

# Association of *Gaeumannomyces graminis* var. *graminis* with a St. Augustinegrass Root Rot Disease

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## ABSTRACT

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.

A patch disease of St. Augustinegrass (*Stenotaphrum secundatum*) characterized by a severe root rot was observed during the summer and fall on residential lawns and sod production fields in Florida during 1988-1991 and in Alabama during the summer of 1991. *Gaeumannomyces graminis* var. *graminis* was consistently isolated from symptomatic roots and stolons. Koch's postulates were fulfilled for this pathogen with St. Augustinegrass grown in a greenhouse. The disease was observed on a range of cultivars growing on numerous soil types, suggesting the pathogen is widely distributed. In addition, the disease was observed on lawns that had been established for only 1 yr as well as lawns established for over 10 yr.

*Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier has been reported on many members of the Poaceae family and causes a number of root and crown rot diseases (17). *G. graminis* var. *graminis* is currently known to cause three diseases in this plant family: spring dead spot (8) and bermudagrass decline (1) of bermudagrass (*Cynodon* spp.) and crown (black) sheath rot of rice (*Oryza sativa* L.) (17). Related variety *G. graminis* var. *tritici* J. Walker causes take-all of wheat (*Triticum aestivum* L.), and *G. graminis* var. *avenae* (E. M. Turner) Dennis causes take-all of oats (*Avena sativa* L.) and take-all patch of creeping bentgrass (*Agrostis stolonifera* L.) (17).

St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) is the primary turfgrass used for lawns in residential and commercial landscapes in Florida and, to a lesser extent, in other southeastern states, especially the coastal regions. During the summer of 1988, large chlorotic patches of St. Augustinegrass were observed in a sod production field in southern Florida. Plants associated with these patches exhibited severe symptoms of a root rot. Both *G. graminis* and *G. incurstans* Landschoot & Jackson were isolated and identified from affected root tissue (3). Since that initial observation, St. Augustinegrass sod fields and residential lawns in both Florida and Alabama have been observed with these symptoms. This paper describes symptoms observed in landscapes and sod production fields, iso-

lation and identification of *Gaeumannomyces* spp. associated with these symptoms, and completion of Koch's postulates with *G. g. graminis*. We propose that this disease should be called "take-all root rot" of St. Augustinegrass.

## MATERIALS AND METHODS

**Isolation and identification.** Symptomatic plants were collected from residential lawns and sod production fields in Florida during the summer and late fall of 1988-1991 and from Alabama home lawns in the summer of 1991. Symptomatic roots and stolons were washed thoroughly under tap water. Small pieces (<2.5 cm) of tissue were soaked for 30 sec in 1% silver nitrate solution, rinsed for 30 sec in sterile water, blotted dry on filter paper, and placed on a medium selective for *Gaeumannomyces* (2). Plates were incubated in the dark at 28 C and examined periodically for up to 14 days. Mycelial growth typical of *Gaeumannomyces* was selected from these plates and transferred to Difco potato-dextrose agar (PDA). Selected isolates were stored on PDA slants at 2 C and in sterile glycerol at -70 C.

The method of Speakman (15) was used to aid in the identification process. Surface-sterilized and germinated wheat seeds were placed on water agar (1.5% Bacto agar) and inoculated with a 5-mm-diameter agar plug from a PDA culture of the test isolate. Plates were sealed with Parafilm and allowed to incubate at 28 C with 12 hr of light for 24 days. Plants and the bottom of the petri dish were then examined for the presence and shape of hyphopodia. Plants were also examined for presence of perithecia; if perithecia were present, then ascospore size was determined. Isolates that did not produce lobed hyphopodia or mature perithecia were used again to inoculate wheat seedlings on water agar but were

also paired with "tester" strains of *G. incurstans* and *Magnaporthe poae* Landschoot & Jackson (3). Isolates were also used in a polymerase chain reaction technique, which amplifies mitochondrial DNA specific for *G. graminis* (11). The template DNA for amplification was obtained from mycelia grown on PDA and then boiled in 8 mM Tris buffer (pH 8.5) (4).

**Pathogenicity tests.** *G. g. graminis* isolate FL-39 was initially used to produce oat-infested inoculum. This isolate originated from a St. Augustinegrass sod production field with root rot symptoms and had previously been demonstrated to be pathogenic on hybrid bermudagrass (1). Inoculum was prepared by mixing a 250-ml volume of whole oats with 125 ml of deionized water in a glass jar. The oat mixture was autoclaved for 90 min on each of two consecutive days. A PDA plate, covered with growth of the isolate, was chopped into small pieces and mixed with the oats. Inoculated oats were incubated in the dark for 4 wk at 30 C, dried, and then stored at room temperature in a heat-sealed bag.

Pathogen-infested oat kernels were macerated with a food processor and incorporated into a topsoil mix (80% sand and 20% peat moss, pH 6.0) at the rate of 10, 5, and 1% inoculum (v/v) for use in 10-cm-square pots. Check pots contained topsoil mix only. Each pot was planted with one St. Augustinegrass plant (sprig) with seven to eight leaves and five to six clean roots 4-8 cm long. These plants had been started from stolons with leaves but no roots and placed on a clean surface (no soil) under intermittent mist to produce *Gaeumannomyces*-free roots. Five replicate pots per inoculum rate were used.

Pots were fertilized with 2.5 g of granular fertilizer (Par Ex 16-4-8, Vigoro Industries, Inc., Winter Haven, FL) and then placed as a randomized complete block design in a greenhouse with ambient temperatures between 30 and 35 C. Pots were misted with water for 5 sec every 4 min during daylight hours. Experiments were terminated after 10 wk. Roots washed free of soil were evaluated for symptoms, using the following rating scale: 1 = no disease symptoms, with roots completely white; 2 = roots with a general tan discoloration but no isolated lesions or blackened roots; 3 = 1-25% of roots with black, coalescing lesions; 4 = 26-50% black roots, caused

by coalescing lesions; 5 = 51–75% black roots; and 6 = 76–100% black roots. For reisolation, symptomatic root pieces were placed on the *Gaeumannomyces*-selective medium after surface sterilization. Analysis of variance and mean comparisons were made using SAS statistical programs (10).

This test was repeated with *G. g. graminis* isolate FL-163 isolated from a home lawn in Montgomery, Alabama. However, inoculum was produced with perennial ryegrass rather than oats. All other procedures and evaluations were exactly the same.

## RESULTS

**Symptoms.** Disease symptoms viewed aboveground in sod production fields consisted of chlorotic, thinning turf in large irregular patches (over 5 m in diameter). Roots of plants in these patches were short and rotted, and stolons could be readily lifted from the ground. Nodes were usually rotted also, and black lesions were occasionally observed on stolons. Leaf symptoms of general chlorosis or necrosis were evident. Unlike *Rhizoctonia* blight caused by *R. solani* Kühn, the leaves did not separate easily from the plant, because of a basal leaf and sheath rot. Sod producers were unable to harvest symptomatic sod, because the sod strength had been severely weakened by the root rot. These symptoms were observed on sod produced on Lauderhill muck soil in Palm Beach County, Florida, and on Myakka and Smyrna fine sand soils in DeSoto County, Florida.

Symptoms similar to those on commercially produced sod were also observed on St. Augustinegrass residential lawns, except that affected areas were circular or irregular in shape and varied in diameter from less than 1 m to over 5 m (Fig. 1). In some lawns, the overall appearance was similar to *Rhizoctonia* blight (Fig. 2). However, upon closer inspection, no individual plant symptoms of *Rhizoctonia* blight were evident. Necrotic patches of grass were observed, and in some cases, the grass had thinned to bare ground (Fig. 3). The root rot was so severe at one lawn site in Alabama that leaves were folded up, a symptom typical of drought-stressed turfgrass. Symptomatic turf was associated with both muck soils (Palm Beach County) and fine sand soils (Broward and Hendry Counties) in Florida. In Alabama, the disease was observed on the coastal plain soils of Orangeburg fine sandy loam (Houston County), Malbis fine sandy loam (Baldwin County), and blackbelt soil of Sumter blackland prairie (Montgomery County). Age of the lawns varied extensively, from 1 or 2 yr to at least 10 yr. Cultivars affected in Florida included Floratam and Jade. Raleigh and common St. Augustinegrass were affected in Alabama.

**Isolations and identification.** Samples were obtained from symptomatic turfgrass from six home lawns and three sod production farms (five different fields) in southern Florida and three home lawns in Alabama. The deeply lobed hyphopodia typical of *G. g. graminis* were clearly visible on affected stolons. On some samples, mature perithecia with ascospores typical of *G. g. graminis* (17) were also observed.

A total of 20 isolates, with morphological characteristics of *Gaeumannomyces* on PDA and the selective medium (3), were selected for identification. Of these, 15 were identified as *G. g. graminis*, based on either production of deeply lobed hyphopodia and perithecia with appropriately sized ascospores (17) or production of lobed hyphopodia and

amplification of a 188-bp product by the polymerase chain reaction technique (4,11) (Table 1). One isolate was identified as a presumptive *G. g. graminis* on the basis of production of lobed hyphopodia. Three isolates were identified as *G. incrustans*, and one isolate has not been identified, since it did not produce hyphopodia or conidia and has not produced fertile perithecia despite matings with both *G. incrustans* and *M. poae* mating types (6,7). Voucher isolates of *G. g. graminis* have been sent to the American Type Culture Collection in Rockville, Maryland.

**Pathogenicity tests.** All St. Augustinegrass plants grown in the topsoil mix with *G. g. graminis* isolate FL-39-infested oat kernels or FL-163-infested ryegrass kernels had an average root rot rating



Fig. 1. Thinning of St. Augustinegrass lawn canopy and chlorosis and necrosis of the leaf tissue caused by root infection by *Gaeumannomyces graminis* var. *graminis*.



Fig. 2. Characteristic patch symptom observed on St. Augustinegrass lawn. *Gaeumannomyces graminis* var. *graminis* was isolated from the rotted roots.

of 5.0 or greater. The average ratings for FL-39 were 5.5, 5.2, and 5.0 for 10, 5, and 1% inoculum, respectively. These values were not significantly different from each other but were significantly different from the average root rot rating for the check plants, which was 2.0 ( $P=0.05$ , Waller-Duncan  $k$ -ratio  $t$  test). The average root rot ratings for FL-163 were 5.8, 5.2, and 5.2 for 10, 5, and 1% inoculum, respectively. Again, these values were not significantly different from each other but were significantly different from the average root rot rating

for the check plants, which was 1.8 ( $P=0.05$ ).

Plants from check pots had an extensive root system that was tan but had no lesions. In addition to the coalescing black lesions on *G. g. graminis*-infected roots, there was a noticeable lack of secondary roots. These symptoms were similar to those observed on samples from sod fields and lawns. Some leaf chlorosis was exhibited by infected plants, but it was not as extensive as that observed in the field. This was probably due to the fact that the only stress placed

on the St. Augustinegrass grown in pots was the pathogen.

Perithecia with mature ascospores typical of *G. g. graminis* were observed on a few roots, but only at the soil line, of plants grown in infested soil. Lobed hyphopodia were observed on roots, crowns, or basal leaf sheaths of plants grown in infested soil but were not observed on plants from noninfested soil. *Gaeumannomyces* growth was reisolated from roots grown in all three levels of pathogen-infested soil (all pots for both isolates) but not from noninfested check pots.

## DISCUSSION

*G. g. graminis* has been found on *S. secundatum* in New South Wales, Australia (16), but Koch's postulates were not completed with this grass species. Elliott and Landschoot (3) provided the first report of this fungus on St. Augustinegrass in the United States with its isolation and identification in Florida. *G. g. graminis* has also been recently observed on symptomatic St. Augustinegrass in Texas (5). The present study extends the geographic range of the fungus on St. Augustinegrass to Alabama, and it completes Koch's postulates with this fungus on St. Augustinegrass for the first time. Given the isolations from symptomatic turfgrass and pathogenicity tests, we conclude that *G. g. graminis* is an etiological agent of a severe root rot of St. Augustinegrass. This is probably not a new disease per se, but one in which the causal agent has eluded proper identification and confirmation as a pathogen until this study. For future clarity and simplicity, we propose that the common name of the disease should be "take-all root rot" of St. Augustinegrass.

Given our observations thus far, there does not appear to be a relationship between take-all root rot incidence and soil type, cultivar, or age of the St. Augustinegrass. In Florida, symptoms indicative of take-all root rot were observed during the summer and fall months, when the greatest proportion of annual precipitation occurs. This may contribute to disease development, as has been noted for other diseases caused by *G. graminis* (9,13). As with other turfgrass patch diseases (12,14), it has been observed that the take-all patch symptoms on St. Augustinegrass recur in the same areas each summer or fall.

Necrotic ringspot is a turfgrass patch disease that is most prevalent on lawns established with sod (12). Summer patch and bermudagrass decline are known to be spread by infected sod (12). Like the pathogens associated with these turfgrass diseases, *G. g. graminis* is an ectotrophic, root-colonizing fungus. Since St. Augustinegrass is vegetatively propagated, any pathogen associated with sprigs or sod will likely be spread at planting.

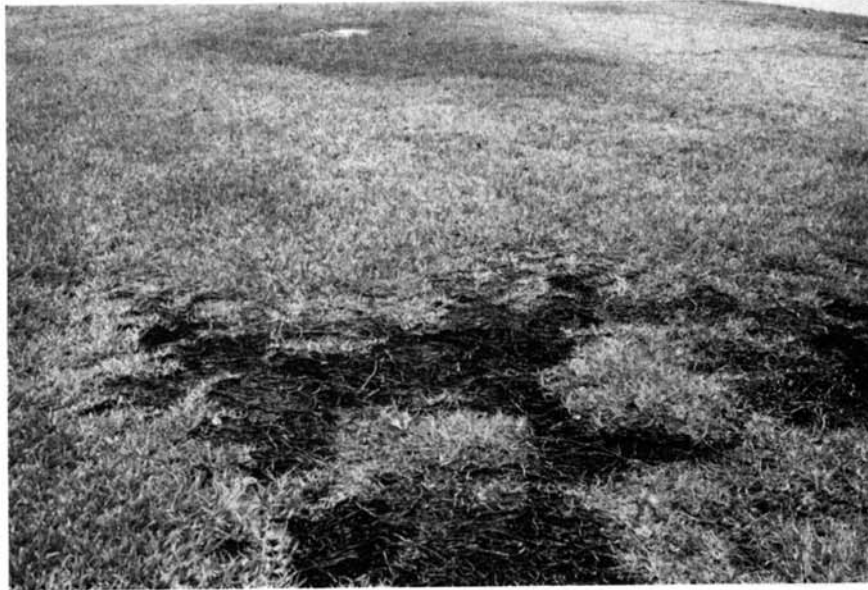


Fig. 3. Severe patch symptom observed on St. Augustinegrass. Note the bare ground and rotting stolons from which *Gaeumannomyces graminis* var. *graminis* was isolated.

Table 1. Characteristics of *Gaeumannomyces*-like isolates from symptomatic St. Augustinegrass

Isolate <sup>a</sup>	Location <sup>b</sup>	Year	Lobed		PCR <sup>c</sup>
			hyphopodia	Perithecia	
FL-38 <sup>d</sup>	South Bay Growers, FL (sod)	1988	—	+	—
FL-39	South Bay Growers, FL (sod)	1988	+	+	+
FL-101	South Bay, FL (lawn)	1989	+	+	+
FL-102	South Bay, FL (lawn)	1989	+	—	+
FL-103	South Bay, FL (lawn)	1989	+	+	+
FL-104	Mace 68-K-19, FL (sod)	1990	+	+	+
FL-105	Mace 68-N-29, FL (sod)	1990	+	+	+
FL-106	Mace 68-N-36, FL (sod)	1990	+	—	+
FL-125	Bethel Farms, FL (sod)	1990	+	—	NT <sup>e</sup>
FL-155	Montgomery 1081, AL (lawn)	1991	+	—	+
FL-156 <sup>d</sup>	Montgomery 1081, AL (lawn)	1991	—	+	—
FL-160 <sup>f</sup>	Ft. Lauderdale, FL (lawn)	1991	—	—	NT <sup>e</sup>
FL-161	Montgomery 1082, AL (lawn)	1991	+	—	+
FL-162 <sup>d</sup>	Montgomery 1082, AL (lawn)	1991	—	+	—
FL-163	Montgomery 1083, AL (lawn)	1991	+	+	+
FL-195	Royal, South Bay, FL (lawn)	1991	+	+	+
FL-196	Dodger, South Bay, FL (lawn)	1991	+	—	+
FL-197	Dodger, South Bay, FL (lawn)	1991	+	+	+
FL-198	Royal, South Bay, FL (lawn)	1991	+	+	+
FL-199	Royal, South Bay, FL (lawn)	1991	+	+	+

<sup>a</sup> Isolates were identified as *G. graminis* var. *graminis* except as noted.

<sup>b</sup> Location description includes state and sod farm or homeowner name, if known; otherwise, only the city and sample designation are provided.

<sup>c</sup> Polymerase chain reaction.

<sup>d</sup> Identified as *G. incrustans* on the basis of perithecia production with appropriate mating strain.

<sup>e</sup> Not tested, because of inability to recover isolate from storage for PCR test.

<sup>f</sup> Identity unknown.

Although the overall importance of take-all root rot in St. Augustinegrass landscapes needs to be determined, an immediate concern is dispersal of *G. g. graminis*-infested sod from sod production fields to landscapes throughout the southeastern United States. In Florida alone, there are over 16,000 ha in St. Augustinegrass sod production. Although sod producers were prevented because of the disease from harvesting infested fields during the summer, they were able to harvest these fields in winter months when the turfgrass had recovered. However, the pathogen was still present on roots (M. L. Elliott, unpublished). Florida currently does not have a sod certification program, so there is no monitoring of pathogens associated with sod. Therefore, an extensive survey of sod production fields in the southern United States should be initiated, with breeding material and foundation material also examined, to clarify the source of *G. g. graminis*.

#### ACKNOWLEDGMENT

We thank Barbara Center for providing the St. Augustinegrass plants.

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