Title of Thesis: Growth, Pseudothecia Production, and Ascospore Germination of *Ophiostoma agrostis* and Cultivar Susceptibility and Geographic Distribution of Bentgrass Dead Spot

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ABSTRACT

Title of Thesis: GROWTH, PSEUODOTHECIA PRODUCTION, AND ASCOSPORE GERMINATION OF OPHIOSPHAERELLA AGROSTIS AND CULTIVAR SUSCEPTIBILITY AND GEOGRAPHIC DISTRIBUTION OF BENTGRASS DEAD SPOT

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Bentgrass dead spot (BDS) is a newly described disease of creeping bentgrass (Agrostis palustris) putting greens caused by a previously undescribed fungal species, Ophiosphaerella agrostis. The prominent use of creeping bentgrass on golf courses in the U.S. makes this disease a potentially serious problem. There is no information regarding the biology of O. agrostis or the geographic distribution of BDS. Studies were conducted to determine the growth rate of isolates over a range of temperatures and to study the ability of the pathogen to produce pseudothecia and ascospores. The optimum temperature for growth of O. agrostis occurred equally at 25 and 30 °C. Studies showed
that BDS reactivated within 2 to 3 weeks in winter-dormant diseased plants when temperatures were sustained within the optimum growth range for *O. agrostis*. To study factors affecting ascospore germination, a method had to be devised to produce spore-bearing pseudothecia. Pseudothecia with mature ascospores were produced by infesting a tall fescue (*Festuca arundinacae*) seed/wheat (*Triticum aestivum*) bran mix with the pathogen and incubating it in constant light for 7 days. Ascospores obtained from pseudothecia produced *in vitro* began to germinate within 2 hours. A large percentage of ascospores germinated in light in the presence of bentgrass leaves or roots after 4 hours of incubation. Ascospores incubated 12 hours under constant light generally had greater percentages of germination, regardless of the presence or absence of plant tissues. Over 68% of the ascospores, however, germinated in darkness or light and in the absence of tissue within 18 hours of incubation in water. Ascospore, pseudothecia and asci measurements for *O. agrostis* are presented. Information on the geographic distribution and bentgrass cultivar susceptibility to the disease and cultural factors associated with BDS also are discussed.
GROWTH, PSEUDOTHECIA PRODUCTION, AND ASCOSPORE GERMINATION

OF OPHIOSPHAERELLA AGROSTIS AND CULTIVAR SUSCEPTIBILITY AND

GEOGRAPHIC DISTRIBUTION OF BENTGRASS DEAD SPOT

by

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DEDICATION

To my parents, John and Elizabeth Kaminski, and my sister, Kristin Murphy
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I. LITERATURE REVIEW

Creeping bentgrass (*Agrostis palustris* Huds; synonym *A. stolonifera* L.) is a commonly used turfgrass species on golf course putting greens throughout the United States because of its high putting quality and ability to withstand low mowing heights and intense cultural practices. Demoeden et al. (1999) discovered a new disease of creeping bentgrass incited by an unidentified species of *Ophiosphaerella*. Through morphological and molecular study, it was shown that the pathogen constituted a new species, *Ophiosphaerella agrostis* Demoeden, M. P. S. Câmara, N. R. O’Neill, van Berkum et M. E. Palm (Câmara et al., 2000). The disease was named bentgrass dead spot (BDS) (Demoeden, 2000).

Spegazzini (1909) described *O. graminicola* Speg., the type species of the genus, which he found to be a pathogen of sprangletop (*Leptochloa virgata* (L.) P. Beauv.) in Argentina. The type species is characterized as having pleosporaceous (many spored) ascocarps with bitunicate asci containing brown scolecospores (filiform spores) lying parallel, or more often slightly twisted, near their middle; and having no swollen cells, gelatinous sheaths, or appendages (Walker, 1980). Other species of *Ophiosphaerella* occur on *Gramineae* or *Cyperaceae* and have thin-walled pseudothecia (20-40 μm) made up of radially flattened cells (Walker, 1980). Species of *Ophiosphaerella* produce long, bitunicate asci containing eight, pale-brown, filiform, multiseptate ascospores. Ascospores are produced in pseudothecia and range in length from 100-200 x 1.5-3 μm (Walker, 1980). Three other turfgrass pathogens in the genus *Ophiosphaerella* have been described. *Ophiosphaerella herpotricha* J. C. Walker, *O. korrae* Walker and Smith
(formerly *Leptosphaeria korrae*), and *O. narmari* Wetzel, Hulbert and Tisserat (formerly *Leptosphaeria narmari*) were determined to cause spring dead spot of bermudagrass (*Cynodon dactylon* (L.) Pers) (Crahay et al., 1988; Endo et al., 1985; Smith 1965; Tisserat et al., 1989; Walker and Smith, 1972; and Wetzel et al., 1999). *Ophiostoma herpotricha* also causes spring dead spot in buffalograss (*Buchloe dactyloides* (Nutt.) Engelm) (Tisserat et al. 1999). Necrotic ring spot of creeping red fescue (*Festuca rubra* var. *rubra*), and Kentucky (*Poa pratensis* L.) and annual (*Poa annua* L.) bluegrasses is incited by *O. korrae* (Dernoeden et al., 1995; Landschoot, 1996; and Worf et al., 1986).

All of the aforementioned *Ophiostoma* species, except for *O. graminicola*, are turfgrass root pathogens. The three root pathogens are characterized by the production of darkly pigmented hyphae on roots, and none have been reported to infect creeping bentgrass.

On close-mown creeping bentgrass grown on golf course putting greens, the disease appears initially as small, reddish-brown spots approximately 1.0 cm in diameter and increasing to about 8.0 cm in diameter (Dernoeden et al., 1999). During early stages of disease development, the spots are reddish-brown or copper-colored and mimic improperly repaired ball-mark injury. As the disease progresses, grass in the center of the spots becomes tan, while leaves in the outer edge appear reddish-brown. Patches may be localized or distributed throughout the putting green, but the spots and patches generally do not coalesce. Sometimes the spots form depressions or pits in the putting surface. Spots recover very slowly, as stolon growth into dead patches appears restrained or inhibited. Foliar mycelium is not observed in the field, but when diseased plants are incubated under high humidity for 3 to 5 days a white to pale pink foliar mycelium may
develop. Numerous pseudothecia can be found on necrotic leaf, sheath, and stolon tissues. ITS sequencing data demonstrated that the fungus belonged in the genus *Ophiosphaerella*, and that all isolates from creeping bentgrass were genetically distinguishable from *O. herpotricha*, *O. korrae*, and *O. narmari* (Câmara et al., 2000).

The fungus therefore represented a new species, which was named *O. agrostis*.

In 1998, isolates of *O. agrostis* were obtained from creeping bentgrass golf greens in Maryland (n=6), Virginia (n=1), Ohio (n=1), Pennsylvania (n=1), and Illinois (n=1). The pathogen was isolated from leaves, stems, and roots, and single-spore isolates were obtained from pseudothecia. When grown on potato dextrose agar nearly all isolates were identical and the colonies appeared rose quartz or pinkish brown, but turned gray as they aged (Demoeden et al., 1999). An exception was an isolate from Ohio, which appeared gray-green rather than rose quartz. In pathogenicity tests, pseudothecia developed on inoculum and sometimes on necrotic tissue within 20 days of inoculation (Demoeden et al., 1999).

A review of the literature has revealed no other reports of *Ophiosphaerella* spp. in association with creeping bentgrass. The prominent use of creeping bentgrass on golf greens makes this disease potentially a serious problem as it disrupts the quality and playability of the putting surface. In extreme cases, putting greens are rendered nearly unplayable and may be closed temporarily. There is little or no information regarding the biology of the pathogen or the epidemiology of the disease. The goal of these studies was to determine some basic biological information about the pathogen. Hence, the primary objectives of this research were to: 1) to determine the cardinal temperatures for growth of *O. agrostis* and to describe colony morphology at each temperature; 2) to assess
reactivation of the disease from winter-dormant, field infected samples at various temperatures; 3) to develop a technique to produce pseudothecia and ascospores in *vitro* for further research studies; and 4) to evaluate factors that promote ascospore germination. Secondary objectives were to determine the susceptibility of various field-grown bentgrass cultivars, and to evaluate the sensitivity of *O. agrostis* to fungicides *in vitro*. Additional information on size of pseudothecia, asci and ascospores, as well as observations of BDS outbreaks on golf courses in several states was obtained. Furthermore, a survey was sent to golf course superintendents at clubs where BDS was confirmed. The survey was designed to obtain additional field information relating to the influence of cultural practices on bentgrass dead spot.