Disease Response of Bermudagrasses to *Gaeumannomyces graminis* var. *graminis*

M. L. Elliott, Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale 33314

ABSTRACT

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A greenhouse study evaluated 28 bermudagrass accessions, 27 from the National Turfgrass Evaluation Program plus the commonly used cultivar Tifdwarf, for their response to the root rot fungus *Gaeumannomyces graminis* var. *graminis,* which causes bermudagrass decline of golf course putting greens in Florida. Four isolates of the fungus at three different inoculum levels were used. As evaluated, there appears to be no resistance or tolerance to this fungus among the bermudagrass germ plasm tested. More than one *G. g.* var. *graminis* isolate at more than one inoculum level was necessary to make adequate evaluations.

Additional keyword: *Cynodon* spp.

Bermudagrass decline is a destructive root rot disease of bermudagrasses (*Cynodon* spp.) used on golf course putting greens in Florida. The disease has been observed on all putting green types (old, new, poorly-drained soil, and well-drained sand) during the summer and fall when the largest proportion of annual precipitation is received, and the weather is typically very warm and humid (16). It is caused by the ectotrophic root pathogen *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *graminis* (6) which also causes root rot diseases of two other warm-season turfgrasses, St. Augustinegrass (9) and zoysiagrass (21).

Tifgreen and Tifdwarf are the two most common bermudagrass cultivars used on putting greens in Florida. Tifgreen (Tifton 328) is a sterile F_1 hybrid from *C. dactylon* (L.) Pers. and *C. transvaalensis* Burtt-Davy, and Tifdwarf is a natural mutant of Tifgreen (1). Both cultivars have been available and used since the late 1960s. While other bermudagrass cultivars have been introduced, none have been as well adapted or accepted by the golf course industry for use on putting greens in the subtropical climate of southern Florida or the more temperate climate of northern Florida.

Although cultural control methods have been successful in managing bermudagrass decline (7), the primary method of control, increasing the mowing height, is not acceptable to the golfing public. Systemic fungicides do not appear to provide control of the disease (7), but contact fungicides are used to control secondary problems that develop (e.g., algal scum and crusts). Therefore, use of disease resistant or tolerant cultivars would be both economically and ecologically beneficial. Bermudagrass cultivars or clones have been examined for their response to other ectotrophic root rotting pathogens, specifically *Ophiosphaerella korrae* (J. Walker & A. M. Smith) R. Shoemaker and C. Babcock (= *Leptosphaeria korrae* J. Walker and A. M. Smith) and *O. herpotricha* (Fr.:Fr.) J. C. Walker (4,17,18). These fungi cause spring dead spot disease but only in regions where the bermudagrass becomes dormant due to cold temperatures. The disease does not occur in Florida. *Gaeumannomyces graminis* var. *graminis* has also been identified as a causal agent of spring dead spot in the southeastern U.S. (12), but it has not been used in cultivar evaluation studies. Therefore, a study was initiated to examine the susceptibility of bermudagrass accessions from the National Turfgrass Evaluation Program using a greenhouse evaluation system designed initially to test pathogenicity of *G. g.* var. *graminis* on Tifgreen bermudagrass (6).

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MATERIALS AND METHODS

Fungal isolates and inoculum preparation. Four isolates of *G. g.* var. *graminis* were used in this study. FL-19 and FL-36 were isolated from bermudagrass golf course putting greens in Florida in 1987 and 1988, respectively. FL-167 was isolated in 1991 from a bermudagrass putting green in New Providence, Bahamas. FL-39 was isolated in 1988 from a St. Augustinegrass sod production farm in Florida. Pathogenicity studies on hybrid bermudagrass were conducted previously with isolates FL-19, FL-36, and FL-39 (6). All three isolates were pathogenic. Isolates were maintained on potato-dextrose agar (PDA) slants at 2°C and as PDA plugs in glycerol at –70°C.

Inoculum was prepared for each isolate by mixing a 250-ml volume 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 2 consecutive days. For each jar of each isolate, one colonized PDA plate was chopped into small pieces and mixed with the oats. Inoculated oats were incubated for 4 weeks at room temperature, air dried, tested for viability and purity, and then stored in heat sealed pouches at room temperature. Only pure, viable inoculum was stored. Inoculum purity and viability was determined before each experiment. All inoculum remained viable, and the same inoculum source was used throughout the 2-year study.

Evaluation of bermudagrass accessions. Three levels of oat kernel inoculum were used: approximately 1.5 g, 0.15, and 0.015 g (one oat kernel) per conetainer (2.5 × 18 cm tapered plastic cones; Ray Leach Cone-Tainers, Stewe and Sons, Inc., Corvallis, Oreg.). The highest inoculum level had been used previously in pathogenicity studies (6) and represented a 2-cm-deep layer of inoculum. The control treatment was 10 noninfested oat kernels, autoclaved dry for 90 min.

The bermudagrasses evaluated included 27 accessions obtained from A. Dudeck, University of Florida, from the National Bermudagrass Test (1986 to 1992) conducted in Gainesville, Fla., and sponsored by the National Turfgrass Evaluation Program (NTEP), USDA, Beltsville, Md. NTEP maintains information and data on these accessions. The cultivar Tifdwarf, which was not part of the National Bermudagrass Test, also was included in the study and was obtained locally. Each bermudagrass accession (28 total) was grown in small pots filled with a sterile topsoil mix, allowing stolons to grow over the edges of the pots. This resulted in aerial stolons that did not touch soil. These soil-free stolons were used as sources for the sprigs used for planting.

Conetainers were plugged with nonabsorbent cotton and filled with a topsoil mix composed of 80% sand and 20% Canadian peat moss (pH 5.8 to 6.3) that had been autoclaved for 90 min. The level of the topsoil mix in the conetainer was dependent on the inoculum amount. The topsoil mix was saturated with water, and the appropriate amount of oat kernel inoculum was added and then covered with a 1-cm-deep layer of topsoil mix. After saturating the inoculum and additional soil with water, each conetainer was planted with one sprig (one node, 3 to 6 leaves and no roots) of the appropriate bermudagrass accession.

Experiments were conducted between April and November of 1992 and 1993. In each year, each bermudagrass accession was evaluated using each *G. g.* var. *graminis* isolate. One experiment consisted of all 28 bermudagrass accessions subjected to four treatments: one *G. g.* var. *graminis* isolate at the three inoculum levels plus the control treatment. Each experiment was a completely randomized block design with four replicate plants per treatment.

Plants were grown as before (6) in an open-sided greenhouse with an average temperature between 25 to 35°C, relative humidity greater than 80% and a natural photoperiod. Plants were misted with water for 7 s every 16 min during daylight hours for the duration of the experiment. After 3 weeks, plants were maintained at 5-cm height via weekly clippings and fertilized with quarter-strength Hoagland's solution (10) once each week. The experiments were terminated and roots evaluated 6 weeks after planting. Each plant had 4 to 6 roots ranging in length from 10 to 15 cm.

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 to 25% of roots with isolated black lesions or a general tan discoloration of the entire root system; 3 = 26 to 50% of roots with black coalescing lesions; 4 = 51 to 75% of roots were black due to coalescing lesions; 5 = 76 to 100% of roots were black.

After root evaluation, selected roots were cut into 2-cm pieces, surface sterilized with a 1% silver nitrate solution, and placed on a selective medium (5) or PDA amended with streptomycin sulfate to confirm the presence of the pathogen.

Statistical analysis. Data were analyzed using the analysis of variance procedure (ANOVA) (SAS Institute Inc., Cary, N.C.). The control treatment in each experiment was analyzed first to determine if there were any significant differences among bermudagrass accessions. Since none were observed, the ANOVA for each year included only the three inoculum levels for each isolate. The Waller-Duncan *k*-ratio *t* test was used, when appropriate, to separate means in each experiment. The Wilcoxon matched-pairs signed ranks test was used to compare similarity between years

Table 1. Analysis of variance for combined means of root rot ratings of 28 bermudagrass accessionns inoculated with four *Gaeumannomyces graminis* var. *graminis* isolates at three inoculum levels

Source	df	1992 Mean square	1992 $P > F$	1993 Mean square	1993 $P > F$
Replications	3	0.62	0.1008	0.36	0.2278
Isolates	3	224.80	0.0001	279.22	0.0001
Inoculum levels	2	1,028.49	0.0001	944.88	0.0001
Bermudagrass sources	27	0.47	0.0310	0.50	0.0018
Isolates × levels	6	127.98	0.0001	110.45	0.0001
Isolates × bermudagrasses	81	0.48	0.0007	0.32	0.0442
Levels × bermudagrasses	54	0.37	0.1049	0.28	0.2577
Isolates × levels × bermudagrasses	162	0.45	0.0001	0.27	0.2042
Error	1,005	0.30	...	0.25	...
Total	1,343	...	...	...	...

Table 2. 1992 and 1993 root rot ratings of 28 bermudagrass accessions inoculated with four *Gaeumannomyces graminis* var. *graminis* isolates at three inoculum levels

	1992					1993	
	FL-19[y]	FL-36	FL-39		FL-167	FL-19	FL-167
Bermudagrass	Low[z]	High	High	Medium	Low	Low	Low
MSB10	1.5 cde	5.0 a	5.0 a	3.5 abc	3.5 ab	2.3 abc	4.3 abc
MSB20	1.8 b–e	5.0 a	4.8 ab	3.0 bc	3.5 ab	1.5 cd	3.8 a–d
MSB30	1.0 e	4.3 ab	5.0 a	3.5 abc	3.0 ab	1.3 d	3.0 cd
NMS1	2.8 ab	5.0 a	5.0 a	3.3 abc	2.8 b	1.8 bcd	3.3 bcd
NMS2	1.0 e	5.0 a	5.0 a	3.8 ab	4.3 ab	1.3 d	4.0 a–d
NMS3	1.3 de	5.0 a	5.0 a	3.8 ab	3.3 ab	1.0 d	3.5 a–d
NMS4	1.8 b–e	5.0 a	4.5 b	3.8 ab	3.8 ab	1.8 bcd	3.5 a–d
NMS14	2.0 a–e	5.0 a	4.5 b	3.5 abc	4.0 ab	1.8 bcd	4.3 abc
NM43	1.3 de	5.0 a	5.0 a	3.3 abc	3.5 ab	1.5 cd	3.3 bcd
NM72	1.8 b–e	5.0 a	4.8 ab	3.8 ab	3.0 ab	1.8 bcd	3.0 cd
NM375	2.5 a–c	5.0 a	5.0 a	3.5 abc	3.3 ab	1.8 bcd	2.8 d
NM471	2.0 a–e	5.0 a	5.0 a	3.5 abc	3.5 ab	1.8 bcd	3.5 a-d
NM507	1.0 e	5.0 a	5.0 a	4.0 a	3.0 ab	1.3 d	3.0 cd
A22	3.0 a	5.0 a	5.0 a	3.8 ab	3.5 ab	2.8 a	3.3 bcd
A29	1.5 cde	4.5 ab	5.0 a	4.0 a	4.0 ab	2.5 abe	4.0 a-d
RS1	1.3 de	5.0 a	5.0 a	4.0 a	3.3 ab	1.3 d	3.5 a-d
E29	1.3 de	3.5 b	5.0 a	3.5 abc	3.5 ab	1.5 cd	3.5 a-d
CT23	2.3 a–d	5.0 a	5.0 a	3.5 abc	4.0 ab	2.3 abc	4.0 a-d
FB119	1.3 de	4.3 ab	5.0 a	3.5 abc	2.8 b	1.8 bcd	3.0 cd
Vamont	1.0 e	5.0 a	5.0 a	3.0 bc	3.3 ab	1.3 d	3.5 a-d
Tifway	1.0 e	3.5 b	4.8 ab	3.5 abc	3.8 ab	1.0 d	3.5 a-d
Guymon	1.5 cde	5.0 a	5.0 a	3.0 bc	3.0 ab	1.5 cd	3.8 a-d
Tifgreen	1.5 cde	4.8 ab	5.0 a	3.0 bc	4.8 a	1.3 d	4.8 a
Tifway II	1.3 de	5.0 a	5.0 a	3.3 abc	4.5 ab	1.5 cd	4.5 ab
Tifdwarf	1.5 cde	5.0 a	5.0 a	3.3 abc	4.3 ab	1.5 cd	4.0 a-d
Midiron	1.5 cde	5.0 a	5.0 a	3.3 abc	4.3 ab	1.5 cd	4.3 abc
Tufcote	1.3 de	5.0 a	5.0 a	2.8 c	3.3 ab	1.0 d	3.5 a-d
Texturf 10	1.0 e	4.5 ab	5.0 a	3.3 abc	4.3 ab	1.3 d	4.0 a-d

[y] Based on a scale of 1-5, with 1 = 0% root discoloration and 5 = 75 to 100% blackened roots. Means within a column and followed by the same letter are not significantly different ($P < 0.05$) according to the Waller-Duncan *k*-ratio *t* test. Only those treatment combinations with significant differences among means are presented. In both years, all bermudagrass accessions at high and medium inoculum levels of FL-19 had means of 5.0, and at low inoculum levels of FL-36 and FL-39 had means of 1.0. Overall means for the control treatment were 1.3 and 1.4 in 1992 and 1993, respectively. Means and range for treatment combinations which were not significantly different are as follows. 1992: FL-36 medium = 1.5 (1.3 to 1.8), FL-167 high = 4.4 (3.0 to 5.0), FL-167 medium = 4.9 (4.5 to 5.0); 1993: FL-36 high = 4.3 (3.8 to 4.8), FL-36 medium = 1.4 (1.3 to 1.8), FL-39 high = 4.9 (4.5 to 5.0), FL-39 medium = 3.5 (3.0 to 4.0), FL-167 high = 4.4 (3.3 to 5.0), FL-167 medium = 4.9 (4.5 to 5.0).

[z] Inoculum levels were approximately 1.5 g (high), 0.15 g (medium) and 0.015 g (low) infested oat kernels.

on the overall means for each bermudagrass accession.

RESULTS

In both years, there were no significant differences among bermudagrass accessions for the control treatment in any of the experiments. The combined means for the control treatment were 1.3 (range from 1.1 to 1.6) and 1.4 (range from 1.1 to 1.6) in 1992 and 1993, respectively. The control treatment did cause minor discoloration of the roots. For isolates FL-36 and FL-39, the lowest inoculum level (one infested oat kernel) resulted in less root discoloration than the control treatment. The primary organisms isolated from check roots were *Trichoderma* and bacteria, usually *Bacillus*. *Gaeumannomyces graminis* var. *graminis* was only isolated from bermudagrass roots grown in the presence of *G. g.* var. *graminis* infested oat kernels.

In both years, the majority of variation (90%) was associated with *G. g.* var. *graminis* isolates ($P < 0.001$), inoculum levels ($P < 0.001$) and the interaction between these treatments ($P < 0.001$) (Table 1). Although there was a significant difference ($P < 0.05$) in root symptoms observed among bermudagrass accessions and the interaction between bermudagrasses and isolates, less than 1% of the variation within the experiments was associated with either of these factors.

Results for individual bermudagrass accessions as affected by *G. g.* var. *graminis* isolates and inoculum levels are presented in Table 2. In both years of the study, the root rot values were exactly the same for each replicate of each bermudagrass accession for *G. g.* var. *graminis* isolate FL-19 at the high and medium inoculum levels (root rot value = 5.0) and isolates FL-36 and FL-39 at the low inoculum level (root rot value = 1.0). While differences in root rot values were observed for all other isolate and inoculum level combinations, significant differences ($P < 0.05$) among bermudagrass accessions were only observed for five of these combinations in 1992 and two of these combinations in 1993 (Table 2).

Across all 28 bermudagrass accessions, the highly significant variation in root rot values observed among *G. g.* var. *graminis* isolates and among inoculum levels did not follow a discernible pattern. Only isolate FL-39 displayed a classic inoculum dilution effect with root rot ratings decreasing as inoculum level decreased. The means were very similar between years except for FL-36 at the highest inoculum level; root rot values for this isolate did decrease in 1993. However, a ranking comparison between years of the overall means for each bermudagrass accession demonstrated there was no significant difference between years ($P < 0.05$).

The overall response of the bermudagrass accessions to *G. g.* var. *graminis* demonstrated limited differences among them. When the root rot ratings for each accession were averaged across all isolates at the three inoculum levels, the difference in both years between the lowest and highest root rot value was less than 0.5 with a range from 3.229 to 3.667 in 1992 and 3.188 to 3.646 in 1993.

DISCUSSION

It is evident from this study that it is necessary to use more than one *G. g.* var. *graminis* isolate at more than one inoculum level to determine disease response of bermudagrass accessions. There were no consistent responses from the bermudagrass accessions to the isolates, inoculum levels, or even isolate by inoculum level interactions. For example, four accessions had significantly lower root rot values for the highest level of either isolate FL-36 (Tifway and E29) or isolate FL-39 (NMS4 and NMS14) but not for any other isolate or inoculum level. Tifgreen had a significantly higher root rot value for the lowest level of isolate FL-167 but not for any other treatment combination. However, all bermudagrass accessions were highly susceptible to all *G. g.* var. *graminis* isolates at one of the inoculum levels, usually the highest level. Even with the large amount of variation in the current study, the overall response of the bermudagrass accessions to *G. g.* var. *graminis* demonstrated limited differences among accessions. There would appear to be minimal prospects for resistance among the bermudagrasses as evaluated.

Greenhouse studies with other turfgrass ectotrophic root pathogens have produced similar results. In one experiment, five bermudagrass cultivars responded quite differently to each of the three *O. korrae* isolates used (4). In a study examining Kentucky bluegrass cultivar response to *Magnaporthe poae* Landschoot & Jackson, causal agent of summer patch, significant isolate by cultivar interactions were observed (11). Research concerning natural levels of *G. graminis* in the field indicates that inoculum levels and initial disease incidence are not the critical factors for disease development, but that weather, primarily rainfall, is the primary factor (3,8,15).

The lack of resistance to *G. g.* var. *graminis* by the bermudagrass accessions is consistent with responses of other grasses (cereals, pasture, and turf) to *G. graminis* varieties. There are no wheat or barley cultivars resistant to *G. g.* var. *tritici*, which causes take-all (20). No cultivars of *Agrostis* spp. (bentgrasses) are resistant to *G. g.* var. *avenae*, causal agent of take-all patch (13,16). Nilsson (13) found all cultivars of thirty cereal and grass species tested were severely infected by presumed isolates of *G. g.* var. *graminis*. However, differences in susceptibility of rice cultivars to crown sheath rot, caused by *G. g.* var. *graminis*, do exist (19).

A problem associated with long-term studies using ectotrophic root pathogens is maintaining isolate pathogenicity and virulence. In this study, only the most recently obtained *G. g.* var. *graminis* isolate, FL-167, was pathogenic across all three inoculum levels. This isolate had not been stored as long (less than 6 months) or subcultured as often as the other three isolates. Loss of pathogenicity or change in virulence was observed previously for *G. graminis* obtained from wheat when the isolates were repeatedly subcultured (2). Landschoot et al. (11) noted that a decline in virulence of *M. poae* during a study with Kentucky bluegrass was probably due to repeated subculturing between experiments. In an attempt to prevent this phenomenon in the current study, the same inoculum source was used over the 2-year study period to eliminate subculturing. However, a decline in virulence was still observed for inoculum of isolate FL-36, indicating a possible change in the fungus during inoculum storage.

A further dilemma in evaluating bermudagrasses for disease response to ectotrophic root pathogens would be the inconsistency between greenhouse and field study results as has been observed previously with *O. korrae* (4). Differences between cultivars or germ plasm may not be observed in a greenhouse study if cultural practices, such as mowing height or fertility requirements, are critical for disease development. All bermudagrasses may be susceptible to fungal attack of the root system, but their ability to tolerate loss of root system may be the differential and critical factor for maintaining turf quality. With spring dead spot (either *O. korrae* or *O. herpotricha*), bermudagrasses that were more cold tolerant also appeared to be more tolerant of the pathogens (4,14,17, 18). Therefore, it may be necessary to evaluate bermudagrass accessions under normal putting green conditions to obtain grasses tolerant of these stressful conditions. The amount of material to be screened for disease resistance or tolerance could be reduced by first screening for adaptation to normal putting green stresses.

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