Geographic Distribution and Genetic Diversity of Three *Ophiosphaerella* Species that Cause Spring Dead Spot of Bermudagrass

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ABSTRACT

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

The distribution of three *Ophiosphaerella* spp. that cause spring dead spot (SDS) of bermudagrass was studied by systematically sampling two golf courses in Oklahoma and one in Kansas. *O. herpotricha* was isolated from all three locations and was the most abundant species. It was the only SDS pathogen found at Jenks, Oklahoma. *O. korrae* was isolated from Afton, Oklahoma, and Independence, Kansas, whereas *O. narmari* was only detected in samples from Afton. This is the first report of all three *Ophiosphaerella* species on bermudagrass at the same location. Amplified fragment length polymorphism (AFLP) marker analysis was used to investigate inter- and intraspecific genetic diversity of *Ophiosphaerella* isolates from North America and Australia. A majority of the *O. herpotricha* and *O. narmari* isolates from Afton were distinct haplotypes, suggesting that sexual recombination was occurring within the population. Conversely, the presence of multiple isolates of *O. herpotricha* and *O. narmari* with the same haplotype also indicated that asexual propagation was occurring. The genetic diversity among *O. herpotricha* isolates from Afton was not distinctly different from that of isolates collected throughout the southern United States. In contrast, *O. narmari* isolates from Afton were distinct from those collected in Australia. The genetic diversity in *O. korrae* was markedly different than that in the other *Ophiosphaerella* spp. The population at Afton was dominated by just a few haplotypes, and these were nearly identical to isolates collected from bermudagrass and Kentucky bluegrass throughout western, central, and northern North America. However, *O. korrae* isolates collected in the southeastern United States were only distantly similar to other North American isolates.

Additional keywords: *Leptosphaeria korrae*, *L. narmari*

Spring dead spot (SDS) is a disease of bermudagrass (*Cynodon dactylon*) and bermudagrass hybrids (*C. dactylon* × *C. transvaalensis*) in Australia (7,23,28) and the United States (3,5,9,26). Sunken, circular dead patches develop within the turfgrass stand as bermudagrass breaks winter dormancy. The patches are generally well-defined and range in size from a few centimeters to 0.5 m or more in diameter (5).

SDS is caused by the three ectotrophic root-infecting fungi *Ophiosphaerella narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (= *Leptosphaeria narmari* J. Walker & A.M. Smith), *O. herpotricha* (Fr.:Fr.) J. Walker, and *O. korrae* (J. Walker & A.M. Smith) R.A. Shoemaker

& C.E. Babcock (= *L. korrae* J. Walker & A.M. Smith). These fungi can be differentiated on the basis of their ascospore morphology (21,27), but ascocarps rarely are seen in nature and cannot be easily induced in certain isolates (2). More recently, these species have been differentiated on the basis of selective PCR amplification with species-specific primers derived from the ITS regions of the rDNA (16,25,32).

Limited information is available on the geographic distribution of SDS pathogens. *O. korrae* has been documented as the cause of SDS in Maryland (3), California (5), and Australia (28). It also causes necrotic ring spot (NRS) disease of Kentucky bluegrass (*Poa pratensis*), annual bluegrass (*P. annua*), and creeping red fescue (*Festuca rubra*) (4,12,33). NRS is limited primarily to the northern United States and Canada (19). *O. narmari* causes SDS in Australia (28) and New Zealand (9) and has recently been reported in the United States (32). *O. herpotricha* is a cause of SDS in Kansas, Oklahoma, and other areas of the United States (26), but it has not been reported on bermudagrass in Australia. Because these reports were based on

relatively few isolations at each location, the true distribution of these fungi remains ambiguous.

Wetzel et al. (32) demonstrated that the three SDS pathogens clustered into a monophyletic group distinct from other tested *Leptosphaeria* and *Phaeosphaeria* species based on nucleotide sequence comparisons of the ITS regions of the rDNA. These results supported the taxonomic transfer of *L. korrae* to *O. korrae* proposed by Shoemaker and Babcock (21) and the assignment of *L. narmari* to *O. narmari*.

Less is known about the intraspecific diversity in these three SDS pathogens. Based on random amplified polymorphic DNA (RAPD) analysis, Raffle and Hsiang (19) reported a relatively low level of genotypic diversity among isolates of *O. korrae* collected from Canada and the state of Washington. They hypothesized that low numbers of genotypes were introduced into these regions, possibly on contaminated sod, and then were dispersed asexually as mycelia or by ascospores of selfed isolates. In contrast, preliminary surveys in Oklahoma and Kansas suggested a higher level of genotypic diversity among *O. herpotricha* isolates (14,31).

The objectives of our research were to examine the distribution and genetic diversity of the three SDS pathogens collected from systematically sampled bermudagrass golf course fairways in Kansas and Oklahoma. The genetic diversity observed on a local and regional level was then compared with *Ophiosphaerella* isolates collected throughout North America and Australia.

MATERIALS AND METHODS

Fungal isolates. We systematically sampled SDS-affected bermudagrass golf course fairways at three locations (Shangri-La Resort in Afton, Oklahoma, South Lakes Golf Club in Jenks, Oklahoma, and Independence Country Club in Independence, Kansas) in May 1994 to assess the distribution and genetic diversity of SDS pathogens. The sampling pattern consisted of delineating two to three equally spaced, 6-m-radius circles on each of three fairways at the Afton and Jenks locations and two fairways at the Independence golf course. An 11-cm-diameter × 10.2-cm-deep turfgrass core was removed from the margin of each of 20 SDS patches in each circle. Most SDS patches were less than 40

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Contribution of the Kansas Agricultural Experiment Station 99-394-J.

Accepted for publication 6 September 1999.

Publication no. D-1999-1018-02R
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cm diameter. An additional 10 SDS patches were randomly sampled outside the circles on each fairway. Two fairways on the Afton course were resampled in May 1996 by removing cores from 100 SDS patches at approximate 5-m intervals along a W-shaped transect.

Turfgrass cores were washed with water to remove soil from roots. Root segments, 5 to 10 mm long and colonized with ectotrophic hyphae, were surface-sterilized in 0.5% NaOCl for 5 min and blotted dry on paper towels. Five segments from each soil core were placed on quarter-strength potato dextrose agar (PDA; Difco, Detroit, MI) amended with 100 mg each of streptomycin sulfate and chloramphenicol per liter and incubated at room temperature (22 to 26°C) for approximately 7 days. Putative SDS pathogens initially were selected based on morphological growth characteristics (26,28,30). In most cases, a single isolate from each core was maintained on a PDA slant at 4°C for further testing. However, two to five isolates were collected from 50 of the 200 cores sampled in 1996 to detect potential diversity of SDS pathogens within individual patches.

We also collected or obtained 61 *O. herpotricha* and 15 *O. narmari* isolates from bermudagrass, and 66 *O. korrae* isolates from bermudagrass and Kentucky bluegrass, from various regions and sources in the United States and Australia to assess their genetic diversity on a larger geographic scale. Isolation and maintenance procedures were similar to those previously described.

DNA isolation. Fungal isolates were grown in modified yeast extract broth, and DNA was extracted and resuspended for amplification in polymerase chain reaction (PCR) assays as described by Wetzal et al. (32). Oligonucleotide primers specific for *O. herpotricha*, *O. korrae*, and *O. narmari* (25,32) were used to verify the identity of all SDS isolates. PCR conditions and species-specific primer sequences are described elsewhere (25,32).

AFLP analysis. Amplified fragment length polymorphism (AFLP) assays were performed with AFLP Analysis System II (Life Technologies, Inc., Gaithersburg, MD). Fungal isolate template DNA was prepared in a one-step restriction-ligation reaction. Fungal genomic DNA (100 ng in 5 µl of dH₂O) was digested at 37°C for 2 h with 4 U of *Eco*RI and 1.6 U of *Mse*I in 1.0 µl of acetate buffer (10 mM Tris-acetate, pH 7.5, 10 mM Mg-acetate, and 50 mM K-acetate) in a final reaction brought to a volume of 10 µl with dH₂O. Then, a 10-µl solution containing 0.4 µl each of 5 pmol/µl *Eco*RI-adapters and 50 pmol/µl of *Mse*I-adapters, 80 U of T4 DNA ligase

(New England Biolabs, Beverly, MA), 1.0 µl of T4 DNA ligase buffer (1 mM ATP, 50 mM Tris-HCl, pH 7.8, 10 mM MgCl₂, 10 mM DTT, and 25 µg of BSA per ml), and 1.0 µl of acetate buffer were added to the reaction tube and incubated at 20°C for 3 h. *Eco*RI and *Mse*I adapters were prepared by adding equal molar amounts of both strands in a microcentrifuge tube, raising the temperature to 95°C, and allowing the tube to cool to 25°C over the course of 45 min in a programmable thermal cycler (MJ Research, PTC-100, Watertown, MA). Following ligation, reactions were diluted with 180 µl of TE and stored at -20°C until further use.

Fig. 1. Estimates of genetic similarity among 26 *Ophiostoma herpotricha* isolates collected from the fourth fairway at Afton, Oklahoma (OK), based on amplified fragment length polymorphisms using the selective primer pair EAA/MCA. Bootstrap values (2,000 replicates) for branch nodes ranged from 3.8 to 42% (average = 21%).

Table 1. Abundance of *Ophiostoma herpotricha*, *O. korrae*, and *O. narmari* isolates associated with spring dead spot patches systematically sampled at three golf courses

Golf course, city, state	Collection date	Fairway number	Number of turf cores collected	Number of isolates ^a		
				Oh	Ok	On
Shangri-La Resort, Afton, OK	1994	4	50	34	0	0
Shangri-La Resort, Afton, OK	1994	11	70	52	3	2
Shangri-La Resort, Afton, OK	1994	16	70	50	1	2
Shangri-La Resort, Afton, OK	1996	11	100	34	23	5
Shangri-La Resort, Afton, OK	1996	16	100	31	11	12
South Lakes G.C., Jenks, OK	1994	9	50	44	0	0
South Lakes G.C., Jenks, OK	1994	14	70	64	0	0
South Lakes G.C., Jenks, OK	1994	16	70	65	0	0
Independence C.C., Independence, KS	1994	13	70	48	0	0
Independence C.C., Independence, KS	1994	14	70	23	9	0
Total cores			720			
Total isolates				445	47	21

^a Based on the number of *Ophiostoma herpotricha* (Oh), *O. korrae* (Ok), and *O. narmari* (On) isolates recovered from turfgrass cores sampled from each golf course fairway in 1994 and 1996, respectively. *Ophiostoma* spp. were positively identified with species-specific polymerase chain reaction primers.

PCR preamplification of the ligated template DNA was performed as described in the AFLP Analysis System II instructions, with the exception that the preamplification PCR primers were as follows: *EcoRI* core plus adapter 5'-CTCGTAGAC-TGCGTACCAATTC-3' and *MseI* core plus adapter 5'-GACGATGAGTCCTGA-GTAA-3'. Individual preamplification PCR reactions consisted of the following: 51- μ l solution (46 μ l from a stock mix of reagents and 5 μ l of diluted ligated template DNA); 1.3 μ l each of *EcoRI* and *MseI* core plus adapter primers (50 ng/ μ l); 0.41- μ l solution of 100 mM each dATP, dCTP, dGTP, and dTTP; 5.1 μ l of 10 $\times$ PCR buffer (200 mM Tris, pH 8.41; 500 mM KCl; and 15 mM MgCl₂); and 0.2 μ l of Amplitaq DNA polymerase (5 U/ μ l) (PE Applied Biosystems, Branchburg, NJ). The reaction mixture was overlaid with 50 μ l of mineral oil. The following thermal cycler temperature regime was employed: 20 cycles of 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s. Following preamplification, 3 μ l of the

reaction was diluted with 147 μ l of TE and stored at -20°C until further use.

Selective amplification of the preamplification template DNA was performed as described in the AFLP Analysis System II instructions with the exception that the selective amplification primers were designed as described by Liscum and Oeller (*personal communication*). The basic *EcoRI*-primer is 5'-AGACTGCGTACC-AATTCxyz-3' and the basic *MseI*-primer is 5'-GATGAGTCCTGAGTAAxyz-3' where x, y, and z represent the selective bases on the 3' end of the oligonucleotide. Twelve selective AFLP PCR *EcoRI/MseI* primer pairs were assessed against 10 isolates each of three SDS fungal species including AA/AA, AA/AC, AA/AG, AA/AT, AA/CA, AA/CC, AA/CG, AA/CT, AA/GA, AA/GC, AA/GG, and AA/GT. Selective AFLP PCR reactions were composed as described in the AFLP Analysis System II instructions, except that the total reaction volume was reduced to 10 μ l. The selective *EcoRI* primer was end labeled with [γ -³²P] ATP (specific activity = 2,000 Ci/mmol) (NEN

Life Science Products, Inc., Boston, MA) using T4 polynucleotide kinase supplied with the kit. The reaction mixture was overlaid with 25 μ l of mineral oil. The following selective AFLP PCR thermal cycler temperature regime was employed: initial cycle of 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s with the following 12 cycles lowering the annealing temperature 0.7°C per cycle and the remaining 23 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s.

Following the selective AFLP PCR amplifications, reaction DNA was denatured at 90°C for 3 min with an equal vol (10 μ l) of gel loading buffer (98% formamide, 10 mM EDTA (pH 8.0), 0.1% bromophenol blue, and 0.1% xylene cyanol). Then, 1.7 μ l of this mixture was applied to shark-tooth comb-formed wells in a 0.4-mm-thick 6% Long Ranger (FMC BioProducts, Rockland, ME) polyacrylamide gel (38 by 50 cm) resolved at constant power (100 W) in 1 $\times$ TBE running buffer for 2.5 h. Gels were then blotted to 0.33 mm gel blot paper (Midwest Scientific, Valley Park, MO) and dried using a Bio-Rad Model 583 gel dryer (Bio-Rad Life Science Group, Hercules, CA). Autoradiographs were obtained by exposing Classic BX film (Midwest Scientific, Valley Park, MO) to the dried gel for 72 h at 22 to 26°C.

AFLP fragment analysis. DNA bands were scored from AFLP autoradiographs by assigning a number in relation to their migration within the gel. Each gel contained the low mass DNA ladder (Life Technologies) with markers of 2,000, 1,200, 800, 400, 200, and 100 bp on both sides of the isolates. AFLP bands between 1,300 and 200 bp were scored and included in the analyses. The DNA band with the highest molecular weight was assigned number 1 and so on in ascending order until the band of lowest molecular weight was assigned. It was assumed that bands of the same molecular weight in different isolates were identical. For each isolate, the presence or absence of each band was determined and designated 1, present, or 0, absent. AFLP amplification was repeated on six isolates of each fungal species with identical results. The genetic similarity among isolates was estimated using the simple matching coefficient (24) based on the presence or absence data of each band. Calculations on these data were performed using algorithms written in the Interactive Matrix Language (Proc IML) of SAS (version 6, SAS/IML Software: Usage and Reference; SAS/STAT User's Guide, SAS Institute, Inc., Cary, NC). The output of this analysis was written into a genetic similarity matrix. Cluster analysis, using the UPGMA method, was performed on each genetic similarity matrix with the program JMP (version 3.1, SAS), and the clustering among genetically similar isolates was graphically displayed in a dendrogram. To determine the robustness of

Fig. 2. Estimates of genetic similarity among *Ophiostoma herpotricha* isolates from Kansas (KS), Kentucky (KY), North Carolina (NC), Oklahoma (OK), and Texas (TX) based on amplified fragment length polymorphisms using the selective primer pair EAA/MCA. Isolate KS204 also represents seven additional isolates collected from the sixteenth fairway at Afton, Oklahoma. Isolates from Afton with the letters A to E following the identification number represent cultures collected from the same turf core. Bootstrap values (2,000 replicates) for branch nodes ranged from 0.1 to 58% (average 19%).

each dendrogram, the presence or absence data were resampled by replacement with 2,000 bootstrap replicates using the program WinBoot (34).

An additional test was performed to determine if the three populations of *Ophiosphaerella* species exhibited genetic linkage disequilibrium (D) based on locus-by-locus comparisons of the AFLP marker data. AFLP markers are dominant and usually have two alleles per locus (15). A D value was calculated using Hill's (8) haploid identification equation (equation 21, p. 235). A D value not significantly different from zero would support the null hypothesis that the population was in genetic equilibrium, the loci were not linked, and the population did not depart from panmixia. A D value significantly greater than zero was evidence for the presence of disequilibrium, suggesting linkage among loci and a departure from random mating. A chi-square test as described by Zhan et al. (35) was used to test if the D value departure from zero was statistically significant. The chi-square had one degree of freedom based on each locus-by-locus comparison made. Calculations of D and chi-square tests were performed using algorithms written in the Interactive Matrix Language (Proc IML) of SAS.

RESULTS

Distribution of SDS pathogens. SDS pathogens were isolated from 70% of all soil cores collected from golf courses in 1994 and 1996 (Table 1). Recovery of *Ophiosphaerella* spp. from cores collected from individual fairways ranged from 46 to 93%. *O. herpotricha* was isolated from each fairway at all three locations and accounted for 87% of the total number of isolates obtained. At Jenks, *O. herpotricha* was the only SDS pathogen isolated. *O. korrae* was isolated from cores collected at Afton and Independence, whereas *O. narmari* was detected only in samples from Afton (Table 1).

Although *O. narmari* and *O. korrae* were not as abundant as *O. herpotricha*, they were widely distributed on those fairways where they were detected. The three SDS pathogens were often found within 5 m of one another, and in nine samples, two *Ophiosphaerella* species were isolated from different roots in the same turfgrass core.

Genetic diversity in *O. herpotricha*. One hundred and thirty-eight haplotypes (i.e., an isolate or group of isolates with a unique AFLP banding pattern following amplification with the selective primer pair EAA/MCA) of *O. herpotricha* were detected among the 239 isolates collected from 201 turf cores on three different fairways at Afton in 1994 and 1996. Thirty-five of these haplotypes were represented by at least two, but no more than 15, isolates. An example of the genotypic variation found at Afton was illustrated from the isolates collected from the fourth fairway (Fig. 1).

Of the 26 isolates analyzed, 22 exhibited different AFLP banding patterns. These haplotypes had genetic similarities ranging from 69 to 98%.

At Afton, isolates of *O. herpotricha* with the same haplotype were often recovered from turf cores collected from SDS patches within a 6-m radius, but identical haplotypes also were found at opposite ends of the same fairway (>300 m) and on different fairways. The same haplotype was also isolated at least twice from 27 of the 34 turf cores in which more than one isolate was collected. In two cases, all five isolates from the same turf core were the same haplotype. Conversely, two or three different haplotypes were recovered from 19 of the 34 turf cores in which multiple isolates were collected.

Twenty representative isolates of *O. herpotricha* from the three systematically sampled golf courses were compared with 61 other isolates collected in Kansas, Oklahoma, North Carolina, Kentucky, and Texas (Fig. 2). The genetic similarity among all isolates (range 60 to 98%) was similar to that found for isolates collected on the fourth fairway at Afton. Isolates

from the same geographic region were generally not tightly clustered, and bootstrap values for most branch nodes in the dendrogram were low (range 0.1 to 58%). Five haplotypes were represented by isolates collected in both Kansas and Oklahoma. In one case, an isolate from Kentucky and Oklahoma had the same AFLP banding pattern.

Genetic diversity in *O. narmari*. *O. narmari* isolates were divided into two distinct groups based on AFLP banding patterns (Fig. 3). Isolates from Australia and California exhibited a high degree of genetic similarity even though they were collected from widely diverse geographic regions. Likewise, isolates from Afton and one Kansas isolate were genetically similar. Similar results were observed in AFLP banding patterns among isolates following amplification with another primer pair, EAA/MAG (data not shown).

Nineteen haplotypes were detected among 26 *O. narmari* isolates collected at Afton. Isolates collected from the same turf core exhibited the same AFLP pattern when amplified with the EAA/MCA and EAA/MAG primer pair combinations in five of the six samples. Two isolates from

Fig. 3. Estimates of genetic similarity among *Ophiosphaerella narmari* isolates from California (CA), Oklahoma (OK), and Australia (AUST) based on amplified fragment length polymorphisms using the selective primer pair EAA/MCA. Isolates from Afton, Oklahoma, with the letters A to C following the identification number represent cultures collected from the same turf core. Bootstrap values (2,000 replicates) for branch nodes ranged from 4.1 to 100% (average 41%).

one turf core differed by 11 and 6 bands for the two primer pair combinations.

Genetic diversity in *O. korrae*. Isolates of *O. korrae* collected over a wide geographic area were clustered into two distinct genetic similarity groups (Fig. 4). Isolates from the southern United States, including those from Arkansas, Georgia, Mississippi, and Tennessee, exhibited a high degree of genetic similarity (average 88%), whereas isolates from northern and

western regions of North America and from Australia also were very similar (average genetic similarity 91%). Some of the haplotypes in each group were represented by more than one isolate. These isolates often were collected from widely dispersed geographic regions. For example, OKA16-3-2 was identical to isolates from nine different locations in the United States. In contrast, genetic similarity between the two groups of isolates was low (average 38%).

Fig. 4. Estimates of genetic similarity among *Ophiosphaerella korrae* isolates based on amplified fragment length polymorphisms using the selective primer pair EAA/MCA. Isolate OKA11-84A represents 13 additional isolates from Afton, Oklahoma (OK). Isolate OKA16-3-2 represents 18 additional isolates (eight from Independence, Kansas [KS], one from Colorado [CO], one from Idaho [ID], three from Maryland [MD], one from Michigan [MI], one from Minnesota [MN], one from Ohio [OH], one from Rhode Island [RI], and one from New Jersey [NJ]). Isolate OKA11-6A represents 42 isolates (41 from Afton and one from Australia [AUST]). Isolate CAN263 represents 13 isolates (two from Canada, two from Washington [WA], six from Idaho, one from New Jersey, one from New York [NY], and one from Pennsylvania). Isolates from Arkansas (AR), Georgia (GA), Illinois (IL), Mississippi (MS), Nebraska (NE), Tennessee (TN), and Wisconsin (WI) also are represented in the dendrogram. Bootstrap values (2,000 replicates) for branch nodes ranged from 0.9 to 100% (average 27%).

There was also a high degree of genetic similarity among isolates collected from different turf cores at Afton (Fig. 4). Only nine haplotypes were detected among the 65 isolates assayed, and 43 of these isolates were the same haplotype. Similarly, multiple isolates collected from the same turf core were identical haplotypes in 15 of these 16 samples.

Genetic linkage disequilibrium (D). A test for genetic linkage disequilibrium (D) was performed on the AFLP fragment data collected from isolates (one isolate of each haplotype) of *O. herpotricha*, *O. korrae*, and *O. narmari* (Table 2). Only 9% of the D values for *O. herpotricha* were significant, indicating there was only slight linkage disequilibrium. Thirty-seven and 55% of the D values were significant when all isolates within *O. narmari* and *O. korrae* were compared (Table 2). However, when *O. narmari* and *O. korrae* isolates were first split into their genetic similarity clusters (Figs. 3 and 4) and then analyzed, evidence for linkage disequilibrium was diminished (Table 2). Within the two groups of *O. narmari*, 11 and 5% of the D values were significant. Within the two groups of *O. korrae*, only 1% of the D values were significant, suggesting sexual recombination within the groups.

DISCUSSION

The distribution of SDS pathogens in North America is apparently more complex than previously described (10). Although *O. herpotricha* was the most abundant SDS pathogen isolated in this study, *O. korrae* also was recovered at two locations. *O. korrae* has been isolated from Kentucky bluegrass with necrotic ring spot disease in Kansas (N. A. Tisserat, *unpublished data*), but this is the first report of this pathogen on bermudagrass in this region. *O. narmari*, which was previously reported only in Australia and New Zealand (9,28), was isolated from sites in Kansas and Oklahoma. We also determined that the sterile isolates CA46 and CA48 (originally designated as SDS1 and SDS3) collected by

Table 2. Test for genetic linkage disequilibrium (D) within isolate collections of the three *Ophiosphaerella* spp. using amplified fragment length polymorphisms (AFLP)

Isolates ^a	No. of isolates	No. of haplotypes ^b	No. of AFLP markers	Total no. of comparisons	% of D values > 0 (P < 0.001) ^c
<i>Ophiosphaerella herpotricha</i>	300	186	45	990	9
<i>O. narmari</i>	41	33	137	9,316	37
Group A	14	13	96	4,560	11
Group B	27	20	90	4,005	5
<i>O. korrae</i>	129	32	44	946	55
Group A	118	22	27	351	1
Group B	11	10	33	528	1

^a Selective primer pair EAA/MCA was used to assess *O. herpotricha* and *O. korrae*, and the primer pairs EAA/MCA and EAA/MAG were used to assess *O. narmari* isolates, respectively. *O. narmari* isolate group A represents individuals from Australia and California, and B represents individuals from Oklahoma and Kansas. *O. korrae* isolate group A represents individuals from Australia, California, Canada, Colorado, Idaho, Illinois, Kansas, Maryland, Michigan, Minnesota, Nebraska, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, Rhode Island, Washington, and Wisconsin, and B represents individuals from Arkansas, Georgia, Mississippi, and Tennessee.

^b Each isolate used in the analysis represents a distinct haplotype, based on its unique AFLP fingerprint.

^c Percentage of D values that deviate from absolute zero in that their chi-square value was ≥ 10.83 ($P < 0.001$) among locus-by-locus comparisons. Each chi-square test was carried out using a single degree of freedom.

Endo et al. (5) from bermudagrass in California were *O. narmari*. This suggests that *O. narmari* is widely distributed in the United States.

O. herpotricha was the most frequently isolated SDS pathogen, and on one golf course, it was the only pathogen isolated during the survey. Whether this is a reflection of the native distribution of SDS pathogens, their differential immigration on roots and stolons during bermudagrass establishment, a reproductive or fitness advantage of *O. herpotricha* on bermudagrass, or a combination of these factors is not known. *O. herpotricha* has been reported from a number of plants in the Poaceae, whereas *O. korrae* and *O. narmari* have a more limited host range (20,21,27,28). A relatively large native population of *O. herpotricha* might explain its higher frequency on bermudagrass. Alternatively, the distribution and frequency of SDS pathogens at a given location may be a function of their abundance in the original bermudagrass source. Most bermudagrass fairways in Kansas and northern Oklahoma are established from vegetatively propagated roots or stolons of cultivars grown in sod farms. Movement of SDS pathogens on colonized stolons previously has been demonstrated (17). Multiple introductions of bermudagrass cultivars, possibly from different farms and infected with different SDS pathogens, could have resulted in the pathogen mixture observed at the Afton golf course. Systematic sampling in other regions of the United States and from bermudagrass sod farms might elucidate the origin and distribution of SDS pathogens.

The presence of more than one SDS pathogen at a given location was reported in Australia (22). However, this is the first report of all three pathogens at the same location. Since multiple isolates were collected from only a small percentage of the turf cores, the frequency of two SDS pathogens present in individual patches may actually be higher. The association and interaction of these pathogens during root colonization and symptom development requires further study.

A relatively large number of genotypes was detected among *O. herpotricha* and *O. narmari*, but not among *O. korrae* isolates collected at Afton. A high level of genotypic diversity in a fungal population suggests that sexual recombination may be occurring (15). Pseudothecia of *O. korrae* and *O. narmari* were reported to be occasionally present on infected bermudagrass in Australia (23,28), whereas ascocarps of *O. herpotricha* were detected in inoculated bermudagrass in the greenhouse (26). Ascocarps also can be induced in some isolates of *O. korrae* (2,3). Although we have not observed ascocarps of these SDS pathogens in turf samples collected in Kansas or Oklahoma, they might be overlooked since they tend to form on decaying

stolons in the thatch layer. While *Ophiosphaerella* spp. are homothallic, the relatively large number of genotypes and the lack of genetic disequilibrium, particularly within genetic similarity groups (Table 2), suggests that some sexual recombination is occurring. Sexual recombination through outcrossing has been demonstrated in homothallic fungi such as *Aspergillus nidulans* (18) and *Gibberella zeae* (1). Nevertheless, Raffle and Hsiang (19) reported no genetic variation among 10 ascospore progeny of one *O. korrae* isolate with RAPD analysis, and they suggested that methods of recombination such as parasexuality or mutations were responsible for generating different haplotypes in this fungus. However, their sampling size was likely too small to detect outcrossing. Further studies are needed to ascertain the mechanisms of genetic diversity in *Ophiosphaerella* species.

The relatively large number of isolates with identical haplotypes in each *Ophiosphaerella* species also indicates that asexual propagation of genotypes is occurring. It is unlikely this is a result of asexual spores, since no anamorph has been reported for *O. narmari* or *O. korrae*. An anamorph was described for *O. herpotricha* (29), but we have never observed it in the field or in the hundreds of diseased turf cores or cultures processed. Clonal propagation may have occurred via dispersion of ascospores resulting from inbreeding or by movement of persistent mycelium on bermudagrass roots and stolons. Bermudagrass roots or stolons with ectotrophic hyphae could easily be moved locally by machinery such as mowers and soil aerators. The discovery of the same haplotype of *O. herpotricha* in different regions in different states suggests that haplotypes may be dispersed over large geographic areas, possibly during bermudagrass establishment. Similarly, the presence of identical haplotypes of *O. korrae* in Oklahoma and Australia and the confirmation of *O. narmari* isolates with nearly identical haplotypes from Australia and California strongly suggest that transcontinental movement of these species can occur. Raffle and Hsiang (19) reported the presence of two unique *O. korrae* haplotypes following RAPD analysis. One haplotype was collected in British Columbia and Quebec, Canada, and in the state of Washington, and the other was collected in Ontario, Canada, and Washington. Continental or panglobal distributions of clones or single clonal lineages for other fungal pathogens have also been documented (6,11).

The level of genetic diversity among *O. herpotricha* isolates collected throughout the United States was similar to that found among isolates collected on an individual fairway at Afton. There was no evidence that isolates from a given location (e.g., Afton) were genetically more similar to

one another than to isolates from other regions. The probable immigration of isolates on bermudagrass stolons coupled with recombination, presumably from meiotic division, would tend to have a homogenizing effect on genetic diversity. This pattern of high genotypic diversity with low levels of genetic differentiation among geographical locations is similar to that described in *Microdochium nivale* (13).

The genotypic diversity in *O. korrae* was markedly different from that in *O. herpotricha*. The population at Afton was dominated by just a few haplotypes, and these were nearly identical to isolates collected throughout western and northern North America and Australia (one isolate only). Some of the isolates in this group were originally isolated from bermudagrass, while others were collected from Kentucky bluegrass, indicating that genetic similarity was not necessarily host-related. Several haplotypes were also represented by isolates collected over a wide geographic area. These results are consistent with Raffle and Hsiang (19), who, by using RAPD analysis, found only six haplotypes of *O. korrae* among 71 isolates collected from Kentucky bluegrass in North America. They speculated that the low level of genetic diversity in *O. korrae* was a result of the introduction to the continent of a limited number of genotypes on their major hosts (presumably Kentucky bluegrass and bermudagrass), followed by their distribution over a wide geographic area by asexual propagation. The low level of genetic diversity we found within groups of North American isolates supports this hypothesis.

ACKNOWLEDGMENTS

We thank Percy Wong for supplying additional *O. narmari* isolates from Australia and Kevin McCann for helping collect isolates from Oklahoma. This research was sponsored in part by the GCSAA Foundation's Chapter Cooperative Research Program, Heart of America Golf Course Superintendents Association, Kansas Golf Course Superintendents Association, Kansas Turfgrass Foundation, and the United States Golf Association.

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