# Use of Polymerase Chain Reaction to Detect *Gaeumannomyces graminis* DNA in Plants Grown in Artificially and Naturally Infested Soil

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## ABSTRACT

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The polymerase chain reaction was used to specifically amplify DNA of *Gaeumannomyces graminis*, a filamentous soilborne fungus that causes root and crown disease of cereals and turfgrasses. Nested primers were used to amplify a 188-bp fragment of mitochondrial DNA from fungal and infected-plant samples, which were simply boiled to release target DNA. Fungal DNA was amplified from single boiled hyphal tips or ascospores. *G. graminis* DNA was also detected in boiled crowns and roots from experimentally and naturally infected wheat, rice, and St. Augustinegrass and experimentally infected bermudagrass. Inhibition of DNA amplification by soil was observed in reactions with relatively low copy numbers of target DNA sequences (i.e., up to 40 ascospores) but not in reactions with large numbers of target DNA sequences (i.e., entire perithecia).

*Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier, a filamentous soilborne fungus, parasitizes the roots and crowns of susceptible hosts, which include many members of the Poaceae (grass) plant family reviewed elsewhere (31,37-39). *G. g.* var. *tritici* J. Walker is the etiologic agent of take-all disease of wheat and barley, a major root disease that limits yields in many areas of the world. *G. g.* var. *avenae* (E. M. Turner) Dennis causes take-all of oats as well as barley and wheat but is uncommon on cereals and is more often the cause of take-all patch disease of bentgrass. *G. g.* var. *graminis* parasitizes wheat, but in contrast to *G. g. tritici* or *G. g. avenae*, it is rarely pathogenic on this host. However, *G. g. graminis* causes bermudagrass decline (9), a destructive root rot on golf course putting greens in Florida, and spring dead spot (20), observed in areas of the southeastern United States where bermudagrass has a winter dormancy period because of cold temperatures. *G. g. graminis* was also recently described as a root pathogen of St. Augustinegrass grown as a lawn turfgrass in the southern United States (17; and M. Elliott, A. Hagan, and J. Mullen, *unpublished*). In addition, *G. g. graminis* is the etiologic agent of crown (black) sheath rot disease of rice, a rarely reported disease until recent outbreaks in Texas and Florida (6,37,39).

Diagnosis of take-all disease of cereals is partly based on disease symptoms that include blackened roots; poorly filled, bleached (white) heads; and stunted growth of host plants, all of which are consequences of damaged root tissue. However, these symptoms, as well as those of bermudagrass decline (irregular, chlorotic, thinning patches of grass), spring dead spot of bermudagrass (circular or arclike patches of dead grass), and crown sheath disease of rice (black leaf sheath, crown rot, and plant lodging), can be caused by other pathogens or environmental conditions (4,31,40). Hence, definitive identification of *G. graminis* as the etiologic agent in diseased plants requires culturing the fungus to examine its colony morphology and development of its teleomorph. Unfortunately, this fungus does not always grow from diseased tissue (5; and Henson et al, *unpublished*), and its sexual stage can take weeks to complete in the laboratory and may not be produced by some isolates.

DNA probes provide additional methods for detection and identification of pathogens. For example, hybridization with pathogen-specific DNA probes and DNA extracted from infected plant tissue was used for detecting the phytopathogenic fungi *Leptosphaeria korrae* and *Phytophthora parasitica* in diseased plants (13,34). A 4.3 kbp mitochondrial DNA probe for *G. graminis* hybridized specifically with fungal DNA when the fungus was first cultured from infected wheat seedlings, but it did not hybridize directly with diseased tissue or with DNA extracted from diseased tissue (14). Bateman et al could detect *G. graminis* by direct hybridization of the same probe with DNA extracted from artificially inoculated plants, but not from naturally infected plants (2).

The polymerase chain reaction (PCR) allows detection of extremely small quantities of specific DNA in complex

environments such as blood or soil (12,25,27,30,32,35,36). A PCR test was developed for *G. graminis* that allows detection of all three varieties of the fungus (29). Without first culturing the fungus, *G. g. tritici* DNA was detected in infected wheat seedlings grown aseptically in the laboratory; however, the DNA extraction procedure from plant tissue was laborious. Herein, we describe the use of PCR to detect *G. graminis* DNA in infected plants after a simple boiling procedure. To better determine its sensitivity, this test was also conducted with purified target DNA and specific numbers of hyphal tips and ascospores.

## MATERIALS AND METHODS

**Fungal strains and media.** Fungi used in this study included FL-39 and FL-36, *G. g. graminis* isolates from Florida St. Augustinegrass and bermudagrass, respectively. Strain JH2033, a *G. g. graminis* strain originally isolated from soybean in Georgia by C. Rothrock, and JH2016 and JH2025, phleomycin-resistant and benomyl-resistant transformants of JH2033, respectively (23), were also used. *G. g. tritici* isolates included a Montana wheat isolate, DM528, and an Australian wheat isolate, DAR65020. *G. graminis* cultures were grown on L medium (22) at room temperature (22–26 C). Solid L medium included 1% agar.

**Plant infections.** Laboratory infections with *G. graminis* (strains DM528 or JH2033) of spring wheat (cv. Pondera) seedlings grown hydroponically from surface-sterilized seeds were conducted as previously described (23,29). Seedlings were sampled after 2 wk, when take-all symptoms were observed.

A *G. g. tritici* isolate DM528 was used to produce oat kernel inoculum for artificially infesting field plots planted with winter wheat (cv. Winridge). Inoculum was introduced concurrently with the wheat seed at 1.5 or 3.5 g per 3-m row. Winter wheat was planted at 5 g per 3-m row. Control plots were planted with seed and autoclaved fungal inoculum. Experimental plots were located at the A. H. Post Research Farm near Bozeman, Montana. This site has an Amsterdam silty clay loam soil and a previous cropping history of 1 yr of cereal, 2 yr of alfalfa, and 1 yr fallow. Plants were pulled at Zadoks growth stage 72–75 (early milk development). Roots were washed, scored for incidence of take-all infection, and then processed for PCR tests.

Mature Pondera spring wheat plants (near Toston, Montana) were sampled in September 1991 from a field with a history of take-all. Plants were transported to Bozeman, kept refrigerated overnight, examined the next day for take-all symptoms, and processed for PCR tests.

As previously described (9), hybrid bermudagrass (cultivar Tifgreen 328) was grown for 6 wk in the greenhouse in a sterile soil mix infested with *G. g. graminis* isolate FL-36 oat kernel inoculum, and control bermudagrass was also grown in non-infested soil. Roots were washed, evaluated for disease, and processed for PCR tests. Bermudagrass plants with bermudagrass decline symptoms were collected from golf course putting greens in southeastern Florida.

St. Augustinegrass (cv. FX-313) was grown in the greenhouse in a sterile soil mix infested with *G. g. graminis* isolate FL-39 oat kernel inoculum. Control plants were grown in noninfested soil mix. After 10 wk, plants were evaluated for disease and roots processed for PCR tests. St. Augustinegrass plants with root rot symptoms were also sampled from home lawns in Alabama and provided by A. K. Hagan of Auburn University.

Rice plants with and without symptoms of crown sheath rot were collected from a Texas field (cv. Gulfmont) and provided by J. Krausz of Texas A&M University.

**Preparation of samples for PCR.** Plasmid pMSU315 was purified according to standard procedures (28). For tests of cultured hyphae, two blocks of freshly grown *G. g. tritici* hyphae were cut directly from L medium agar plate cultures using the open end of a 13-mm dry heat-sterilized glass test tube. The blocks were pushed into the test tube with a dry heat-sterilized glass pipet. Buffer (2 ml of 10 mM Tris, pH 8.0) was added, and the sample was boiled for 20 min. Dead (unculturable) hyphae from plates were similarly prepared for PCR.

Perithecia of strains JH2025 and JH2016 were produced as described elsewhere (24). Ascospores were squashed from perithecia and spread onto an agar surface. Isolated single ascospores were picked immediately into 1 ml of buffer (10 mM Tris, pH 8.0) and boiled for 20 min. Agar plugs containing hyphal tips were also cut from the surface of L medium agar cultures, placed into 1 ml of buffer, and boiled for 20 min. Negative controls were plugs cut from sterile L medium agar plates.

Approximately 2 g (wet weight) of plant crown or root tissue was washed free of soil and boiled for 20 min in 2 ml of buffer (10 mM Tris, pH 8.0). Single whiteheads (containing many empty seed cases) of field plants were boiled in 2 ml of buffer. All boiled samples were kept at 4 C during use or frozen at −20 C for storage.

**PCR and PCR product analysis.** Unless otherwise specified, 50-$\mu$l reactions contained 50 pmol of each outside primer (KS1F and KS2R) (29), 1.25 units (U) of *Taq* DNA polymerase, 0.125 mM deoxynucleotide triphosphates, 1× PCR buffer (50 mM KCl, gelatin at 10 mg/ml, and 10 mM Tris, pH 8.0 (28) and $MgCl_2$ (1.5–10.0 mM, as indicated in Table 1). Unless otherwise indicated in Table 1, 10 $\mu$l of boiled sample solution or 10 mM Tris (pH 8.0) for negative control reactions was added. Reactions were cycled 20 times (for 1 min at 92 C, 2 min at 52 C, and 3 min at 72 C), and then 2 $\mu$1 of the reaction was added to a 50-$\mu$l reaction with fresh *Taq* polymerase (1.25 U), 1.5 mM $MgCl_2$, 0.125 mM deoxynucleotide triphosphates, 50 pmol of inside primers (KS4F and KS5R) (29), and 1× PCR buffer (28) and amplified for an additional 35 cycles (1 min at 92 C, 2 min at 52 C, and 3 min at 72 C). Thermal cycler wells were previously shown not to vary significantly in the amounts of *G. graminis* products amplified from purified template DNA.

To reduce the possibility of false positives due to slight amounts of contaminating DNA, several precautions were included in our procedures. Positive-displacing pipets, or tips with filters to prevent aerosol contamination, were used for dispensing template DNA. Other pipets were reserved for PCR reagents and never used for pipetting DNA. Eppendorf reaction tubes were handled as little as possible during addition of reaction reagents. Frequent negative controls (reactions without added template DNA) were included in all experiments. Reactions listed in Table 1 were repeated at least twice. Using boiled samples directly instead of extracting DNA from tissue eliminated procedures that could leave laboratory glassware contaminated with *G. graminis* DNA.

PCR products were separated by electrophoresis for 3 h (65 mA) in 3% agarose gels or 3:1 NuSieve (3% NuSieve and 1% SeaPlaque agarose; FMC Bioproducts, Philadelphia, PA) with Tris-borate buffer (28), stained with ethidium bromide, and photographed with a Polaroid camera (type 57 film) while illuminated with ultraviolet light.

## RESULTS

This PCR procedure differed from our earlier one (29) as follows. The annealing temperature of the first round of amplification with outside primers was raised from 42 C to 52 C. First-round reaction products were not diluted before their addition to the second-round reaction mixture. Eliminating the additional dilution decreased sample handling between rounds and the possibility of sample contamination with extraneous DNA. In addition, we started reactions at the denaturing temperature, and all samples to be amplified were kept on ice until they were transferred to the preheated thermal cycler. This modification helped reduce the amount of "primer dimers" in PCR reactions (11).

Hyphae cut from solid agar medium and boiled in buffer produced positive PCR results across a range of magnesium ion concentrations (Fig. 1, lanes 3 and 4; and Table 1). Even mycelium from which we could no longer recover live hyphae produced positive reactions if relatively large samples (two 13-mm disks cut from agar plates) were collected (Table 1). Low molecular weight RNA, but not high molecular weight (chromosomal and mitochondrial) DNA, from boiled samples was visible on agarose

gels stained with either ethidium bromide or acridine orange (data not shown).

Typically, two fragments were amplified in positive PCR tests, examples of which are shown in Figure 1. One fragment was the expected size of approximately 188 bp, and the second was approximately 287 bp. The 188-bp fragment was always observed with *G. graminis* samples, but the second fragment was not always present (15). Both fragments were also amplified from plasmid pMSU315, which carries a 4.3-kb mitochondrial DNA fragment cloned from *G. g. tritici* (strain DM528). Restriction and sequencing analyses suggested that the 287-bp fragment contains the 188-bp sequence and resulted from annealing of one of the primers at another sequence about 100 bp from the primary annealing site (29; and data not shown).

In order to estimate the efficiency of the PCR test, purified plasmid pMSU315 DNA (14) was also diluted and tested. We estimate that under conditions described in Materials and Methods, approximately 195 copies of plasmid DNA are reproducibly detected (visible on agarose gels) without the use of radioisotopes (data not shown).

A single *G. g. graminis* ascospore, which has up to 12 cells when mature (1), released enough template DNA to produce amplified products which could be visualized on gels (Fig. 1, lanes 6 and 7). Similar PCR results, summarized in Table 1, were obtained with single hyphal tips (5–10 cells) from both *G. g. graminis* (Fig. 1, lanes 10 and 11) and *G. g. tritici*.

Addition of soil to fungal samples was inhibitory for amplification. In the presence of 1 g of soil, up to 40 ascospores gave negative PCR tests; this inhibition, however, could be over-

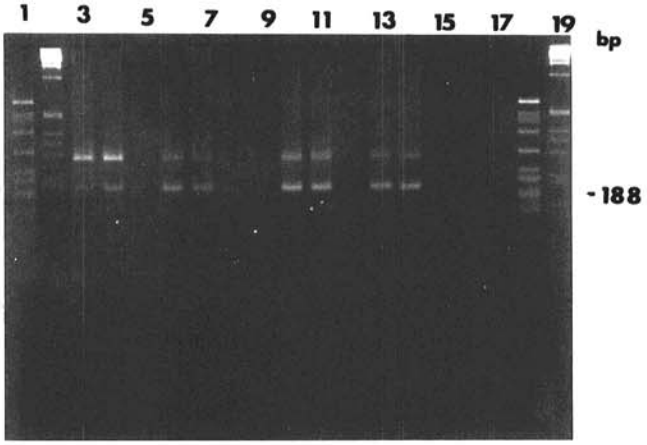


**Fig. 1.** Examples of PCR reactions with boiled samples. Hyphae of *G. g. graminis* strain 2016 (lanes 3 and 4); a single ascospore of strain 2016 (lanes 6 and 7); a single hyphal tip of strain 2033 (lanes 10 and 11); crown and root tissue from a wheat field plant artificially infected with *G. g. tritici* (lanes 13 and 14); uninoculated plant tissue from the same field (lanes 15 and 16). Each sample was tested with two magnesium ion concentrations (1.5 and 3.0 mM). Lanes 5, 8, 9, 12, and 17 were negative controls (without template DNA) with either 1.5 or 3.0 mM $MgCl_2$. Lanes 1 and 18 had pBR322 digested with *Msp*I for molecular weight markers, and lanes 2 and 19 had 1-kb ladder molecular weight markers from Bethesda Research Laboratories.

TABLE 1. Polymerase chain reaction test results of samples boiled to detect *Gaeumannomyces graminis* DNA

| Sample[a] | Visible 188-bp fragment | $MgCl_2$ concentrations tested (mM) |
|---|---|---|
| Purified plasmid (pMSU315) DNA 30 μg/ml solution diluted $10^{-8}$ [b] | + | 1.5, 3.0 |
| Buffer (10 mM Tris, pH 8.0) | − | 1.5, 2.5, 3.0, 5.0, 7.5, 9.0 |
| *G. g.* var. *tritici* (strain DAR65020) viable hyphae cut from agar | + | 1.5, 2.5, 5.0, 9.0 |
| *G. g. tritici* (strain DAR65020) nonviable (unculturable) hyphae cut from agar | + | 1.5, 2.5, 5.0, 9.0 |
| Sterile agar plugs | − | 1.5, 3.0, 5.0, 7.5, 9.0 |
| *G. g.* var. *graminis* (strain JH2025) ascospore (total tested = 5) | + | 1.5, 3.0 |
| *G. g. graminis* (strain JH2016) ascospore (total tested = 6) | + | 1.5, 3.0 |
| *G. g. graminis* (strain JH2025) ascospore with 1 g of added soil[c] | − | 1.5, 3.0, 5.0, 7.5, 9.0 |
| *G. g. graminis* (strain JH2025) ascospores (up to 40) with 1 g of added soil[c] | − | 1.5, 3.0, 5.0, 7.5, 9.0 |
| *G. g. graminis* (strain JH2025) perithecium with 1 g of added soil[c] | + | 1.5, 3.0, 5.0, 7.5, 9.0 |
| 1 g of soil[c] | − | 1.5, 3.0, 5.0, 7.5, 9.0 |
| *G. g. tritici* (strain DAR65020) hyphal tip (total tested = 5) | + | 1.5, 3.0 |
| *G. g. graminis* (strain JH2016) hyphal tip (total tested = 5) | + | 1.5, 3.0 |
| *G. g. graminis* (strain JH2033)-infected, hydroponically grown wheat plants | + | 0.5, 2.5, 5.0, 9.0 |
| *G. g. tritici* (strain DM528)-infected, hydroponically grown wheat plants | + | 0.5, 2.5, 5.0, 9.0 |
| Uninfected, hydroponically grown wheat plants | − | 0.5, 2.5, 5.0, 9.0 |
| Roots and crowns of *G. g. tritici* (strain DM528)-infected greenhouse-grown wheat plants | + | 0.5, 2.5, 5.0 |
| Roots and crowns of greenhouse-grown wheat plants | − | 0.5, 2.5, 5.0 |
| Roots and crowns of experimental field wheat plants infected with *G. g. tritici* (1990,[d] 1991,[d] 1992) | + | 1.5, 3.0, 5.0 |
| Roots and crowns of uninoculated, experimental wheat plants (1990,[d] 1991,[d] 1992) | − | 1.5, 3.0, 5.0 |
| Whiteheads (three tested) of experimental field wheat plants with take-all symptoms | − | 1.5, 3.0, 5.0, 9.0 |
| Roots and crowns of wheat plants displaying take-all symptoms (near Toston, MT) naturally infected with *G. g. tritici* (1991) | + | 1.5, 3.0, 5.0, 7.5 |
| Roots and crowns of wheat plants without take-all symptoms (near Toston, MT, 1991) | − | 1.5, 3.0, 5.0, 7.5 |
| Roots of greenhouse-grown St. Augustinegrass infected with *G. g. graminis* (isolate FL-39) | + | 1.5, 3.0, 5.0, 10.0 |
| Roots of uninfected greenhouse-grown St. Augustinegrass | − | 1.5, 3.0, 5.0, 10.0 |
| Roots of St. Augustinegrass naturally infected with *G. g. graminis* (collected from three different lawns in Montgomery, AL) | + | 1.5, 2.5, 5.0, 9.0 |
| Roots of greenhouse-grown bermudagrass infected with *G. g. graminis* (isolate FL-36) | + | 2.5, 5.0 |
| Roots of uninfected, greenhouse-grown bermudagrass | − | 2.5, 5.0 |
| Roots of symptomatic, *G. g. graminis*-infected bermudagrass from five different Florida golf courses | − | 1.5, 2.5, 5.0, 9.0 |
| Crowns from *G. g. graminis*-infected Texas rice field plants displaying symptoms | + | 1.5, 3.0, 5.0, 9.0 |
| Crowns from *G. g. graminis*-infected Texas rice field plants without symptoms | + | 1.5, 3.0, 5.0, 9.0 |

[a] Unless otherwise stated, 10 μl of boiled samples was tested.
[b] 5 μl tested.
[c] Amsterdam silty clay loam.
[d] DNA from these samples was extracted as previously described (29).

come with large amounts of target DNA (e.g., an entire perithecium).

*G. g. tritici*- and *G. g. graminis*–infected crowns and roots from laboratory- and greenhouse-grown wheat seedlings tested positive in PCR reactions throughout a range of $MgCl_2$ concentrations, whereas uninoculated control plants were negative. Symptomatic wheat roots and crowns from field plots artificially infested with *G. g. tritici* were also positive with the PCR test (Fig. 1, lanes 13 and 14), whereas uninoculated control plants (Fig. 1, lanes 15 and 16) and whiteheads from wheat plants with severe take-all disease were negative. As take-all disease of wheat is not known to be a seedborne disease (4), the lack of detectable *G. graminis* DNA in whiteheads was not unexpected.

Crown and root tissue from symptomatic wheat plants of a Toston, Montana, field naturally infested with *G. g. tritici* was positive, but asymptomatic plants from this field were negative. Attempts to culture *G. g. tritici* from both asymptomatic and symptomatic plants failed, even though this fungus was isolated the previous year from plants in the same field.

*G. g. graminis*–infected bermudagrass and St. Augustinegrass grown in the greenhouse were positive in PCR tests, whereas control (uninfected) plants were negative. Positive PCR reactions were also obtained from St. Augustinegrass plants with root rot symptoms collected from three different locations in Alabama. However, positive PCR reactions were not obtained with naturally infected bermudagrass samples, even when *G. g. graminis* could be cultured from them. These samples probably had an inhibitor of DNA polymerization, because boiled aliquots from them inhibited amplification in reactions with added template DNA (data not shown).

Positive PCR reactions were obtained from symptomatic and asymptomatic crown tissue of Texas rice field plants naturally infested with *G. g. graminis*. *G. graminis* was also isolated from both symptomatic and asymptomatic plants, and fungal perithecia were apparent on the crowns of plants with disease symptoms.

## DISCUSSION

Previously, template DNA for *G. graminis* PCR tests was phenol-extracted from fungal hyphae or from infected laboratory-grown plants by a procedure that took several hours and required laboratory glassware, mortar, pestles, and plastic ware that could not be sterilized by dry heat (29). In this study we found that boiling *G. graminis* hyphae, ascospores, or infected tissue released enough mitochondrial DNA to produce a positive PCR test. Boiling samples also reduced much of the handling of fungal DNA samples and, consequently, reduced the time required for DNA processing and limited the possibility of cross-contamination of DNA in PCR reactions.

A single boiled ascospore reproducibly gave positive results. We did not count the numbers of mitochondria in single ascospores or hyphal tips, but from their studies of *G. g. tritici* DNA sequence complexities, McFadden and Buck estimated approximately 100 copies of mitochondrial DNA per hyphal compartment (21). Thus, each *G. graminis* ascospore or hyphal tip probably contained 500–1,200 copies of target DNA. Mitochondrial DNA, as well as being located in another cellular compartment, is probably complexed with different proteins than nuclear DNA (3). Hence, target mitochondrial DNA sequences may be more accessible than nuclear DNA for amplification by *Taq* DNA polymerase. This PCR test also used nested primers and two rounds of amplification, which increases the specificity of this PCR test; reactions using only the outside primers or only the inside primers resulted in more amplified fragments, some of which had little homology with the original 4.3-kb mitochondrial fragment (29; and data not shown). In addition, the use of nested primers dilutes DNA polymerase inhibitors (e.g., phenolic compounds) that may be present in plant samples or agar (8,16,32,35,36).

Positive PCR results were also obtained with nonculturable hyphae, suggesting that *G. graminis* mitochondrial DNA may persist in injured or nonviable cells. Nonviable hyphae or free *G. graminis* mitochondrial DNA in field plants or soil may also produce positive PCR tests. Hence, a positive PCR field test does not necessarily indicate that viable fungal cells are present in plants or soil. Indeed, we could not culture *G. graminis* from all samples that were PCR-positive. PCR tests should prove useful for determining the survival rate of free *G. graminis* mitochondrial DNA or DNA entombed in dead hyphae in soil or plant debris. If free DNA or DNA in dead hyphae does not survive long in the field, then a positive PCR test may correlate well with the presence of viable *G. graminis*, but this remains to be determined.

Positive PCR tests were obtained with *G. g. tritici*–infected wheat plants grown in naturally and artificially infested fields. PCR diagnostic tests with diseased plants could be helpful in identifying *G. graminis* in fields showing symptoms of take-all disease but having an unculturable, or unknown, etiologic agent. PCR testing for *G. graminis* is relatively expensive in comparison to culturing the fungus on selective media, but it may be more sensitive and rapid.

Another potential problem with PCR tests for *G. graminis* in wheat fields is that many *Phialophora* isolates, some of which are closely related to *G. graminis* and also colonize wheat and barley, produced positive PCR tests (15). However, *Phialophora* isolates are nonpathogenic and would not produce take-all symptoms. In addition, although *Phialophora* isolates are commonly cultured from roots of wild grasses, they are not frequently isolated from wheat plants (7,18,19,33; and Henson et al, *unpublished*) and were not recovered from any wheat plants in this study. Uninfected control wheat plants in experimental fields were always negative in PCR tests, which also suggested that *Phialophora* spp. were not present.

Several organisms have been implicated as causative agents of spring dead spot and bermudagrass decline, and studies are complicated by the finding that more than one organism may be cultured from diseased turf (10). For example, *G. g. graminis* causes spring dead spot of bermudagrass in the southeastern United States (20), but *Leptosphaeria korrae*, which was negative with our PCR test for *G. graminis* (15), was determined to be the etiologic agent of spring dead spot of bermudagrass in Australia and some geographic areas of the United States (31). Perhaps this PCR test, or a modified version of it, will be helpful in determining which organisms are present in diseased patches of bermudagrass and other warm-season turfgrasses.

Symptomless plants in a naturally infested rice field produced positive tests. Because rice fields are flooded and *G. g. graminis* perithecia release ascospores when soaked in water, it is possible that most plants in an infested field will be positive for the fungus (J. Krausz, *personal communication*). Although *G. g. graminis* was isolated from these plants, positive PCR results may have been due to the presence of *Magnaporthe grisea*, which is commonly found in Texas rice fields (J. Krausz, *personal communication*). Although *M. grisea* only hybridized weakly with the 4.3-kb mitochondrial DNA probe from *G. g. tritici*, several *M. grisea* isolates produced positive PCR tests with higher $MgCl_2$ concentrations (15), in which primers have less specificity for target DNA (11,32). Presently, we are sequencing amplified products, so that primers can be designed that will better distinguish between *M. grisea* and *G. graminis* in PCR tests.

Although this PCR test is probably sensitive enough for detection of *G. graminis* in wheat and rice plants, some samples (e.g., naturally infested bermudagrass and soil) apparently inhibit amplification under the PCR conditions described here. Plants and soil contain polymerase-inhibiting substances, including polysaccharides (8) and polyphenolic compounds (16,35,36). Although Rollo et al were able to specifically amplify *Phoma tracheiphila* fungal DNA from boiled mycelia collected from infected lemon trees (26), others have usually found it necessary to extract target bacterial and fungal DNA from soil and plants before amplifying it by PCR (11,25,30,32,35). It may be necessary to perform similar extractions of *G. graminis* DNA for maximum detection of the fungus in turfgrasses or soil. We are also testing other methods (16,36), reviewed elsewhere (11,32), for increasing sensitivity and decreasing inhibition of DNA amplification with soil and turfgrass samples.

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