Determination of an Etiological Agent of Bermudagrass Decline

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ABSTRACT

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Gaeumannomyces graminis var. *graminis*, *G. incrustans* and *Phialophora* spp. have been isolated in Florida from bermudagrass putting greens with bermudagrass decline symptoms. Using a containerized test system, *G. g. graminis* isolates, but not *G. incrustans* or *Phialophora* spp. isolates, were pathogenic to hybrid bermudagrass. A *Magnaporthe poae* isolate, obtained from bentgrass turf grown in Florida, also was pathogenic to bermudagrass. However, *M. poae* has not been identified from bermudagrass in nature. For both *G. g. graminis* and *M. poae*, the plant growth conditions and turfgrass host and geographic location of the isolates did not affect pathogenicity. Wheat pathogenicity assays also were conducted for all the isolates to develop a quick assay for determining pathogenicity. Results from the wheat and bermudagrass pathogenicity assays correlated well for *G. g. graminis*, *M. poae*, and *Phialophora* spp. but not for *G. incrustans*. This wheat assay also permitted tentative identification of the fungi. Preliminary results indicate that these ectotrophic fungi are introduced into new putting greens on the bermudagrass sprigs used for green establishment.

Additional keywords: *Cynodon* spp., root rot.

Bermudagrass decline is a destructive root rot disease of hybrid bermudagrass (*Cynodon dactylon* (L) Pers. × *C. transvaalensis* Burtt-Davy) used for golf course putting greens in Florida. This disease is most prevalent during the summer and fall, when the largest proportion of annual precipitation is received, and the weather typically is very warm and humid. Symptoms of the disease have been described elsewhere (3,4). When first described, the sterile ectotrophic fungus consistently associated with diseased root tissue was postulated to be either a species of *Gaeumannomyces* or *Leptosphaeria* (4). Later, one fungal isolate was identified as *Phialophora radicicola* Cain (5), but it was not an aggressive pathogen of healthy bermudagrass (6).

Recently, *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *graminis*, *G. incrustans* Landschoot and Jackson, and *Phialaphora* sp. were isolated from bermudagrass putting greens with bermudagrass decline symptoms (3) in Florida. For some locations, more than one of these organisms was identified in association with the symptomatic plant roots. *G. g. graminis* has been isolated from *C. dactylon* in Australia (17) and *C. transvaalensis* in Australia and South Africa (15,17). In addition, *G. g. graminis* was implicated as a causal agent of spring dead spot of bermudagrass in the southeastern United States, where a winter dormancy is induced by cold temperatures (11). *G. incrustans* has been isolated from a *Cynodon* sp. (8), but its pathogenicity has not been evaluated on this host. In general, *Phialophora* species are not considered pathogens of grasses (1,18).

The objective of this study was to determine the pathogenicity of *G. g. graminis*, *G. incrustans*, *Phialophora* sp. and *Magnaporthe poae* Landschoot and Jackson to hybrid bermudagrass roots. The first three organisms have been isolated from hybrid bermudagrass and from two other warm-season turfgrasses grown in Florida, St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) and centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) (3). *M. poae* is the causal agent of summer patch, a root rot disease, on Kentucky bluegrass (*Poa pratensis* L.) and annual bluegrass (*Poa annua* L.) (9,10). *M. poae* was included in this study because it had been isolated from a creeping bentgrass (*Agrostis stolonifera* L.) putting green in central Florida (3) and potentially could spread to bermudagrass. The pathogenicity of these Florida isolates was compared to ATCC-designated isolates of these fungi from other turfgrass hosts and locations.

MATERIALS AND METHODS

Fungal isolates. Isolates used in the study have been described previously (3). All isolates were maintained on potato-dextrose agar (PDA) slants at 2 C.

In-vitro pathogenicity study on wheat. Speakman's (16) method was used as a simple in vitro pathogenicity assay. Seeds of spring wheat cultivar Pondera were surface sterilized with a 1% silver nitrate solution for 10 min, rinsed once with sterile 0.5% NaCl solution, rinsed three times with sterile deionized water, and allowed to dry under a sterile air stream. Seeds were germinated on water agar (1.5% agar) with three seeds per plate. After the seeds germinated, only plates with three clean seedlings were selected for the assay. For each isolate, a 5-mm-diameter agar plug from a PDA culture was placed next to the emerging roots of each seedling with two plates per isolate. Controls were seedlings with noncolonized PDA plugs and seedlings with no agar plugs. Plates were sealed with Parafilm (American National Can Co., Greenwich, CT) and incubated at 30 C with 12 h of light each day. After 24 days, plants were rated as follows: 1 = plants healthy with white roots; 2 = plants healthy but roots discolored (tan not white); 3 = majority of roots black in color, basal stem white, <50% chlorotic leaves; 4 = all roots black in color, basal stem black, 50–75% chlorotic or necrotic leaves; 5 = roots and basal stem black, >75% chlorotic or necrotic leaves. This experiment was conducted three times.

After the disease evaluation, the plants were examined for presence of perithecia and lobed hyphopodia of *G. g. graminis* or crusts of *G. incrustans*. One plate of each isolate was used for reisolation of the fungus. For each plant on this plate, the seed was removed, and the plant was cut 2 cm above and below the crown. These pieces were surface sterilized with 1% silver nitrate for 30 s, rinsed for 30 s in sterile deionized water, blotted dry on sterile filter paper, and placed on a selective medium for *Gaeumannomyces* (SM-GGT7) (2). Plates were incubated at 28 C. After 4 and 7 days, plates were examined for *Gaeumannomyces* growth from the plant tissue.

Pathogenicity study on hybrid bermudagrass. Inoculum was prepared for each isolate by mixing a 250-ml vol of whole oats with 125 ml of deionized water in a glass jar. The oat mixture was autoclaved twice for 90 min on each of two consecutive days. One PDA plate of each isolate was chopped into small pieces and mixed with the oats. Inoculated oats were incubated for 4 wk at 30 C.

A topsoil mix, commonly used in constructing putting greens, was autoclaved for 90 min. The topsoil was composed of 80% sand and 20% peat moss and had a pH of 5.8. Tapered plastic containers (Ray Leach Cone-Tainer Co., Canby, OR) (2.5 × 18 cm) were plugged at the tip with nonabsorbent cotton and filled to within 5 cm of the top with the topsoil mix. The soil was saturated with water, a 2-cm-deep layer of oat kernel inoculum was added, and the inoculum was covered with more topsoil mix. The two check treatments were autoclaved noninfested oat kernels and no oat kernels added to the cones. Each cone was planted with one sprig of Tifgreen 328 hybrid bermudagrass. Each plant sprig had one node and three to six leaves, but all roots had been removed. Plant material was thoroughly washed to remove all soil and debris before cutting the material into sprigs. The sprigs were not disinfested with sodium hypochlorite because even a dilute solution was slightly phytotoxic to the bermudagrass and did not effectively kill saprophytic fungi (M. L. Elliott, *unpublished data*).

The first experiment (experiment 1) was initiated 17 July 1990. Inoculated plants were placed in a greenhouse with an average temperature of 35 C, relative humidity greater than 80%, a maximum photo flux density of 760 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and a natural photoperiod. The experiment was a completely randomized block design with four replicate plants per treatment. The plants received 10 ml of water on alternate days and were misted with water for 5 s every 4 min during daylight hours for the duration of the experiment. After 3 wk, plants were maintained at 5-cm height via weekly clippings and fertilized with quarter-strength Hoagland's solution (7) once each week. The experiment was terminated, and roots were evaluated after 6 wk.

For the second experiment, initiated 6 September 1990, twelve replicate plants were established for each isolate treatment. All inoculated plants were placed in the greenhouse with temperatures, relative humidity, and light conditions similar to those of the first experiment. Initially, all plants received 10 ml of water on alternate days and were misted with water for 5 s every 4 min during daylight hours. After two weeks, all plants continued to receive 10 ml of water on alternate days, but the misting frequency was altered. One set of four replicate plants per isolate treatment continued to receive mist for 5 s every 4 min for the duration of the experiment (experiment 2A). This was a duplication of experiment 1. A second set of four replicate plants was moved to a bench that received mist for 5 s every 30 min for the duration of the experiment (experiment 2B). The final set of four replicate plants was moved to a bench that received no mist (experiment 2C). Each experiment had a completely randomized block design with four replicate plants per isolate treatment. After 3 wk, plant height and fertility were maintained as in experiment 1. The experiments were terminated, and roots were evaluated after 6 wk.

For all experiments, roots were evaluated by thoroughly washing soil from roots under running tap water and rating symptoms on the following scale: 1 = no disease symptoms with roots completely white; 2 = 1–25% of roots with isolated black lesions or a general tan discoloration of the entire root system; 3 = 25–50% of roots with black, coalescing lesions or entire root

TABLE 1. Disease ratings from in vitro wheat pathogenicity tests of fungal isolates obtained from root rot symptomatic turfgrasses

Fungus/isolate	Original source	Disease rating[z]		
		Test 1	Test 2	Test 3
Gaeumannomyces graminis var. *graminis*				
ATCC 64419	VA, zoysiagrass	5.0 a	4.5 bc	3.5 d
FL-19	FL, bermudagrass	5.0 a	5.0 a	5.0 a
FL-25	FL, bermudagrass/ryegrass mix	5.0 a	5.0 a	4.7 ab
FL-36	FL, bermudagrass	5.0 a	5.0 a	5.0 a
FL-39	FL, St. Augustinegrass	5.0 a	5.0 a	5.0 a
FL-46	FL, bermudagrass	5.0 a	5.0 a	4.8 ab
G. incrustans				
ATCC 64418	RI, Kentucky bluegrass	4.7 ab	4.4 bc	3.7 cd
FL-28	FL, centipede grass	4.7 ab	4.3 c	4.0 c
FL-32	FL, bermudagrass	4.8 ab	4.7 abc	4.7 ab
FL-38	FL. St. Augustinegrass	4.5 b	3.4 de	4.0 c
FL-52	FL, bermudagrass	4.5 b	4.6 abc	4.8 ab
FL-53	FL, bermudagrass	4.5 b	3.7 d	4.7 ab
Magnaporthe poae				
ATCC 64413	NY, Kentucky bluegrass	5.0 a	4.8 ab	4.7 ab
FL-4	FL, creeping bentgrass	4.8 ab	4.5 bc	4.5 b
Phialophora graminicola				
ATCC 64414	RI, Kentucky bluegrass	2.0 i	2.0 h	2.7 fgh
P. radicicola				
JI3	FL, bermudagrass	4.5 b	3.8 d	3.3 de
Phialophora sp.				
FL-7	FL, creeping bentgrass	2.7 efg	3.0 fg	4.0 c
FL-11	FL, bermudagrass	2.5 fgh	2.5 g	2.5 ghi
FL-15	FL, bermudagrass	2.8 def	2.5 g	2.3 hi
FL-18	FL, bermudagrass	2.0 i	3.0 ef	3.0 ef
FL-23	FL, bermudagrass/ryegrass mix	2.3 ghi	2.4 gh	2.7 fgh
FL-24	FL, bermudagrass/ryegrass mix	3.7 c	3.0 ef	3.0 ef
FL-37	FL, bermudagrass	3.0 de	2.7 fg	2.7 fgh
JI2	FL, bermudagrass	4.0 c	3.6 d	3.7 cd
Gaeumannomyces type, sterile				
FL-14	FL, bermudagrass	3.2 d	2.7 fg	2.8 fg
FL-47	FL, bermudagrass	2.2 hi	2.7 fg	2.2 i
Check with PDAS plugs		1.0 j	1.0 i	1.0 j
Check without plugs		1.0 j	1.0 i	1.0 j

[z]Based on a scale of 1–5, with 1 = healthy plant and 5 = dead plant. For each assay, values are the mean for six plants. Means within a column and followed by the same letter are not significantly different ($P = 0.05$) according to Waller-Duncan k-ratio t-test. LSD = 0.4 for all three tests.

system was a brown (not black) color; 4 = 51–75% of roots were black due to coalescing lesions; 5 = 76–100% of roots were black. After evaluating the roots for the experiments conducted in the greenhouse (experiments 1, 2A, 2B, and 2C), roots were cut into 2-cm pieces, surface sterilized, and placed on selective medium SM-GGT7 as described previously. After 5 and 10 days, plates were examined for growth of *Gaeumannomyces*-like fungi from the plant tissue.

Detection of *Gaeumannomyces*-like fungi on symptomless bermudagrass. A 0.2-ha field of Margate fine sand soil was fumigated with methyl bromide and planted with Tifgreen 328 hybrid bermudagrass sprigs in April 1989 at the Fort Lauderdale Research and Education Center. This area was maintained as a golf course fairway. In November 1989, random samples of the established turfgrass were obtained to determine if *Gaeumannomyces*-like fungi were present on the roots, even though symptoms of bermudagrass decline were not apparent. Roots were washed thoroughly under tap water, cut into 2-cm pieces, surface sterilized, and placed on selective medium SM-GGT7 as described previously. By August 1990, symptoms of bermudagrass decline were evident in one portion of the field. Samples were obtained from this area and processed as before.

In August 1990, a 1,860-m^2 putting green was built at the Fort Lauderdale Research and Education Center. The topsoil mix was 80% sand and 20% peat moss. Twenty-five percent of the green was planted with Tifgreen 328, and the remaining portion planted with Tifdwarf. Before the bermudagrass sprigs were planted in the metam-sodium fumigated topsoil, the sprigs were sampled for *Gaeumannomyces*-like fungi. Random samples of both cultivars were obtained and processed as described above. Six months later in February 1991, root samples were randomly obtained from throughout the green and processed as before.

RESULTS

In vitro pathogenicity study on wheat. *G. g. graminis* and *M. poae* were pathogenic to wheat. Average root disease ratings were 4.5 or greater for all three tests, with the exception of plants inoculated with *G. g. graminis* isolate ATCC 64419 in the third test (Table 1). Each isolate of *G. incrustans* also was pathogenic to wheat, resulting in an average root disease rating across all three tests of 4.0 or greater. *G. incrustans* isolates ATCC 64418, FL-38, and FL-53 were the only isolates of *G. incrustans* that resulted in an individual test average of less than 4.0. *P. radicicola* isolate JI3 and *Phialophora* sp. isolate JI2 consistently yielded root disease ratings above 3.0 with overall averages of 3.9 for JI3 and 3.8 for JI2. The other *Phialophora* sp. and sterile *Gaeumannomyces* isolates yielded root disease ratings equal to or less than 3.0 with the exception of isolates FL-14 and FL-24 for test 1, and isolate FL-7 for test 3. All isolates were recovered from the wheat plant tissue. No growth similar to *Gaeumannomyces* was associated with the check treatments.

Pathogenicity study on hybrid bermudagrass. The check treatment with autoclaved oats as inoculum caused a general root discoloration that did not occur with the check treatment with no oat inoculum. However, no root lesions were observed and no mycelia similar to *Gaeumannomyces* were observed on or isolated from the roots. Therefore, root disease ratings for the autoclaved oat check treatment were considered the base value for evaluating pathogenicity of the fungal isolates examined.

Results for all the bermudagrass experiments are provided in Table 2. Although only experiments 1 and 2A had exactly the same environmental conditions, the results for all experiments were similar. With only two exceptions, *G. g. graminis* isolates were pathogenic on hybrid bermudagrass in all four experiments, and resulted in root disease ratings that were at least 3.8. The majority of root disease ratings for plants inoculated with *G. g. graminis* isolates were at least 4.5. The root disease ratings of plants inoculated with *M. poae* isolates were not significantly different from the *G. g. graminis* ratings except for FL-4 in experiment 2A.

In general, the root disease ratings for plants inoculated with the *G. incrustans* isolates and the *Phialophora* isolates were significantly less than root disease ratings for plants inoculated with *G. g. graminis* and were not significantly different from the check treatment with autoclaved oats. The exceptions for *G. incrustans* were isolate FL-28 in experiment 2A and isolate ATCC 64418 in experiment 2C. Plants inoculated with *Phialophora* isolate FL-7 had root disease ratings that were significantly different from the check treatment in three of the four experiments. In experiment 2A, plants inoculated with four of the *Phialophora* isolates had root disease ratings that were significantly different from the check treatment but were still significantly less than the ratings for plants inoculated with the *G. g. graminis* isolates.

All *G. g. graminis* isolates were consistently recovered from the infected plant roots. *M. poae* isolates were recovered from the first experiment but not the second experiment due to fungal contamination in the latter. None of the *G. incrustans* isolates were recovered on the selective medium. Reisolation of *Phialophora* sp. was variable. *P. radicicola* JI3 was recovered from each experiment, isolates FL-7, FL-11, and FL-18 were recovered in only one of the experiments, and the remaining *Phialophora* and sterile *Gaeumannomyces* isolates were never recovered. No growth similar to *Gaeumannomyces* was isolated from the roots of either check treatment.

Detection of *Gaeumannomyces*-like fungi. Of the 35 bermudagrass roots sampled in November 1989 from the Tifgreen 328 field area established in April 1989, 24 (63%) roots yielded *Gaeumannomyces*-like fungi. Of the six isolates selected for identification, one isolate was *G. incrustans* as determined by production of fertile perithecia with the appropriate mating strain. None of the other isolates produced hyphopodia or perithecia and could not be identified as to their teleomorphic state. Roots sampled in August 1990 from the bermudagrass decline symptomatic area of this field resulted in growth similar to *Gaeumannomyces*-like fungi. Two isolates selected for identification were tentatively identified as *G. incrustans* based on pathogenicity to wheat and production of crusts on wheat roots.

When the putting green was planted in August 1990, 96 roots from Tifgreen 328 and 99 roots from Tifdwarf bermudagrass sprigs were sampled as the sprigs arrived from the sod farm. From these samples, 9% of the Tifgreen 328 and 22% of the Tifdwarf roots yielded *Gaeumannomyces*-like fungi. For the samples obtained in February 1991, 50% of the Tifgreen 328 and 35% of the Tifdwarf roots yielded *Gaeumannomyces*-like fungi. The sprigs for this putting green were provided by a different sod farm than was used for the field established in 1989.

DISCUSSION

The *G. g. graminis* isolates were highly virulent to wheat and caused the distinctive root rot associated with bermudagrass decline on bermudagrass in the containerized test system. Therefore, Koch's postulates were fulfilled for *G. g. graminis* indicating it is an etiological agent of bermudagrass decline. Confirmation of Koch's postulates for *G. g. graminis* has not been obtained in the field to date. In general, *G. g. graminis* has not been considered a serious pathogen of species in the Poaceae (1). However, Nilsson documented the occurrence of *G. graminis* isolates with lobed hyphopodia that were highly pathogenic to wheat, barley, oats, rye, maize, Kentucky bluegrass, and annual bluegrass (12,13). With an average ascospore length of 81 μm, these isolates were identified as *G. g. graminis*. These particular isolates did not produce the lobed hyphopodia at temperatures below 13–14 C and instead produced simple, unlobed hyphopodia. *G. g. graminis* was identified from bermudagrass with spring dead spot symptoms and produced spring dead spot disease symptoms on bermudagrass grown in pots and inoculated with this fungus (11). In addition, black sheath rot of rice caused by *G. g. graminis* recently has become a major problem in Texas with perithecia readily produced in the flooded rice fields (19). It should also be noted that *G. g. graminis* has been isolated

from healthy soybean pods in Indiana and that these isolates were pathogenic to wheat (14).

M. poae also was pathogenic to wheat and bermudagrass. The *M. poae* isolates did not originate from bermudagrass, so Koch's postulates were not fulfilled for this pathogen. *M. poae* isolate ATCC 64413, originally identified as *P. graminicola* by R. W. Smiley (10,) was obtained from Kentucky bluegrass in New York and previously had demonstrated the ability to cause a root rot of Kentucky bluegrass, annual bluegrass, creeping bluegrass, Chewing's fescue, tall fescue, and perennial ryegrass (10). *M. poae* isolate FL-4 was obtained from a creeping bentgrass putting green in central Florida. The putting green was bentgrass all year and was not bermudagrass overseeded with bentgrass, as is the common practice in much of Florida during the winter months. Thus, *M. poae* also may be an etiological agent of bermudagrass decline.

Bermudagrass decline occurs in southern Florida when the temperature and relative humidity remain fairly constant (average daily temperature greater than 27 C and relative humidity greater than 75%) and precipitation occurs almost daily. The amount of rain received each day is the primary weather variable. The three misting regimes were an attempt to reflect this variable and resulted in soil that was perpetually wet (5 s mist every 4 min) to soil that was subjected to cyclic wet and dry periods (no supplemental mist). However, the plants in the latter environment never approached drought conditions. Overall, pathogenicity of *G. g. graminis* to bermudagrass was not affected by the misting regimes, the turfgrass source of the *G. g. graminis* isolates (bermudagrass, St. Augustinegrass, and zoysiagrass), or the location of the source (Florida and Virginia). Pathogenicity of *M. poae* was also not affected by these factors.

The *Phialophora* spp. and the sterile *Gaeumannomyces* isolates were not pathogenic to wheat or bermudagrass, which confirmed them as saprophytes on grasses (1,10,18). Although the *G. incrustans* isolates were pathogenic to wheat, bermudagrass was unaffected under the conditions evaluated. *G. incrustans* isolate ATCC 64418 previously had been determined to be only weakly virulent to 8-wk-old Kentucky bluegrass and annual bluegrass (10). Since the original survey work was completed (3), 39 isolates with characteristics similar to *Gaeumannomyces* have been obtained from putting greens in Florida with bermudagrass decline symptoms. Of these isolates, one has been identified as *G. g. graminis* and eight have been identified as *G. incrustans*. The remaining isolates in this collection, three of which produce the distinctive hyphopodia of *G. g. graminis*, have yet to produce fertile perithecia that would confirm their identity. In addition, *G. incrustans* was isolated from roots before and after bermudagrass decline symptoms developed in the bermudagrass

TABLE 2. Root rot ratings from pathogenicity studies on hybrid bermudagrass

Fungus/isolate	Root rot rating[x]			
	Experiment 1[y]	Experiment 2A	Experiment 2B	Experiment 2C
Gaeumannomyces graminis var. *graminis*				
ATCC 64419	2.0 defg	4.8 a	4.3 a	5.0 a
FL-19	5.0 a	5.0 a	5.0 a	4.8 a
FL-25	3.8 bc	5.0 a	4.0 a	4.0 bc
FL-36	5.0 a	5.0 a	2.0 bcd	4.3 ab
FL-39	4.8 ab	5.0 a	5.0 a	5.0 a
FL-46	3.8 bc	5.0 a	4.5 a	4.3 ab
G. incrustans				
ATCC 64418	2.0 defg	1.8 ghi	1.7 bcd	4.3 ab
FL-28	1.3 fg	3.5 b	1.0 d	3.0 cdef
FL-32	1.5 fg	1.5 hi	1.8 bcd	1.8 g
FL-38	1.8 efg	2.0 fgh	1.8 bcd	3.0 cdef
FL-52	1.5 fg	1.8 ghi	1.8 bcd	3.0 cdef
FL-53	NT[z]	1.5 hi	1.5 bcd	1.5 g
Magnaporthe poae				
ATCC 64413	5.0 a	4.5 a	4.3 a	3.3 bcde
FL-4	4.3 ab	3.0 bcd	4.3 a	4.0 abc
Phialophora graminicola				
ATCC 64414	1.8 efg	2.0 fgh	2.3 bc	2.3 defg
P. radicicola				
JI3	2.0 defg	2.0 fgh	2.3 bc	3.0 bcdef
Phialophora sp.				
FL-7	2.9 cde	3.3 bc	4.3 a	4.0 abc
FL-11	3.0 cd	2.0 fgh	2.3 bc	3.3 bcde
FL-15	1.5 fg	2..5 def	2.3 bc	2.5 cdefg
FL-18	2.0 defg	2.3 efg	2.5 b	3.3 bcde
FL-23	1.3 fg	2.8 cde	1.8 bcd	2.0 fg
FL-24	2.3 def	1.8 ghi	2.0 bcd	2.3 efg
FL-37	2.3 def	2.8 cde	2.0 bcd	3.5 bcd
JI2	1.5 fg	1.5 hi	2.0 bcd	2.3 efg
Gaeumannomyces type, sterile				
FL-14	1.5 fg	2.0 fgh	1.0 d	2.3 efg
FL-47	2.0 defg	1.5 hi	1.5 bcd	2.0 fg
Check autoclaved oats	2.0 defg	1.8 ghi	1.5 bcd	2.3 defg
Check no oats	1.0 g	1.3 i	1.3 cd	1.5 g
LSD	1.1	0.8	1.0	1.2
F Value	10.1	5.8	21.3	10.9

[x]Based on a scale of 1–5, with 1 = 0% root discoloration and 5 = 75–100% blackened roots. Means within a column and followed by the same letter are not significantly different ($P = 0.05$) according to Waller-Duncan k-ratio *t*-test.

[y]Experiments 1 and 2 conducted in a greenhouse with an average temperature of 35 C from July through October. Experiments 1 and 2A received supplemental water as mist for 5 s every 4 min. Experiment 2B received supplemental water as mist for 5 s every 30 min. Experiment 2C received no supplemental water.

[z]Not tested.

field area established in April 1989. Thus, the role of *G. incrustans* in bermudagrass decline remains questionable.

Hybrid bermudagrass is vegetatively propagated, so establishment of a simple pathogenicity assay with seeds, as with wheat or seed-propagated turfgrasses, is not possible. Thus, the wheat pathogenicity study was conducted with the same isolates used for the bermudagrass pathogenicity experiments to determine if a correlation between the two grass hosts existed. There was a correlation between the two assays for *G. g. graminis*, *M. poae*, and *Phialophora* spp. but not *G. incrustans*.

The species evaluated in this study can not be easily separated without an extensive process of identification. In normal culture on PDA, the species are morphologically indistinguishable. Sexual stages must be produced to confirm teleomorphic identification. Thus, the wheat assay did provide a method for tentative identification of an isolate within 3 wk. Based on the results of this study, if an isolate with *Gaeumannomyces*-like characteristics in vitro is not pathogenic on wheat, the isolate is probably a saprophytic *Phialophora* sp. If the isolate is pathogenic and lobed hyphopodia are observed, the isolate is identified tentatively as *G. g. graminis*. Production of appropriate fertile perithecia would confirm *G. g. graminis*. If the isolate is pathogenic and crusts are present on the roots, the isolate is identified tentatively as *G. incrustans*. Otherwise, the isolate could be *M. poae*. Identification of the latter two heterothallic species must be determined using mating strains to produce fertile perithecia (8,9).

Because hybrid bermudagrass is vegetatively propagated, the causal agents of bermudagrass decline could not be seedborne. Although no perithecia have been observed on samples obtained from putting greens, ascospores could be one method of disease spread. The field detection portion of this study demonstrated another possible source of the natural disease inoculum. Two different sod producers provided the hybrid bermudagrass sprigs used for planting the fumigated field and putting green described herein. *Gaeumannomyces*-like fungi were easily recovered from the bermudagrass roots from both sources, either from sprigs before planting (putting green) or from 6-mo-old plants established with the sprigs (field area and putting green). Therefore, *G. g. graminis* and related ectotrophic fungi probably were associated with roots of the bermudagrass sprigs used for planting. Sprigs are normally planted into fumigated soil, so *Gaeumannomyces* spp. and similar fungi may be able to spread rapidly on the new root systems, because populations of antagonistic organisms probably have insufficient time to reach suppressive levels. This may explain why putting greens that are only 2 or 3 yr old can exhibit bermudagrass decline symptoms. This propagation method provides an opportunity for introducing biological control agents or instituting cultural control practices at the time of planting to prevent bermudagrass decline. This line of research is being pursued.

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