Gaeumannomyces graminis Associated with Spring Dead Spot of Bermudagrass in the Southeastern United States

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ABSTRACT

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Three isolates of a dark-gray, slow-growing fungus isolated from bermudagrass cultivar Tifway with spring dead spot (SDS) symptoms were tested for pathogenicity on bermudagrass. One isolate produced typical SDS symptoms on bermudagrass when potted plants were inoculated in the fall and grown outside during the winter. Of plants inoculated with isolate B, top weight was 92% and root weight was 71% less than for the control. Of those inoculated with isolate A, top weight was 55% and root weight was 42% less than for the control. Isolate B produced asci and ascospores in perithecia on the dead stolons and was identified as *Gaeumannomyces graminis* var. *graminis*. The same fungus was identified from perithecia and ascospores on naturally SDS-infected bermudagrass from North Carolina and Alabama in May 1987. This is the first report of an association of *G. graminis* as a causal agent of SDS on bermudagrass.

Spring dead spot (SDS) is the most important disease of bermudagrass (*Cynodon dactylon* (L.) Pers.) used for turf in the northern range of adaptation of this grass in the United States (2) and in Australia (5). SDS was first described in Oklahoma in the 1950s and was reported in North Carolina in the late 1960s (2,3). The disease occurs in most of the southern areas of the United States where the winters are cold enough to induce bermudagrass winter dormancy.

SDS appears as circular dead spots, from 0.2 to 1 m in diameter, that occur in the spring as bermudagrass resumes growth (Fig. 1A). The disease usually develops in intensively managed turf 2–3 yr following establishment. High nitrogen fertilization, excessive thatch, and the use of hybrid bermudagrass cultivars are factors most often associated with the disease (2). Diseased areas usually enlarge for 3–5 yr and develop into rings with live grass in the center by the third and fourth years. Bermudagrass recovery in SDS areas is slow, and invasion of annual weeds such as crabgrass (*Digitaria* spp.) often occurs.

Plants in affected spots have rotted stolons and roots. Dark-colored, septate hyphae and black, disk-shaped sclerotia are usually found on affected bermudagrass basal leaf sheaths, stolons, and rhizomes. Numerous fungi have been isolated from SDS-affected turf in the southeastern United States, but none have been shown to cause SDS symptoms. These include species of *Helminthosporium*, *Pythium*, *Fusarium*, and *Curvularia* (3). Mycoplasma and five genera of nematodes are considered not to be involved in SDS (2).

Leptosphaeria narmari Walker & Smith and *L. korrae* Walker & Smith have been demonstrated to cause SDS in Australia (7), whereas *L. korrae* has been reported to cause SDS of bermudagrass in California (1) and necrotic ring spot of bluegrass (*Poa pratensis* L.) in the northern United States (4). Previous isolation attempts in North Carolina have not associated a causal agent with SDS.

Isolation and establishment of an SDS causal agent would speed progress in laboratory and greenhouse studies and in field evaluation of management practices and chemicals for control of this disease. This research was conducted to isolate fungi from SDS-affected areas of bermudagrass in North Carolina and to determine the pathogenicity of these fungi.

MATERIALS AND METHODS

Isolations. Plants of bermudagrass cultivar Tifway (*C. dactylon* × *C. transvaalensis* Burt-Davy) were collected in October from golf course fairways that had typical SDS symptoms the previous spring. Soil was removed by washing roots under tap water. Plants were subjected to 6 hr of rinsing in order to remove many of the contaminating organisms commonly associated with bermudagrass roots. Sclerotia and dark, septate mycelium similar to those shown in Figure 1B were removed and placed on one-quarter strength potato-dextrose agar (PDA) to which 100 ppm of streptomycin sulfate (0.25× PDAS) had been added. Plates were then incubated on a laboratory bench at 25 C in either light (plastic bags) or darkness (paper bags).

Inoculations. Stolons of Tifway bermudagrass, approximately 3 cm long, from previously non-SDS affected areas were surface-disinfested in 0.5 NaOCl for 10 min and transplanted into 10-cm-diameter plastic pots containing a steam-treated sand and Cecil clay loam mixture (4:1, v/v). The bermudagrass was grown in the greenhouse for 2 mo, at which time complete grass cover of the soil surface was achieved.

Mycelium from the 0.25× PDAS was transferred to autoclaved oat (*Avena sativa* L.) seeds and allowed to grow for 1 mo. Fungal growth on oat seeds was primarily black mycelium similar to growth observed on SDS-affected stolons and roots. A 75-cm sample of inoculated oat seed from each isolate was transferred to the crown and shoot areas at or just below the soil line of bermudagrass plants in each pot. In order to allow for ample fungal dispersal and growth, inoculated plants were grown in a greenhouse for 1 mo and shoots were trimmed periodically to 2.5 cm high. Inoculated plants were then placed outside in a sandbox with the top of the pots at sand level from January to May 1987. Pots were placed outside to induce winter dormancy of the bermudagrass. A randomized complete block design with six single-pot replications was used with a noninoculated oat treatment serving as a control. In May, after green-up and SDS symptoms developed, pots were removed from the sandbox and soil was washed from the roots. Green shoots were clipped and then dried at 115 C for 24 hr in a forced-air oven and weights were recorded. Roots below the thatch layer were removed, dried, and recorded in a similar manner. Treatment effects on shoot and root weight were evaluated by analysis of variance, and means were separated by Waller-Duncan's k-ratio t test ($P = 0.01$). Two sets of the replications were not dried and were examined for

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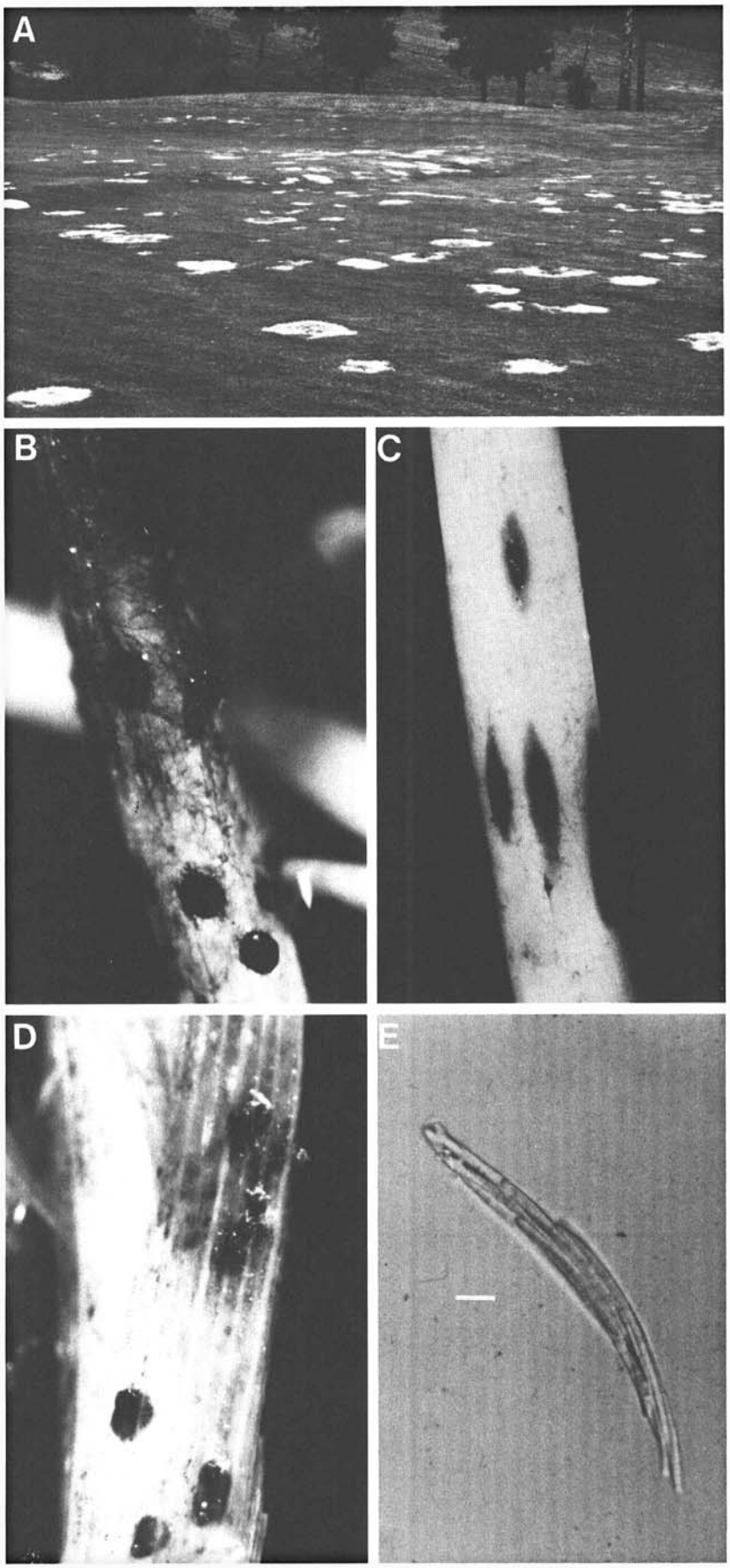

Fig. 1. (A) Cultivar Tifway bermudagrass golf course fairway with symptoms of spring dead spot (SDS). **(B)** Sclerotia and dark, septate mycelium on SDS-affected lower stem bases and sheaths. **(C)** Tan to black-colored SDS lesions on a stolon of bermudagrass. **(D)** Perithecia of *Gaeumannomyces graminis* erumpent within bermudagrass leaf sheath. **(E)** Clavate and unitunicate asci of *G. graminis* formed a narrow, footlike base and contained ascospores (bar = 14 μm).

fungal development on the plants. SDS-affected bermudagrass from North Carolina and Alabama was examined for perfect stages of fungi during the spring of 1987.

RESULTS AND DISCUSSION

Tifway bermudagrass roots, stolons, rhizomes, and shoots from field-grown turf had the typical brown lesions (Fig. 1C) that eventually coalesced to produce blackened, dead tissue. In the fall, the bermudagrass that had regrown over the previous SDS spots had the black, septate hyphae growing throughout roots, stolons, and rhizomes. Black disk-shaped sclerotia were located periodically in these regions, especially underneath basal leaf sheaths (Fig. 1B).

Fungi that were isolated with large black mycelium grew slowly, requiring 10–14 days to cover the 9-cm-diameter agar surface. Fungi were first white, gradually darkened from the center of the colony, and then turned dark gray to black by the end of 14 days. Three isolates were recovered from SDS-affected bermudagrass and are hereafter referred to as SDSA, SDSB, and SDSC.

Tifway bermudagrass inoculated with isolate SDSB produced symptoms similar to those seen on SDS-affected, field-grown bermudagrass. Root and shoot regrowth of bermudagrass inoculated with SDSB was much less than the control. Average shoot weight for SDSB and SDSA isolates was 92 and 55% less for inoculated plants than for the control, respectively (Table 1). Root growth was 71 and 42% less for the SDSB and SDSA inoculated plots, respectively. Results of analysis of variance indicated treatment effects were highly significant.

Brown to black lesions associated with SDS-affected stolons and roots of Tifway bermudagrass were observed (Fig. 1C). Similar dark, septate mycelium and black, disk-shaped sclerotia initially associated with SDS-affected bermudagrass on golf course fairways were observed and isolated from the SDSB-inoculated plants (Fig. 1B). Mycelium identical to that used to inoculate oat seeds grew from the sclerotia when placed on 0.25× PDAS.

Upon further examination of plants inoculated with isolate SDSB, erumpent perithecia within basal leaf sheaths (Fig. 1D) were seen in May following fall inoculation. Asci containing eight ascospores emerged from the perithecia that had thickened lateral necks and contained cylindrical to clavate unitunicate asci (175 × 14 μm). Each ascus had a minute apical ring and a narrowed footlike base (Fig. 1E). Each ascus contained eight hyaline and septate (seven to 10 septa) ascospores. Ascospores measured (78) 94–125 (140) × 3.9 μm, which are shorter than those described for *L. korrae* ([120] 140–170 [180] × 4–5 [5.5] μm) (7). Mycelium on

diseased tissue and associated with perithecia had lobed hyphopodia. The type of ascus and the length of ascospores produced by the fungus isolated from bermudagrass and used in this study indicates that the fungus was not a *Leptosphaeria* spp. Mycelium produced from single-spore isolations was identical in appearance to that grown from sclerotia. Based on the above description and the examination of additional perithecia, asci, and ascospores, the fungus was identified as *Gaeumannomyces graminis* (Sacc.) v. Arx. & Olivier var. *graminis* by J. Walker, a mycologist with the New South Wales Department of Agriculture, Australia, and L. F. Grand and C. S. Hodges, North Carolina State University. The same fungus was identified in May from perithecia and ascospores that formed on naturally SDS-affected bermudagrass from golf course fairways in North Carolina and Alabama.

Gaeumannomyces spp. are associated with take-all of cereals, which is very similar in symptom expression to SDS. Walker reports that under sterile conditions, *L. narmari* and *L. korrae* can produce symptoms similar to those of take-all (6). Other fungi that cause or have been associated with take-all and related diseases include species of *Ophiobolus* and *Phialophora*. The causal fungi of SDS appear to grow most actively when temperatures are cool and the soil is moist. Soil temperatures of 10–20 C are most favorable for the growth of *Gaeumannomyces* spp. Bermudagrass root growth is extremely slow at 15 C and most rapid at 35 C. Therefore, the fungus has a distinct ecological advantage in North Carolina in the fall.

Cold weather has been associated with the development of SDS of bermudagrass (2) and may be the factor that actually kills the grass. Bermudagrass remains green later in the fall in SDS-affected areas, which could predispose bermudagrass to damage by cold temperatures (2). The injury of roots by fungi with low virulence could also predispose bermudagrass to low temperature damage. Information is needed to determine if SDS fungi actually kill the bermudagrass or if the fungi predispose the plants to injury or death by low temperatures. Additional research is needed on which specific environmental and management parameters trigger disease expression because similar fungi have been observed on healthy as well as diseased bermudagrass.

This is the first report of an association of *G. graminis* with SDS and the development of SDS-like symptoms on bermudagrass after inoculation with the fungus. More observations of the teliomorph of this fungus are needed to determine if this *G. graminis* is regularly associated with SDS throughout the southeastern United States. Because *Leptosphaeria* spp. were not isolated or observed in these studies, additional research is needed to determine if SDS of bermudagrass is caused by different fungi in different areas of the world.

Table 1. Shoot and root weights of bermudagrass 4 mo after inoculation with fungi isolated from spring dead spot- (SDS) affected bermudagrass

Isolate	Weight (g)[z]	
	Shoots	Roots
Control	1.31 a	2.46 a
SDSC	1.11 a	2.16 ab
SDSA	0.59 b	1.44 bc
SDSB	0.11 c	0.72 c

[z] Means of four replications. Means separation was by Waller-Duncan's *k*-ratio *t* test at the 1% level.

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