	Laurens	Tall fescue	Rebel III	2	42	0	85
5	Cherokee	Tall fescue	Rebel III	0	61	5	82
6	Floyd	Tall fescue	Confederate	3	63	0	70
7	Paulding	Tall fescue	Bonzai	0	10	0	68
8	Paulding	St. Augustinegrass	Raleigh	16	0	85	0

Table 2.5. Fertility status of *Magnaporthe grisea* isolates obtained from tall fescue in Cherokee County, GA in 2000

		Perithecia barren			Perithecia with asci		
Test isolate	**No reaction**	**Male fertile**	**Female fertile**	**Hermaph-roditic**	**Male fertile**	**Female fertile**	**Hermaph-roditic**
2539	40	19	0	0	28	0	0
Guy 11	87	0	0	0	0	0	0
1213-8	87	0	0	0	0	0	0
533-78	87	0	0	0	0	0	0

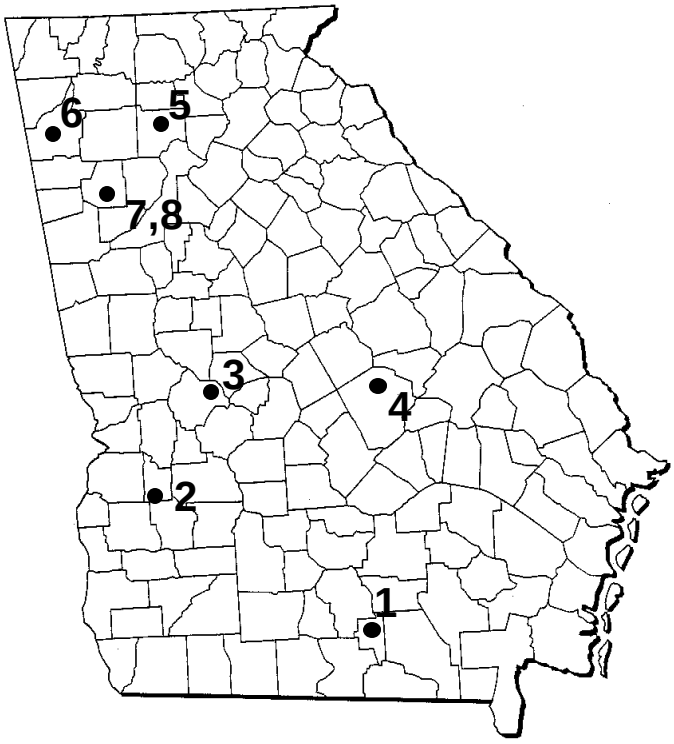

Figure 2.1. Location of *Magnaporthe grisea* populations sampled in 1999 and 2000 and analyzed for mating type distribution. See Table 2.1 for specific information on each population.

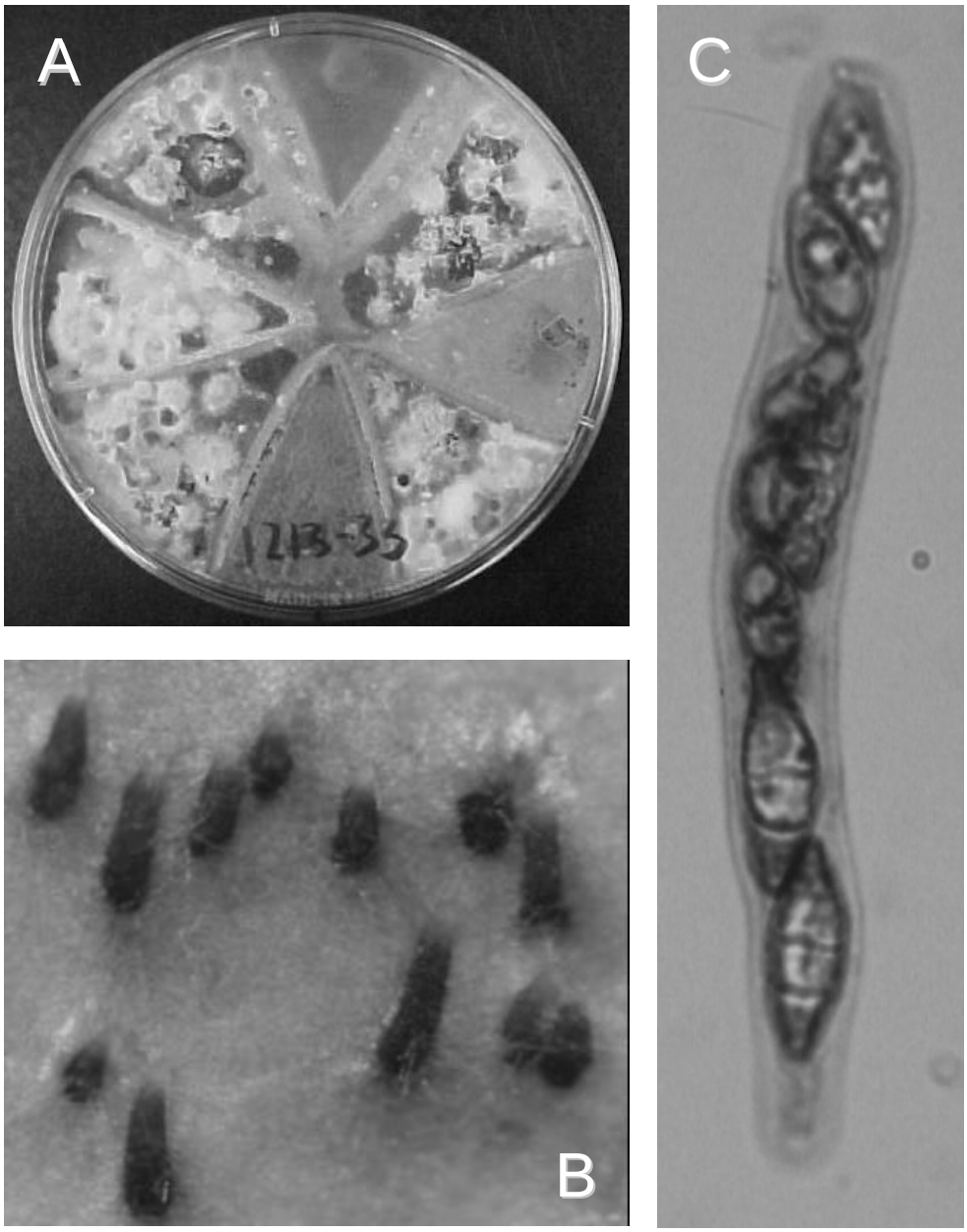

Figure 2.2. Determination of fertility status of *Magnaporthe grisea* isolates using a modification of the three-point culture method described by Itoi et al. (15); (A) Petri dishes containing oatmeal agar were divided into eight equal sectors, with unknown and tester isolates alternated among the eight sectors; (B) Fully developed perithecia were observed on the agar surface in fertile crosses after 4 weeks of incubation; (C) In positive control crosses, asci and ascospores were observed within perithecia.

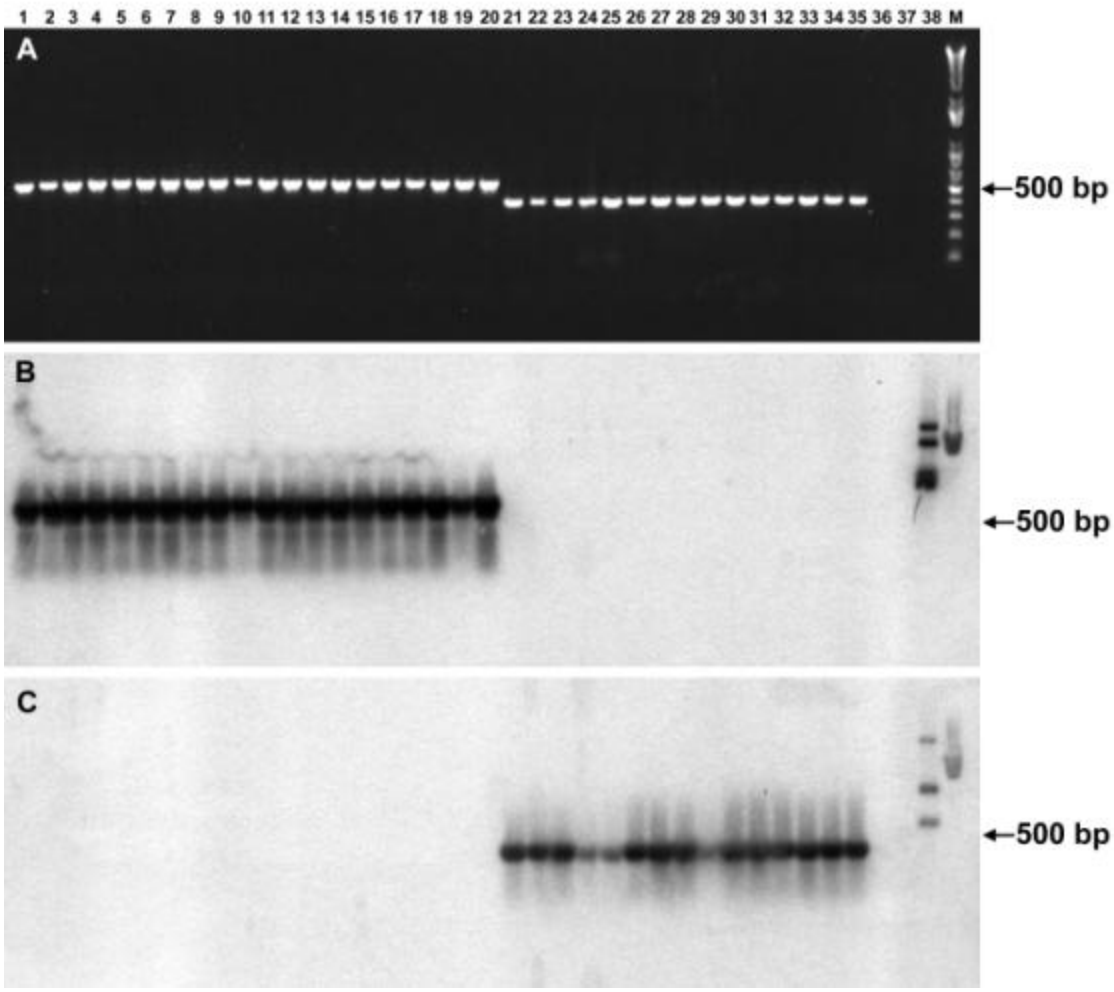

Figure 2.3. Application of the PCR-based mating type assay to a diverse collection of *Magnaporthe grisea* isolates of known and unknown mating type. Lanes 1-35, isolates listed in Table 2.1. Lanes 36 and 37, negative PCR controls containing no template. Lane 38, positive hybridization control containing the *Mat*1-1 idiomorph (SK43) digested with *Kpn*I/*Sal*I or the *Mat*1-2 idiomorph (SK44) digested with *Kpn*I/*Hind*III. Lane M, molecular size standard (100-bp ladder); (A) PCR products separated in a 1% agarose gel and stained with ethidium bromide; (B) Southern analysis of the PCR products in A using the cloned *Mat*1-1 idiomorph (SK43) as a hybridization probe; (C) Southern analysis of PCR products in A using the cloned *Mat*1-2 idiomorph (SK44) as a hybridization probe.

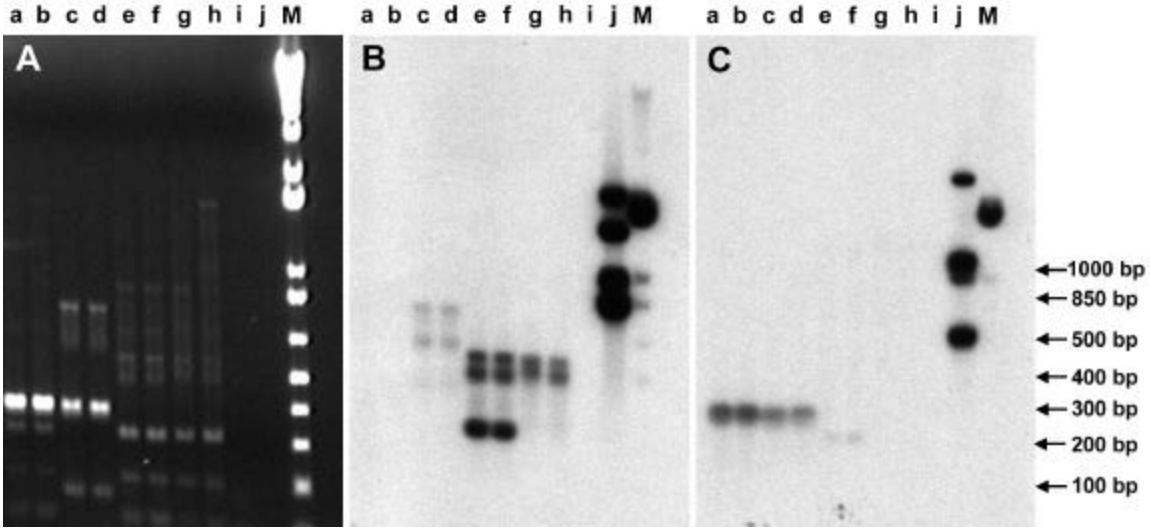

Figure 2.4. Analysis of *Magnaporthe grisea* isolates that yielded no PCR products under standard PCR conditions for the mating type assay. Lanes a-h, isolates of *M. grisea* listed in Table 2.3. Lane i, negative PCR control containing no template. Lane j, positive hybridization control containing digested SK43 or SK44. Lane M, molecular size standard (100-bp ladder); (A) PCR products from reactions with 45°C annealing temperature separated in a 1% agarose gel and stained with ethidium bromide; (B) Southern analysis of the PCR products in A using the cloned *Mat*1-1 idiomorph (SK43) as a hybridization probe; (C) Southern analysis of PCR products in A using the cloned *Mat*1-2 idiomorph (SK44) as a hybridization probe.

CHAPTER 3

GENETIC STRUCTURE OF *MAGNAPORTHE GRISEA* POPULATIONS ASSOCIATED WITH ST. AUGUSTINEGRASS AND TALL FESCUE IN GEORGIA[1]

[1]Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2002. To be submitted to Phytopathology.

ABSTRACT

Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2002. Genetic structure of *Magnaporthe grisea* populations associated with St. Augustinegrass and tall fescue in Georgia. To be submitted to Phytopathology.

The genetic structure of *Magnaporthe grisea* populations associated with St. Augustinegrass and tall fescue in Georgia was described using Amplified Fragment Length Polymorphisms (AFLPs). Compared to rice populations, molecular diversity was low in turfgrass populations, but diversity was generally higher in tall fescue populations than in St. Augustinegrass populations. All populations of *M. grisea* from turfgrasses were dominated by single clonal lineages, with other lineages present in low frequencies in some locations. Host species had a significant effect on population structure, such that there were no lineages common to St. Augustinegrass and tall fescue. Differences were observed among populations that could not be explained by host species, host cultivar, or geographic region. *M. grisea* populations associated with tall fescue and perennial ryegrass appear to have been established from a common source, but no alternative host for the dominant tall fescue lineage was identified. Weeping lovegrass was an alternative host for lineage E from tall fescue, and isolates from wheat were also closely related to lineages from tall fescue. Turfgrass populations of *M. grisea* may have been established from populations associated with weeping lovegrass or wheat. In growth chamber experiments, wheat cultivar 'Roberts' exhibited a hypersensitive response when inoculated with isolates from lineage G4, but other *M. grisea* isolates from tall fescue induced typical wheat blast symptoms. The clonal lineages associated with tall fescue in Georgia may correspond to distinct pathogenic races of *M. grisea*.

Introduction

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 limited to St. Augustinegrass (*Stenotaphrum secundatum*), a warm-season grass grown in subtropical and tropical climates (21). In 1971, a severe epidemic occurred on annual ryegrass (*Lolium multiflorum*) pastures in Mississippi and Louisiana (1,3). *M. grisea* was first reported as a pathogen of perennial ryegrass (*Lolium perenne*) 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 (4,11,26,29,32). 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 working to transfer this resistance into commercial cultivars.

M. grisea also causes blast disease of rice (*Oryza sativa* L.), which is one of the most important plant diseases worldwide (24). 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 (24). Recently, studies of *M. grisea* population structure and genetics have led to the development of novel methods for developing rice cultivars with durable blast resistance (37).

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. (30) 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 RFLPs.

M. grisea exhibits a clonal population structure in association with rice. The majority of *M. grisea* isolates from rice are male fertile or completely sterile, and populations are composed of one to several clonal lineages (17,36). Evidence collected to date indicates that *M. grisea* exhibits a similar population structure in association with turfgrasses. Viji et al. (30) identified 3 clonal lineages among 194 *M. grisea* isolates from perennial ryegrass in 33 locations across 4 states. Mating studies in vitro demonstrated that 56.3% of isolates from perennial ryegrass were male fertile, but no female fertile isolates were identified (31). In a tall fescue population from Georgia, 54% of *M. grisea* isolates from tall fescue were male fertile, but no isolates were female fertile (Chapter 2). A single isolate from St. Augustinegrass was male fertile, whereas all others were completely sterile (Chapter 2). Based on these results, the potential for sexual recombination in turfgrass populations of *M. grisea* appears to be low. The role of other evolutionary forces, such as selection, gene flow, and genetic drift, in shaping population structure has yet to be determined.

Restriction Fragment Length Polymorphisms (RFLPs) 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 (5,7,30) and are consequentially of limited value for analysis of turfgrass populations. A new technique, called Amplified Fragment Length Polymorphisms (AFLP), may be useful for analysis of population structure and genetics in plant pathogenic fungi (2,20,35). With the AFLP technique, a potentially unlimited number of molecular markers can be generated using multiple PCR primer combinations or sets of restriction enzymes (35). As discussed by Brown (2), AFLPs meet the criteria

for selection of molecular markers for population genetics, 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 estimate the genetic relationships among *M. grisea* isolates from diverse hosts, describe the genetic structure of *M. grisea* populations associated with turfgrass hosts in Georgia, and determine which evolutionary forces are important in shaping the observed population structure. *M. grisea* populations were selected to allow the separation of factors that may influence population structure, such as geographical region, host species, and host cultivar. AFLP markers were utilized to estimate genetic relationships among *M. grisea* isolates and conduct analyses of population structure and genetics.

Materials and Methods

Populations, collection, and isolation. Isolates of *M. grisea* were collected from St. Augustinegrass and tall fescue turf stands throughout Georgia in 1999 and 2000 (Fig. 3.1). Populations were selected to allow the separation of factors that may influence population structure, such as geographical region, host species, and host cultivar (Table 3.1). Populations 1 through 6 were from sod production fields, and populations 7 and 8 were from a research farm with stands of tall fescue and St. Augustinegrass approximately 100 m apart. 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-m^2 area. The leaf samples were allowed to dry overnight in paper bags at room temperature (23 to 25^{o}C),

and then stored at 4ºC for further use. Isolates were obtained and prepared for long-term storage as described in Chapter 2. A diverse collection of *M. grisea* isolates were included as standards for comparison (Table 3.2) and were provided by J. C. Correll (University of Arkansas, Fayetteville, AR), M. L. Farman (University of Kentucky, Lexington, KY), and B. B. Clarke (Rutgers University, New Brunswick, NJ). Crabgrass (*Digitaria sanguinalis*), pearl millet (*Pennisetum glaucum*), and weeping lovegrass (*Eragrostis curvula*) leaves exhibiting gray leaf spot symptoms were also collected in Georgia, and isolates were obtained as described above.

AFLP fingerprinting. Each isolate was grown for 7 days at room temperature (23 to 25ºC) in 2 ml of potato dextrose broth. The mycelial suspension was transferred to a 1.5-ml microcentrifuge tube, harvested by centrifugation for 5 min at 13,200 rpm, and genomic DNA was extracted using the Easy-DNA Kit (Invitrogen Corp., Carlsbad, CA). The DNA solutions 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.

AFLP reactions were conducted with AFLP Analysis System II (Life Technologies, Inc., Gaithersburg, MD), as described by Vos et al. (33), with modifications. Template DNA (125 ng) was digested with 1.25 U *Eco*RI, 1.25 U *Mse*I, and 2.5 µl 5X reaction buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 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 *Eco*RI and *Mse*I adapters were ligated to the sticky ends of template DNA in the same reaction tube with 0.5 U T4 DNA ligase and 12 µl adapter/ligation solution [*Eco*RI/*Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate] at 20°C for 2 h, then diluted 10X for use in preamplification reactions. Preamplification

reactions were performed with primers complimentary to the adapter sequences with no selective nucleotides. Diluted ligation reaction (2 µl) was added to 2 µl 10X PCR buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl], 1.5 mM $MgCl_2$, 1 U Taq DNA polymerase, 50 ng *Eco*RI primer, 50 ng *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 94ºC for 1 min, followed by 30 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min, and a final extension step at 72ºC for 1 min. The preamplification products were diluted 20X 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 PCR reactions (4-µl volume) contained 1 µl diluted preamplification product, 1.5 ng *Eco*RI primer, 9 ng *Mse*I primer, 0.15 U Taq DNA polymerase, 0.4 µl 10X PCR buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl], 1.5 mM $MgCl_2$, and 0.2 mM each dNTPs. Thermal cycling conditions involved an initial cycle of 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 of 94°C for 30 s, at 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 formamide, 0.30 µl GS-500 ROX internal size standard (Applied Biosystems Inc., Foster City, CA), and 0.35 µl loading dye (Applied Biosystems Inc., Foster City, CA). The mixture was denatured at 95ºC for 5 min, then separated in a 5% polyacrylamide gel on a Perkin-Elmer ABI Prism 377 DNA

sequencer (Applied Biosystems Inc., Foster City, CA). The molecular weight of amplified restriction fragments was determined in Genescan v. 3.1 (Applied Biosystems Inc., Foster City, CA) by comparison to the GS-500 ROX internal size standard. Restriction fragments were scored in Genotyper v. 2.5 (Applied Biosystems Inc., Foster City, CA) by visual examination and recorded as present or absent for each taxa. Each marker was named using its molecular weight in base pairs and the *Eco*RI primer which amplified the marker. For example, the designation '281AA' denotes a 281 bp marker amplified by primer E-AA. Preamplification 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 assumed to represent background contamination and excluded from further analysis. The mating type of each isolate was determined using the PCR-based assay as described in Chapter 2.

Phylogenetic relationships among *M. grisea* isolates from diverse hosts. Dice Similarity Coefficients (D_s) were calculated for pairwise comparisons among all isolates in Windist (34), 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 (34) based on 1000 random samplings of the data set.

Genetic structure of *M. grisea* populations from turfgrass hosts in Georgia. The Genetic Diversity Index (GDI) and Haplotype Diversity Index (HDI) in each population were measured with the Shannon Information Statistic, calculated using the formula $h_0=-\Sigma p_i \ln p_i$, where p_i is equal to the frequency of the i^{th} allele (for calculation of GDI) or multilocus genotype (for calculation of HDI) (10). GDI and HDI were 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).

Measurement of selection and recombination in *M. grisea* populations. The analysis of molecular variance (AMOVA) procedure (6) was used to compare groups of *M. grisea* populations differing in certain characteristics (host species, host cultivar, and region) and test the significance of those characteristics as selective forces. AMOVA calculations were conducted in Arlequin v. 2.0 (25). 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 covariance (V_T). Non-parametric permutation of the data set was used to determine probability values and test the significance of each covariance component (6).

Populations of *M. grisea* were tested for evidence of linkage disequilibrium using two-locus gametic disequilibrium (18) and the Index of Association (22). Linkage disequilibrium tests were conducted on uncensored (containing all individuals) and censored (containing one representative of each haplotype) data sets to correct for "epidemic" population structure as discussed by Maynard-Smith et al. (22) and Milgroom (23). Two-locus gametic disequilibrium was calculated for all pairs of loci in each population in Arlequin v. 2.0 (25), using the formula $D_{ij}=p_{ij}-p_i p_j$, where p_{ij} is the frequency of the haplotype having alleles i and j, p_i is the frequency of allele i, and p_j is the frequency of allele j. The standardized statistic $D`_{ij}$ was obtained by dividing by the maximum value possible for $D_{ij}$, given the allele frequencies. Significant values of $D`_{ij}$ were detected using Fisher's exact probability test on contingency tables (27). The Index of Association (I_a) was calculated as described by Maynard-Smith et al. (22) using the formula $I_a=(V_{obs}/V_{exp})-1$, where V_{obs} is the observed variance in genetic distance

among individuals, and V_{exp} is the variance expected if the population is randomly mating. A null distribution was created by random sampling of the data set for each population and used to test for significant departure of I_a from 0 (random mating).

Host range of selected *M. grisea* isolates. The host range of *M. grisea* isolates 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 (*Digitaria 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 into 946.4-cm^3 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 4 times per day to encourage rapid germination. After establishment, the grass was irrigated to field capacity twice per day and fertilized weekly with 1.3 g N/m^2 with Peters 20-10-20 (Scotts-Sierra Horticultural Products Co., Marysville, OH). Turfgrass species were cut to a height of 7 cm weekly, whereas wheat and weeping lovegrass were left uncut.

One to four *M. grisea* isolates representing each clonal lineage associated with turfgrasses in Georgia were arbitrarily selected. When possible, isolates representing multiple *M. grisea* populations or haplotypes within a lineage were selected. Isolates from wheat and weeping lovegrass were also included due to their similarity to tall fescue isolates according to AFLP markers. The isolates were stored at –80ºC on sterilized rye (*Secale cereale* L.) as described in Chapter 2. Isolates were revived by placing three to five infested grains on the surface of a 90-mm petri dish containing potato dextrose agar (PDA), amended with 50 mg/liter each of tetracycline, streptomycin, and chloramphenicol. After 1 week of incubation at 20ºC with no light,

each isolate was transferred to five 90-mm petri dishes containing 1.5% water agar. The agar surface was overlaid with 15 to 20 sterilized alfalfa stem sections (6 cm long) with leaves and petioles removed. The cultures were incubated at room temperature (23 to 25°C) with continuous fluorescent illumination. After 3 weeks, conidia were harvested by transferring the alfalfa stem sections to 50-ml conical centrifuge tubes containing 20 ml H_2O, vortexing for 10 sec, then filtering the suspension through a single layer of cheesecloth. The conidial suspension was adjusted to a concentration of 2×10^5 conidia ml^{-1} using a hemacytometer.

The turfgrass species were inoculated 10 to 12 weeks after seeding, whereas wheat and weeping lovegrass were inoculated 6 weeks after seeding. The pots were arranged in plastic containers (41.2 × 28.7 × 25.3 cm; Rubbermaid, Wooster, OH), which had a single 6.35-mm diameter hole at a height of 2.54 cm to permit water drainage. The grass in each pot was inoculated with 5 ml conidial suspension from a single *M. grisea* isolate applied with an air-brush (Model 350, Badger Air-Brush Co., Franklin Park, IL) powered with compressed CO_2 at 2.2 kg m^{-2}. Immediately after inoculation, the plastic containers were covered and transferred to a growth chamber and arranged in a randomized complete block with three replications. Incubation conditions for the first 24 h were 24°C, 100% RH, and no light. Subsequently, the containers were uncovered, and the growth chamber was programmed for 12-h days at 30°C and 75% RH and 12-h nights at 24°C and 100% RH. An auxiliary humidifier (Herrmidifier 500, Trion Inc., Sanford, NC) was used to maintain 100% RH in the growth chamber during the initial incubation and each night cycle. The plants were observed for symptoms induced by *M. grisea* 7 days after inoculation and the isolates were rated as pathogenic or non-pathogenic for each replication. Each isolate-host combination was repeated in two independent experiments, with 3 replications per experiment. Non-inoculated 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 947 *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. Phylogenetic analysis revealed the presence of 12 AFLP lineages, designated A-L (Fig. 3.2). Significant bootstrap support values, ranging from 84 to 100, were observed for most lineages, but not for lineages I and L. Similarities among groups of lineages were evident in the phylogram. Lineages A-H form one such group, joined by a single node at the 83% similarity level. Within the lineage A-H group, two smaller groups of lineages were also observed. Lineages A-D are joined by a single node at the 91% similarity level, whereas lineages E-G are joined by a single node at the 89% similarity level. Lineage G was composed of 4 distinct groups of isolates, designated G1-G4. Compared to groups G2-G4, group G1 is characterized by absence of markers of 121AA and 202AA and presence of markers of 380AA, 456AA, and 205TA. Compared to other groups in lineage G, group G2 is characterized by presence of markers 154AA, 274AA, and 274TA and absence of marker 272TA. Group G3 is unique from other groups due to the presence of 87AA, 308AA, 353AA, and 157AC. Because these groups of isolates are characterized by several unique molecular markers, they are hereafter referred to as distinct lineages.

Each AFLP lineage identified in the collection was associated with a single host or small number of hosts (Table 3.3). Ten 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. All *Mat*1-1 isolates from tall fescue were contained within lineage G1, whereas the *Mat*1-2 isolates from tall fescue were contained within lineages E, G2, G4, and H.

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 2 AFLP lineages and between 2 and 4 haplotypes (Table 3.4). The GDI ranged from 0.00 to 1.13, and the HDI ranged from 0.00 to 0.18. For St. Augustinegrass, molecular variation was only detected in the Taylor population in 1999, which was characterized by the highest GDI (1.13) and a low HDI (0.03). This is indicative of a population dominated by a single haplotype (low HDI), but containing other distantly related haplotypes (high GDI). For tall fescue, the highest GDI and HDI in 1999 were observed in the Cherokee population, however, the Laurens and Floyd populations also contained 2 lineages and 4 haplotypes. Similar results were obtained in 2000, but some differences were noted (Table 3.4). The Lanier, Webster, Laurens, Floyd, and Paulding-SA populations were comprised of the same number of lineages in 1999 and 2000. One additional lineage was found in the Paulding-TF population, 3 additional lineages were found in the Cherokee population, and only 1 lineage was found in the Taylor population in 2000. The GDI and HDI were highest in the Cherokee population in 2000, which was composed of 5 AFLP lineages and 10 distinct haplotypes.

All populations of *M. grisea* sampled from Georgia turfgrasses were dominated by single AFLP lineages (Table 3.5). All isolates from St. Augustinegrass populations belonged to lineage C, except a single isolate (455-2) from the Taylor population in 1999, which was placed in lineage K. According to AFLP markers, isolate 455-2 was identical to a haplotype commonly isolated from large crabgrass in Georgia. No lineage

K isolates were obtained from St. Augustinegrass in 2000, even though levels of crabgrass infestation were higher at the Lanier, Webster, and Taylor locations than in 1999 (unpublished observations). However, one isolate (1203-2) obtained 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 in contrast to St. Augustinegrass, most tall fescue populations also contained other lineages. 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.

Measurement of selection and recombination in *M. grisea* populations. 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 3.6). 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 geographical region or cultivar were compared, differences within populations accounted for 89.55% to 100% of the genetic variation. Differences among groups were not significant for region or cultivar in either tall fescue or St. Augustinegrass.

In 1999, two-locus gametic disequilibrium and I_A could not be determined for the Lanier, Webster, Paulding-TF, and Paulding-SA populations because no polymorphic AFLP markers were detected in these populations (Table 3.7). For the other populations, between 46.4% and 100% of loci pairs were linked in uncensored data sets.

I_a ranged from 3.40 to 77.12 in uncensored data sets and was significantly greater than 0 in all cases, rejecting the null hypothesis of random mating. For censored data sets, no loci pairs were linked, and I_a was significantly greater than 0 only for the Cherokee population. Because calculation of I_a requires three or more individuals, this parameter could not be calculated for the Taylor population with clonal censoring.

In 2000, between 48.3% and 100% of loci pairs were linked in uncensored data sets (Table 3.8). In uncensored data sets, I_a was significantly greater than 0 in the Laurens, Cherokee, Floyd, and Paulding-TF populations, but not the Lanier population. For censored data sets, evidence of gametic disequilibrium was only detected in the Cherokee population, for which 27.6% of loci pairs were linked and I_a was significantly greater than 0.

Host range of selected *M. grisea* isolates. Isolates within AFLP lineages exhibited similar host ranges (Table 3.9). All *M. grisea* isolates were pathogenic to their host of origin, with the exception 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, and tall fescue. These isolates also induced symptoms on Kentucky bluegrass, which is not known to be a host of *M. grisea* in nature. However, the virulence of *M. grisea* isolates on Kentucky bluegrass was 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 (Fig. 3.3). In addition, secondary inoculum was not produced in visible quantities on Kentucky bluegrass as it was on other hosts (data not shown). The majority of 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. 3.4). Isolates from lineage G4 were weakly virulent to wheat, or non-pathogenic, inducing a hypersensitive response (Fig. 3.5), but were pathogenic to weeping lovegrass. All isolates from lineages E, F, G1, G2, G4, and H

were non-pathogenic to crabgrass, but several were weakly virulent to St. Augustinegrass (Fig. 3.6). All isolates from lineage C were pathogenic to St. Augustinegrass, most were also weakly virulent to annual ryegrass, and one was weakly virulent to weeping lovegrass. Isolates from lineage K were pathogenic to crabgrass and annual ryegrass, and one isolate was pathogenic to weeping lovegrass. Lineage K isolates were also weakly virulent to perennial ryegrass and tall fescue in some cases.

Discussion

The phylogenetic relationships among *M. grisea* isolates estimated from AFLP markers in this study were similar to those previously estimated from ITS sequences (15) and transposon-detected RFLPs (5,15). Lineages A-H in this study correspond to groups r1-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. Twelve lineages of isolates with significant bootstrap support were identified by AFLP markers, and each clonal lineage was associated with a discrete group of host species. Within lineage G, four additional groups of isolates with distinct AFLP fingerprint profiles were identified that may represent additional lineages of the pathogen.

Molecular diversity was low in populations of *M. grisea* from turfgrasses in Georgia. The HDI ranged from 0.00 to 0.29 in turfgrass populations, considerably lower than the range of 0.89 to 0.92 observed in populations from rice in the Indian Himalayas (13). No reports of GDI in *M. grisea* populations are available in the literature for comparison. In general, genetic and haplotypic diversity was lower in populations from St. Augustinegrass than in populations from tall fescue. This result was unexpected, since *M. grisea* has been associated with St. Augustinegrass in the United States much longer than with tall fescue. Host diversity, rather than time, may influence the diversity in *M.*

grisea populations. Tall fescue is an outbreeding species and cultivars 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 higher genetic diversity in tall fescue cultivars may favor increased genetic diversity in the pathogen population.

Host species appears to be the dominant selection force influencing *M. grisea* population structure in turfgrasses. All populations of *M. grisea* sampled 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 appear to occur in nature. Host range studies demonstrated that some *M. grisea* isolates from tall fescue infect and induce symptoms on St. Augustinegrass, but the virulence of these isolates on St. Augustinegrass was very low. 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 could not account for this genetic variation according to AMOVA. Other selection forces, gene flow, genetic drift, or founder effects may be responsible for the differences observed among populations and among growing seasons. Further research is required to investigate the role of these evolutionary forces.

The results of this study illustrate the weaknesses of methods for measuring gametic disequilibrium discussed by Milgroom (23). In many cases, the appearance of gametic disequilibrium is the result of an “epidemic” population structure, where a small number of progeny from a sexual cross proliferate asexually, masking the occurrence of recombination. To correct for this, data sets may be censored to include only a single representative of each haplotype, as recommended by Maynard-Smith et al. (22). However, clonal censoring may drastically reduce the sample size and, consequently,

the power of significance tests for gametic disequilibrium. In this study, a large proportion of loci pairs were linked and I_a was significantly greater than 0 in most uncensored data sets. However, in most censored data sets, no evidence of gametic disequilibrium was detected. The exception was the Cherokee County tall fescue population in 2000. This population had the highest genetic and haplotype diversity in the study, and therefore provides the highest power for detection of gametic disequilibrium. A large percentage of loci pairs were linked, and I_a was significantly greater than 0, in both uncensored and censored data sets. Based on the evidence available, we conclude that sexual recombination is not an important factor in turfgrass populations of *M. grisea*. The use of a larger number of isolates from *M. grisea* turfgrass populations, or more AFLP primer combinations, may reveal more genotypes and enable more powerful tests for gametic disequilibrium.

Alternative hosts for the dominant lineages associated with tall fescue and St. Augustinegrass were not identified in this study, so the origin of these *M. grisea* strains remains unknown. A single lineage C isolate was obtained from large crabgrass, and a single lineage K isolate was obtained from St. Augustinegrass; therefore, a low frequency of cross-infection among these hosts may occur in nature. We were unable to reproduce cross-infection among large crabgrass and St. Augustinegrass in growth chamber experiments, so these isolates may represent opportunistic infection of wounds or damaged tissue rather than true pathogenic relationships. All *M. grisea* isolates from perennial ryegrass were placed in lineage G4, except for one isolate which was placed in lineage G3. The *M. grisea* populations associated with tall fescue and perennial ryegrass appear to have been established from a common source.

Weeping lovegrass was confirmed to be an alternative host of one *M. grisea* lineage associated with tall fescue. Isolates belonging to lineage E were obtained from both tall fescue and weeping lovegrass, and the majority of isolates in this group were

indistinguishable by AFLP markers. All lineage E isolates tested were highly virulent to both tall fescue and weeping lovegrass. In addition, isolates from all other tall fescue lineages of *M. grisea* were also pathogenic to weeping lovegrass. Weeping lovegrass was introduced to the United States from Africa in 1927 (19), and has been established on more than 0.4 million ha since 1985 as part of the Conservation Reserve Program administered by the United States Department of Agriculture (28). The introduction of weeping lovegrass and its increased use in recent years may be involved in the sudden appearance of gray leaf spot in tall fescue and perennial ryegrass. Isolates of *M. grisea* obtained from wheat in Brazil were also closely related to, but distinct from, isolates from tall fescue. The genetic structure of *M. grisea* populations associated with weeping lovegrass and wheat must be characterized in order to determine if these grasses are hosts of lineages G1, G2, G3, G4, or H.

Based on the results of this study, we conclude that *Magnaporthe grisea* populations associated with turfgrasses are structured similarly to those associated with rice. The absence of female fertile isolates in *M. grisea* turfgrass populations indicates that the potential for sexual recombination is low (Chapter 2), and evidence of gametic disequilibrium was detected in AFLP marker data in this study. The distinct groups of *M. grisea* isolates associated with turfgrasses 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 cultivar 'Roberts' suggests that this may be the case. Additional research is needed to determine the relationship between clonal lineage and race in turfgrass populations because of its important implications to management of *M. grisea* with resistant cultivars.

Literature Cited

1. Bain, D. C., Patel, B. N., and Patel, M. V. 1972. Blast of ryegrass in Mississippi. Plant Dis. Rep. 56:210.
2. Brown, J. K. M. 1996. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol. 133:183-195.
3. Carver, R. B., Rush, M. C., and Lindberg, G. D. 1972. An epiphytotic of ryegrass blast in Louisiana. Plant Dis. Rep. 56:157-159.
4. Dernoeden, P. H. 1996. Perennial ryegrass and gray leaf spot. Golf Course Manage. 64:49-52.
5. 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.
6. 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.
7. 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.
8. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.
9. Fraser, M. L. 1996. Susceptibility of tall fescues to gray leaf spot, 1995. Biological and Cultural Tests 12:130.
10. 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.

11. 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.
12. Hofmann, N. E., and Hamblin, A. M. 2000. Reaction of perennial ryegrass to gray leaf spot following inoculation in the greenhouse, 1999. Biological and Cultural Tests 16:55.
13. 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.
14. Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: Molecular Evolutionary Genetics Analysis, Version 1.01. The Pennsylvania State University, University Park, PA.
15. 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. Phytopath. Soc. Japan 65:588-596.
16. Landschoot, P. J., and Hoyland, B. F. 1992. Gray leaf spot of perennial ryegrass turf in Pennsylvania. Plant Dis. 76:1280-1282.
17. 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.
18. Lewontin, R. C. 1988. On measures of gametic disequilibrium. Genetics 120:849-852.
19. Magness, J. R., Markle, G. M., and Compton, C. C. 1971. Food and feed crops of the United States. Interregional Research Project IR-4, Bul. 828. New Jersey Agricultural Experiment Station, New Brunswick, NJ.
20. 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.

21. Malca, I., and Owen, J. H. 1957. The gray leaf spot disease of St. Augustinegrass. Plant Dis. Rep. 41:871-875.
22. Maynard-Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G. 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. 90:4384-4388.
23. Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Ann. Rev. Phytopathol. 34:457-477.
24. Ou, S. H. 1980. A look at worldwide rice blast control. Plant Dis. 64:439-445.
25. Schneider, S., Roessli, D., and Excoffier, L. 2000. Arlequin Version 2.0: A Software for Population Genetics Data Analysis. University of Geneva, Geneva, Switzerland.
26. 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.
27. Slatkin, M. 1994. Linkage disequilibrium in growing and stable populations. Genetics 137:331-336.
28. 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.
29. 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.
30. 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.
31. Viji, G., and Uddin, W. 2001. Mating behavior of *Magnaporthe grisea* isolates causing gray leaf spot (blast) of perennial ryegrass turf. Phytopathology 91:S92.
32. Vincelli, P. 1999. Gray leaf spot: An emerging disease of perennial ryegrass. Turfgrass Trends 7:1-8.

33. Vos, P., Hogers, R., Bleeker, M., Reijans, 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.

34. 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.

35. Zeigler, R. S. 1998. Recombination in *Magnaporthe grisea*. Ann. Rev. Phytopathol. 36:249-275.

36. 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 resisance breeding. Pages 267-292 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.

Table 3.1. *Magnaporthe grisea* populations from Georgia sampled in 1999 and 2000 for analysis of mating type distribution

				1999		2000	
Population	**County**	**Host species**	**Cultivar**	**Date**	**N**	**Date(s)**	**N**
1	Lanier	St. Augustinegrass	Mercedes	7/13/99	65	7/18/00	78
2	Webster	St. Augustinegrass	Palmetto	7/14/99	55	7/17/00	79
3	Taylor	St. Augustinegrass	Palmetto	7/29/99	43	7/31/00	64
4	Laurens	Tall fescue	Rebel III	8/16/99	44	8/14/00, 8/24/00	85
5	Cherokee	Tall fescue	Rebel III	9/1/99	61	9/14/00	87
6	Floyd	Tall fescue	Confederate	9/1/99	66	9/14/00	70
7	Paulding	Tall fescue	Bonzai	8/13/99	10	9/7/00	68
8	Paulding	St. Augustinegrass	Raleigh	8/13/99	16	8/9/00	85

Table 3.2. Host and geographic origin of *Magnaporthe grisea* isolates analyzed with AFLP markers for comparison to Georgia turfgrass isolates

Host	Common name	Isolates (#)	Origin(s)
Oryza sativa	rice	9	Arkansas, Cote d'Ivoire, France, French Guiana, India
Digitaria sanguinalis	large crabgrass	58	Georgia, Kentucky
Cyperus sp.	sedge	2	Philippines
Eleusine coracana	finger millet	5	Cote d'Ivoire, Japan, Philippines, Rowanda
Eragrostis curvula	weeping lovegrass	6	Georgia
Festuca arundinacea	tall fescue	429	Georgia
Pennisetum glaucum	pearl millet	2	Georgia
Pennisetum sp.	n/a	1	Philippines
Lolium perenne	perennial ryegrass	11	Kentucky, Maryland, New Jersey, Pennsylvania, Virginia
Setaria faberii	giant foxtail	1	Kentucky
Setaria italica	foxtail millet	1	Kentucky
Setaria viridis	yellow foxtail	8	Kentucky
Stenotaphrum secundatum	St. Augustinegrass	411	Georgia, Florida
Triticum aestivum	wheat	2	Brazil
Zingiber sp.	ginger	1	unknown

Table 3.3. Host origin and mating type of *Magnaporthe grisea* isolates belonging to lineages identified with AFLP markers

AFLP lineage	Host(s)	Mating type(s)
A	rice	*Mat* 1-1, *Mat* 1-2
B	giant foxtail, foxtail millet, yellow foxtail	*Mat* 1-1, *Mat* 1-2
C	St. Augustinegrass, large crabgrass	*Mat* 1-1
D	finger millet	*Mat* 1-1, *Mat* 1-2
E	weeping lovegrass, tall fescue	*Mat* 1-2
F	wheat	*Mat* 1-1
G1	tall fescue	*Mat* 1-1
G2	tall fescue	*Mat* 1-2
G3	perennial ryegrass	*Mat* 1-2
G4	tall fescue, perennial ryegrass	*Mat* 1-2
H	tall fescue	*Mat* 1-2
I	ginger	*Mat* 1-1
J	pearl millet	nd[x]
K	large crabgrass, St. Augustinegrass, *Pennisetum* sp.	*Mat* 1-1, *Mat* 1-2
L	sedge	nd

[x] Mating type not determined.

Table 3.4. Measures of diversity in populations of *Magnaporthe grisea* associated with tall fescue and St. Augustinegrass in Georgia

				1999					2000				
Population	**County**	**Host**	**Cultivar**	**N[t]**	**L[u]**	**H[v]**	**GDI[w]**	**HDI[x]**	**N**	**L**	**H**	**GDI**	**HDI**
1	Lanier	SA[y]	Mercedes	56	1	1	0.00	0.00	36	1	2	0.05	0.04
2	Webster	SA	Palmetto	50	1	1	0.00	0.00	72	1	1	0.00	0.00
3	Taylor	SA	Palmetto	37	2	2	1.13	0.03	54	1	2	0.01	0.04
4	Laurens	TF[z]	Rebel III	34	2	3	0.18	0.08	80	2	3	0.17	0.06
5	Cherokee	TF	Rebel III	45	2	4	0.62	0.18	76	5	10	1.74	0.29
6	Floyd	TF	Confederate	55	2	4	0.22	0.11	61	2	2	0.12	0.04
7	Paulding	TF	Bonzai	11	1	1	0.00	0.00	66	2	2	0.16	0.04
8	Paulding	SA	Raleigh	15	1	1	0.00	0.00	82	1	1	0.00	0.00

[t] Number of isolates.

[u] Number of lineages idenfied by AFLP markers.

[v] Number of haplotypes idenfied by AFLP markers.

[w] Genetic Diversity Index as determined by Shannon's information statistic formula.

[x] Haplotypic Diversity Index as determined by Shannon's information statistic formula.

[y] St. Augustinegrass (*Stenotaphrum secundatum*).

[z] Tall fescue (*Festuca arundinacea*).

Table 3.5. Frequency of AFLP lineages in populations of *Magnaporthe grisea* associated with tall fescue and St. Augustinegrass in Georgia

				1999							2000						
Population	**County**	**Host**	**Cultivar**	**C**	**E**	**G1**	**G2**	**G4**	**H**	**K**	**C**	**E**	**G1**	**G2**	**G4**	**H**	**K**
1	Lanier	SA[x]	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[y]	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	--	--	--	--	--	--

[x] St. Augustinegrass (*Stenotaphrum secundatum*).

[y] Tall fescue (*Festuca arundinacea*).

Table 3.6. Summary statistics from analysis of molecular variance (AMOVA) used to test the significance of potential selection factors in Georgia turfgrass populations of *Magnaporthe grisea*

		1999						2000					
		Among groups		Within groups		Within populations		Among groups		Within groups		Within populations	
Selection factor	Groups[v]	V_a/V_T (%)[w]	*P* value[x]	V_b/V_T (%)	*P* value	V_c/V_T (%)	*P* value	V_a/V_T (%)	*P* value	V_b/V_T (%)	*P* value	V_c/V_T (%)	*P* 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[y]	(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[z]	(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

[v] Groups of populations compared with AMOVA to test significance of selection factors. Population numbers are from Table 3.1.

[w] Percentage of genetic variation partitioned into components (among groups, within groups, and within populations) by AMOVA.

[x] *P* values for covariance components obtained by comparison to random data sets created by non-parametric permutations.

[y] St. Augustinegrass

[z] Tall fescue

Table 3.7. Gametic linkage disequilibrium in populations of *Magnaporthe grisea* associated with turfgrass hosts in Georgia, 1999

Population	County	Host	Cultivar	Polymorphic loci	Pairwise comparisons	Linked loci pairs(%)[v]		Index of association	
						Uncensored	Censored	Uncensored	Censored
1	Lanier	SA[w]	Mercedes	0	0	n/a[y]	n/a	n/a	n/a
2	Webster	SA	Palmetto	0	0	n/a	n/a	n/a	n/a
3	Taylor	SA	Palmetto	78	3003	100	0	77.12[z]	n/a
4	Laurens	TF[x]	Rebel III	8	28	46.4	0	5.76[z]	2.56
5	Cherokee	TF	Rebel III	20	190	81	0	12.38[z]	18.21[z]
6	Floyd	TF	Confederate	7	21	100	0	3.40[z]	2.26
7	Paulding	TF	Bonzai	0	0	n/a	n/a	n/a	n/a
8	Paulding	SA	Raleigh	0	0	n/a	n/a	n/a	n/a

[v] Percentage of linked loci pairs determined by two-locus gametic disequilibrium and Fisher's exact test on contingency tables.

[w] St. Augustinegrass (*Stenotaphrum secundatum*).

[x] Tall fescue (*Festuca arundinacea*).

[y] Value could not be calculated due to insufficient number of polymorphic loci.

[z] Index of association significantly greater than 0 based on 1000 bootstrap replications.

Table 3.8. Gametic linkage disequilibrium in populations of *Magnaporthe grisea* associated with turfgrass hosts in Georgia sampled in 2000

Population	County	Host	Cultivar	Polymorphic loci	Pairwise comparisons	Linked loci pairs(%)[v]		Index of association	
						Uncensored	Censored	Uncensored	Censored
1	Lanier	SA[w]	Mercedes	2	1	100	0	1.00	n/a
2	Webster	SA	Palmetto	0	0	n/a[y]	n/a	n/a	n/a
3	Taylor	SA	Palmetto	1	0	n/a	n/a	n/a	n/a
4	Laurens	TF[x]	Rebel III	6	15	66.7	0	3.76[z]	1.66
5	Cherokee	TF	Rebel III	43	903	48.3	27.6	13.73[z]	8.29[z]
6	Floyd	TF	Confederate	5	10	100	0	4.00[z]	n/a
7	Paulding	TF	Bonzai	5	10	100	0	4.00[z]	n/a
8	Paulding	SA	Raleigh	0	0	n/a	n/a	n/a	n/a

[v] Percentage of linked loci pairs determined by two-locus gametic disequilibrium and Fisher's exact test on contingency tables.

[w] St. Augustinegrass (*Stenotaphrum secundatum*).

[x] Tall fescue (*Festuca arundinacea*).

[y] Value could not be calculated due to insufficient number of polymorphic loci.

[z] Index of association significantly greater than 0 based on 1000 bootstrap replications.

Table 3.9. Partial host range of *Magnaporthe grisea* isolates belonging to clonal lineages associated with large crabgrass, St. Augustinegrass, weeping lovegrass, tall fescue, and wheat

			Inoculated Host							
Lineage	**Host Origin**	**Isolate**	**annual ryegrass**	**perennial ryegrass**	**Kentucky bluegrass**	**tall fescue**	**wheat**	**weeping lovegrass**	**large crabgrass**	**St. Aug[x]**
C	large crabgrass	1203-2	---/++-[y]	---/---	---/---	---/---	---/---	--+/---	---/---	+++/+-+
C	St. Aug	1173	++-/+-+	---/---	---/---	---/---	---/---	---/---	---/---	+++/+++
C	St. Aug	1345	-+-/--+	---/---	---/---	---/---	---/---	---/---	---/---	+++/-++
C	St. Aug	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. Aug	455-2	+++/+++	---/---	---/---	---/---	---/---	---/---	+++/+++	---/---

[x] St. Augustinegrass

[y] Reaction observed for each host-isolate combination. A '+' indicates a pathogenic response, whereas a '-' indicates a non-pathogenic response. Each data point represents a single replication. Sets of data points separated by a '/' represent independent experiments.

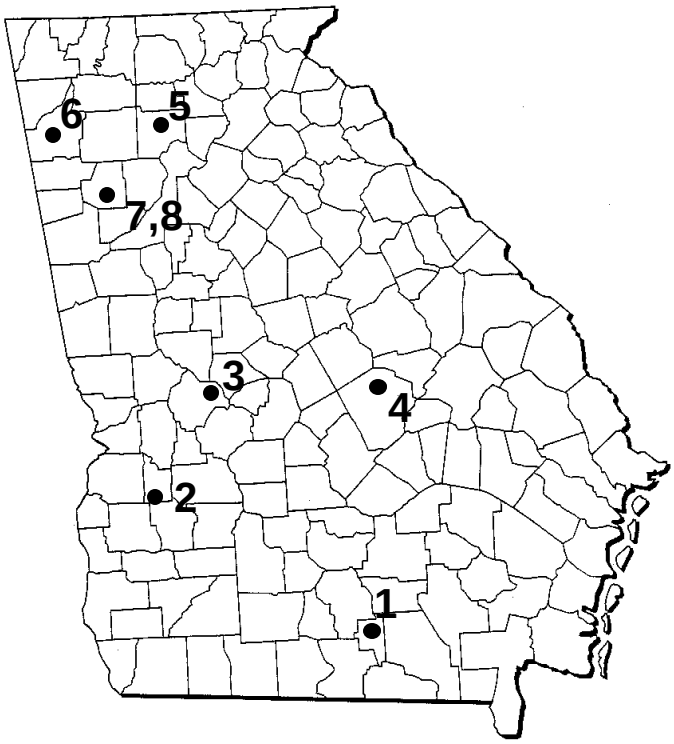

Figure 3.1. Location of *Magnaporthe grisea* populations sampled in 1999 and 2000 for analysis of population structure. See Table 3.1 for specific information on each population.

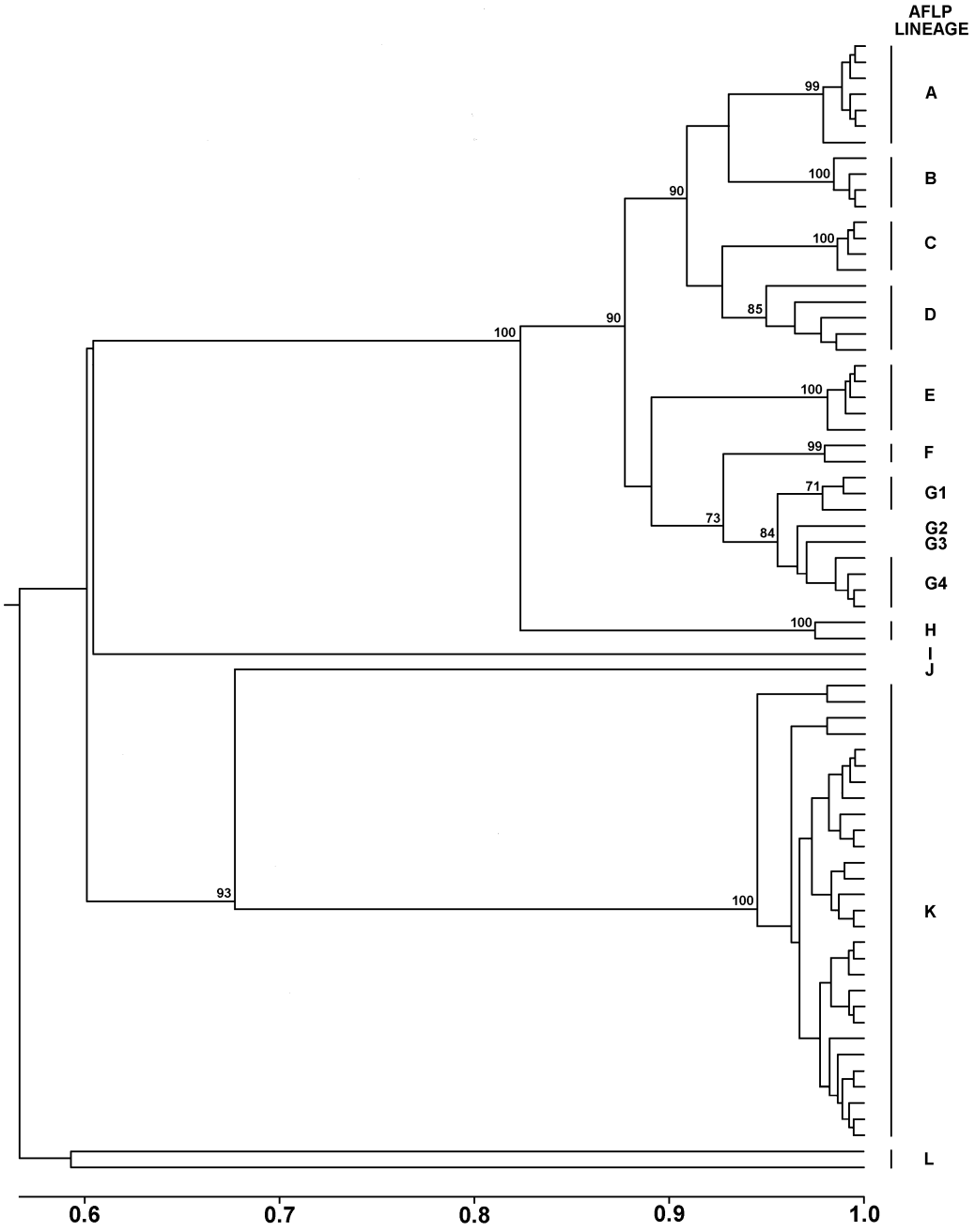

Figure 3.2. UPGMA phylogram produced from AFLP fingerprint patterns observed in *Magnaporthe grisea* isolates. Scale bar indicates the horizontal distance corresponding to genetic similarity as measured by the Dice Similarity Coefficient. Bootstrap values greater than 70 are indicated adjacent to the nodes and are based on 1000 replications. Clonal lineages with significant bootstrap support are indicated.

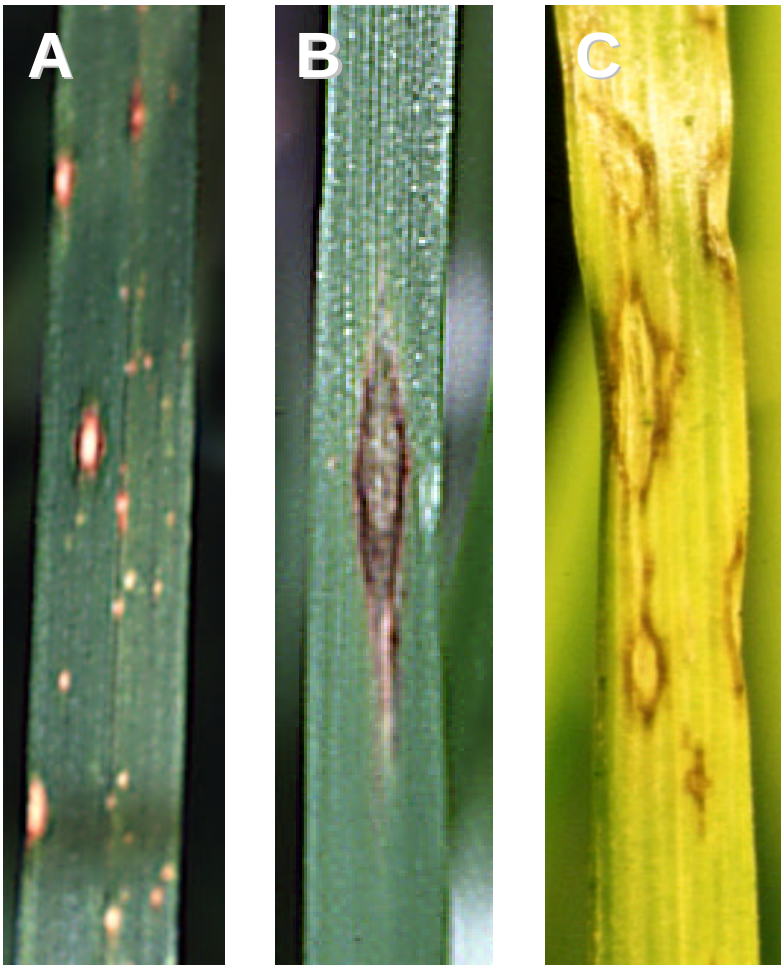

Figure 3.3. Symptoms observed on (A) Kentucky bluegrass, (B) annual ryegrass, and (C) tall fescue 7 days after inoculation with *Magnaporthe grisea* isolates obtained from tall fescue in Georgia.

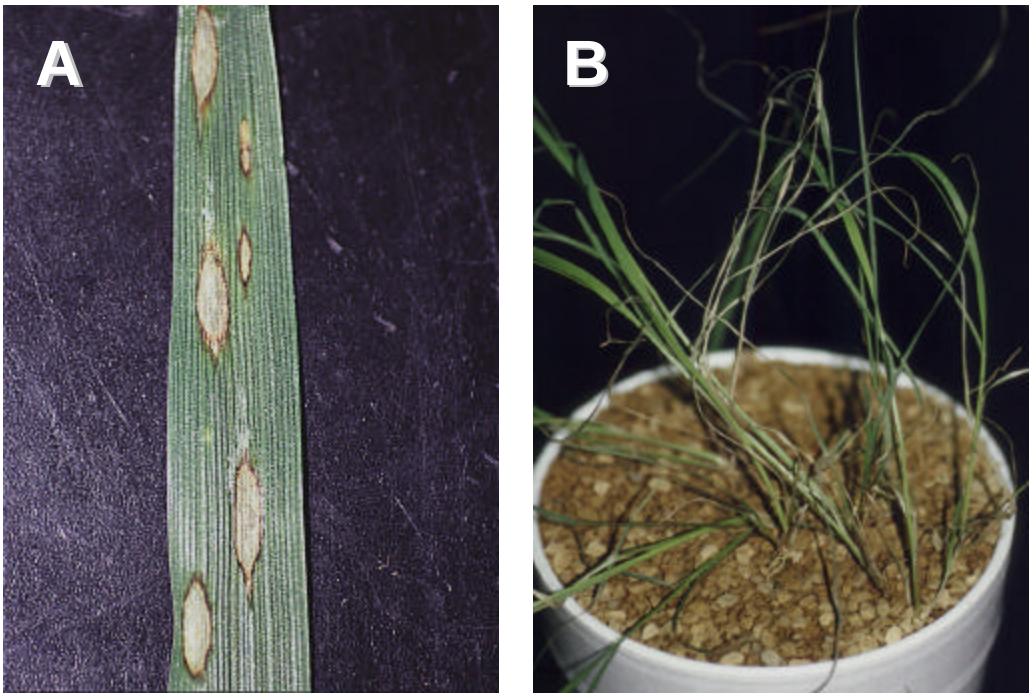

Figure 3.4. Symptoms induced on (A) wheat by *Magnaporthe grisea* isolate BR32 (lineage F) and (B) weeping lovegrass by *M. grisea* isolate 1208-2 (lineage E). These symptoms are typical of those induced by *M. grisea* isolates belonging to lineages E, F, G1, G2, and H.

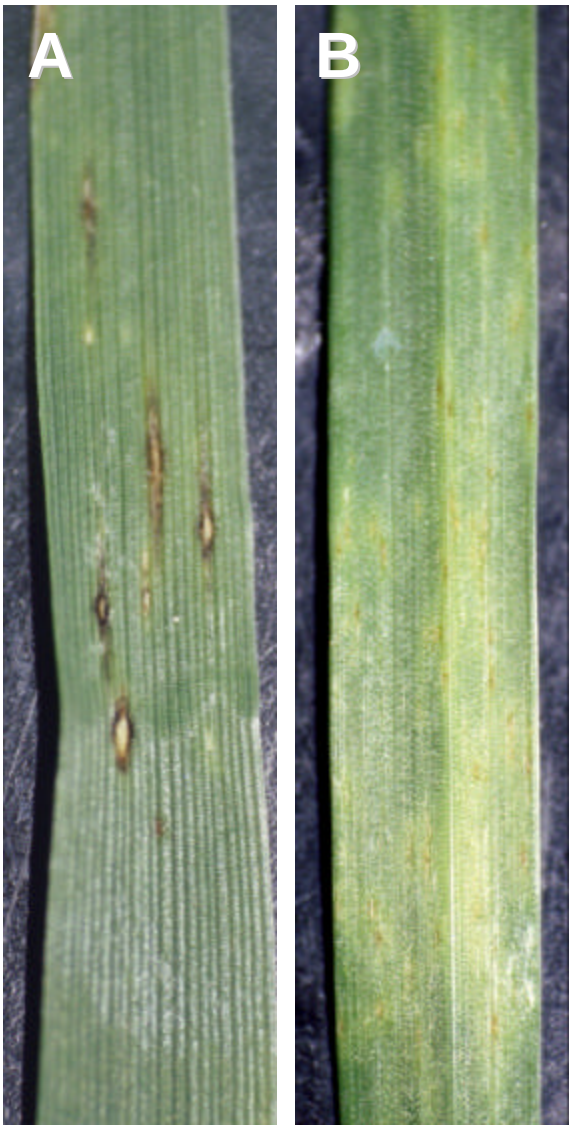

Figure 3.5. Response of wheat to inoculation with *Magnaporthe grisea* isolates belonging to lineage G4: (A) weakly virulent response; (B) hypersensitive response.

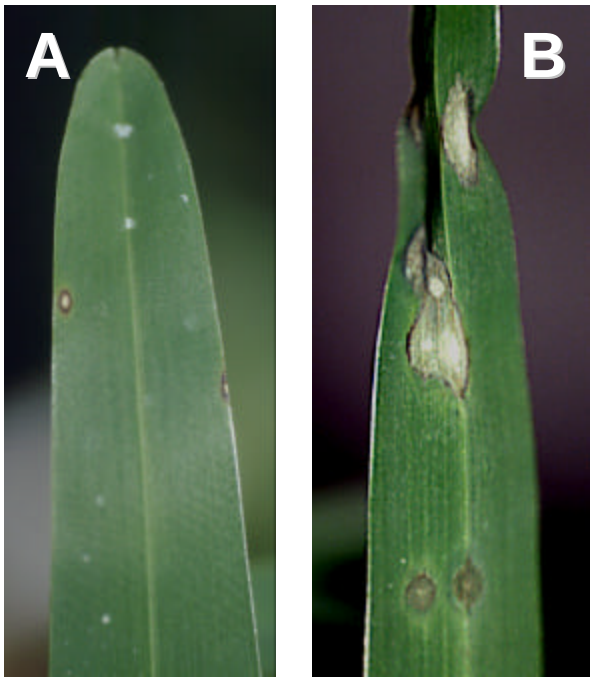

Figure 3.6. Symptoms observed on St. Augustinegrass 7 days after inoculation with *Magnaporthe grisea* (A) isolate 1213-71 from lineage G1; (B) isolate 1204-50 from lineage C.

CHAPTER 4

COMPONENTS OF RESISTANCE TO CLONAL LINEAGES OF *MAGNAPORTHE GRISEA* IN 'COYOTE' AND 'CORONADO' TALL FESCUE[1]

[1]Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2002. To be submitted to Plant Disease.

ABSTRACT

Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2002. Components of resistance to clonal lineages of *Magnaporthe grisea* in 'Coyote' and 'Coronado' tall fescue. To be submitted to Plant Disease.

The components of resistance in tall fescue to *Magnaporthe grisea*, the causal agent of gray leaf spot, were measured in growth chamber experiments. Cultivars ranging in resistance to *M. grisea* were selected: 'Kentucky 31' (susceptible), 'Rebel III' (moderately susceptible), 'Coronado' (resistant), and 'Coyote' (resistant). Plants were inoculated with *M. grisea* isolates representing the five clonal lineages associated with tall fescue in Georgia. Compared to 'Kentucky 31', 'Coyote' and 'Coronado' exhibited longer incubation and latent periods, reduced rates of disease progress and lesion expansion, and lower final disease incidence, foliar blight incidence, and final lesion length. The resistance of 'Rebel III' varied between two experiments, performing similarly to the resistant cultivars 'Coronado' and 'Coyote' in one experiment and similarly to 'Kentucky 31' in a second experiment. Lineages of *M. grisea* differed significantly in virulence, but no relationship between virulence and frequency in field populations was evident. No consistent interactions among cultivar and isolate were detected for any resistance component. Because several components are responsible for the resistance of 'Coyote' and 'Coronado', and the resistance is equally effective against all lineages of the pathogen, it is concluded that these cultivars represent sources of partial resistance to *M. grisea*. These sources of partial resistance will be a valuable component of integrated programs for management of gray leaf spot.

Introduction

Gray leaf spot, caused by *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa (anamorph *Pyricularia grisea* (Cooke) Sacc.), is a disease of increasing importance in the turfgrass industry. Before 1991, gray leaf spot was limited to St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), a warm-season grass grown in subtropical and tropical climates (15). Since 1991, the occurrence of gray leaf spot has become a persistent problem in the management of tall fescue (*Festuca arundinacea* Schreb.) in the Southeastern United States (9) and perennial ryegrass (*Lolium perenne* L.) in the Northeastern, Mid-Atlantic, and Midwestern United States (5).

Variation in *M. grisea* resistance among tall fescue cultivars was identified in North Carolina in 1995 (8,9). Two cultivars, 'Coyote' and 'Coronado', exhibited no gray leaf spot symptoms, whereas 'Kentucky 31' tall fescue was severely defoliated (9). However, 'Coyote' and 'Coronado' are highly susceptible to *Rhizoctonia solani* Kühn, causal agent of brown patch, the most severe disease of tall fescue in the Southeastern United States. In order to produce a cultivar that will be commercially viable for this region, the resistance to *M. grisea* in Coyote and Coronado must be integrated into a cultivar with resistance to *R. solani*.

Resistance to *M. grisea* in rice may be complete or partial (17). Complete resistance is qualitative in nature, rendering the plant immune to pathogen attack through a hypersensitive response. In general, complete resistance is controlled by single genes, insensitive to environmental conditions, effective against some races of the pathogen, and therefore is unstable over time (17). Conversely, partial resistance is quantitative, reducing the rate of epidemic development so that the disease has less impact over the entire epidemic (17). Partial resistance is controlled by several to many genes, is sensitive to environmental conditions, and is effective against all races of the pathogen (17,22).

Three problems have limited the use of partial resistance in rice breeding. First, a single lesion induced by *M. grisea* on a rice culm can inhibit grain production (33). As a result, rice cultivars with partial resistance may sustain unacceptable damage under periods of severe disease pressure (33). During intensive selection for complete resistance over many years, genes for partial resistance have been lost from rice breeding lines, a phenomenon described by Vanderplank as the "Vertifolia effect" (26). Second, the genetic basis for partial resistance is poorly understood, making the transfer of this characteristic into commercial cultivars difficult (28,29). Third, detection of partial resistance in breeding lines in the presence of genes for complete resistance is complicated due to epistasis (22).

Partial resistance may be an effective tool for management of gray leaf spot. In turfgrasses, single, isolated lesions induced by *M. grisea* do not cause significant reductions in stand quality. Visible damage to the stand occurs when lesions expand and coalesce to girdle the leaf blade, producing a foliar blight symptom. Therefore, if the number of lesions or the rate of lesion expansion could be reduced, the impact of *M. grisea* on turfgrass quality may be reduced to acceptable levels.

The high level of resistance in 'Coronado' and 'Coyote' tall fescue indicates that these cultivars may have complete resistance to *M. grisea* (8,9). However, inheritance studies have shown that the resistance in 'Coronado' and 'Coyote' is controlled by several genes, indicating that these cultivars may have partial resistance (11). To further characterize these sources of *M. grisea* resistance, we measured the components of resistance associated with 'Coronado' and 'Coyote' in growth chamber experiments. The specific objectives of this study were to: (1) determine which components are responsible for the resistance observed in 'Coyote' and 'Coronado' tall fescue; (2) determine if the resistance in 'Coronado' and 'Coyote' is partial (and race non-specific) or complete (and race-specific); and (3) investigate the relationship

between virulence and frequency of clonal lineages in *M. grisea* populations associated with tall fescue.

Materials and Methods

Turfgrass establishment and maintenance. Tall fescue cultivars ranging in susceptibility to *M. grisea* were selected: 'Coyote' (resistant), 'Coronado' (resistant), 'Rebel III' (moderately susceptible), and 'Kentucky 31' (susceptible). Each cultivar was seeded at a rate of 440 kg ha^{-1} into 946.4-cm^3 styrofoam cups containing calcined clay (Turface Allsport, Profile Products LLC, Buffalo Grove, IL). The cups were covered with paper and misted 4 times per day to encourage rapid seed germination. After establishment, the grass was irrigated to field capacity 2 times per day, cut to a height of 9 cm weekly, and fertilized weekly with 1.3 g N m^{-2} (Peters 20-10-20, Scotts-Sierra Horticultural Products Co., Marysville, OH). Prior to inoculation, the average number of leaves per cup for each cultivar was determined for calculation of disease incidence by counting the number of leaves in 10 cups of each cultivar.

Inoculum production. Nine *M. grisea* isolates representing the five clonal lineages associated with tall fescue in Georgia were selected arbitrarily. The isolates were stored at –80ºC on sterilized rye (*Secale cereale* L.) as previously described (Chapter 2). Isolates were revived by placing three to five infested grains on the surface of a 90-mm petri dish containing potato dextrose agar (PDA), amended with 50 mg/liter each of tetracycline, streptomycin, and chloramphenicol. After 1 week of incubation at 20ºC with no light, each isolate was transferred to five 90-mm petri dishes containing 1.5% water agar. The agar surface was overlaid with 15 to 20 sterilized alfalfa stem sections (6 cm long), with leaves and petioles removed. The cultures were placed at room temperature (23 to 25ºC) with continuous fluorescent illumination. After 3 weeks, conidia were harvested by transferring the alfalfa stem sections to 50-ml conical centrifuge tubes

(Corning Inc., Corning, NY) containing 20 ml H_2O, vortexing for 10 sec, then filtering the suspension through a single layer of cheesecloth. The conidial suspension was adjusted to a concentration of 2×10^5 conidia ml^{-1} using a hemacytometer.

Inoculation and incubation conditions. A factorial treatment design and a split-plot, randomized complete block experimental design was used in this experiment, with the tall fescue cultivars representing subplots and individual *M. grisea* isolates applied to main plots. Ten weeks after seeding, one cup of each tall fescue cultivar was randomly arranged in plastic containers (41.2 × 28.7 × 25.3 cm; Rubbermaid, Wooster, OH) 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 20 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 CO_2 at 2.2 kg m^{-2}. Immediately after inoculation, the lids were placed on the plastic containers, which were transferred to a single growth chamber and arranged in a randomized complete block with three replications. Incubation conditions for the first 24 h were 24ºC, 100% RH, and no light. Subsequently, the growth chamber was programmed for 12-h days at 30ºC and 75% RH and 12-h nights at 24ºC and 100% RH. The lids were removed from the plastic containers for each day cycle. At the beginning of each night cycle, the cups were misted with water and the lids were placed on the containers to maintain continuous and uniform humidity and leaf wetness. An auxiliary 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.

Disease development. Incubation period was determined by examining the foliage in each cup daily for gray leaf spot symptoms. Two distinct symptoms of gray leaf spot were observed in this study. The initial symptoms were distinct leaf spots that were tan in color with a dark brown border. In some cases, lesions expanded and coalesced to

girdle the leaf blade and induce a foliar blight symptom, where the distal portions of the leaf blade became twisted, shriveled, and necrotic. Disease incidence, foliar blight incidence, and average lesion length were recorded daily for 7 days. Disease incidence was determined by counting the number of symptomatic leaves (spotted or blighted) in each cup and dividing by the average number of leaves per cup for the appropriate cultivar. The incidence of foliar blighting was determined by counting the number of leaf blades that were shriveled, twisted, and necrotic in each cup and dividing by the average number of leaves per cup for the appropriate cultivar. Average lesion length was determined by measuring the length of up to ten individual lesions per cup using a handheld digital caliper (Series 500, Mitutoyo Corp., Aurora, IL). Latent period was determined by examining lesions daily for a distinct change in color from tan to gray. Once a color change was detected, the presence of conidia was confirmed microscopically.

Secondary inoculum production was assessed for each cup 3 days after the latent period had elapsed. Up to 10 symptomatic leaves from each cup were placed in screw cap vials (17 × 60 mm) containing 2 ml of a 1% $CuSO_4$, 1% Triton X-100 (Sigma-Aldrich Co., St. Louis, MO) solution, then vortexed for 1 min to disperse the conidia. The concentration of conidia was estimated with a hemacytometer, and the total number of conidia per sample was calculated. The symptomatic leaves were removed from the conidial suspension, blotted dry with paper towels, arranged on a piece of black construction paper, and affixed to the construction paper with clear packing tape. A digital image of each sample was obtained using a flatbed scanner (Astra 2200, UMAX Technologies, Inc., Fremont, CA). The total lesion surface area in each sample was measured using UTHSCSA ImageTool v. 2.03 (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, Texas). The number of conidia produced per unit lesion surface area was calculated for each sample.

Viability of conidia. The viability of conidia produced by each isolate was assessed in vitro. Conidial suspensions were produced as described above, adjusted to 2×10^5 conidia/ml H_2O using a hemacytometer, and spread in 100-µl aliquots on 4 petri dishes containing 1.5% water agar. The cultures were incubated for 24 h at 20º C with no light. Between 100 and 150 conidia were examined under a compound microscope and rated as either germinated or not germinated based on the presence or absence of a germ tube.

Data analysis. All statistical analyses were conducted using SAS v. 8.2 (SAS Institute, Cary, NC). Simple linear regression was used to estimate the rates of disease progress and lesion expansion over time from disease incidence and average lesion length data, respectively. Area Under the Disease Progress Curve (AUDPC) and Area Under the Lesion Expansion Curve (AULEC) were calculated from the disease incidence and average lesion length data, respectively. Analysis of variance (ANOVA) was used to test the significance of main effects (cultivar and isolate) and the first-order interaction (cultivar × isolate). The frequency of viable conidia was determined by dividing the number of germinated conidia by the total number examined, and ANOVA was used to test for significant differences among isolates. Means separations were conducted using the Waller-Duncan k-ratio t-test (k=100). The experiments were repeated twice, and the results of each experiment were analyzed separately.

Results

Disease development. In Experiment 1, a significant cultivar × isolate interaction was detected for mean lesion length, but no other interactions were significant (Table 4.1). On 'Kentucky 31', isolates 1213-59 and 1213-37 induced lesions that were significantly longer than those induced by several other *M. grisea* isolates (Fig. 4.1). However, the

lesions induced by these isolates were generally shorter on ‘Coronado’, ‘Coyote’, and ‘Rebel III’ relative to other isolates.

In Experiment 1, significant differences among cultivars were detected for all parameters measured except disease progress rate, lesion expansion rate, and secondary inoculum density (Table 4.1). Compared to other cultivars, ‘Kentucky 31’ was characterized by shorter incubation and latent periods and higher AUDPC, final disease incidence, foliar blight incidence, AULEC, and mean final lesion length (Table 4.2). Overall, ‘Coronado’, ‘Coyote’, and ‘Rebel III’ were similar in their responses to *M. grisea* inoculation. Incubation period was significantly shorter for ‘Coronado’ than ‘Coyote’ and ‘Rebel III’, whereas AUDPC and final disease incidence was significantly lower for ‘Coyote’ than for ‘Rebel III’ and ‘Coronado’.

In Experiment 1, significant differences among isolates were detected in disease progress rate, AUDPC, final disease incidence, foliar blight incidence, final average lesion length, and secondary inoculum density (Table 4.1). Isolates within a lineage tended to be similar in virulence, except for lineage E, for which significant differences among isolates were detected for AUDPC, and lineage G4, for which significant differences among isolates were detected for mean lesion length and secondary inoculum density (Table 4.3). Inoculation with isolates 1210-17, 1213-26, and 533-78 resulted in lower AUDPC and final disease incidence compared to other isolates. Isolate 1207-59 exhibited significantly greater mean final lesion length and secondary inoculum production than all other isolates, but the other lineage G4 isolate (1210-57) did not exhibit increased virulence.

Overall, the differences among cultivars were greater in Experiment 2 than in Experiment 1. In Experiment 2, a significant cultivar × isolate interaction was detected for foliar blight incidence, but no other interactions were significant (Table 4.1). Isolate 1210-57 induced a moderate incidence of foliar blight on ‘Kentucky 31’, whereas this

isolate induced the highest foliar blight incidence on ‘Coronado’ and ‘Rebel III’ (Fig. 4.2). Significant differences among cultivars were detected for all parameters measured except secondary inoculum density in Experiment 2 (Table 4.1). ‘Kentucky 31’ was more susceptible to *M. grisea* than the other cultivars according to all resistance components except incubation period and secondary inoculum density (Table 4.4). Compared to ‘Rebel III’ and ‘Kentucky 31’, ‘Coronado’ and ‘Coyote’ exhibited longer incubation and latent periods, as well as reduced disease progress rate, AUDPC, final disease incidence, AULEC, and mean lesion length (Fig. 4.3). Lesion expansion rates were similar in ‘Coronado’ and ‘Rebel III’, but significantly greater than in ‘Coyote’. In addition, ‘Coyote’ had significantly lower symptom expression rate, AUDPC, final disease incidence, AULEC, and mean final lesion length than all other cultivars. Foliar blight incidence was similar in ‘Coronado’, ‘Coyote’, and ‘Rebel III’, but significantly less than in ‘Kentucky 31’ (Fig. 4.4).

In Experiment 2, significant differences were detected among isolates in latent period, AUDPC, foliar blight incidence, lesion expansion rate, AULEC, and mean lesion length (Table 4.1). As in Experiment 1, isolates within a lineage tended to be similar in virulence, with the exception of isolates in lineage G1, which differed significantly in AUDPC, foliar blight incidence, and AULEC (Table 4.5). Isolates in lineage G4 were not consistently more virulent than isolates belonging to other lineages.

Viability of conidia. Significant differences in conidial viability were detected among isolates in this study (Table 4.6). In Experiment 1, conidial viability of isolates 533-78 and 1210-17 was significantly lower than all other isolates. Viability of conidia produced by other isolates ranged from 94.9% to 98.1%. In Experiment 2, conidial viability of isolate 533-78 was significantly lower than all other isolates, which ranged in viability from 91.3% to 96.7%.

Discussion

Based on the results of this study, we conclude that 'Coyote' and 'Coronado' tall fescue have partial resistance to *M. grisea*. The following specific results support this conclusion. First, every *M. grisea* isolate completed the disease cycle on 'Coyote' and 'Coronado' by producing secondary inoculum in both experiments. Completion of the disease cycle is a fundamental characteristic of partial resistance, which slows the rate of epidemic development rather than preventing it (17). Second, the resistance in 'Coyote' and 'Coronado' was equally effective against 9 isolates representing 5 clonal lineages associated with tall fescue in Georgia, which may correspond to distinct races of the pathogen (Chapter 3). Two significant interactions among cultivar and isolate were detected, but the interactions were not the result of differential resistance in 'Coronado' and 'Coyote' and were not consistent among two independent experiments. Third, several components contributed to the observed resistance of Coronado and Coyote tall fescue to *M. grisea*. Compared to 'Kentucky 31', 'Coyote' and 'Coronado' consistently exhibited longer incubation and latent periods and lower AUDPC, disease incidence, foliar blight incidence, AULEC, and mean final lesion length. Previous component analyses have determined that latent period, incubation period, infection efficiency, rate of lesion expansion, and secondary inoculum density are involved in partial resistance to *M. grisea* in rice (1,22,24,28,33), whereas reduced infection efficiency is primarily involved in complete resistance (17,29).

Differences in the response of tall fescue cultivars to *M. grisea* were observed among the two independent experiments conducted. In Experiment 1, 'Rebel III' was similar to 'Coronado' and 'Coyote' for most resistance components measured. However, 'Rebel III' was intermediate between the resistant cultivars and the susceptible 'Kentucky 31' in Experiment 2. Varying conditions in the greenhouse during the 10 weeks between seeding and inoculation may be responsible for differences among the two experiments.

The pots for Experiment 1 were seeded on 20 July 2001 and inoculated on 25 Sept 2001, whereas the pots for Experiment 2 were seeded on 20 August 2001 and inoculated on 30 Oct 2001. Lower night temperatures, reduced photoperiod, and/or reduced light quality in the greenhouse between 25 Sept 2001 and 30 Oct 2001 may have influenced cultivar susceptibility. The cultivar Rebel III appears to have some partial resistance to *M. grisea*, but this resistance may be influenced by environmental conditions to a greater extent than the resistance in 'Coyote' and 'Coronado'.

Isolates within a clonal lineage were similar in their virulence to tall fescue cultivars, however, some differences within lineages were observed. One isolate from lineage G1 (533-78) and both isolates from lineage G2 (1210-17 and 1213-26) exhibited reduced virulence compared to isolates from other lineages for most parameters measured. Reduced conidium viability may be involved in the lower virulence of isolate 533-78, but isolates 1210-17 and 1213-26 did not exhibit reduced conidium viability compared to other isolates. Isolates in lineage G4 were among the most virulent isolates for each parameter measured, but were not significantly more virulent than isolates in other lineages. Therefore, the dominance of lineage G4 in *M. grisea* populations from tall fescue in Georgia cannot be explained by increased virulence of these isolates. Other factors, such as founder effects or enhanced overwintering ability may be responsible for the dominance of lineage G4 in Georgia.

The results of this study may facilitate efforts to develop tall fescue cultivars with resistance to *M. grisea*. Now that the specific resistance components associated with 'Coyote' and 'Coronado' have been determined, these parameters should be measured in breeding populations (17). Alternatively, the loci that confer each resistance component could be located on a genetic map, enabling the use of marker-assisted selection to monitor the inheritance of resistance loci and ensure that all are transferred into a commercial cultivar (29). Because 'Coyote' and 'Coronado' tall fescue represent

sources of partial resistance, they may retain their effectiveness in gray leaf spot management for extended periods of time. However, cultivars with partial resistance may sustain unacceptable damage during periods of severe disease pressure (33). To prevent this, partial resistance may be supplemented with genes for complete resistance. Due to epistasis of complete resistance over partial resistance (22), marker-assisted selection will be an essential tool for breeding cultivars with genes conferring partial and complete resistance.

Literature Cited

1. Castani, J., MacKenzie, D. R., and Nelson, R. R. 1989. Components analysis of race non-specific resistance to blast disease of rice caused by *Pyricularia oryzae*. J. Phytopathol. 127:89-99.
2. Correa-Victoria, F. J., and Zeigler, R. S. 1993. Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" breeding site in eastern Colombia. Plant Dis. 77:1029-1035.
3. Correa-Victoria, F. J., Zeigler, R. S., and Levy, M. 1994. Virulence characteristics of genetic families of *Pyricularia grisea* in Colombia. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
4. Correll, J. C., Harp, T. L., Guerber, J. C., Zeigler, R. S., Liu, B., Cartwright, R. D., and Lee, F. N. 2000. Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. Phytopathology 90:1396-1404.
5. Dernoeden, P. H. 1996. Perennial ryegrass and gray leaf spot. Golf Course Management 64:49-52.

6. Don, L. D., Kusaba, M., Urashima, A. S., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Population structure of the rice blast fungus in Japan examined by DNA fingerprinting. Ann. Phytopath. Soc. Japan 65:15-24.
7. Don, L. D., Tosa, Y., Nakayashiki, H., and Mayama, S. 1999. Population structure of the rice blast pathogen in Vietnam. Ann. Phytopath. Soc. Japan 65:475-479.
8. Fraser, M. L. 1996. Susceptibility of tall fescues to gray leaf spot, 1995. Biological and Cultural Tests 12:130.
9. Fraser, M. L. 1997. Tall fescues vary in leaf spot susceptibility. Golf Course Management 65:64-66.
10. Hayashi, N., Li, C., Li, J., Iwano, M., Naito, H., Yoshino, R., and Kato, H. 1997. Distribution of fertile *Magnaporthe grisea* fungus pathogenic to rice in Yunnan Province, China. Ann. Phytopath. Soc. Japan 63:316-323.
11. Hofmann, N. E., and Hamblin, A. M. 2001. Segregation of gray leaf spot resistance in tall fescue. 2001 Annual Meeting Abstracts [ASA/CSSA/SSSA].
12. Itoi, S., Mishima, T., Arase, S., and Nozu, M. 1983. Mating behavior of Japanese isolates of *Pyricularia oryzae*. Phytopathology 73:155-158.
13. Kato, H., and Yamaguchi, T. 1982. The perfect state of *Pyricularia oryzae* CAV. in culture. Ann. Phytopath. Soc. Japan 48:607-612.
14. 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.
15. Malca, I., and Owen, J. H. 1957. The gray leaf spot disease of St. Augustinegrass. Plant Dis Rep. 41:871-875.
16. Manibhushanrao, K., and Day, P. R. 1972. Low night temperature and blast disease development on rice. Phytopathology 62:1005-1007.

17. Marchetti, M. A. 1994. Race-specific and rate-reducing resistance to rice blast in US rice cultivars. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
18. Mekwatanakarn, P., Kositratana, W., Phromraksa, T., and Zeigler, R. S. 1999. Sexually fertile *Magnaporthe grisea* rice pathogens in Thailand. Plant Dis. 83:939-943.
19. Notteghem, J. L., and Silue, D. 1992. Distribution of the mating type alleles in *Magnaporthe grisea* populations pathogenic on rice. Phytopathology 82:421-424.
20. Ou, S. H. 1980. A look at worldwide rice blast control. Plant Dis. 64:439-445.
21. Priyadarisini, V. B., Viji, G., and Gnanamanickam, S. S. 1999. Mating type distribution, fertility and pathogenicity of rice isolates of *Magnaporthe grisea* in four rice growing regions of India. Indian Phytopathol. 52:28.
22. Roumen, E. C. 1994. A strategy for accumulating genes for partial resistance to blast disease in rice within a conventional breeding program. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
23. Roumen, E., Levy, M., and Notteghem, J. L. 1997. Characterisation of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. European J. Plant Pathol. 103:363-371.
24. Seebold, K. W., Kucharek, T. A., Datnoff, L. E., Correa-Victoria, F. J., and Marchetti, M. A. 2001. The influence of silicon on components of resistance to blast in susceptible, partially resistant, and resistant cultivars of rice. Phytopathology 91:63-69.
25. Urashima, A. S., Igarashi, S., and Kato, H. 1993. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Phytopathology 77:1211-1216.
26. Vanderplank, J. E. 1984. Disease Resistance in Plants. Academic Press, New York.

27. Viji, G., and Gnanamanickman, S. S. 1998. Mating type distribution and fertility status of *Magnaporthe grisea* populations from various hosts in India. Plant Dis. 82:36-40.

28. Wang, Z., Mackill, D. J., and Bonman, J. M. 1989. Inheritance of partial resistance to blast in indica rice cultivars. Crop Sci. 29:848-853.

29. Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C., and Nelson, R. J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434.

30. Xia, J. Q., Correll, J. C., Lee, F. N., Marchetti, M. A., and Rhoads, D. D. 1993. DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) population in two rice fields in Arkansas. Phytopathology 83:1029-1035.

31. Xia, J. Q., Correll, J. C., and Rhoads, D. D. 2000. Regional population diversity of *Pyricularia grisea* in Arkansas and the influence of host selection. Plant Dis. 84:877-884.

32. Yaegashi, H., and Yamada, M. 1986. Pathogenic race and mating type of *Pyricularia oryzae* from Soviet Union, China, Nepal, Thailand, Indonesia and Colombia. Ann. Phytopath. Soc. Japan 52:225-234.

33. Yeh, W. H., and Bonman, J. M. 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathol. 35:319-323.

34. Zeigler, R. S., Scott, R. P., Leung, H., Bordeos, A. A., Kumar, J., and Nelson, R. J. 1997. Evidence of parasexual exchange of DNA in the rice blast fungus challenges its exclusive clonality. Phytopathology 87:284-294.

35. 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.

Table 4.1. *P*-values for cultivar, isolate and cultivar × isolate interactions according to analysis of variance conducted on components of resistance of tall fescue to *Magnaporthe grisea*

	Experiment 1			Experiment 2		
Resistance component	**Cultivar**	**Isolate**	**Cultivar × isolate**	**Cultivar**	**Isolate**	**Cultivar × isolate**
Incubation period	<0.0001	0.7455	0.8632	<0.0001	0.1618	0.1871
Latent period	<0.0001	0.4842	0.2357	<0.0001	0.007	0.1447
Disease progress rate	0.1485	0.0425	0.4825	<0.0001	0.2283	0.683
AUDPC[x]	<0.0001	<0.0001	0.4435	<0.0001	0.0081	0.4013
Disease incidence, 7 DAT[y]	<0.0001	0.0011	0.4475	<0.0001	0.1954	0.7602
Foliar blight incidence, 7 DAT	0.0007	0.0271	0.1256	<0.0001	0.0017	0.0025
Lesion expansion rate	0.5575	0.0932	0.8665	<0.0001	0.0016	0.2558
AULEC[z]	<0.0001	0.4472	0.8852	<0.0001	0.007	0.5115
Mean lesion length, 7 DAT	<0.0001	0.0121	0.0456	<0.0001	0.0182	0.2761
Secondary inoculum density	0.2152	<0.0001	0.1153	0.6796	0.1243	0.7563

[x] Area under the disease progress curve.

[y] Days after treatment.

[z] Area under the lesion expansion curve.

Table 4.2. Components of resistance in tall fescue cultivars varying in susceptibility to *Magnaporthe grisea*, Experiment 1

Cultivar	Incubation period (days)	Latent period (days)	Disease progress rate (%/day)	AUDPC $(\%^2)^x$	Disease incidence (%)	Foliar blight incidence (%)	Lesion expansion rate (mm/day)	AULEC $(mm^2)^y$	Mean lesion length (mm)	Secondary inoculum (conidia/mm^2)
Coronado	3.9 a[z]	6.2 a	2.0 a	0.24 bc	8.9 bc	0.56 b	0.73 a	8.5 b	3.5 b	277 a
Coyote	3.5 b	6.0 a	1.9 a	0.23 c	7.7 c	0.59 b	0.73 a	9.4 b	3.5 b	270 a
Kentucky 31	2.5 c	5.3 b	2.6 a	0.59 a	16.6 a	1.89 a	0.81 a	13.9 a	4.8 a	370 a
Rebel III	3.3 b	6.0 a	2.0 a	0.31 b	10.9 b	0.54 b	0.74 a	9.6 b	3.2 b	384 a

[x] Area under the disease progress curve.

[y] Area under the lesion expansion curve.

[z] Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

Table 4.3. Virulence of *Magnaporthe grisea* isolates belonging to clonal lineages associated with tall fescue in Georgia, averaged over four tall fescue cultivars, Experiment 1

Lineage	Isolate	AUDPC (%^{2})[y]	Disease incidence (%)	Foliar blight incidence (%)	Mean lesion length (mm)	Secondary inoculum (conidia/mm^2)
E						
	1213-2	0.40 b[z]	11.6 ab	0.5 b	3.5 b	142 d
	1213-59	0.53 a	15.4 a	0.6 b	3.7 b	199 cd
G1						
	533-78	0.25 cd	8.4 bc	0.8 ab	3.8 b	316 bcd
	1213-90	0.34 bc	12.3 ab	1.5 ab	3.7 b	441 b
G2						
	1210-17	0.22 cd	8.4 bc	0.5 b	3.7 b	314 bcd
	1213-26	0.19 d	7.0 c	0.3 b	3.6 b	293 bcd
G4						
	1207-59	0.38 b	12.2 ab	2.1 a	4.7 a	697 a
	1210-57	0.37 b	12.7 a	1.3 ab	3.8 b	356 bc
H						
	1213-37	0.39 b	11.5 ab	0.5 b	3.3 b	170 cd

[y] Area under the disease progress curve.

[z] Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

Table 4.4. Components of resistance in tall fescue cultivars varying in susceptibility to *Magnaporthe grisea*, Experiment 2

Cultivar	Incubation period (days)	Latent period (days)	Disease progress rate (%/day)	AUDPC $(\%^2)^x$	Disease incidence (%)	Foliar blight incidence (%)	Lesion expansion rate (mm/day)	AULEC $(mm^2)^y$	Mean lesion length (mm)	Secondary inoculum (conidia/mm^2)
Coronado	3.4 a[z]	5.9 a	1.0 c	0.15 c	5.2 c	0.09 b	0.55 b	7.8 c	2.6 c	315 a
Coyote	3.7 a	6.3 b	0.4 d	0.08 d	2.7 d	0.00 b	0.43 c	5.5 d	2.0 d	385 a
Kentucky 31	2.7 b	5.3 d	4.6 a	0.79 a	22.3 a	4.49 a	0.68 a	12.5 a	3.7 a	314 a
Rebel III	2.7 b	5.6 c	1.7 b	0.28 b	8.6 b	0.47 b	0.57 b	9.9 b	3.0 b	296 a

[x] Area under the disease progress curve.

[y] Area under the lesion expansion curve.

[z] Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

Table 4.5. Virulence of *Magnaporthe grisea* isolates belonging to clonal lineages associated with tall fescue in Georgia, average over four tall fescue cultivars, Experiment 2

Lineage	Isolate	Latent period (Days)	AUDPC ($\%^2$)[x]	Foliar blight incidence (%)	Lesion expansion rate (mm/day)	AULEC (mm^2)[y]	Mean lesion length (mm)
E							
	1213-2	5.4 bc[z]	0.40 a	0.8 bc	0.49 bc	9.1 a-d	2.6 bc
	1213-59	5.2 c	0.38 a	0.5 c	0.50 bc	9.1 a-d	2.5 c
G1							
	533-78	5.9 ab	0.26 bc	0.9 bc	0.54 abc	8.2 cd	2.7 abc
	1213-90	5.9 ab	0.40 a	2.4 a	0.56 abc	9.9 a	2.9 abc
G2							
	1210-17	6.1 a	0.22 c	1.2 bc	0.62 a	8.3 cd	3.1 ab
	1213-26	5.8 ab	0.28 abc	1.0 bc	0.58 ab	8.5 bcd	2.8 abc
G4							
	1207-59	5.8 ab	0.34 ab	2.4 a	0.63 a	9.7 ab	3.2 a
	1210-57	5.8 ab	0.34 ab	1.8 ab	0.63 a	9.5 abc	3.0 abc
H							
	1213-37	5.8 ab	0.33 abc	0.6 c	0.48 c	7.9 d	2.6 c

[x] Area under the disease progress curve.

[y] Area under the lesion expansion curve.

[z] Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

Table 4.6. Viability of conidia produced by *Magnaporthe grisea* isolates in vitro

		Germinated conidia (%)			
Lineage	**Isolate**	**Experiment 1**		**Experiment 2**	
E					
	1213-2	98.1	a[x]	96.6	a
	1213-59	96.9	ab	94.3	bc
G1					
	533-78	91.7	d	81.1	e
	1213-90	95.5	bc	92.5	cd
G2					
	1210-17	87.9	e	91.3	d
	1213-26	95.3	bc	95.5	ab
G4					
	1207-59	96.3	abc	93.1	cd
	1210-57	94.9	c	91.5	d
H					
	1213-37	95.0	c	96.7	a

[x] Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

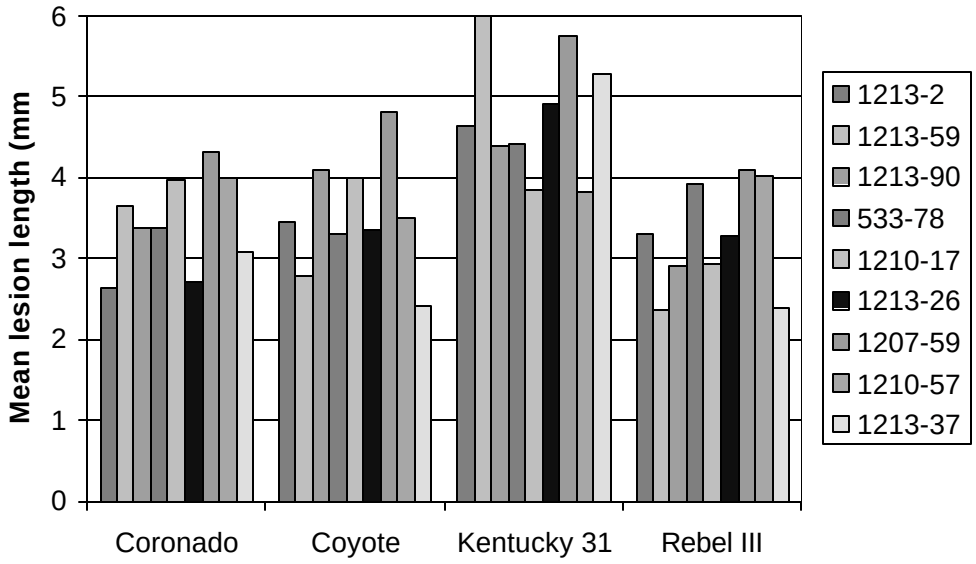

Figure 4.1. Effects of tall fescue cultivar and *Magnaporthe grisea* isolate on mean lesion length in Experiment 1. The minimum significant difference (MSD) according to the Waller-Duncan k-ratio t-test (k=100) is 0.79.

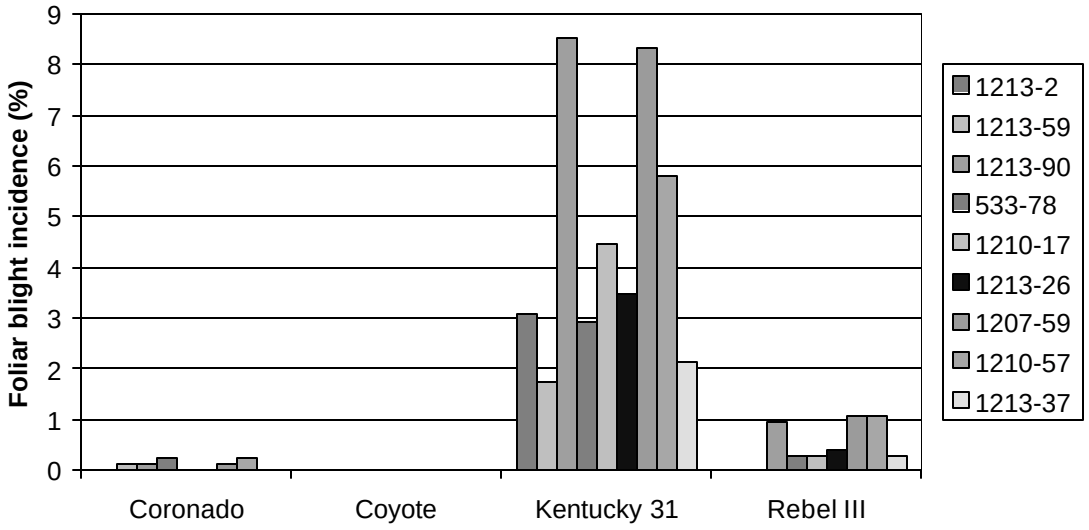

Figure 4.2. Effects of tall fescue cultivar and *Magnaporthe grisea* isolate on foliar blight incidence in Experiment 2. The minimum significant difference (MSD) according to the Waller-Duncan k-ratio t-test (k=100) is 1.2.

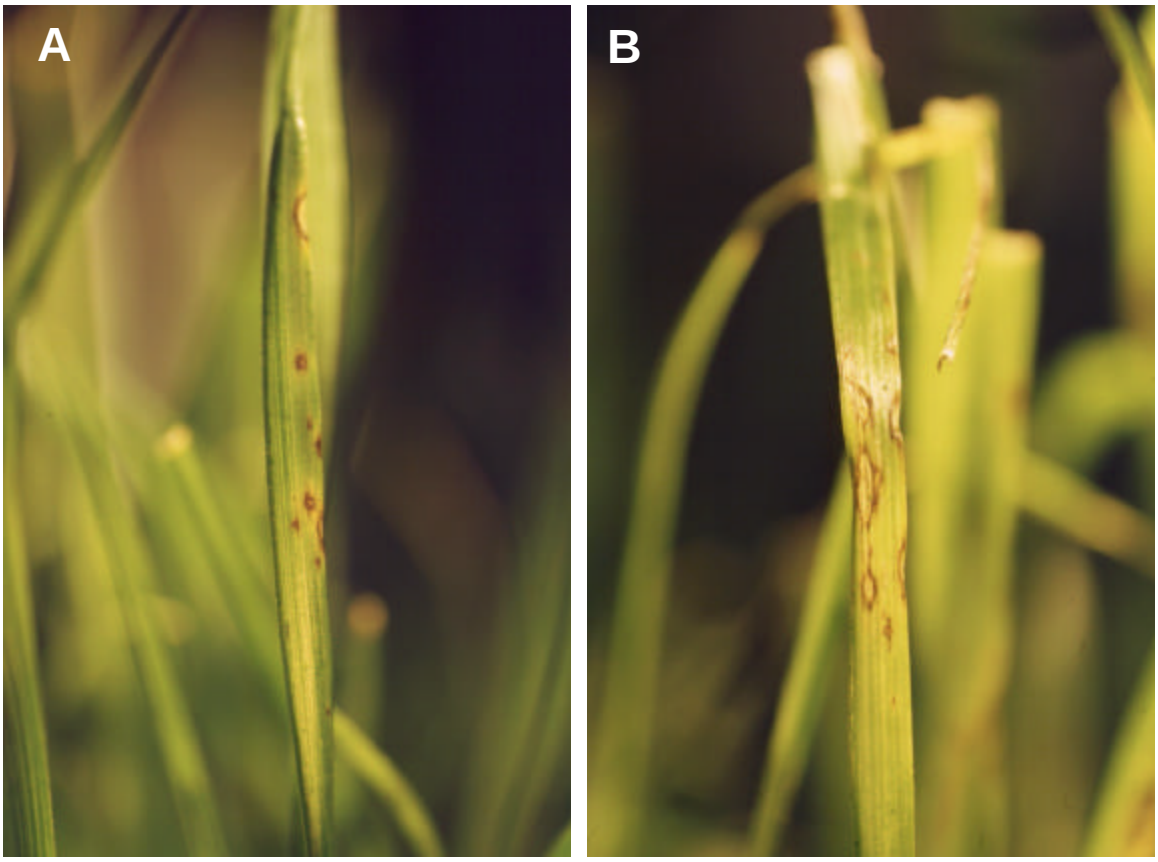

Figure 4.3. Differences in lesion length observed between tall fescue cultivars varying in resistance to *Magnaporthe grisea*: (A) ‘Coronado’ and (B) ‘Kentucky 31’ tall fescue 6 days after inoculation with isolate 1213-59.

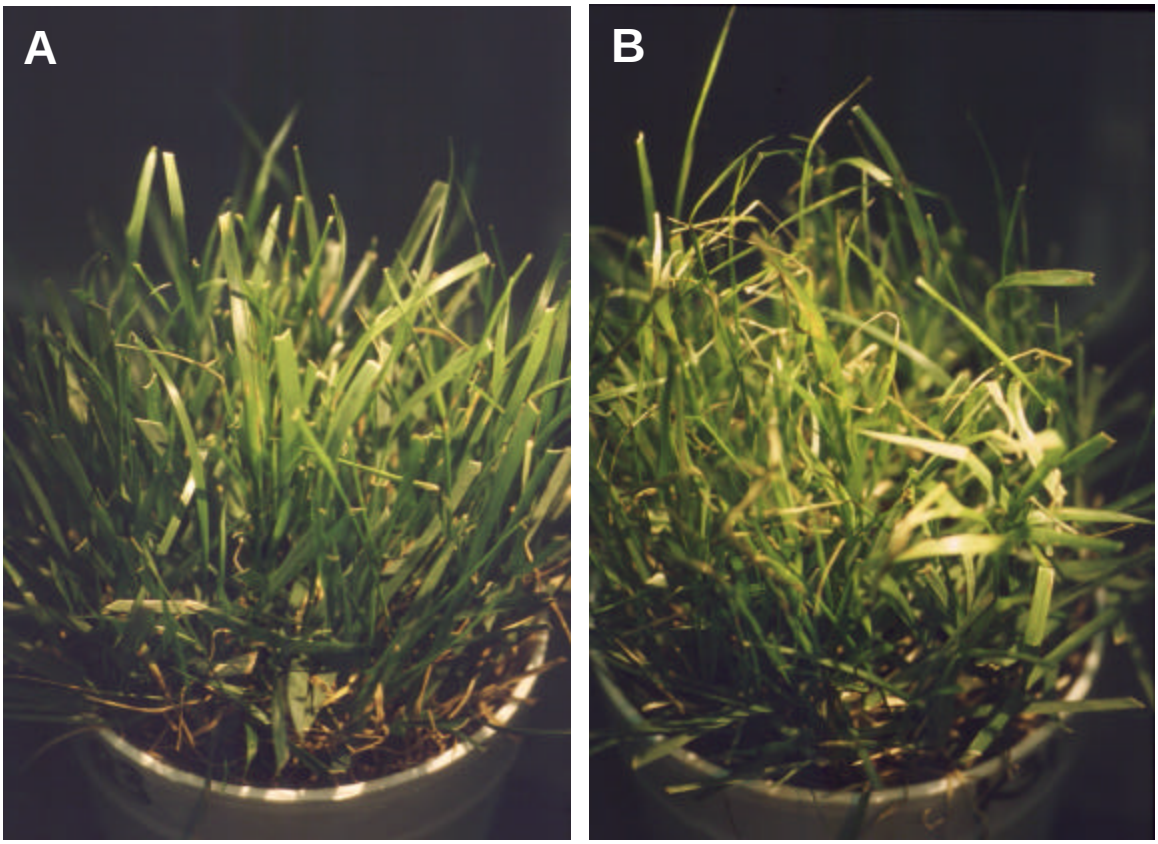

Figure 4.4. Differences in foliar blight incidence observed between tall fescue cultivars varying in resistance to *Magnaporthe grisea*: (A) 'Coronado' and (B) 'Kentucky 31' tall fescue 6 days after inoculation with isolate 1213-90.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Among turfgrasses, the host range of *Magnaporthe grisea* in the United States has expanded rapidly over the last 30 years. Gray leaf spot has been chronic since the 1950s on St. Augustinegrass, a warm-season grass grown in tropical and sub-tropical climates (8). Since 1971, this disease has become persistent in annual ryegrass (11), perennial ryegrass (6), and tall fescue (2) turfgrasses wherever these species are grown. The sources of *M. grisea* strains associated with turfgrasses are unknown, but may have arisen through introduction from abroad or from mutation of endemic strains. The source of pathogen strains is important because additional genetic variation may be present at the source, serving as a reservoir for new races of the pathogen. The genetic structure of *M. grisea* populations may provide evidence of which process, mutation or introduction, led to the establishment of populations pathogenic to turfgrasses.

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 responsible for the observed population structure, and explore the implications of the observed population structure to gray leaf spot management with pathogen resistant cultivars. Populations of *M. grisea* associated with St. Augustinegrass and tall fescue in Georgia were sampled in 1999 and 2000. The populations were selected to allow the separation of factors that may influence population structure, such as host species, host cultivar, and geographic region. To facilitate mating type determination in the populations, a new PCR-based assay was developed for *M. grisea*. Amplified Fragment Length Polymorphisms (AFLPs) were utilized to describe the genetic structure of the sample populations. The host range of *M. grisea* clonal lineages associated with turfgrass hosts in Georgia was determined in growth chamber experiments. The response of tall fescue cultivars to inoculation with clonal lineages of *M. grisea* was measured to determine if the lineages correspond to distinct races of the pathogen.

Populations of *M. grisea* from turfgrass hosts in Georgia were dominated by a single mating type. Populations from St. Augustinegrass were dominated by *Mat*1-1, whereas populations from tall fescue were dominated by *Mat*1-2. However, the opposite mating type was found associated with each host in low frequency. The fertility status of isolates from selected populations were determined using controlled crosses in vitro. All *Mat*1-1 isolates from St. Augustinegrass were sterile, and the single *Mat*1-2 isolate from St. Augustinegrass was male-fertile. Approximately half of the *Mat*1-2 isolates from tall fescue were male-fertile, but the *Mat*1-1 isolates from tall fescue were sterile. Although isolates of opposite mating type have the opportunity to interact, the potential for sexual reproduction in M. grisea populations from turfgrasses appears to be low due to the absence of female-fertile isolates.

Populations of *M. grisea* associated with St. Augustinegrass were predominantly composed of a single strain according to AFLP markers. Out of 411 isolates from St. Augustinegrass, only 4 isolates were distinguished from this dominant strain, one of which appears to have been involved in an opportunistic infection. The other three isolates were distinguished from the dominant strain by only one or two AFLP markers. Based on the level of genetic diversity observed, it appears that the *M. grisea* population associated with St. Augustinegrass was established by a single strain. This strain may have arisen as a mutant from within endemic populations of *M. grisea*, but also may have been introduced from abroad. Isolates of *M. grisea* from other hosts in this study did not exhibit significant genetic similarity to St. Augustinegrass isolates, therefore, the source of this strain remains unknown.

Even though *M. grisea* has been associated with tall fescue for only 8 years, levels of genetic diversity are generally higher in populations associated with this host than with St. Augustinegrass. Five distinct clonal lineages were identified from tall fescue in Georgia, with additional genetic variation present within 4 of the 5 lineages. This level of

genetic variation is not likely to have arisen through mutation in only 8 years; therefore, it is likely that these strains were introduced from abroad. An alternative host for the dominant *M. grisea* lineage associated with tall fescue and perennial ryegrass was not identified in this study; however, weeping lovegrass (*Eragrostis curvula*) was confirmed to be an alternative host for lineage E from tall fescue. Weeping lovegrass was introduced from Africa in 1927 (7), and has been planted on 0.4 million ha since 1985 as part of the Conservation Reserve Program administered by the United States Department of Agriculture (10). The introduction and recent increased use of weeping lovegrass may be involved in the recent appearance of gray leaf spot on tall fescue. Lineages G1, G2, G4, and H were pathogenic to weeping lovegrass and may be a minor component of the *M. grisea* population associated with this host, just as lineage E is a minor component of populations from tall fescue. Populations of *M. grisea* from weeping lovegrass, both in Africa and the United States, should be analyzed with AFLP markers in search of these tall fescue lineages and additional lineages that are pathogenic to tall fescue.

According to AFLP markers, *M. grisea* isolates from tall fescue and weeping lovegrass were closely related to, but distinct from, isolates from wheat collected in Brazil. This agrees with the results of Viji et al. (12), which were based on ITS sequences and TD-RFLPs. *M. grisea* populations from wheat, therefore, may also serve as a source of strains infecting tall fescue and perennial ryegrass. Wheat blast, caused by *M. grisea*, was first observed in 1985 in Brazil (5), but has not been reported as a significant problem in the United States. Populations of *M. grisea* from wheat in South America should also be analyzed for the presence of lineages that are pathogenic to turfgrass hosts.

Repeated attempts to locate and isolate *M. grisea* from annual ryegrass pastures in Georgia failed, therefore, no isolates from annual ryegrass were included in this study.

Annual ryegrass, perennial ryegrass, and tall fescue are closely related, and even have been proposed to belong to the same genus (3). Therefore, it is reasonable to hypothesize that the populations of *M. grisea* associated with these grasses share a common origin. In this study, the majority of tall fescue and perennial ryegrass strains were indistinguishable, and all tall fescue isolates tested were pathogenic to both annual ryegrass and perennial ryegrass. However, *M. grisea* isolates from lineage C and K were also pathogenic to annual ryegrass, but not to perennial ryegrass or tall fescue. Because little host specialization is apparent in annual ryegrass, populations of *M. grisea* associated with this host may be composed of a diverse collection of lineages from varying sources. The role of annual ryegrass as a source of strains pathogenic to tall fescue and perennial ryegrass also must be investigated further.

In rice populations, clonal lineages of *M. grisea* correspond to distinct groups of pathogenic races with similar virulence profiles (1). The relationship between lineage and race in *M. grisea* lineages from tall fescue is unknown, but the results of this study provide evidence that such a relationship exists. In growth chamber inoculation experiments, *M. grisea* isolates from lineages E, G1, G2, and H were pathogenic to wheat cultivar 'Roberts', whereas lineage G4 was avirulent or weakly virulent. Because no information is available concerning *M. grisea* resistance genes in 'Roberts', additional research is required to verify that a relationship between lineage and race exists in the *M. grisea* population from tall fescue.

The resistance in 'Coyote' and 'Coronado' tall fescue is effective against all clonal lineages of *M. grisea* identified thus far in tall fescue populations, despite evidence that these lineages correspond to distinct pathogenic races. Several resistance components, including incubation period, latent period, infection efficiency, and lesion expansion rate, are responsible for the reduced impact of *M. grisea* on these cultivars. In addition, every *M. grisea* isolate tested completed the disease cycle on 'Coyote' and 'Coronado' by

producing secondary inoculum. We conclude that these cultivars have partial resistance to *M. grisea*, which is consistent with the polygenic control found by Hamblin and Hofmann (4). Therefore, these sources of *M. grisea* resistance may be durable after gaining widespread use in the turfgrass industry. Unfortunately, 'Coyote' and 'Coronado' are both highly susceptible to *Rhizoctonia solani*, another important pathogen of tall fescue in the Southeastern United States. The gray leaf spot resistance in 'Coyote' and 'Coronado' must be integrated into a cultivar with brown patch resistance in order to create a cultivar that will be commercially viable for this region. This may be a difficult task, since the genetic control of partial resistance to *M. grisea* is poorly understood (9). Genomic mapping of loci conferring resistance in tall fescue may facilitate this process by enabling turfgrass breeders to use marker-assisted selection for breeding *M. grisea* resistance (13).

Future Research

Based on the results of this study, several research projects can be developed to expand upon our knowledge of *M. grisea* populations associated with turfgrasses:

1. **Origin of *M. grisea* lineages associated with turfgrass hosts.** AFLP markers should be used to analyze populations of *M. grisea* associated with weeping lovegrass and wheat, both in the United States and abroad, in an effort to identify the source of *M. grisea* strains infecting tall fescue and perennial ryegrass. In addition, populations of *M. grisea* associated with annual ryegrass in Mississippi and Louisiana should be characterized to determine their relationship to populations from tall fescue and perennial ryegrass.
2. **Increased resolution of *M. grisea* lineages associated with tall fescue.** In this study, evidence of additional lineages within lineage G was observed. Isolates in these proposed lineages were characterized by several unique AFLP markers,

different mating types, and differing virulence profiles. However, due to the small amount of genetic variation identified within these groups, significant bootstrap support for lineages G1-G4 was not detected in the phylograms. Additional AFLP primer combinations may reveal more polymorphic markers among these groups of isolates and provide significant statistical support for lineages G1-G4.

3. **Relationship between lineage and race in *M. grisea* associated with turfgrasses.** Race identification is critical to the development of cultivars with durable resistance to *M. grisea*. Although genes for complete resistance to *M. grisea* have not been identified in turfgrasses, other hosts may be able to differentiate races among turfgrass lineages. Lineage G4 from tall fescue is avirulent or weakly virulent to wheat cultivar 'Roberts', whereas other tall fescue lineages are highly virulent. Because no information is available on resistance genes in 'Roberts', further research is required to verify that there is a relationship between lineage and race in *M. grisea* populations from turfgrasses. Once *M. grisea* races are identified, then turfgrass breeders can use them to identify genes for complete resistance in tall fescue and perennial ryegrass germplasm.
4. **Molecular mapping of loci conferring *M. grisea* resistance in 'Coyote' and 'Coronado' tall fescue.** The resistance to *M. grisea* in 'Coyote' and 'Coronado' tall fescue appears to be partial and controlled by several genes. This type of resistance is highly desirable because it is effective against all races of the pathogen and is therefore stable over time. However, partial resistance is difficult to breed because its genetics are poorly understood. Molecular mapping of loci controlling partial resistance will enable turfgrass breeders to use marker-assisted selection to efficiently transfer this characteristic into a cultivar with resistance to *M. grisea* and *R. solani*. In addition, marker-assisted selection will enable breeders to detect genes

for partial resistance in the presence of genes for complete resistance, avoiding the problems associated with epistasis of complete resistance over partial resistance.

5. **Population dynamics of *M. grisea* associated with resistant tall fescue and perennial ryegrass cultivars.** Although 'Coyote' and 'Coronado' are resistant to all lineages of *M. grisea* identified in populations from tall fescue thus far, races may exist that are capable of infecting these cultivars. Populations of *M. grisea* associated with 'Coronado' and 'Coyote', and other resistant cultivars developed in the future, should be monitored occasionally for the appearance of new races. If a relationship between race and lineage can be verified for turfgrass populations of *M. grisea*, a molecular technique such as AFLP markers would be useful for population monitoring. Early race identification will enable turfgrass breeders to develop cultivars with resistance to new races before they become dominant in the pathogen population.

Literature Cited

1. Correa-Victoria, F. J., Zeigler, R. S., and Levy, M. 1994. Virulence characteristics of genetic families of *Pyricularia grisea* in Colombia. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
2. Fraser, M. L. 1997. Tall fescues vary in leaf spot susceptibility. Golf Course Manage. 65:64-66.
3. Gaut, B. S., Tredway, L. P., Kubik, C., Gaut, R. L., and Meyer, W. A. 2000. Phylogenetic relationships and genetic diversity among members of the *Festuca-Lolium* complex (poaceae) based on ITS sequence data. Plant Syst. Evol. 224:33-53.

4. Hofmann, N. E., and Hamblin, A. M. 2001. Segregation of gray leaf spot resistance in tall fescue. 2001 Annual Meeting Abstracts [ASA/CSSA/SSSA].
5. Igarashi, S. 1990. Update on wheat blast (*Pyricularia oryzae*) in Brazil. Pages 25-33 in: Proc. Int. Conf. Wheat for the Nontraditional Warm Areas. D. A. Saunders, ed. United Nations Development Programme International Maize and Wheat Improvement Center, Mexico.
6. Landschoot, P. J., and Hoyland, B. F. 1992. Gray leaf spot of perennial ryegrass turf in Pennsylvania. Plant Dis. 76:1280-1282.
7. Magness, J. R., Markle, G. M., and Compton, C. C. 1971. Food and Feed Crops of the United States. Interregional Research Project IR-4, Bul. 828 New Jersey Agr. Expt. Sta., New Brunswick, NJ.
8. Malca, I., and Owen, J. H. 1957. The gray leaf spot disease of St. Augustinegrass. Plant Dis. Rep. 41:871-875.
9. Roumen, E. C. 1994. A strategy for accumulating genes for partial resistance to blast disease in rice within a conventional breeding program. Pages 211-229 in: Rice Blast Disease. R. S. Zeigler, S. A. Leong and P. S. Teng, eds. CAB International, Oxon, UK.
10. 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.
11. Trevathan, L. E., Moss, M. A., and Blasingame, D. 1994. Ryegrass blast. Plant Dis. 78:113-117.
12. 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.

13. Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C., and Nelson, R. J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434.