Fungi Similar to *Gaeumannomyces* Associated with Root Rot of Turfgrasses in Florida

M. L. ELLIOTT, Assistant Professor, University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale 33314, and P. J. LANDSCHOOT, Assistant Professor, Agronomy Department, The Pennsylvania State University, University Park 16802

ABSTRACT

Elliott, M. L., and Landschoot, P. J. 1991. Fungi similar to *Gaeumannomyces* associated with root rot of turfgrasses in Florida. Plant Dis. 75:238-241.

Dark-pigmented, ectotrophic fungi were isolated from the roots of hybrid bermudagrass with symptoms of bermudagrass decline, a root rot disease. Similar fungi were also isolated from the roots of St. Augustinegrass, centipedegrass, bentgrass, and perennial ryegrass that exhibited root rot symptoms. *Gaeumannomyces graminis* var. *graminis* was identified from the roots of bermudagrass, St. Augustinegrass, and a bermudagrass-perennial ryegrass mix. *G. incrustans* was identified from centipedegrass, bermudagrass, and St. Augustinegrass roots, whereas *Magnaporthe poae* was identified only from bentgrass roots. *Phialophora* sp. and sterile fungi similar to *Gaeumannomyces* were identified from bermudagrass, bermudagrass-perennial ryegrass mix, and bentgrass roots. For some locations, more than one of the organisms were identified in association with the symptomatic plant roots. Methods of isolation and identification are described. Pathogenicity of these isolates and known isolates of *Gaeumannomyces, Phialophora,* and *Magnaporthe* was examined on wheat seedlings in vitro.

At least four turfgrass patch diseases are caused by fungi with dark-pigmented hyphae and an ectotrophic growth habit on roots. *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *avenae* (E. M. Turner) Dennis is the causal agent of take-all patch of bentgrass (*Agrostis* spp.) (16). Spring dead spot is a disease of bermudagrass (*Cynodon dactylon* (L.) Pers.) grown in areas of the United States and Australia where a winter dormancy is induced because of cold temperatures. Four fungi have been documented as causal agents of this disease, *Leptosphaeria korrae* Walker & Smith, *L. narmari* Walker & Smith (22), *G. g.* var. *graminis* (13), and *Ophiosphaerella herpotricha* (Sr.) Walker (20). *L. korrae* also causes necrotic ringspot of Kentucky bluegrass (*Poa pratensis* L.), whereas *Magnaporthe poae* Landschoot & Jackson causes summer patch of this same grass (12,17,24). Other species of *Gaeumannomyces* associated with turfgrasses are *G. cylindrosporus* Hornby, Slope, Gutteridge & Sivanesan, the probable teleomorph of *Phialophora graminicola* (Deacon) Walker (8), and *G. incrustans* Landschoot & Jackson, a recently described species of *Gaeumannomyces* that is heterothallic (11). The host range and pathogenicity of these latter two species of *Gaeumannomyces* has not been fully determined.

Bermudagrass decline is a root rot disease of hybrid bermudagrass (*C. dactylon* × *C. transvaalensis* Burtt-Davy) used for golf greens in Florida (5–7). It is most prevalent during the summer and fall, when the largest proportion of annual precipitation is received, and the weather is typically very warm and humid. Initial symptoms of bermudagrass decline include the appearance of irregular, chlorotic patches ranging in diameter from 0.2 to 1 m. Chlorosis and necrosis are first observed on the lower leaves. Foliar lesions are absent. The root systems of these plants are short and discolored, with dark-colored lesions on the roots. As the disease progresses, roots and associated rhizomes and stolons become completely rotted (Fig. 1). Entire plants may die, resulting in a thinning of the grass. If the disease is not controlled, bare patches may develop and coalesce. Although the outer margins of a golf green often exhibit the disease symptoms first, symptoms may be expressed across an entire green.

When Freeman and Augustin (5,6) first described the disease, the only fungus consistently associated with diseased root tissue from plants with bermudagrass decline symptoms was a fungus with brown, ectotrophic, sterile hyphae. *Rhizoctonia* and *Pythium* were not consistently isolated from plants with bermudagrass decline symptoms. It was postulated that the ectotrophic fungus was either a species of *Gaeumannomyces* or *Leptosphaeria.* As stated previously, these organisms are known to cause patch diseases characterized by root rot symptoms. Later, one fungal isolate was identified as *Phialophora radicicola* Cain (7), an organism previously associated only with wheat and corn (1,15).

The purpose of this study was to determine if dark-pigmented, ectotrophic fungi, other than the previously identified *P. radicicola*, were associated with the roots of bermudagrass plants exhibiting bermudagrass decline symptoms. During the period that these plants were collected, other turfgrasses were obtained that exhibited root rot symptoms. These samples were also included in the study to help determine the turfgrass host range of fungi similar to *Gaeumannomyces* in Florida.

MATERIALS AND METHODS

Isolations. Hybrid bermudagrass plants symptomatic for bermudagrass decline were collected from golf greens

Florida Agricultural Experiment Station Journal Series R-00531.

Accepted for publication 22 August 1990 (submitted for electronic processing).

in southeastern Florida from July 1987 through September 1988. Plants with root rot symptoms were also obtained from a bentgrass golf green in central Florida, a bermudagrass golf green overseeded with perennial ryegrass (*Lolium perenne* L.) in southeastern Florida, a St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) sod production field in southeastern Florida and a centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) sod production field in northern Florida. The centipedegrass sample was provided by G. Simone, University of Florida, Gainesville.

Soil was removed from symptomatic plants by washing them thoroughly under tap water. Leaf tissue was severed from the plant and discarded. The remaining crown tissue with attached roots or individual root tissue pieces were then soaked for 30 sec in 1% silver nitrate solution, rinsed for 30 sec in sterile water, blotted dry on filter paper and placed on potato-dextrose agar (PDA) with 100 μg/ml streptomycin sulfate (PDAS) and SM-GGT3, a selective medium for *Gaeumannomyces* (10). Plates were incubated at 28 C and examined after 5, 7, and 10 days. Growth typical of *Gaeumannomyces* was selected from these plates and purified on fresh PDAS plates. Each fungal isolate selected was stored on a PDA slant at 2 C.

Wheat seedling pathogenicity assay. The method of Speakman (19) was used as a simple in vitro pathogenicity assay. Seeds of spring wheat cultivar Pondera, surface sterilized with 0.1% silver nitrate solution, were germinated on water agar (1.5% Bacto agar) with three seeds per plate. After the seeds germinated, only plates with three clean seedlings were selected for the assay. A 5-mm-diameter agar plug from a PDAS culture of the test isolate was placed next to the emerging roots of each seedling with two plates per test isolate. Two check treatments were included—seedlings with PDAS plugs placed next to the roots and seedlings with no agar plugs. Plates were sealed with Parafilm and allowed to incubate at room temperature in natural light for 4 wk. Plants were then examined for root rot symptoms and fungal structures characteristic of *Gaeumannomyces*, i.e., hyphopodia, perithecial initials or perithecia, and mycelial crusts on roots and stems.

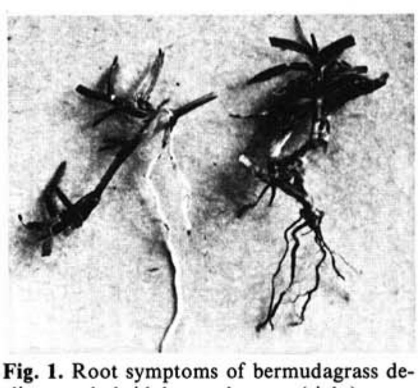

Fig. 1. Root symptoms of bermudagrass decline on hybrid bermudagrass (right) compared with healthy roots (left).

In addition to the fungal isolates obtained in this study, the following fungi were also used in the assay. They were *G. g.* var. *tritici* Walker (MT-528), *P. radicicola* (J13), *G. g.* var. *graminis* (ATCC 64419), *G. g.* var. *avenae* (LL), *G. incrustans* (ATCC 64418), *M. poae* (ATCC 64413), *G. cylindrosporus* (ATCC 64420), and *P. graminicola* (ATCC 64414). The isolate designated *P. radicicola* was provided by T. E. Freeman, University of Florida, Gainesville. Except for *G. g.* var. *tritici* (MT-528), which originated from spring wheat, these fungi had been isolated from turfgrasses.

Identification. Twenty-two isolates were selected for identification. Each isolate selected was inoculated individually and paired with 'tester' isolates of *M. poae* and *G. incrustans* on wheat seedlings in water agar, again using the method described by Speakman (19). The tester isolates of *M. poae* (ATCC 64412, 'a' mating type and ATCC 64411, 'A' mating type), and *G. incrustans* (ATCC 64416, 'a' mating type and ATCC 64417, 'A' mating type) were selected for this assay based on their prolific production of perithecia when combined with isolates of compatible mating types (P. J. Landschoot, *unpublished*). Plates with seedlings were incubated at room temperature in natural light for 5–6 wk. Isolates that produced mature perithecia were identified based on characteristics of the ascospores. Ascospores were stained with 1.0% cotton blue solution and measured with an ocular micrometer under a ×40 objective. Hyphopodia of *G. g.* var. *graminis* were produced on wheat leaves, leaf sheaths, and Parafilm.

Conidia were produced by seeding isolates on one-fifth strength PDA (R. E. Wagner, *personal communication*) or a dilute rabbit food agar (9) and placing the plates in the dark for 2–5 days.

RESULTS AND DISCUSSION

Fifty fungal isolates similar to *Gaeumannomyces*, representing 20 field locations and five turfgrass species, were collected over 15 mo. All isolates had distinct characteristics in culture that were indicative of *Gaeumannomyces*. These characteristics included hyphae that curled back at the colony margins and colonies that darkened with age on PDAS and produced a diffuse melanin pigment on SM-GGT3 (10,18). Twenty-two isolates were selected for identification based on their point of origin (e.g., golf course location), turfgrass host, and additional cultural characteristics such as aerial hyphae, rhizomorphlike structures, or sunken centers in PDAS or SM-GGT3 plates. For example, isolates FL-45, FL-46, and FL-47 were from the same golf course and exhibited characteristics indicative of *Gaeumannomyces* but had additional cultural characteristics that distinguished them individually.

Three genera of fungi were identified: one species of *Magnaporthe*, two species of *Gaeumannomyces*, and an unidentified species of *Phialophora* (Table 1). Two isolates produced neither asexual or sexual structures nor any other distinguishable taxonomic characteristics. These isolates, FL-14 and FL-47, originated from bermudagrass. FL-04 was the only isolate identified as *M. poae* having ascospores that were fusoid and dark-pigmented. The mean length of 50 ascospores was 29 μm with a range of 25–33 μm and a standard deviation of 2 μm. Isolate FL-04 was obtained from a bentgrass golf green. *G. g.* var. *graminis* was identified from samples of St. Augustine-

Table 1. Fungi isolated from turfgrass plants symptomatic for root rot in Florida

Isolate	Location	Turfgrass common name	Identification
FL-04	Isleworth	Bentgrass	*Magnaporthe poae*
FL-07	Isleworth	Bentgrass	*Phialophora* sp.
FL-11	Riviera	Bermudagrass	*Phialophora* sp.
FL-14	Bocaire	Bermudagrass	*Gaeumannomyces* type; sterile
FL-15	Bocaire	Bermudagrass	*Phialophora* sp.
FL-18	Miami Lakes	Bermudagrass	*Phialophora* sp.
FL-19	Miami Lakes	Bermudagrass	*G. graminis* var. *graminis*
FL-23	Hollywood Lakes	Bermudagrass & ryegrass mix	*Phialophora* sp.
FL-24	Hollywood Lakes	Bermudagrass & ryegrass mix	*Phialophora* sp.
FL-25	Hollywood Lakes	Bermudagrass & ryegrass mix	*G. g.* var. *graminis*
FL-28	Sod Farm	Centipedegrass	*G. incrustans*
FL-32	Rolling Hills	Bermudagrass	*G. incrustans*
FL-36	Doral	Bermudagrass	*G. g.* var. *graminis*
FL-37	Doral	Bermudagrass	*Phialophora* sp.
FL-38	Sod Farm	St. Augustinegrass	*G. incrustans*
FL-39	Sod Farm	St. Augustinegrass	*G. g.* var. *graminis*
FL-45	Riomar	Bermudagrass	*G. incrustans*
FL-46	Riomar	Bermudagrass	*G. g.* var. *graminis*
FL-47	Riomar	Bermudagrass	*Gaeumannomyces* type; sterile
FL-49	Bent Pine	Bermudagrass	*G. incrustans*
FL-52	Miami	Bermudagrass	*G. incrustans*
FL-53	Miami	Bermudagrass	*G. incrustans*

grass, bermudagrass, and the bermudagrass-ryegrass mix. All isolates produced lobed hyphopodia (Fig. 2) and typical perithecia (Fig. 3). The mean length of a sample population of 250 ascospores of *G. g.* var. *graminis* was 86 μm with a range of 60–100 μm and a standard deviation of 6 μm (Fig. 4). *G. incrustans* was isolated from bermudagrass, St. Augustinegrass, and centipedegrass. Both mating types of *G. incrustans* were identified in this study. A sample population of 350 ascospores ranged from 35 to 55 μm with a mean of 45 μm and a standard deviation of 4 μm (Fig. 5). *Phialophora* spp. were associated with bentgrass, bermudagrass, and the bermudagrass-ryegrass mix (Fig. 6).

Although the isolates designated as *Phialophora* sp. have characteristics similar to other species of *Phialophora* from the roots of Gramineae (1,3,15), species epithets were not determined at this time. Schol-Schwarz (14) cautioned that considerable variation and lack of morphological differentiation exist between conidial states of *Phialophora*, particularly in closely related species. She also noted that variation in spore shape as well as conidiophore branching and size occurs within the same species depending on the age of the isolate, the culture medium used, and the amount of illumination. The possibility also exists that the *Phialophora* sp. found in this study are anamorphs of *G. incrustans* or *M. poae* that have lost the capacity to produce perithecia.

In general, the majority of *Gaeumannomyces* species but not of *Phialophora* species are pathogenic on grasses (2,21). Because hybrid bermudagrass is vegetatively propagated, the wheat seedling assay was used as a simple and general pathogenicity asssay. Check seedlings were healthy with only leaf tips exhibiting a slight chlorosis at the end of the incubation period. Roots and basal stems remained white. Seedlings inoculated with *P. radicicola* (JI3), *G. cylindrosporus* (ATCC 64420), and *P. graminicola* (ATCC 64414) were similar in appearance to the check seedlings. *G. g.* var. *avenae* (LL) caused a general leaf chlorosis but did not appear to affect the roots. Although the *Phialophora* isolates and the sterile fungal isolates from Florida resulted in slightly discolored and sometimes even superficially blackened roots with ectotrophic hyphae, leaves of the wheat seedlings were only slightly chlorotic. All the identified isolates of *M. poae, G. incrustans,* and *G. g.* var. *graminis* from Florida and the known isolates of the same species plus *G. g.* var. *tritici* resulted in severe chlorosis (>50%) and, with some isolates, necrosis of the wheat seedlings. These seedlings had rotted roots and basal stems with ectotrophic hyphae easily observed on the roots. Plant growth chamber and field pathogenicity studies are currently being conducted on bermudagrass and perennial ryegrass.

Although isolate FL-25 of *G. g.* var. *graminis* and isolates FL-23 and FL-24 of *Phialophora* were obtained from a bermudagrass-perennial ryegrass mix and not from one specific grass, fungi similar to *Gaeumannomyces* were obtained 1 yr later at the same location from both bermudagrass and ryegrass roots. No additional samples of St. Augustinegrass, centipedegrass, or bentgrass with root rot symptoms have been obtained since September 1988. An additional 39 isolates similar to *Gaeumannomyces* have been obtained from 13 bermudagrass golf courses and one baseball field with symptoms of bermudagrass decline (M. L. Elliott, *unpublished*). Of these 14 locations, 10 were new locations not represented in this study. St. Augustinegrass, bermudagrass, perennial ryegrass, bentgrass, and centipedegrass have not been listed as hosts of *G. g.* var. *graminis*, *G. incrustans*, or *M. poae* in Florida (4) and, to our knowledge, this is the first report of these organisms from turfgrass in Florida. St. Augustinegrass and centipedegrass have not previously been reported as hosts for *G. incrustans* (11).

Bermudagrass decline is known to occur throughout Florida (6). Both 'Tifdwarf' and 'Tifgreen' cultivars of hybrid bermudagrass, the most widely grown cultivars on golf course greens, are affected by bermudagrass decline. Presently, if fungi similar to *Gaeumannomyces* are isolated from the roots of a bermudagrass decline-symptomatic patch of bermudagrass, bermudagrass decline would be confirmed and appropriate control measures implemented. However, isolating these fungi can be difficult because they grow quite slowly in comparison with the many saprophytes that may also be present. Removing the leaf tissue and surface sterilizing the remaining plant tissue with a 1% silver nitrate solution is useful. SM-GGT3 medium is also helpful (10), but

Fig. 2. Lobed hyphopodia of *Gaeumannomyces graminis* var. *graminis* on wheat leaf sheaths. Scale bar = 30 μm.

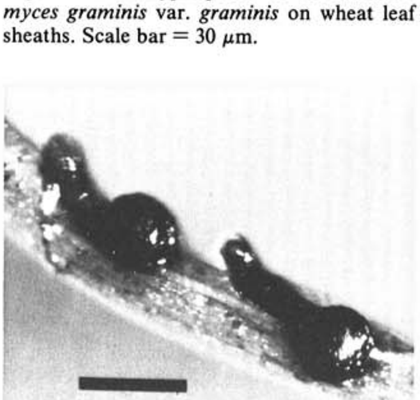

Fig. 3. Perithecia of *Gaeumannomyces graminis* var. *graminis* on wheat leaf. Scale bar = 200 μm.

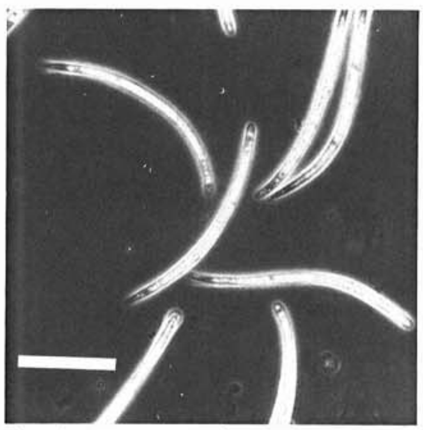

Fig. 4. Ascospores of *Gaeumannomyces graminis* var. *graminis.* Scale bar = 40 μm.

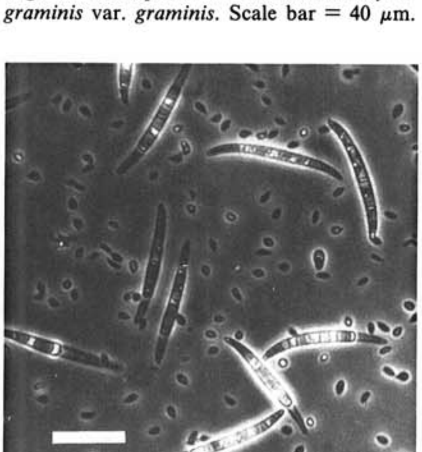

Fig. 5. Ascospores and microconidia of *Gaeumannomyces incrustans.* Scale bar = 25 μm.

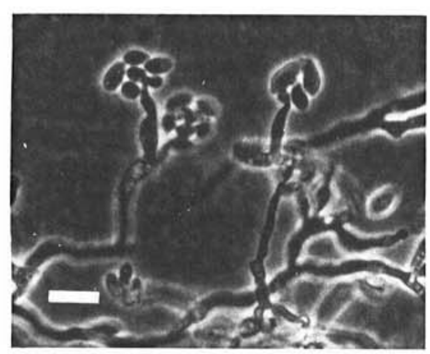

Fig. 6. Phialides and phialospores of an undesignated *Phialophora* sp. growing on one-fifth strength potato-dextrose agar. Scale bar = 8 μm.

it does not completely exclude or inhibit the saprophytes *Curvularia* and *Trichoderma* that are often associated with turfgrasses.

In a clinical situation, determining the exact identification of isolated fungi with characteristics of *Gaeumannomyces* is time consuming, difficult and, in most cases, impractical. Because *G. incrustans* and *M. poae* are heterothallic, the appropriate mating strains are required. In addition, 5–7 wk is required for production of perithecia. Thus far, perithecia of the *Gaeumannomyces* spp. and *M. poae* have not been observed in any field situation where bermudagrass decline was diagnosed. Hyphopodia of *G. g.* var. *graminis* are not commonly observed on clinical samples. However, these structures can be easily observed in culture with the wheat seedling assay.

With the completion of this study, *G. incrustans*, *G. g.* var. *graminis,* and *Phialophora* spp., including *P. radicicola* (7), have been isolated from bermudagrass with bermudagrass decline symptoms. In some locations, more than one of these organisms was identified. This, of course, leads to more questions concerning the etiology of the disease. As indicated previously, the pathogenicity of the different fungal isolates is being determined. The majority of golf course greens in Florida are hybrid bermudagrass. However, a common practice during the winter months is the overseeding of greens with cool-season turfgrasses such as perennial ryegrass or creeping bentgrass. Therefore, it is plausible that the fungal pathogens that cause bermudagrass decline could also infect these turfgrass hosts. It is also possible that bermudagrass decline and other turfgrass root rot diseases in Florida are caused by a complex of *Gaeumannomyces* species or fungal species with similar characteristics. In addition, the role of nonpathogenic *Gaeumannomyces* or *Phialophora* species in these diseases needs to be determined as it may be possible to develop these organisms as biological control agents (23).

LITERATURE CITED

1. Cain, R. F. 1952. Studies of Fungi Imperfecti. I. *Phialophora*. Can. J. Bot. 30:338-343.
2. Deacon, J. W. 1981. Ecological relationships with other fungi: Competitors and hyperparasites. Pages 75-101 in: Biology and Control of Take-all. M. J. C. Asher and P. J. Shipton, eds. Academic Press, London.
3. Deacon, J. W., and Scott, D. B. 1983. *Phialophora zeicola* sp. nov., and its role in the root rot-stalk rot complex of maize. Trans. Br. Mycol. Soc. 81:247-262.
4. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, MN. 1252 pp.
5. Freeman, T. E., and Augustin, B. J. 1983. Bermudagrass decline-progress report. Pages 7-11 in: Report of Turfgrass Research Supported by the Florida Turfgrass Association 1982-83. University of Florida.
6. Freeman, T. E., and Augustin, B. J. 1984. Bermudagrass Decline. Plant Pathology Fact Sheet No. 31. University of Florida. 4 pp.
7. Freeman, T. E., and Augustin, B. J. 1986. Association of *Phialophora radicicola* Cain with declining bermudagrass in Florida. (Abstr.) Phytopathology 76:1057.
8. Jackson, N., and Landschoot, P. J. 1986. *Gaeumannomyces cylindrosporus* associated with diseased turfgrass in Rhode Island. (Abstr.) Phytopathology 76:654.
9. Jong, S. C., and Gantt, M. J. 1987. American Type Culture Collection Catalogue of Fungi/Yeasts. 17th ed. ATCC, Rockville, MD. 532 pp.
10. Juhnke, M. E., Mathre, D. E., and Sands, D. C. 1983. A selective medium for *Gaeumannomyces graminis* var. *tritici*. Plant Dis. 68:233-236.
11. Landschoot, P. J., and Jackson, N. 1989. *Gaeumannomyces incrustans* sp. nov., a root-infecting hyphopodiate fungus from grass roots in the United States. Mycol. Res. 93:55-58.
12. Landschoot, P. J., and Jackson, N. 1989. *Magnaporthe poae* sp. nov., a hyphopodiate fungus with a *Phialophora* anamorph, from grass roots in the United States. Mycol. Res. 93:59-62.
13. McCarty, L. B., and Lucas, L. T. 1989. *Gaeumannomyces graminis* associated with spring dead spot of bermudagrass in the southeastern United States. Plant Dis. 73:659-661.
14. Schol-Schwarz, M. B. 1970. Revision of the genus *Phialophora* (Moniliales). Persoonia 6:59-94.
15. Sivansithamparam, K. 1975. *Phialophora* and *Phialophora*-like fungi occurring in the root region of wheat. Aust. J. Bot. 23:193-212.
16. Smiley, R. W. 1983. Compendium of Turfgrass Diseases. American Phytopathological Society, St. Paul, MN. 102 pp.
17. Smiley, R. W. 1987. The etiologic dilemma concerning patch diseases of bluegrass turfs. Plant Dis. 71:774-781.
18. Smiley, R. W., Kane, R. T., and Craven-Fowler, M. C. 1985. Identification of *Gaeumannomyces*-like fungi associated with patch diseases of turfgrasses in North America. Pages 609-618 in: Proc. Int. Turfgrass Res. Conf., 5th (Avignon). F. Lemaire, ed. INRA Publications, Versailles, France.
19. Speakman, J. B. 1982. A simple, reliable method of producing perithecia of *Gaeumannomyces graminis* var. *tritici* and its application to isolates of *Phialophora* spp. Trans. Br. Mycol. Soc. 79:350-353.
20. Tisserat, N. A., Pair, J. C., and Nus, A. 1989. *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass in Kansas. Plant Dis. 73:933-937.
21. Walker, J. 1981. Taxonomy of take-all fungi and related genera and species. Pages 15-74 in: Biology and Control of Take-all. M. J. C. Asher and P. J. Shipton, eds. Academic Press, London.
22. Walker, J., and Smith, A. M. 1972. *Leptosphaeria narmari* and *L. korrae* spp. nov., two long-spored pathogens of grasses in Australia. Trans. Br. Mycol. Soc. 58:459-466.
23. Wong, P. T. W., and Siviour, T. R. 1979. Control of Ophiobolus patch in Agrostis turf using avirulent fungi and take-all suppressive soils in pot experiments. Ann. Appl. Biol. 92:191-197.
24. Worf, G. L., Stewart, J. S., and Avenius, R. C. 1986. Necrotic ring spot disease of turfgrass in Wisconsin. Plant Dis. 70:453-458.