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Spring dead spot (SDS) of bermudagrass is caused by three closely related fungi:*Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*. These fungi are difficult to distinguish by morphological characteristics in culture, but researchers at Kansas State University and Oklahoma State University have shown these pathogens can be identified by DNA-fingerprinting techniques and are currently developing greenhouse and laboratory-based assays to rapidly screen large numbers of bermudagrass selections for SDS resistance.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 225 projects at a cost of $21 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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

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SUMMARY

Spring dead spot is a destructive disease of common bermudagrass (*Cynodon dactylon*) and bermudagrass hybrids (*C. dactylon* X *C. transvalensis*) throughout the northern range of its adaptation in the United States. For the past few years, researchers at Kansas State University and Oklahoma State University have investigated this serious turfgrass disease. Their findings include:

- Spring dead spot (SDS) of bermudagrass is caused by three closely related fungi: *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*. These fungi are difficult to distinguish by morphological characteristics in culture, but can be identified by selective DNA amplification using species-specific primers in a polymerase chain reaction technique.
- All three SDS pathogens are present in the United States, although there are regional differences in their distribution. *O. herpotricha* is the most common cause of SDS in Kansas and Oklahoma, whereas *O. korrae* appears to be the most prevalent pathogen in the southern United States. *O. narmari* is not common in the eastern or central United States, but may be more widely distributed in California.
- Preliminary data suggest that there are differences in aggressiveness among SDS pathogens to bermudagrass with *O. herpotricha* the most aggressive and *O. narmari* the least. However, further tests in other regions and including additional cultivars are needed to confirm these results.
- Although no bermudagrass cultivars are immune to SDS, the breeding program at Oklahoma State University has identified several selections, including the seeded varieties 'Yukon' and 'Riviera' and the vegetative types 'Midlawn' and 'Patriot' that have increased resistance to the disease.
- Researchers at Kansas State University and Oklahoma State University are currently developing greenhouse and laboratory-based assays to rapidly screen large numbers of bermudagrass selections for SDS resistance.

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Spring dead spot is a destructive disease of common bermudagrass (*Cynodon dactylon*) and bermudagrass hybrids (*C. dactylon* X *C. transvalensis*) 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 three to four 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 (Figure 1).

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 (Figure 2). In some regions, such as Australia and California, patches may be visible on slow growing, but not dormant bermudagrass following wet, cold weather. Bermudagrass slowly fills in the bare areas during the growing season and by late summer,

Figure 1. Spring dead spot symptoms develop in spring as bermudagrass breaks winter dormancy. Most of the roots and stolons within the patch may be killed on susceptible varieties. Symptoms caused by *Ophiosphaerella korrae*, *O. herpotricha* and *O. narmari* may be identical.

Figure 2. Root discoloration and rotting of Arizona common bermudagrass 90 days after inoculation with *Ophiosphaerella korrae* (middle) and *O. herpotricha* (right). Roots on the left were not inoculated.

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 (2, 3, 8, 11,13). 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.

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 (Figure 3). However, these fungi seldom produce fruiting structures (called pseudothecia) containing ascospores on diseased bermudagrass stolons and crowns in nature, 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 *Ophiosphaerella* species (7, 10, 16). 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

Figure 3. Ascospores of *Ophiosphaerella korrae* 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.

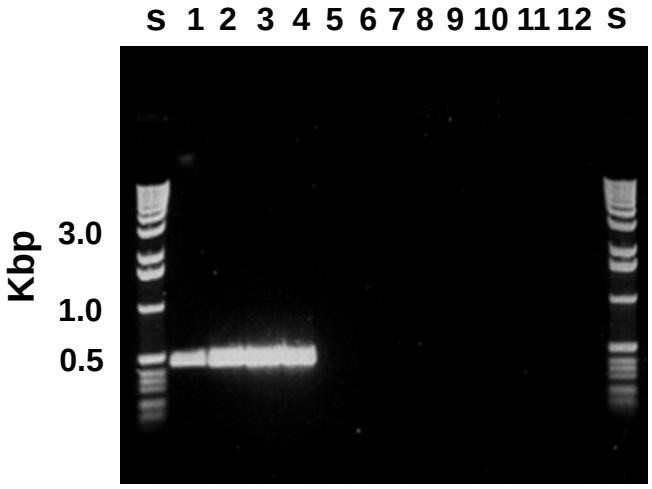

Figure 4. Photograph of a thin gel used to separate and visualize DNA products of *O. herpotricha* following amplification with DNA primers specific to this fungus. The multiple bands in the two standard lanes (labeled 's') contain DNA of different sizes that are used as references to determine the size of amplified products (Lanes 1-12). The bright bands in lanes 1-4 illustrate DNA amplification from *O. herpotricha*-infected bermudagrass root samples. In contrast, no amplification, and subsequently no bands, appears in non-infected bermudagrass roots (lane 5) or roots infected with *O. korrae* (lanes 6-9) or *O. narmari* (lanes 10-12).

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 (Figure 4).

Distribution of SDS Pathogens in the United States

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 (8, 13), but it had not been found in North America. *Ophiosphaerella korrae* also was reported to be widespread in Australia (13) and was documented as the cause of SDS in Maryland and California (2, 3) whereas *O. herpotricha* was recovered from SDS patches in Kansas and Oklahoma (11). 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 (Table 1). 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 (Table 1)

In contrast, *O. korrae* was the only SDS pathogen in samples collected at sites in Mississippi, Alabama, South Carolina, Tennessee, and Virginia, and 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, California, Nevada, 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 established in areas previously grassed to bermudagrass (12), 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

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, Unknown site	1999	0	4	0
West Virginia	1999-2000	0	11	0

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

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. (17) supports this hypothesis. They found that *O. korrae* isolates collected from several southern states were genetically similar based on DNA fingerprinting techniques (amplified fragment length polymorphisms). 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 were also 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 if there were differences in SDS severity following inoculations with *O. herpotricha*, *O. korrae*, and *O. narmari*. A

Fungal species	1999		2000	
	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.

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

two-year-old stand of 'Midlawn' bermudagrass at the KSU John Pair Research Center, Wichita, KS, 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 (Table 2). 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 seven 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 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 (9). Control by fungicide applications has proved to be expensive and inconsistent (14, 15). 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 seed- and vegetatively-propagated bermudagrasses with increased resistance to SDS. Currently the screening process involves inoculating established bermudagrass in replicated field plots (Figure 5). SDS symptoms do not develop with consistency until two years after inoculation (1, 11) and meas-

Figure 5. Bermudagrass selections are screened for spring dead spot resistance in replicated field trials in Stillwater, Oklahoma. Each plot was inoculated at three equally spaced sites (in a line) with *Ophiosphaerella herpotricha* (designated Oh), *O. korrae* (Ok), and *O. narmari* (Ln). Note that on this susceptible selection *O. herpotricha*, but not *O. narmari* and *O. korrae* caused patch development.

urements 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 (4, 5). 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 (1, 2, 11). 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 (6) 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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References

1. Baird, J. H., D. L. Martin, C. M. Taliaferro, M. E. Payton, and N. A. Tisserat. 1998. Bermudagrass resistance to spring dead spot caused by *Ophiosphaerella herpotricha*. *Plant Disease* 82:771-774. (TGIF Record 76975)

2. Crahay, J. N., P.H. Dernoeden, and N.R. O'Neill. 1988. Growth and pathogenicity of *Leptosphaeria korrae* in bermudagrass. *Plant Disease* 72:945-949. (TGIF Record 13541)

3. Endo, R.M., H.D. Ohr, and E. M. Krausman. 1985. *Leptosphaeria korrae*, a cause of spring dead spot disease of bermudagrass in California. *Plant Disease* 69:235-237. (TGIF Record 76982)

4. Martin, D. L., G. E. Bell, C. M. Taliaferro, N. A. Tisserat, J. H. Baird, D. D. Dobson, R. M. Kuzmic, and J. A. Anderson. 2001. Spring dead spot resistance of inter-specific hybrid bermudagrasses. *International Turfgrass Research Journal* 9:685-688. (TGIF Record 74263)

5. Martin, D. L., G. E. Bell. J. H. Baird, C. M. Taliaferrro, N. A. Tisserat, R. M. Kuzmic, D. D. Dobson, and J. A. Anderson. 2001. Spring dead spot resistance and quality of seeded bermudagrasses under different mowing heights. *Crop Science* 41:451-456. (TGIF Record 73367)

6. Nus, J. L., and K. Shashikumar. 1993. Fungi associated with spring dead spot reduces freezing resistance in bermudagrass. *HortScience* 28:306-307. (TGIF Record 27600)

7. O'Gorman, D., B. Xue, T. Hsiang, and P. H. Goodwin. 1994. Detection of *Leptosphaeria korrae* with polymerase chain reaction and primers from the ribosomal internal transcribed spacers. *Canadian Journal of Botany* 72:342-346. (TGIF Record 30638)

8. Smith, A.M. 1971. Spring dead spot of couch grass turf in New South Wales. *Sports Turf Research Institute Journal* 47:54-59. (TGIF Record 2879)

9. Tisserat, N. and J. Fry. 1997. Cultural practices to reduce spring dead spot (*Ophiosphaerella herpotricha*) severity in *Cynodon dactylon*. *International Turfgrass Research Journal* 8:931-936. (TGIF Record 56094)

10. Tisserat, N.A., S.H. Hulbert, and K. M. Sauer. 1994. Selective amplification of rDNA internal transcribed spacer regions to detect *Ophiosphaerella korrae* and *O. herpotricha*. *Phytopathology* 84:478-482. (TGIF Record 91980)

11. Tisserat, N.A., J. C. Pair, and A. Nus. 1989. *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass in Kansas. *Plant Disease* 73:933-937. (TGIF Record 16493)

12. Tisserat, N., H. Wetzel III, J. Fry, and D. L. Martin. 1999. Spring dead spot of buffalograss caused by *Ophiosphaerella herpotricha* in Kansas and Oklahoma. *Plant Disease* 83:199. (TGIF Record 56886)

13. Walker, J., and A. M. Smith. 1972. *Leptosphaeria narmari* and *L. korrae spp*. nov., two long-spored pathogens of grasses in Australia. *Transactions of the British Mycological Society* 58:459-466. (TGIF Record 76985)

14. Walker, N. R, K. E. Jackson, D. L. Martin. 2002. Evaluation of fungicides for the management of spring dead spot of common bermudagrass turf, 2000-2001. *Fungicide and Nematicide Reports* 57:T12. (TGIF Record 91215)

15. Wetzel, H. C., III. 2001. Evaluation of fungicides for control of spring dead spot of bermuda-

grass, 2000. *Fungicide and Nematicide Reports* 56:T3. (TGIF Record 91040)

16. Wetzel, H.C., III, S.H. Hulbert, and N.A. Tisserat. 1999. Molecular evidence for the presence of *Ophiosphaerella narmari* n. comb., a cause of spring dead spot of Bermudagrass, in North America. *Mycological Research* 103:981-989. (TGIF Record 62602)

17. Wetzel, H. C. III, D. Z. Skinner, and N.A. Tisserat. 1999. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. *Plant Disease* 83:1160-1166. (TGIF Record 62601)