Use of a Polymerase Chain Reaction Assay to Aid in Identification of *Gaeumannomyces graminis* var. *graminis* from Different Grass Hosts

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ABSTRACT

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A previously described polymerase chain reaction (PCR) assay results in the amplification of a 188-bp product from *Gaeumannomyces graminis* var. *graminis* fungal template DNA derived from boiled fungal mycelia. Similar results are obtained when mycelia are grown on potato-dextrose agar, Czapek solution agar, and Luria-Bertani agar, but not on a medium selective for *Gaeumannomyces* (SM-7). Amplification of this specific DNA fragment combined with observation of lobed hyphopodia can be used to identify a *Gaeumannomyces*-like isolate as *G. g. graminis*, even if perithecia are never produced by the isolate. All 40 "presumptive" *G. g. graminis* test isolates produced lobed hyphopodia in culture and the 188-bp PCR product, but only 19 of these isolates produced mature perithecia. Additional amplification products (i.e., products other than the expected 188-bp product) were also obtained with this assay. Although the sizes and numbers of these additional products varied among isolates, they correlated with the particular grass host from which the isolates were derived (bermudagrass, St. Augustinegrass, or rice), which suggested that the grass host selected specific pathogen genotypes. For example, isolates from bermudagrass consistently produced a DNA band pattern that was distinct from the DNA band pattern produced by isolates from St. Augustinegrass.

Gaeumannomyces graminis (Sacc.) Arx & Olivier var. *graminis* has been reported on many members of the Poaceae (24) and is currently known to cause four diseases in this plant family: spring dead spot (14) and bermudagrass decline (3) of bermudagrass (*Cynodon* spp.), take-all root rot of St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) (4,12), and crown (black) sheath rot of rice (*Oryza sativa* L.) (24). The related pathogens *G. g. tritici* Walker and *G. g. avenae* (E. M. Turner) Dennis cause take-all disease of wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.), respectively (24). *G. g. avenae* also causes take-all patch of amenity cool-season turfgrasses, primarily *Agrostis* spp. (20,24).

All *G. graminis* varieties produce superficial hyphae on their hosts (24). Hyphopodia, organs of attachment and penetration, are produced from the superficial hyphae. The three varieties of *G. graminis* are distinguished by their hyphopodia shape and morphological characteristics of perithecia and ascospores. Ascospores of *G. g. avenae* are longer than those of *G. g. tritici* and *G. g. graminis*. *G. g. tritici* and *G. g. avenae* produce nonlobed, simple hyphopodia, whereas *G. g. graminis* produces distinctly lobed hyphopodia. These lobed hyphopodia are produced on plant tissue, primarily basal stems and leaf sheaths, but are also produced readily in culture. The presence of lobed hyphopodia is the characteristic used to confirm the identification of *G. g. graminis*.

Although perithecia of *G. g. graminis* are produced on rice in the field, they are infrequently observed on other grasses (23,24; M. L. Elliott, *personal observation*). In laboratory culture with a plant source present, we observed that isolates from rice and St. Augustinegrass usually produced perithecia when first evaluated, whereas isolates from bermudagrass did not, even immediately after isolation. In South Africa, *G. g. graminis* isolates from *C. transvaalensis* Burtt-Davy (African bermudagrass) and *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyugrass) produced perithecia in culture but isolates from *Setaria italica* (L.) P. Beauv. (foxtail millet) did not (19). In addition, a minimum of 3 wk is normally required to produce perithecia with mature ascospores.

Therefore, one must initially describe a *Gaeumannomyces* isolate with lobed hyphopodia as a "presumptive" *G. g. graminis* isolate. Currently, if perithecia and conidia are never produced, verification of the isolate as *G. g. graminis* cannot be accomplished. If the appropriate conidia are observed, the isolate may be described as a *Phialophora* sp. (lobed hyphopodia). However, use of the name *Phialophora* should be used with caution since isolates producing only phialospores are often obtained from grass roots, and many are not anamorphs of *Gaeumannomyces* spp. (24). Also, the three varieties of *G. graminis* cannot be distinguished by their *Phialophora* anamorphs.

For pathologists, especially turfgrass pathologists, even preliminary identification from diseased plant tissue of *Gaeumannomyces*-like fungal colonies as *G. g. graminis* is not possible as this fungus and *G. incrustans* Landschoot & Jackson are often isolated from the same plant (5). Thus far, only *G. g. graminis* has been demonstrated to be pathogenic (3). In addition, *G. incrustans* does not produce lobed hyphopodia (13). Since they are morphologically similar in culture, the two species cannot be distinguished, even on a preliminary basis, until lobed hyphopodia are observed.

The polymerase chain reaction (PCR) (22), used with nested primers initially developed for *G. g. tritici*, results in amplification of a 188-bp product that is common to all three *G. graminis* varieties (9,18). By combining the simplicity and specificity of this PCR assay, which does not require purified DNA, with the unique morphological characteristic of lobed hyphopodia, we proposed that *G. g. graminis* isolates could be identified without producing perithecia and mature ascospores. While conducting this study, we observed that *G. g. graminis* isolates produced additional PCR products (i.e., products other than the expected 188-bp product) that correlated with the grass host of an isolate.

MATERIALS AND METHODS

Isolates and media. *G. g. graminis* isolates used in this study are listed in Table 1. These isolates were obtained by us, except

for the soybean isolates, which were kindly provided by C. Rothrock, University of Arkansas. Other fungal isolates included in the study were *G. g. avenae* isolate "LL" from creeping bentgrass in Rhode Island, kindly provided by P. Landschoot, Pennsylvania State University, and *G. g. tritici* isolate 568 from wheat in Montana. All isolates were stored on potato-dextrose agar (Difco, Detroit, MI) (PDA) slants at 2–4 C and in sterile glycerol at −70 C. For the initial PCR tests, all isolates were grown on PDA supplemented with 100 $\mu g\ ml^{-1}$ streptomycin sulfate (Sigma, St. Louis, MO) (PDAS). For PCR tests comparing results from different growth media, isolates were grown on PDAS, a selective medium for *Gaeumannomyces*-like fungi (SM-7) (2), Czapek solution agar (CSA) (Difco), and Luria-Bertani agar (LB) (17). Cultures were incubated at 28 C for 7–14 days.

PCR and PCR product analysis. Fungal template DNA was prepared by aseptically cutting mycelia (2 by 5 cm) from the surface of an agar culture plate and boiling these agar-mycelia blocks in 3–5 ml of 8 mM Tris-HCl (pH 8.5) for 15 min to release DNA. Fungal DNA solutions were either used immediately upon cooling or were stored frozen at −20 C. Purified DNA of *G. g. tritici,* for use as a positive control, was obtained as described previously (8).

PCR tests were based on the nested primer technique developed for *G. graminis* by Schesser et al (18). Each reaction tube contained 25 μL of fungal template DNA, 1 μM of each outside primer (KS1F and KS2R), 1.25 U of *Taq* DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 0.125 mM deoxynucleotide triphosphates (U.S. Biochemical Corp., Cleveland, OH), 5 μl of amplification buffer with either 1.5, 2.5, 5.0, or 9.0 mM $MgCl_2$ (17), and enough sterile water to obtain a total volume of 50 μl. One drop of mineral oil was added to each tube. Two different thermal cyclers were used in this study, a BIOS Bioscyler Oven (New Haven, CT) in Florida and a COY Thermocycler (Ann Arbor, MI) in Montana. Thermal cycling conditions were as follows: denaturation for 1 min at 92 C, annealing for 2 min at 52 C, and extension for 3 min at 72 C.

After 20 cycles, products were diluted 25-fold with sterile water, and 2 μL of each diluted product was amplified another 35 cycles (same conditions) in a 50-μL reaction with fresh *Taq* polymerase, deoxynucleotide triphosphates, amplification buffer (3.0 mM $MgCl_2$), and 1 μM of each inside primer (KS4F and KS5R). No mineral oil was added to the tubes in Florida as it was not necessary with the oven thermal cycler. Oil was added to the first series of reactions with the outside primers to prevent evaporation since the processed reaction tubes were stored at 2–4 C until the second series of reactions was completed.

Primers used in Florida were synthesized by the Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville. Primers used in Montana were synthesized by the Veterinary Laboratory, Montana State University, Bozeman. For

TABLE 1. Production of perithecia, the expected 188-bp PCR product and additional PCR products by *Gaeumannomyces graminis* var. *graminis* isolates

Isolate	Plant source[a]	Location[b]	Perithecia	PCR products (bp) from PDAS[c]					
				287	250	220	188	160	150
561	Soybean	Georgia	+	+	−	−	+	−	−
562	Soybean	Georgia	+	+	−	−	+	−	−
FL-19	Bermudagrass	Miami Lakes, FL	+	−	+	−	+	+	−
FL-25	Rye/bermudagrass mix	Hollywood Lakes, FL	+	−	+	−	+	+	−
FL-36	Bermudagrass	Doral, FL	+	−	+	−	+	+	−
FL-46	Bermudagrass	Riomar, FL	+	−	+	−	+	+	−
FL-90	Bermudagrass (fairway)	Avila, FL	−	−	+	−	+	+	−
FL-140	Bermudagrass	FLREC, FL	−	−	+	−	+	+	−
FL-145	Bermudagrass	Stonebridge, FL	−	−	+	−	+	+	−
FL-151	Bermudagrass	Weston Hills, FL	−	−	+	−	+	+	−
FL-152	Bermudagrass	Weston Hills, FL	−	−	+	−	+	+	−
FL-167	Bermudagrass	Nassau, Bahamas	−	−	+	−	+	+	−
FL-171	Bermudagrass	Nassau, Bahamas	−	−	+	−	+	+	−
FL-177	Bermudagrass	Delray Dunes, FL	−	−	+	−	+	+	−
FL-179	Bermudagrass	FLREC, FL	−	−	+	−	+	+	−
FL-180	Bermudagrass	FLREC, FL	−	−	+	−	+	+	−
FL-208	Bermudagrass (tee)	Loxahatchee, FL	−	−	+	−	+	+	−
FL-211	Bermudagrass (tee)	Loxahatchee, FL	−	−	+	−	+	+	−
FL-218	Bermudagrass	Forest, FL	−	−	+	−	+	+	−
FL-219	Bermudagrass	Forest, FL	−	−	+	−	+	+	−
FL-220	Bermudagrass	Forest, FL	−	−	+	−	+	+	−
FL-39	St. Augustinegrass (sod)	South Bay Growers, FL	+	+	−	+	+	−	+
FL-101	St. Augustinegrass (lawn)	South Bay, FL	+	+	−	+	+	−	+
FL-102	St. Augustinegrass (lawn)	South Bay, FL	−	+	−	+	+	−	+
FL-104	St. Augustinegrass (sod)	Mace K-19, FL	+	+	−	+	+	−	+
FL-105	St. Augustinegrass (sod)	Mace N-29, FL	+	+	−	+	+	−	+
FL-106	St. Augustinegrass (sod)	Mace N-36, FL	−	+	−	−	+	−	−
FL-155	St. Augustinegrass (lawn)	Montgomery 1081-1, AL	−	+	−	−	+	−	−
FL-161	St. Augustinegrass (lawn)	Montgomery 1082, AL	−	+	−	−	+	−	−
FL-163	St. Augustinegrass (lawn)	Montgomery 1083, AL	+	+	−	+	+	−	+
FL-195	St. Augustinegrass (lawn)	Royal, FL	+	+	−	+	+	−	+
FL-196	St. Augustinegrass (lawn)	Dodger, FL	−	+	−	+	+	−	+
FL-197	St. Augustinegrass (lawn)	Dodger, FL	+	+	−	+	+	−	+
FL-198	St. Augustinegrass (lawn)	Royal, FL	+	+	−	+	+	−	+
FL-199	St. Augustinegrass (lawn)	Royal, FL	+	+	−	+	+	−	+
FL-173	Rice	Belle Glade, FL	+	−	−	−	+	(+)[d]	−
FL-174	Rice	Belle Glade, FL	−	−	−	−	+	(+)	−
FL-175	Rice	Belle Glade, FL	+	−	−	−	+	(+)	−
FL-176	Rice	Belle Glade, FL	+	−	−	−	+	(+)	−
FL-221	Rice	Belle Glade, FL	+	−	−	−	+	(+)	−

[a] All bermudagrass isolates were obtained from golf course putting greens unless otherwise noted.
[b] Location descriptions include state or country and golf course, sod farm, or homeowner name, if known. Otherwise, only the city name and sample designation is provided.
[c] The PCR assay was conducted with mycelia obtained from potato-dextrose agar with streptomycin (PDAS).
[d] (+), band was less visible in comparison to band from bermudagrass isolates.

negative controls in this study, sterile water was substituted for template DNA in reaction mixtures. The Tris-HCl buffer or uninoculated agar, boiled in buffer, may also be used as negative controls (unpublished data). Positive controls used purified *G. g. tritici* DNA (2 μl; approximately 0.2–0.4 μg) as the template DNA. At least three negative controls and two positive controls were included in each set of 36 reactions. The thermal cylers were preheated to 92 C before reaction mixtures were placed in them.

Final reaction products (45 μL) were either electrophoresed for 3–5 h at 60 mA (2.8 V cm^{-1}) in 3% agarose (Low EEO, Fisher Scientific, Pittsburgh, PA) gels or, to enhance separation of products and optimize resolution, for 15 h at 30 mA (1.5 V cm^{-1}) in 3% agarose (Sea Plaque, FMC BioProducts, Rockland, ME) gels with Tris-borate buffer (17). Gels were stained with ethidium bromide and photographed with ultraviolet illumination (17).

Fungal template DNA from all isolates was initially used for PCR tests (first series reactions) at each of four concentrations of $MgCl_2$ (1.5, 2.5, 5.0, and 9.0 mM) in the amplification buffer. PCR tests were repeated at least once more for all isolates, but only at 2.5 mM $MgCl_2$.

Production of hyphopodia and perithecia. For each *G. g. graminis* isolate, three agar plugs were transferred from a PDAS culture to a water agar plate (1.5% Bacto agar, Difco) with three germinated, surface-sterilized wheat seeds per plate (5,21). The 5-mm-diameter agar plugs were placed next to emerging roots. Plates were sealed with Parafilm (American National Can, Greenwich, CT) and incubated at 25 ± 2 C with 12 h light per day for 21–24 days. Plates and plants were then examined for lobed hyphopodia and perithecia with mature ascospores. This test was conducted periodically during a 4-yr period as isolates were obtained from plant material. Isolates were tested once, as soon as possible, after their initial isolation. Many isolates were tested again after placement in storage, especially if they had not produced perithecia in the first test.

RESULTS AND DISCUSSION

Although all of the *G. g. graminis* isolates used in this study produced the distinctly lobed hyphopodia characteristic of this variety in culture, fewer than half (19 of 40 isolates) produced perithecia with mature ascospores that would allow for definitive identification of these isolates (Table 1). However, the expected 188-bp PCR amplification product (9,18) was observed for all isolates, at the 2.5 mM $MgCl_2$ concentration of the amplification buffer, when mycelia grown on PDAS were used as fungal template DNA (Table 1). The plant or geographic source of the isolates did not affect production of this particular product. The 188-bp product was not produced by one isolate (FL-46) at the 1.5 mM concentration, two isolates (FL-198 and Fl-221) at the 5.0 mM concentration and 12 isolates (FL-25, FL-46, FL-101, FL-171, FL-173, FL-198, FL-208, FL- 211, FL-218, FL-219, FL-220, and FL-221) at the 9.0 mM concentration of $MgCl_2$ in the amplification buffer. The remaining 25 isolates produced the product at each of the four $MgCl_2$ concentrations.

The magnesium ion concentration in the PCR reaction mixture affects primer annealing, DNA melting temperatures, and enzyme activity (22). Thus, a range of magnesium concentrations were evaluated to determine the best concentration for our conditions. The use of a nested primer technique provided an additional level of specificity in two ways (22). First, because the sample is diluted after the first series of cycles, potential inhibitors are diluted, and a high rate of target DNA to nontarget DNA results. Second, the inside primer set is designed to recognize DNA regions within the previously amplified product which reduced nonspecific primer annealing.

An example of the PCR assay results obtained is illustrated in Figure 1. It should be noted that the same results were obtained in two different laboratories using two different thermal cyclers and sources of the primers. In addition, some isolates (e.g., FL-19, FL-39, and FL-46) have been used repeatedly in our laboratories, as positive checks in ongoing PCR assay studies, with the same results obtained each time. Athough the *G. g. graminis* population used in this study was restricted to isolates from the southeastern United States, a large number of isolates (40) composed that population. The fluorescence intensity of the 188-bp product appeared greater for rice and St. Augustinegrass isolates than for soybean and bermudagrass isolates, perhaps due to a greater amount of target DNA. The efficiency of this PCR assay had been determined previously using purified plasmid pMSU315 DNA, the source of the primers (10). Approximately 195 copies of plasmid DNA are easily detected by this assay. It should also be noted that clarity and separation of amplification products was greatest with FMC Sea Plaque agarose gels that were run overnight. However, to determine quickly and inexpensively the presence or absence of the 188-bp product, the Fisher Low EEO agarose was used and the gel was run for 5 h or less.

To date, other fungi with *Gaeumannomyces*-like colony morphology that potentially could be associated with diseased bermudagrass, St. Augustinegrass, or rice, include *G. incrustans, Magnaporthe poae, M. grisea, Leptosphaeria korrae,* and *Ophiosphaerella herpotrica* (20). These fungi do not produce lobed hyphopodia, and none of the isolates evaluated have produced the 188-bp PCR amplification product when boiled mycelia have been used in the assay (M. L. Elliott and E. A. Des Jardin, *unpublished data*). Even when purified DNA was used in the PCR assay, potentially providing larger quantities of target DNA, only two of 10 *M. poae* isolates (ATCC 64413 from bentgrass, LM-A17 from mixed turfgrass) and one of 34 *G. incrustans* isolates (FL-28 from centipedegrass) produced the 188-bp PCR amplification product across all assay conditions (9; M. L. Elliott and E. A. Des Jardin, *unpublished data*).

We propose to identify *G. g. graminis* isolates using the described PCR assay in conjunction with observations of lobed hyphopodia in culture. The advantages of using the PCR assay, rather than previously described molecular techniques for identifying *G. graminis* (9,25), include: 1) purified DNA is not required for the assay, 2) DNA does not have to be cut with restriction enzymes before separating fragments on agarose gels, and 3) DNA does not have to be transferred to membranes for hybridization with labeled DNA probes. In situations where

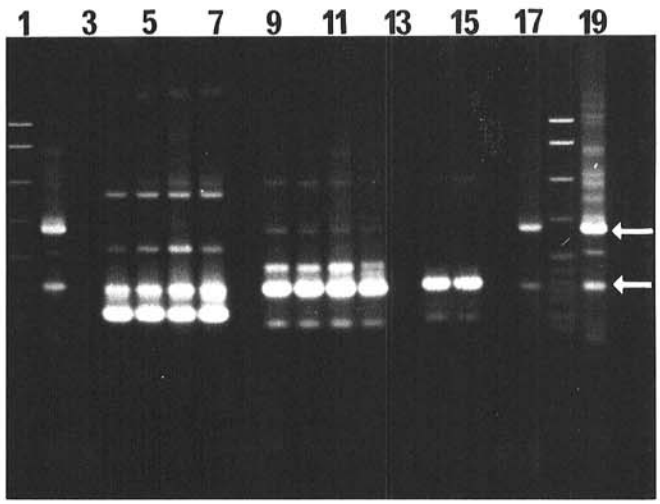

Fig. 1. Examples of polymerase chain reaction (PCR) assay results using mycelia of *Gaeumannomyces graminis* obtained from cultures grown on PDAS. Products were electrophoresed 15 h in a 3% agarose (FMC Sea Plaque) gel. Lanes 1 and 18 are pBR322 digested with *Msp*I for molecular weight standards. Lanes 3, 8, 13, and 16 are negative controls without template DNA, whereas lane 19 is a positive control using purified *G. g. tritici* DNA as template DNA. The arrows indicate the 287- and 188-bp products. Lane 2 is *G. g. graminis* isolate 561 (soybean). Lanes 4–7 are *G. g. graminis* isolates FL-19, FL-90, FL-46, and FL-180 (bermudagrass). Lanes 9–12 are *G. g. graminis* isolates FL-39, FL-102, FL-105, and FL-196 (St. Augustinegrass). Lanes 14 and 15 are *G. g. graminis* isolates FL-173 and FL-174 (rice). Lane 17 is *G. g. avenae* isolate "LL."

isolation of *G. g. tritici* or *G. g. avenae* is unlikely from symptomatic plants (e.g., warm-season turfgrasses and rice) (20,24), a preliminary identification of a colony as *G. g. graminis* may be made based on positive PCR test results, even before lobed hyphopodia are observed. To decrease the chances of obtaining false negatives, it would be advantageous to run the first series reaction of the PCR assay with two $MgCl_2$ concentrations in the amplification buffer, either 5.0 and 2.5 mM or 2.5 and 1.5 mM. Confirmation of an isolate as *G. g. graminis* would necessarily require observation of lobed hyphopodia.

Since a selective medium (SM-7) (2) for isolating *Gaeumannomyces*-like fungi from symptomatic plant tissue is used routinely in our laboratories, PCR assays were conducted for 24 isolates (one from soybean, 11 from bermudagrass, seven from St. Augustinegrass, and five from rice) to determine if mycelia grown on SM-7 agar could be used as template DNA. In addition, mycelia grown on CSA and LB agar, other commonly used media for pure cultures of *G. g. graminis* in our laboratories, were used as sources of template DNA for these isolates. All isolates tested, except FL-176 from rice, produced the 188-bp product from mycelia grown on both CSA and LB agar (Fig. 2). The 188-bp product was not obtained for the bermudagrass isolates and one rice isolate (FL-176) when grown on SM-7 agar, but it was obtained with the remaining rice and St. Augustinegrass isolates tested. However, the fluorescence intensity of the 188-bp product was significantly less than when the isolates were grown on PDAS (Fig. 3). One compound in SM-7 agar is L-3,4-β-dihydroxyphenylalanine (L-DOPA) which is used by *Gaeumannomyces* to produce melanin that is secreted into the agar. It is possible that the melanin, a phenolic compound, is binding the template DNA during the boiling process or during the PCR assay and thus altering the specificity of primer annealing (11).

In general, additional amplification products (i.e., products other than the expected 188-bp product) were dependent on the plant source from which the isolates were initially recovered. These products were most clearly and consistently visualized when FMC Sea Plaque agarose gels were run overnight. Products that were close to 400 bp in size were not observed as consistently as the products described below. This product size corresponds to the product obtained with the first set of primers in the PCR assay (18). Thus, it is possible these primers are present in sufficient quantity in the second reaction mix to result in this 400-bp amplification product.

The two *G. g. graminis* isolates from soybean and three of the St. Augustinegrass isolates (FL-106, FL-155, and FL-161) consistently produced band patterns previously observed for *G. g. tritici* and *G. g. avenae* isolates, primarily a 287-bp product in addition to the 188-bp product (9,10) (Fig. 1). The remaining 11 St. Augustinegrass isolates produced the 287-bp product, although it appeared less intense, but these isolates also yielded strongly visible 220- and 150-bp products. The 150-bp product was also associated with *G. g. tritici* and *G. g. avenae* (9).

All bermudagrass isolates consistently had 250- and 160-bp products amplified (Fig. 1). Interestingly, fluorescence intensity of the 160-bp product was much greater than the expected 188-bp product. In addition, the 160-bp product was still present, whereas the 188-bp product was not, when mycelia of isolates from bermudagrass were grown on SM-7 agar rather than PDAS, CSA, or LB agar (Figs. 2 and 3). This suggests that the primers were annealing at preferred sites of target DNA to amplify the 160-bp product. This 160-bp product, though less intense, was also observed for rice isolates (Fig. 1).

Both the 188- and 287-bp products have strong homology with pMSU315, a 4.3-kb mitochondrial fragment from *G. g. tritici* (8) from which the primers used in this PCR assay were derived (18). DNA hybridizations have yet to be conducted with the other amplification products to determine if they also share homology with the cloned 4.3-kb mitochondrial DNA fragment.

Hamer et al (7) discovered genomic *M. grisea* repeat sequences and demonstrated that distinctive patterns were observed for rice isolates obtained from different rice cultivars. In addition, the number of copies (conservation of sequences) of a particular repeat sequence was significantly greater for *M. grisea* isolates pathogenic on rice than for nonpathogenic rice isolates. These latter isolates, however, were pathogenic on other grass species such as weeping lovegrass or finger millet. Their results suggested that host plants may select particular fungal pathogen genotypes (7).

O'Dell et al (16) observed that the 57 *G. g. tritici* and *G. g. avenae* isolates they examined could be classified into the three host pathogenicity groups (*G. g. tritici*-N isolates pathogenic on wheat and oats; *G. g. tritici*-R isolates pathogenic on wheat, barley, and rye; or *G. g. avenae* isolates pathogenic on wheat and oats) based on restriction fragment length polymorphisms in families

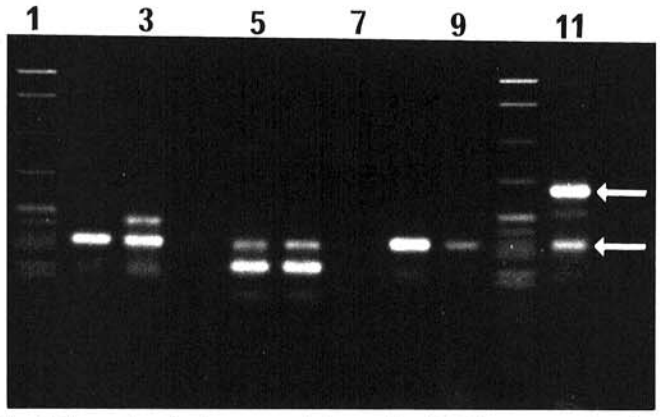

Fig. 2. Examples of polymerase chain reaction (PCR) assay results using mycelia of *G. graminis* obtained from cultures grown on CSA or LB agar. Products were electrophoresed 5 h in a 3% agarose (Fisher Low EEO) gel. Lanes 1 and 10 are pBR322 digested with *Msp*I for molecular weight standards. Lanes 4 and 7 are negative controls without template DNA, whereas lane 11 is a positive control using purified *G. g. tritici* DNA as template DNA. The arrows indicate the 287- and 188-bp products. Lanes 2 and 3, FL-39 (St. Augustinegrass) from CSA and LB, respectively; lanes 5 and 6, FL-19 (bermudagrass) from CSA and LB, respectively; lanes 8 and 9, FL-199 (St. Augustinegrass) from CSA and LB, respectively.

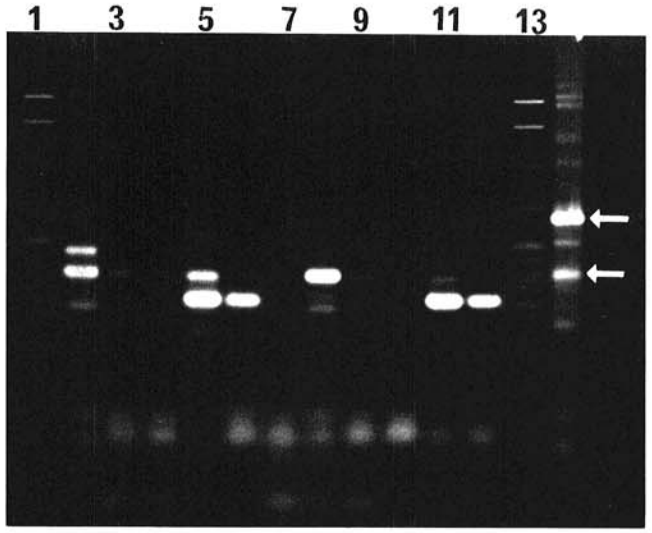

Fig. 3. Examples of polymerase chain reaction (PCR) assay results using mycelia of *G. graminis* obtained from cultures grown on PDAS or SM-7 agar. Products were electrophoresed 5 h in a 3% agarose (Fisher Low EEO) gel. Lanes 1 and 13 are pBR322 digested with *Msp*I for molecular weight standards. Lanes 4, 7, and 10 are negative controls without template DNA, whereas lane 14 is a positive control using purified *G. g. tritici* DNA as template DNA. The arrows indicate the 287- and 188-bp products. Lanes 2 and 3, FL-39 (St. Augustinegrass) from PDAS and SM-7, respectively; lanes 5 and 6, FL-19 (bermudagrass) from PDAS and SM-7, respectively; lanes 8 and 9, FL-199 (St. Augustinegrass) from PDAS and SM-7, respectively; lanes 11 and 12, FL-211 (bermudagrass) from PDAS and SM-7, respectively.

of repeated DNA sequences. However, the N and R isolate types of *G. g. tritici* have not been associated with a particular grass host. Ward and Gray (25) have suggested, based on their results from a limited number of isolates, that there may be an association between the sizes of restriction fragments of amplified ribosomal DNA and grass host of *G. g. avenae* isolates. For the *G. g. graminis* isolates described herein, the pattern of products amplified with this PCR assay was correlated with their grass hosts. More expansive studies must be conducted to determine if selection pressure for host-specific pathogenicity genes has occurred with *G. g. graminis*. Even if no evidence is obtained to support genotype selective pressure, the DNA band patterns observed with our PCR assay may provide a tool for examining genetic variation (6,15), or for conducting complex ecological studies (1) since introduced isolates could be identified based on amplified product profiles.

LITERATURE CITED

1. Chase, T. E., Otrosina, W. H., Spieth, P. T., and Cobb, F. W. Jr., 1991. Use of PCR to distinguish biological species within the *Heterobasidion annosum* complex. (Abstr.) Phytopathology 81:1190.
2. Elliott, M. L. 1991. A selective medium for *Gaeumannomyces*-like fungi. Plant Dis. 75:1075.
3. Elliott, M. L. 1991. Determination of an etiological agent of bermudagrass decline. Phytopathology 81:1380-1384.
4. Elliott, M. L., Hagan, A. K., and Mullen, J. M. 1993. Association of *Gaeumannomyces graminis* var. *graminis* with a St. Augustinegrass root rot disease. Plant Dis. 77:206-209.
5. Elliott, M. L., and Landschoot, P. J. 1991. Fungi similar to *Gaeumannomyces* associated with root rot of turfgrasses in Florida. Plant Dis. 75:238-241.
6. Goodwin, P. H., and Annis, S. L. 1991. Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. Appl. Environ. Microbiol. 57:2482-2486.
7. Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA 86:9981-9985.
8. Henson, J. M. 1989. DNA probe for identification of the take-all fungus, *Gaeumannomyces graminis*. Appl. Environ. Microbiol. 55:284-288.
9. Henson, J. M. 1992. DNA hybridization and polymerase chain reaction (PCR) tests for identification of *Gaeumannomyces, Phialophora* and *Magnaporthe* isolates. Mycol. Res. 96:629-636.
10. Henson, J. M., Goins, T., Grey, W., Mathre, D. E., and Elliott, M. L. Use of polymerase chain reaction (PCR) to detect *Gaeumannomyces graminis* DNA in plants grown in artificially- and naturally-infested soil. Phytopathology. In press.
11. Hill, H. Z., Huselton, C., Pilas, B., and Hill, G. J. 1987. Ability of melanins to protect against radiolysis of thymine and thymidine. Pigment Cell Res. 1:81-86.
12. Krausz, J. P. 1991. Take-all patch suspected in Texas warm-season turf. Southern Turf Manag. 2(12):12.
13. Landschoot, P. J., and Jackson, M. 1989. *Gaeumannomyces incrustans* sp. nov., a root-infecting hyphopodiate fungus from grass roots in the United States. Mycol. Res. 93:55-58.
14. McCarty, L. B., and Lucas, L. T. 1989. *Gaeumannomyces graminis* associated with spring dead spot of bermudagrass in the southeastern United States. Plant Dis. 73:659-661.
15. Nicholson, P., Hollins, T. W., Rezanoor, H. N., and Anamthawat-Jonsson, K. 1991. A comparison of cultural, morphological and DNA markers for the classification of *Pseudocercosporella herpotrichoides*. Plant Pathol. 40:584-594.
16. O'Dell, M., Flavell, R. B., and Hollins, T. W. 1992. The classification of isolates of *Gaeumannomyces graminis* from wheat, rye and oats using restriction fragment length polymorphisms in families of repeated DNA sequences. Plant Pathol. 41:554-562.
17. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18. Schesser, K., Luder, A., and Henson, J. M. 1991. Use of polymerase chain reaction to detect the take-all fungus, *Gaeumannomyces graminis,* in infected wheat plants. Appl. Environ. Microbiol. 57:553-556.
19. Scott, D. B. 1989. *Gaeumannomyces graminis* var. *graminis* on gramineae in South Africa. Phytophylactica 21:251-254.
20. Smiley, R. W., Dernoeden, P. H., and Clarke, B. B. 1992. Compendium of Turfgrass Diseases, 2nd ed. American Phytopathological Society, St. Paul, MN.
21. Speakman, J. B. 1982. A simple, reliable method of producing perithecia of *Gaeumannomyces graminis* var. *tritici* and its application to isolates of *Phialophora*. Trans. Br. Mycol. Soc. 79:350-353.
22. Steffan, R. J., and Atlas, R. M. 1991. Polymerase chain reaction: Applications in environmental biology. Annu. Rev. Microbiol. 45:137-161.
23. Walker, J. 1972. Type studies on *Gaeumannomyces graminis* and related fungi. Trans. Br. Mycol. Soc. 58:427-457.
24. Walker, J. 1981. Taxonomy of take-all fungi and related genera and species. Pages 15-74 in: Biology and Control of Take-all. M. J. C. Asher and P. J. Shipton, eds. Academic Press, London.
25. Ward, E., and Gray, R. M. 1992. Generation of a ribosomal DNA probe by PCR and its use in identification of fungi within the *Gaeumannomyces-Phialophora* complex. Plant Pathol. 41:730-736.