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Identification, Distribution, and Aggressiveness of Spring Dead Spot Pathogens of Bermudagrass

Understanding what organisms cause the disease and their geographic distribution.

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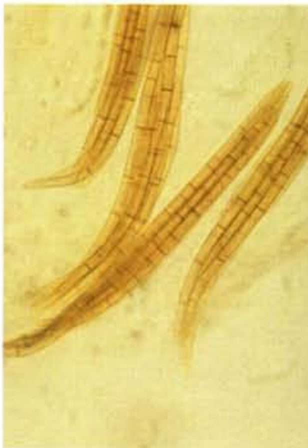
Spring dead spot (SDS) is a destructive disease of common bermudagrass (*Cynodon dactylon*) and bermudagrass hybrids (*C. dactylon X C. transvaalensis*) throughout the northern range of its adaptation in the United States. It may occur on bermudagrass fairways and putting surfaces of all ages, although it typically appears 3-4 years after the turf has been established. The disease results in the formation of circular or arc-shaped patches of dead turf in early spring as bermudagrass breaks winter dormancy.

The dead patches, which are slightly depressed and straw-colored, may range in size from several inches to several feet in diameter. Roots and stolons of affected plants are dark brown to black and are severely rotted. In some regions, such as Australia and California, patches may be visible on slowly growing, but not dormant bermudagrass following wet, cold weather. Bermudagrass slowly fills in the bare areas during the growing season, and by late summer there may be little or no evidence of the disease. Dead patches reappear the following spring in the same locations.

IDENTIFICATION OF SDS PATHOGENS

Identifying the cause(s) of SDS has been an elusive and frustrating process. We now know that three closely related root-rotting fungi called *Ophiosphaerella korrae* (also called *Leptosphaeria korrae*), *O. herpotricha*, and *O. narmari* cause SDS. It is important to determine which *Ophiosphaerella* species is the cause of SDS at a specific location because these pathogens may differ in seasonal development, sensitivity to fungicides, and aggressiveness to individual bermudagrass cultivars.



Ophiosphaerella korrae ascospores are initially grouped together in a sac-like structure called an ascus. Individual ascospores are light brown, long, and cylindrical with multiple septations. The three *Ophiosphaerella* species that cause spring dead spot can be differentiated by spore length.

Unfortunately, these fungi are not easily distinguished in the field because they cause identical symptoms. They can be differentiated in the laboratory by spore (ascospore) length, with those of *O. herpotricha* being the longest, *O. korrae* intermediate, and *O. narmari* the shortest. However, these fungi seldom produce fruiting structures (called pseudothecia) containing ascospores on diseased bermudagrass stolons and crowns in the field, and only certain isolates can be induced to produce them in culture. Therefore, spore morphology cannot routinely be used to distinguish these fungi.

To facilitate rapid identification, we employed a polymerase chain reaction technique (PCR) to selectively amplify small segments of DNA from the three

Ninety days after inoculation with *Ophiosphaerella korrae* (middle) and *O. herpotricha* (right), the Arizona common bermudagrass roots showed discoloration and rotting. Roots on the left were not inoculated.

Ophiosphaerella species. By using species-specific oligonucleotide primers, DNA can be differentially amplified following DNA extraction from fungal cultures or diseased tissue. For example, if a PCR reaction is run using primers specific for *O. narmari*, only DNA of this fungus and not others, including other *Ophiosphaerella* species, will be amplified. Thus, the use of PCR primers specific to the three SDS pathogens can be used to determine which fungus is present in a diseased root. Amplified DNA can be detected in several ways, but commonly it is stained, loaded into an agarose gel placed in a buffer solution, and then subjected to an electrical current. As the amplified DNA migrates through the gel, it can be visualized using ultraviolet light.

DISTRIBUTION OF SDS PATHOGENS IN THE U.S.

One of our objectives was to determine the geographic distribution of SDS pathogens. The fungus *O. narmari* had previously been reported to be the primary cause of SDS in Australia, but it had not been found in North America. *Ophiosphaerella korrae* also was reported to be widespread in Australia and was documented as the cause of SDS in Maryland and California, whereas *O. herpotricha* was recovered from SDS patches in Kansas and Oklahoma. However, these early studies provided an incomplete picture of the geographic distribution of SDS pathogens in the United States because they were based on a limited number of samples collected from just a few geographic locations.

Since 1994 we have intensively sampled golf course fairways in several states and also collected a small number of isolates from widely dispersed geographic locations throughout much of the range of where SDS occurs in the United States. Our survey indicates that there are regional differences in the distribution of SDS pathogens. The majority of isolates we collected in Kansas and Oklahoma were *O. herpotricha*. This confirms that this fungus is the primary cause of SDS in the southern Great Plains. The fungus *O. korrae* was isolated less frequently in this region, with almost all of these isolates collected from a single golf course fairway near Afton, Oklahoma. Although only a few *O. narmari* isolates were recovered, they represented the first report of this fungus in North America. *Ophiosphaerella narmari* has since been found in other regions of the United States.

Bermudagrass selections are screened for spring dead spot resistance in replicated field trials in Stillwater, Oklahoma. Each plot was inoculated with *Ophiosphaerella herpotricha, O. korrae,* and *O. narmari.* Several selections showed good resistance to the disease.

In contrast, *O. korrae* was the only SDS pathogen in samples collected at sites in Mississippi, Alabama, South Carolina, Tennessee, and Virginia, and it was dominant in North Carolina. Although the number of locations sampled in each state was small, these results suggest that *O. korrae* is the most widely distributed SDS pathogen in the southern United States. Both *O. herpotricha* and *O. korrae* were collected in Kentucky, with their isolation frequency dependent on the location that was sampled.

The distribution of SDS pathogens in the western United States is less clear, although both *O. korrae* and *O. narmari* have been isolated from samples collected near Los Angeles, California. Further sampling in Arizona, Nevada, California, New Mexico, and western Texas is needed.

The uneven distribution of SDS pathogens in the United States may reflect regional differences in native ranges of these fungi. While *O. herpotricha* has been isolated from buffalograss lawns exhibiting SDS symptoms, it has not been isolated from this or other native grasses in natural prairie stands. Therefore, we are still uncertain whether this fungus is native to the Great Plains.

It seems unlikely that *O. korrae* is native to North America since bermudagrass and Kentucky bluegrass (the fungus causes necrotic ringspot on this host), the primary hosts for this fungus, are exotic grasses. An alternative explanation is that *O. korrae* was introduced and dispersed on infected bermudagrass roots and stolons. Thus, *O. korrae* might have initially infected an improved bermudagrass cultivar adapted to a specific geographic region and then was dispersed via contaminated sod/sprigs across a wide geographic area.

A study by Wetzel et al. supports this hypothesis. They found that *O. korrae* isolates collected from several southern states were genetically similar based on DNA fingerprinting techniques. These isolates differed substantially from *O. korrae* isolates collected from Kentucky bluegrass and bermudagrass in more northern regions of the United States. These northern isolates also were genetically similar. We believe that *O. korrae* may have been introduced into North America, with one or a few initial introductions occurring on bermudagrass in the southern United States and another introduction in more northern regions on Kentucky bluegrass. Further genetic studies are needed to confirm this hypothesis. Nevertheless, these results are strong evidence that this pathogen has been moved from one location to another on infected stolons or roots.

AGGRESSIVENESS OF SDS PATHOGENS

We were also interested to see if there were differences in SDS severity following inoculations with *O. herpotricha, O. korrae*, and *O. narmari*. A two-year-old stand of Midlawn bermudagrass at the Kansas State University John Pair Research Center, Wichita, Kansas, with no previous history of SDS, was inoculated in September 1997 with the three SDS pathogens. All 14 sites inoculated with *O. herpotricha* developed symptoms in 1999. The patches reappeared and had expanded in diameter in May 2000.

In contrast, only 10 of 14 sites inoculated with *O. korrae* developed symptoms in 1999, and of those only 7 appeared the following year. Patch diameters associated with *O. korrae* were significantly smaller than those caused by *O. herpotricha*. Unfortunately, this study did not include any *O. korrae* isolates from the southern region. Only one of the sites inoculated with *O. narmari* developed SDS, and the patch diameter of this spot was small.

These preliminary results indicate there are differences in aggressiveness of SDS pathogens to the bermudagrass

Table 1
Identification of *Ophiosphaerella* species isolated from bermudagrass cores collected in various states.

Location	Collection Date	Number of Isolates: *O. herpotricha*	*O. korrae*	*O. narmari*
Alabama, Vestavia Hills	1999	0	20	0
Arkansas, sites unknown	1994	0	6	0
California, Los Angeles	1983, 1999	0	2	4
Georgia, sites unknown	1994-1996	0	3	0
Kansas, 20 sites	1984-2000	55	0	1
Kansas, Independence	1994	71	9	0
Kentucky, Mayfield	1998	2	13	0
Kentucky, Henderson	1998	14	5	1
Kentucky, Paducah	1998	0	17	0
Mississippi, Starkville	1999	0	18	2
Missouri, Dunklin County	1996	2	0	0
North Carolina, 12 sites	1999-2000	0	63	1
North Carolina, Raleigh	1999-2000	12	5	0
Oklahoma, Afton	1994-1996	201	38	22
Oklahoma, Jenks	1994	173	0	0
South Carolina, 8 sites	1999-2000	0	16	0
Tennessee, Knoxville	1999	0	15	0
Texas, Dallas	1996	3	0	0
Virginia, Virginia Beach	1999	0	20	0
Virginia, Charlottesville	1999	0	5	0
Virginia, site unknown	1999	0	4	0
West Virginia	1999-2000	0	11	0

Table 2
Development of spring dead spot on Midlawn bermudagrass following inoculation with *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari* in Wichita, Kansas.

	1999		2000	
Fungal Species	**Number of inoculation sites with dead spots**[X]	**Patch area (cm^2)**[Y]	**Number of inoculation sites with dead spots**	**Patch area (cm^2)**
Ophiosphaerella herpotricha	14	374a	14	1120a
Ophiosphaerella korrae	10	78b	7	264b
Ophiosphaerella narmari	1	46c	1	79c
Sterile oats	0	—	0	—

[X]Number of 14 inoculation sites for each species in which spring dead spot symptoms developed. Plots were inoculated in September 1997 with three isolates of each species and were rated in May of 1999 and 2000.

[Y]Average patch area for those inoculation sites in which spring dead spot symptoms developed. Patch diameters not followed by the same letter are significantly different ($P<0.05$) by Fisher's LSD test.

hybrid Midlawn grown under Kansas conditions. Further studies are needed to determine if this pattern holds true for other cultivars and in other locations. If so, it indicates that the pathogen needs to be considered when screening bermudagrass cultivars for regional differences in resistance to SDS.

SCREENING BERMUDAGRASS FOR RESISTANCE TO SPRING DEAD SPOT

Only a limited amount of success has been achieved in controlling SDS by cultural means. Control by fungicide applications has proved to be expensive and inconsistent. A promising approach to SDS control is development and deployment of resistant bermudagrass cultivars. Oklahoma State University has an active bermudagrass breeding program to develop vegetatively and seed-propagated bermudagrasses with increased resistance to SDS. Currently the screening process involves inoculating established bermudagrass in replicated field plots.

SDS symptoms do not develop with consistency until two years after inoculation, and measurements need to be continued for several growing seasons to insure consistent ratings. Thus, screening is an expensive and slow process. Nevertheless, this method has been used successfully to identify several bermudagrass selections with increased resistance to SDS. They include the vegetative selections Midlawn and Patriot and the seeded varieties Yukon and Riviera. These varieties, while not immune to SDS, consistently exhibit smaller dead spots and recover more quickly from the disease than susceptible varieties.

We are attempting to develop a more rapid greenhouse and laboratory method for screening large numbers of bermudagrass selections for SDS resistance. Previous attempts to correlate root discoloration and rotting observed in the laboratory to resistance ratings in the field were unsuccessful. In these studies, bermudagrass was not subjected to a freezing treatment, as would occur in the field, and no shoot mortality transpired. Hence, exposure to cold temperatures appears necessary for complete expression of SDS symptoms. Nus and Shashikumar found that inoculation with *O. herpotricha* and *O. korrae* reduced the ability of bermudagrass to withstand freezing temperatures. We are in the process of refining procedures for exposing inoculated bermudagrass to freezing temperatures to optimize symptom development in the laboratory.

SUMMARY

We now know that at least three closely related root-rotting fungi are responsible for SDS, they have regional distributions in the United States, and they vary in their aggressiveness to bermudagrass. We have also identified bermudagrass selections with increased disease resistance to *O. herpotricha*. Nevertheless, SDS remains a serious and largely uncontrolled disease of bermudagrass on many golf courses throughout the United States. Control of this disease will require a more comprehensive understanding of pathogen biology, disease epidemiology, and host resistance.

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Note: Further information on this topic may be found at: http://usgatero.msu.edu/v02/n20.pdf.

REFERENCES

1. Nus, J. L., and K. Shashikumar. 1993. Fungi associated with spring dead spot reduces freezing resistance in bermudagrass. *HortScience* 31:182–184.

2. Wetzel, H. C., III. 2001. Evaluation of fungicides for control of spring dead spot of bermudagrass, 2000. *Fungicide and Nematicide Reports* 56:T3.

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