Mating Type Distribution and Fertility Status in *Magnaporthe grisea* Populations from Turfgrasses in Georgia

L. P. Tredway and K. L. Stevenson, Department of Plant Pathology, University of Georgia, Athens 30602; and L. L. Burpee, Department of Plant Pathology, Georgia Station, University of Georgia, Griffin 30223

ABSTRACT

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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 polymerase chain reaction based assay for mating type was developed to facilitate population analysis. *M. grisea* populations from St. Augustinegrass in Georgia were dominated by the *Mat1-1* mating type, whereas populations from tall fescue were dominated by *Mat1-2*. The opposite mating type was found in low frequency (0 to 5.7%) associated with each host. The fertility status of isolates from two populations was determined using controlled crosses in vitro. Seventy-eight *Mat1-1* isolates from St. Augustinegrass were sterile in test crosses, but a single *Mat1-2* isolate from St. Augustinegrass was male fertile. Of 87 *Mat1-2* isolates from tall fescue, 47 were male fertile in test crosses, but 19 produced perithecia that were barren. All *Mat1-1* isolates from tall fescue were sterile. Although both mating types exist in *M. grisea* populations from turfgrasses in Georgia, no female fertile isolates were identified in sample populations. The predominance of one mating type in eight sample populations and absence of female fertile isolates in two sample populations indicates that sexual reproduction may not occur with significant frequency in *M. grisea* populations associated with turfgrasses in Georgia.

Additional keywords: *Festuca arundinacea*, gray leaf spot, *Pyricularia*, *Stenotaphrum secundatum*

Magnaporthe grisea (Hebert) Yaegashi & Udagawa (anamorph: *Pyricularia grisea* (Cooke) Sacc.) is a pathogen of 22 genera of grasses (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 sources of resistance 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).

M. grisea is a heterothallic ascomycete, thus it requires the interaction of two mating types, *Mat1-1* and *Mat1-2*, to mate and reproduce sexually (46). The absence of one mating type in a population precludes mating, but other barriers may also prevent mating when both mating types are present. *M. grisea* isolates vary in fertility status, or the ability to produce (as females) or induce (as males) the production of perithecia (46). Isolates may be hermaphroditic (male and female fertile), female fertile (and male sterile), male fertile (and female sterile), or completely sterile (46). Other poorly understood compatibility factors also influence mating, so that the fertility of a cross is determined by an interaction of the parental genotypes (3,5,19,23,30). Finally, in a fertile cross between two isolates of opposite mating type, the perithecia may be barren or lacking asci and ascospores due to unknown factors (46).

Sexual reproduction by *M. grisea* has not been observed in nature. Both mating

types coexist in the majority of *M. grisea* populations from rice, but one mating type is typically dominant, and most isolates are male fertile or completely sterile (5,7,11,15,16,18,20,27,29,31,37,38,45). Evidence of genetic recombination has been detected in *M. grisea* populations from rice in India (5,20), but most *M. grisea* populations from rice appear to be asexual.

The frequency of female fertile and hermaphroditic isolates in *M. grisea* populations associated with other grasses is generally higher than in populations from rice (15,16,37,38,43,44). The presence of female fertile or hermaphroditic isolates in a population indicates that sexual reproduction has occurred in the recent past, and also increases the likelihood that sexual reproduction will occur in the future (46). 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). It is possible that sexual reproduction is occurring in *M. grisea* populations associated with hosts other than rice, however, no extensive surveys of *M. grisea* populations associated with these hosts have been reported.

Little is known about the distribution of mating types and fertility status in *M. grisea* populations from turfgrasses, particularly tall fescue and St. Augustinegrass. A single isolate from meadow fescue (*Festuca elatior* L.) in Japan, analyzed by Yaegashi (43), was sterile and its mating type was undetermined. Among seven isolates from St. Augustinegrass collected in the United States, two were fertile in controlled crosses and were designated mating type *Mat1-1* (43). *M. grisea* populations from perennial ryegrass and St. Augustinegrass have been analyzed by Viji and Uddin (39) and Viji et al. (40). In a collection of 194 *M. grisea* isolates from 33 perennial ryegrass stands in the northeastern and midwestern United States, only *Mat1-2* isolates were found (40). Of 312 isolates from perennial ryegrass collected primarily in the northeastern United States, all were *Mat1-2* and male fertile, but produced perithecia that were barren (39). Of 62 isolates from St. Augustinegrass in Florida, 19 produced perithecia in test crosses and were male fertile or female fertile, but no hermaphrodites were detected (39). Of the 19 fertile isolates from St. Augustinegrass,

Corresponding author: Lane P. Tredway
E-mail: lane_tredway@ncsu.edu

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

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15 were the *Mat1*-1 mating type whereas 4 were *Mat1*-2 (39).

The objectives of this research were to (i) assess the distribution of mating types in *M. grisea* populations associated with turfgrasses in Georgia, and (ii) determine the fertility status of isolates in selected populations.

MATERIALS AND METHODS

Populations, collection, and isolation.

Nine hundred seventy six isolates of *M. grisea* were collected from St. Augustinegrass and tall fescue turf stands throughout Georgia in 1999 and 2000 (Fig. 1; Table 1). Locations were selected to allow the separation of factors that may influence the distribution of mating types in turfgrass populations, such as geography, host species, and host cultivar (Table 1). Populations 1 to 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, leaf blades exhibiting gray leaf spot symptoms were collected on 3 m centers in a 27 × 27 m grid, to yield 100 collection points from a 729-m² area. The leaf samples were allowed to dry overnight in paper bags at room temperature (23 to 25°C).

Leaf sections containing individual lesions were excised from dried symptomatic leaves, rinsed in sterile H₂O for 30 s, then surface disinfested in 70% ethanol for 30 s and 10% Clorox 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 25°C) under continuous fluorescent illumination to induce sporulation of *M. grisea*. The lid of each plate was rotated 90° daily to encourage spore release and deposition on the agar surface. From each leaf section, a

single isolate was obtained by transferring a germinating conidium to a fresh petri dish containing potato dextrose agar amended with tetracycline, streptomycin, and chloramphenicol at 50 mg per kg each. Each culture was overlaid with several sterilized filter paper sections and incubated at 20°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 25°C), and then stored at -80°C. In addition, each isolate was transferred to a 16 × 100 mm culture tube containing sterilized rye (*Secale cereale* L.) grains (2.75 g of rye grain, 0.08 g of CaCO₃, and 5 ml of H₂O), incubated at 20°C for 10 to 14 days, then stored at -80°C.

Mating type assay. *M. grisea* isolates of known and unknown mating type were provided by J. C. Correll (University of Arkansas, Fayetteville), M. L. Farman (University of Kentucky, Lexington), or were isolated from turfgrasses in Georgia (Tables 2 and 3). Each isolate was grown for 7 days at room temperature (23 to 25°C) 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). S. Kang (Pennsylvania State University, University Park) provided sequences of the mating type idiomorphs *Mat1*-1 and *Mat1*-2 (17). Potential 24-mer polymerase chain reaction (PCR) primers with a melting temperature of 60°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'-ACAGGATGTAGGCATT-CGAGGAC-3') are homologous to the *Mat1*-1 idiomorph and yield a product of 552 bp. Primers T1 (5'-ACAAGGCAACCATCTTGGACCCTG-3') and T2 (5'-CCAAAACACCGAGTGCCATCAAGC-3') are homologous to the *Mat1*-2 idio-

Fig. 1. Location of *Magnaporthe grisea* populations sampled in 1999 and 2000 and analyzed for mating type distribution.

Table 1. *Magnaporthe grisea* populations from turfgrasses in Georgia sampled in 1999 and 2000, and distribution of mating types in populations as determined by a polymerase chain reaction based assay

Population	County	Host species	Cultivar	Collection dates	1999		2000	
					Mat1-1	Mat1-2	Mat1-1	Mat1-2
1	Lanier	St. Augustinegrass ^a	Mercedes	7/13/99, 7/18/00	65	0	78	0
2	Webster	St. Augustinegrass	Palmetto	7/14/99, 7/17/00	55	0	79	0
3	Taylor	St. Augustinegrass	Palmetto	7/29/99, 7/31/00	42	1	64	0
4	Laurens	Tall fescue ^b	Rebel III	8/16/99, 8/14/00, 8/24/00	2	42	0	85
5	Cherokee	Tall fescue	Rebel III	9/1/99, 9/14/00	0	61	5	82
6	Floyd	Tall fescue	Confederate	9/1/99, 9/14/00	3	63	0	70
7	Paulding	Tall fescue	Bonzai	8/13/99, 9/7/00	0	10	0	68
8	Paulding	St. Augustinegrass	Raleigh	8/13/99, 8/9/00	16	0	85	0

^a *Stenotaphrum secundatum*.

^b *Festuca arundinacea*.

morph 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 MgCl₂, 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 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min, and a final extension step at 72°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. The plasmids SK43 and SK44, provided by S. Kang, contain fragments of the *Mat1*-1 and *Mat1*-2 idiomorphs, respectively, and were used as hybridization probes. 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 -³²P]dCTP using the Decaprime II Kit (Ambion Inc., Austin, TX). Hybridization was conducted at 65°C for 16 h in a buffer containing 7% sodium dodecyl sulfate (SDS), 1 mM EDTA, and 0.5 M NaHPO₄,

followed by two 20-min washes in buffer containing 2× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 sodium citrate, pH 7.0) and 0.2% SDS.

Determination of fertility status. In 2000, one *M. grisea* population each from tall fescue (Cherokee County) and St. Augustinegrass (Lanier County) was selected for determination of fertility status using controlled crosses in vitro. Each isolate was crossed with isolates 2539 (*Mat1*-1) and Guy11 (*Mat1*-2), which are standard hermaphroditic tester strains used for determining fertility status in *M. grisea* be-

cause of their high fecundity (22). Because the fertility of a cross is dependent on an interaction among the parental genotypes, isolates from turfgrass populations in Georgia were also included as tester strains to more accurately assess fertility status in natural populations. Each isolate from tall fescue was crossed with two *Mat1*-1 isolates from tall fescue (1213-8 and 533-78). Isolates 1213-8 and 533-78 were isolated from tall fescue cv. Rebel III from the Cherokee County and Laurens County populations, respectively. In preliminary studies, all *Mat1*-1 isolates from tall fescue

Table 3. *Magnaporthe grisea* isolates that required reduced annealing temperatures in polymerase chain reaction for successful amplification

Lane ^a	Isolate	Host	Location	Year	Mating type ^b
a	1100-11	<i>Digitaria sanguinalis</i>	Georgia	2000	Mat1-2
b	1100-14	<i>D. sanguinalis</i>	Georgia	2000	Mat1-2
c	1211-05	<i>Pennisetum glaucum</i>	Georgia	2000	nd ^c
d	1211-06	<i>P. glaucum</i>	Georgia	2000	nd ^c
e	1206-13	<i>D. a sanguinalis</i>	Georgia	2000	Mat1-1
f	1206-17	<i>D. sanguinalis</i>	Georgia	2000	Mat1-1
g	PH55	<i>Pennisetum sp.</i>	Philippines	1990	Mat1-1
h	DsKY96-1	<i>D. sanguinalis</i>	Kentucky	1996	Mat1-1

^a Lane assignment in agarose gel depicted in Fig. 3.

^b Tentative mating type assignment based on Southern analysis.

^c nd = Mating type could not be determined.

Table 2. Diverse collection of *Magnaporthe grisea* isolates successfully characterized for mating type using the polymerase chain reaction based assay

Lane ^a	Isolate	Host	Origin	Year	Mating type
1	CD156	<i>Eleusine coracana</i>	Cote d'Ivoire	1992	Mat1-1
2	Jp29	<i>E. coracana</i>	Japan	1991	Mat1-1
3	RW18	<i>E. coracana</i>	Rwanda	1991	Mat1-1
4	533-78	<i>Festuca arundinacea</i>	Georgia	1999	Mat1-1
5	2539	n/a	lab strain	1988 ^b	Mat1-1 ^c
6	75A1	<i>Oryza sativa</i>	Arkansas	1975 ^d	Mat1-1 ^c
7	Fr13	<i>O. sativa</i>	France	1986 ^c	Mat1-1 ^c
8	Fr9	<i>O. sativa</i>	France	1988 ^c	Mat1-1 ^c
9	In17	<i>O. sativa</i>	India	1991	Mat1-1
10	IT2	<i>O. sativa</i>	Italy	1986 ^c	Mat1-1 ^c
11	LO1-4	<i>O. sativa</i>	Arkansas	1991 ^d	Mat1-1 ^c
12	GF9	<i>Setaria feberii</i>	Kentucky	1998	Mat1-1 ^c
13	SiKY97-1	<i>S. italica</i>	Kentucky	1998	Mat1-1
14	GrF6	<i>S. viridis</i>	Kentucky	1998	Mat1-1 ^c
15	YF2	<i>S. viridis</i>	Kentucky	1998	Mat1-1 ^c
16	Pg470	<i>Stenotaphrum secundatum</i>	Georgia	1999	Mat1-1
17	Br32	<i>Triticum aestivum</i>	Brazil	1991	Mat1-1
18	Br80	<i>T. aestivum</i>	Brazil	1991	Mat1-1
19	Ep1	unknown	unknown	1991	Mat1-1
20	RN1	<i>Zingiber sp.</i>	unknown	1986	Mat1-1
21	531-19	<i>Digitaria sanguinalis</i>	Georgia	1999	Mat1-2
22	PH42	<i>E. coracana</i>	Philippines	1991	Mat1-2
23	RW12	<i>E. coracana</i>	Rwanda	1991	Mat1-2
24	669	<i>F. arundinacea</i>	Georgia	1991	Mat1-2
25	LpKY97-1C	<i>Lolium perenne</i>	Kentucky	1997	Mat1-2 ^c
26	GG9	<i>L. perenne</i>	Kentucky	1997	Mat1-2 ^c
27	CR24	<i>L. perenne</i>	Korea	unknown	Mat1-2
28	Guy11	<i>O. sativa</i>	French Guyana	1988 ^b	Mat1-2 ^c
29	LO3-8	<i>O. sativa</i>	Arkansas	1991 ^d	Mat1-2 ^c
30	BM1-24	<i>O. sativa</i>	Arkansas	1991 ^d	Mat1-2 ^c
31	CrF5	<i>S. viridis</i>	Kentucky	1998	Mat1-2 ^c
32	McC2	<i>S. viridis</i>	Kentucky	1998	Mat1-2 ^c
33	ARC2	<i>S. viridis</i>	Kentucky	1998	Mat1-2 ^c
34	CrF3	<i>S. viridis</i>	Kentucky	1998	Mat1-2 ^c
35	455-2	<i>Stenotaphrum secundatum</i>	Georgia	1999	Mat1-2

^a Lane assignment in agarose gel depicted in Fig. 2.

^b Leung et al., 1988 (22).

^c Mating type of isolate previously known.

^d Xia et al., 1993 (42).

^e Roument et al., 1997 (32).

were sterile, so these isolates were selected at random from the *Mat1-1* isolates obtained from tall fescue. Each isolate from St. Augustinegrass was also crossed with a *Mat1-2* tall fescue isolate (1213-30) and a *Mat1-2* isolate from St. Augustinegrass (455-2). Isolate 1213-30 was isolated from tall fescue cv. Rebel III from the Cherokee County population, and isolate 455-2 was obtained from St. Augustinegrass cv. Palmetto at the Taylor County location. In preliminary studies, 1213-30 and 455-2 were male fertile in crosses with isolate 2539 and, therefore selected as tester strains.

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 of oatmeal (Gerber Products Co., Fremont, MI), 15 g of Bacto Agar (Becton Dickinson Co, Sparks, MD) per liter of H₂O] were divided into eight equal sectors, and plugs of PDA containing mycelium of the unknown and tester isolates were transferred to alternating sectors. Cultures were incubated at 25°C under continuous fluorescent illumination and monitored weekly for the presence of perithecia. The fertility status

of unknown isolates was determined by observing the pattern of perithecia production at the interface between the unknown and tester strains as described by Itoi et al. (15). Female fertile isolates produce perithecia on their side of the interface, whereas male fertile isolates induce the production of perithecia on the tester strain side of the interface. Isolates that produce perithecia on both sides of the interface are considered hermaphroditic. When fully developed perithecia were observed, fecundity was measured by counting the number of perithecia per unit area using a dissecting microscope. Fully developed perithecia were excised from the culture and checked for the presence of asci and ascospores microscopically. Each cross was conducted in two to three independent experiments, with one to three replicates per experiment. Positive control crosses between 2539 and Guy11 were included in each experiment to ensure that conditions were favorable for mating.

RESULTS

Mating type assay. PCR products of the expected size were produced from all iso-

lates of known mating type (Fig. 2A). For unknown isolates, the PCR products were readily distinguished as corresponding to *Mat1-1* or *Mat1-2*. Southern analysis of the PCR products by hybridization with the cloned mating type idiomorphs demonstrated that the observed 552-bp bands were homologous to *Mat1-1* (Fig. 2B), whereas the observed 390-bp bands were homologous to *Mat1-2* (Fig. 2C). *M. grisea* isolates from *Pennisetum glaucum*, *Pennisetum* sp., and some isolates from *Digitaria sanguinalis* consistently yielded no PCR products using the procedure described above (Table 3). When the annealing temperature in PCR reactions was lowered to 45°C, four distinct banding patterns were observed (Fig. 3A). Southern analysis with SK43 and SK44 was performed as described above, except that hybridization was conducted at 50°C to detect homology between the PCR products and the mating type idiomorphs. The *Mat1-1* 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. 3B). No ho-

Fig. 2. Application of the polymerase chain reaction (PCR)-based mating type assay to a diverse collection of *Magnaporthe grisea* isolates of known and unknown mating type. Lanes 1 to 35 = isolates listed in Table 1. Lanes 36 and 37 = negative PCR controls containing no template. Lane 38 = positive hybridization control containing the *Mat1-1* idiomorph (SK43) digested with *KpnI/SalI* or the *Mat1-2* idiomorph (SK44) digested with *KpnI/HindIII*. 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 *Mat1-1* idiomorph (SK43) as a hybridization probe. **C**, Southern analysis of PCR products in **A** using the cloned *Mat1-2* idiomorph (SK44) as a hybridization probe.

mologous sequences were detected by the *Mat1-2* idiomorph in these isolates (Fig. 3C). The *Mat1-2* idiomorph hybridized to bands of 300 bp from isolates 1100-11, 1100-14, 1211-05, and 1211-06 (Fig. 3C). The *Mat1-1* 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. 3B).

Mating type distribution in Georgia turfgrass populations. A total of 976 *M. grisea* isolates were obtained in 1999 and 2000. Due to varying rates of isolation success, sample sizes for each population ranged from 16 to 85 isolates. In 1999 and 2000, populations of *M. grisea* from St. Augustinegrass were predominantly *Mat1-1* (97.6 to 100%), whereas populations from tall fescue were dominated by *Mat1-2* isolates (94 to 100%, Table 1). The opposite mating type was found in low frequency (0 to 6%) in some populations from St. Augustinegrass and tall fescue. In 1999, a single *Mat1-2* isolate was obtained from St. Augustinegrass cv. Palmetto in Taylor County, but no *Mat1-2* isolates were obtained from St. Augustinegrass in 2000. In populations from tall fescue, *Mat1-1* 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. All St. Augustinegrass isolates from the 2000 Lanier population failed to form perithecia in crosses with 2539, Guy11, 1213-30, and 455-2. All tall fescue isolates from the 2000 Cherokee population failed to form perithecia in crosses with Guy11, 1213-8, and 533-78, but the fertility status 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 from tall fescue 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 4). 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/cm² was produced in crosses between male fertile tall fescue isolates and isolate 2539, whereas 80 perithecia/cm² 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 fertility status of an unknown isolate is dependent on 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. Using this assay, we correctly determined the mating type of 18 known isolates, and also characterized 976 isolates of unknown mating type from turfgrasses in Georgia.

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

Fig. 3. Analysis of *Magnaporthe grisea* isolates that yielded no polymerase chain reaction (PCR) products under standard PCR conditions for the mating type assay. Lanes a to h = isolates of *M. grisea* listed in Table 2. 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 *Mat1-1* idiomorph (SK43) as a hybridization probe. **C**, Southern analysis of PCR products in **A** using the cloned *Mat1-2* idiomorph (SK44) as a hybridization probe.

Table 4. Fertility status of *Magnaporthe grisea* isolates obtained from tall fescue in Cherokee County, GA in 2000

Test isolate	Sterile	Perithecia barren			Perithecia with asci		
		Male fertile	Female fertile	Hermaphroditic	Male fertile	Female fertile	Hermaphroditic
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

that their mating types can be determined with confidence.

A single mating type dominated each population of *M. grisea* from turfgrasses analyzed in this study. Populations from St. Augustinegrass were predominantly *Mat1-1*, whereas populations from tall fescue were predominantly *Mat1-2*. The opposite mating type was found in low frequencies in association with each host. On the basis of these results, we conclude that selection due to host species influences mating type distribution. Based on comparison of populations differing in cultivar and geographical location, it does not appear that host cultivar or geographical location influence mating type distribution.

The majority of turfgrass isolates analyzed for fertility status in this study failed to produce perithecia and is presumed to be sterile. The single *Mat1-2* isolate obtained from St. Augustinegrass was male fertile in crosses with isolate 2539. A total of 47 (54%) tall fescue isolates (all *Mat1-2*) were also male fertile in crosses with 2539, but 19 (22%) produced barren perithecia. Due to the absence of female fertile or hermaphroditic isolates in two sample populations, no fertile crosses among turfgrass isolates were observed in this study.

On the basis of this research, we conclude that the eight sample populations of *M. grisea* from tall fescue and St. Augustinegrass in Georgia are similar to populations from rice and other hosts with respect to mating type distribution and fertility status (5,11,15,16,18,20,27,29,31,37,38,45). The predominance of one mating type and absence of female fertile or hermaphroditic isolates in sample populations is symptomatic of a clonal population structure (28,46). Sexual reproduction does not appear to have had a significant effect on the genetic structure of our sample populations of *M. grisea* from turfgrasses in Georgia. Other evolutionary forces, such as mutation and gene flow, are alternative sources of genotypic variation that must be considered. Further research is needed to determine the significance of mutation and gene flow in *M. grisea* populations from turfgrasses.

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