Detection and disruption of virulence factors associated with *Ophiosphaerella* spp., the causal agents of spring dead spot of bermudagrass

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- Spring dead spot is the most devastating disease of bermudagrass in the transition zone where it goes dormant in the winter.
- The genomes of three *Ophiosphaerella herpotricha*, five *O. korrae*, and three *O. narmari* isolates were sequenced.
- The transcriptomes from cultures of the same eleven *Ophiosphaerella* isolates were obtained.
- Current efforts are attempting to identify products secreted by the fungi when infecting and colonizing resistant and susceptible hosts.

Bermudagrass is among the predominant turfgrasses used for commercial and residential urban ground cover in the southern United States. Spring dead spot (SDS) is the most devastating disease of bermudagrass in the transition zone where it goes dormant in the winter and is caused by a complex of three species of *Ophiosphaerella*. To develop effective, durable resistance to SDS in bermudagrass cultivars, a thorough understanding of how the pathogen induces necrosis of host tissues is necessary. Based on our recent insights into the spring dead spot host/pathogen interaction and how they differ for resistant and susceptible cultivars (Figure 1), we are now using a bioinformatics approach to identify the fungal gene(s) encoding the necrosis-inducing effectors. The first objective of this study is: to produce a genome sequence of *O. korrae*. In a previous study, a preliminary draft genome sequence of *Ophiosphaerella herpotricha* was generated using one 2nd generation sequencing approach. Since producing that previous genome sequence, higher throughput 3rd generation sequencing approaches have been commercialized. This latter technology is less expensive than previous approaches and can produce extremely long reads i.e. tens of thousands bases versus the hundreds generated by 2nd generation sequencing approaches.

We sent total genomic DNA samples from three *Ophiosphaerella herpotricha*, five *O. korrae*, and three *O. narmari* to Novogene Corp., Hong Kong, for Illumina sequencing (Table 1) Total genomic DNA of one isolate of each species was sent to Research and Testing Laboratory,

Lubbock, TX, for Pacific Biosciences Technology (PacBio) sequencing to generate very long DNA sequence reads that can serve as the scaffolds for the genome assemblies. We have received back the PacBio sequences for *O. korrae* and *O. narmari* and are still awaiting the *O. herpotricha*; however, the Novogene sequences were of such high quality the PacBio data only slightly improved the *O. korrae* and *O. narmari* assemblies. Currently, analysis of the genomes and gene function is ongoing. Most genes identified thus far appear to be orthologous (genes in different species that evolved from a common ancestral gene, and retained their original function) and are shared between *Ophiosphaerella* spp. (figure 2).

We also used Novogene Corp to sequence total RNA (RNA transcripts of expressed genes, or transcriptome) from cultures of the eleven *Ophiosphaerella* isolates. This will be compared to the fungal RNA expressed when the fungi are infecting the plants and will be used in the next two objectives of the study. Current efforts are directed at identifying compounds secreted by the fungi when infecting and colonizing resistant (U3) or susceptible hosts (Tifway 419). To do this, secreted products will be extracted and dialyzed against pure water with dialysis cassettes of different molecular weight cutoff sizes. Products in the dialyzed water will be digested and identified by tandem mass spectrometry. The secreted products will be analyzed in the Orbitrap Fusion Tribid mass spectrometer coupled with an electrospray ion source detector and compared to customized databases using Mascot (Matrix Science, Inc. Boston, MA). The databases will be generated from the data obtained in objective one from other plant pathogenic fungi characterized in the literature.

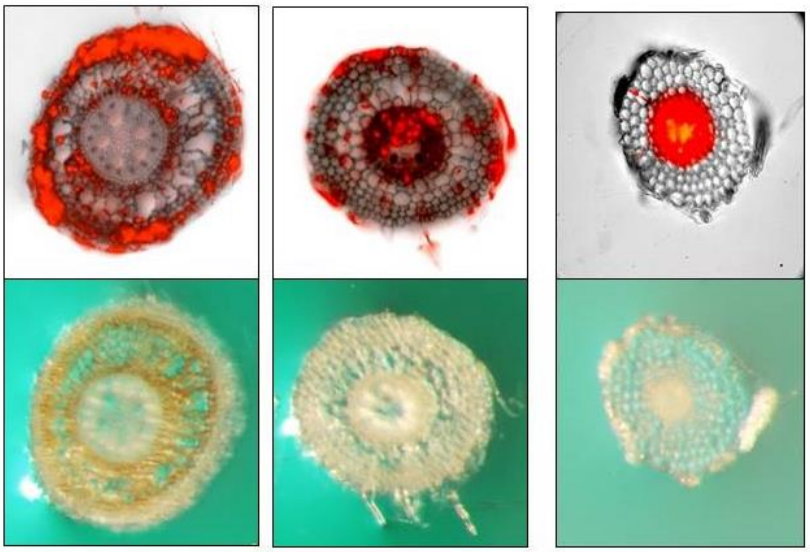

Figure 1. Colonization of a spring dead spot susceptible bermudagrass and cortical necrosis by *Ophiosphaerella korrae* (left), a tolerant bermudagrass (Center) exhibiting vascular colonization by *O. korrae* and no necrosis, and *O. korrae* colonization of a grass which does not produce disease (right). Pictures by F. Flores.

Genome Metric	*Ophiosphaerella herpotricha*			*Ophiosphaerella korrae*					*Ophiosphaerella narmari*		
	KS28	TX2.5A	16FISCC	14BISCC	OW11	TX1.4	KY162	HCW2	BCGCC2	AUS58	ATCC202719
Genome Assembly Size	66,125,271	67,214,859	63,855,078	68,185,353	67,389,091	72,180,653	71,419,005	71,157,136	47,063,809	47,000,963	47,756,967
n50	61,683	54,308	50,289	37,869	40,856	31,766	51,429	47,026	213,028	221,777	1,524,584
Largest Contig	914,685	1,067,401	686,546	458,646	516,330	423,522	484,841	479,782	1,060,903	1,885,537	1,378,950
Number of Contig	27,846	29,099	20,526	26,403	14,402	34,418	13,696	12,155	7,233	7,094	5,309
GC%	41.4	41.1	40.1	39.2	38.1	41.5	39.2	38.6	46.5	46.4	45.8
Numer of Protein Coding Genes	14,588	14,511	13,364	14,156	12,701	14,468	12,988	12,720	12,115	14,215	13,462
Number of Complete Genes (AUG+Stop)	13,901	14,001	13,285	13,460	12,576	13,880	12,602	12,615	12,006	14,091	13,384
Number of Introns	23,959	23,220	23,812	22,492	21,039	23,577	21,071	12,263	21,426	23,711	23,069
Number of Exons	38,182	37,455	37,108	36,203	33,606	37,660	33,841	33,875	33,684	37,913	36,448
Number of Ge ies without Int ons	3,452	3,739	3,118	3,791	3,393	3,623	3,446	3,367	3,139	3,599	3,374
Intergenic ases	45,295,214	45,658,788	42,898,131	46,297,742	47,072,483	50,543,721	50,791,964	50,737,579	26,776,106	25,855,809	26,602,284
Bases Non oding	47,152,037	47,648,742	44,763,120	48,214,060	48,622,005	52,409,605	52,594,436	51,716,166	28,404,482	27,633,830	28,450,111
Intragenic Bases	20,830,057	21,556,071	20,956,947	21,887,611	20,316,608	21,636,932	20,627,041	20,419,557	20,287,703	21,145,154	21,154,683
Average Gene Size	1,471	1,485	1,568	1,546	1,600	1,495	1,543	1,377	1,657	1,488	1,571
Exons per gene	2.6	2.6	2.8	2.6	2.6	2.6	2.6	2.7	2.8	2.7	2.7
Introns Per Gene	1.6	1.6	1.8	1.6	1.7	1.6	1.6	1.0	1.8	1.7	1.7
Average Exon Size	506.0	521.1	516.8	540.3	558.9	523.9	558.6	551.5	557.0	502.1	528.4
Average Intron Size	77.5	85.7	78.3	85.2	73.7	79.1	85.5	79.8	76.0	75.0	80.1

Table 1. Genomic statistics for three isolates of *Ophiosphaerella herpotricha*, five isolates of *O. korrae* and three isolates of *O. narmari*.

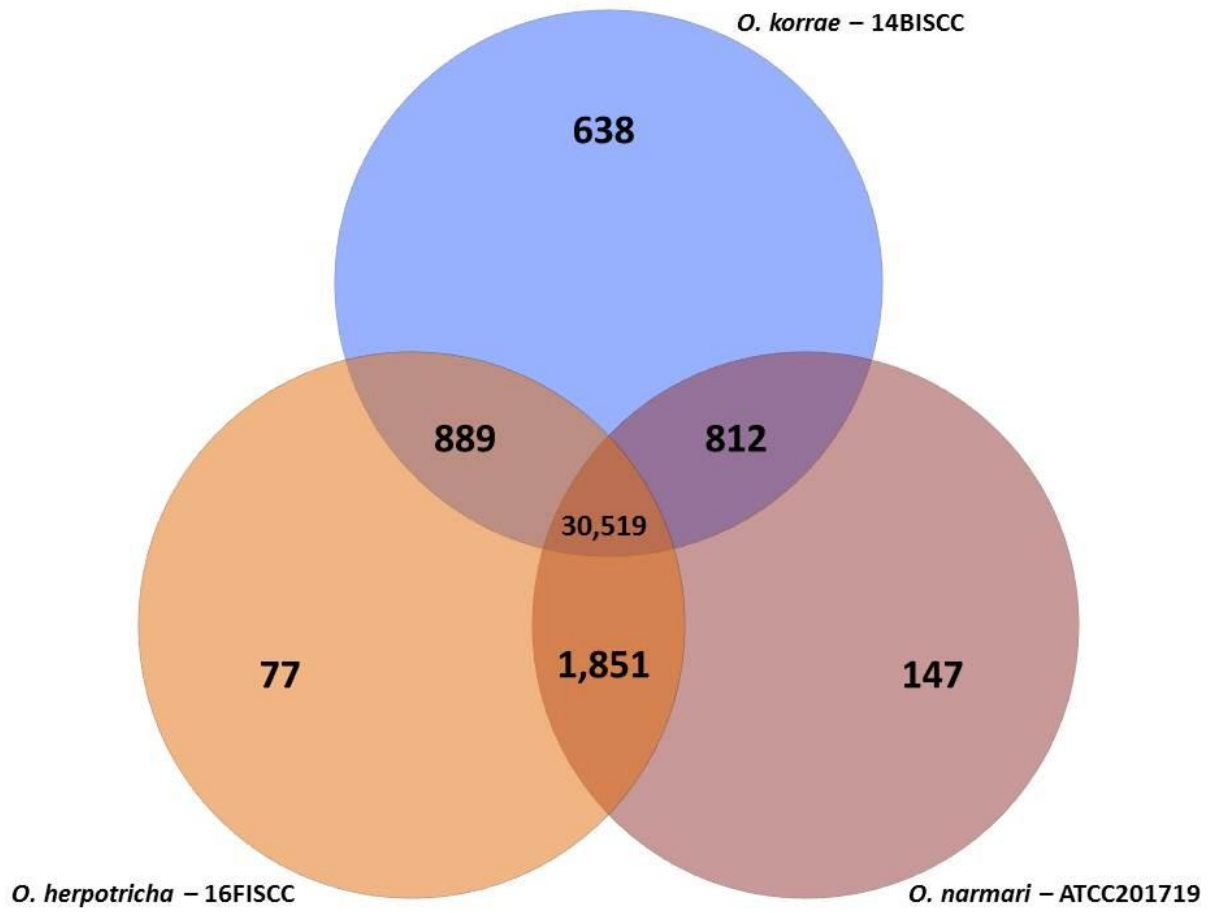

Figure 3. . Venn diagram of the orthologous genes (genes in different species that evolved from a common ancestral gene, and retained function) of *Ophiosphaerella korrae*, *O. herpotricha*, *O. narmari*. The three species share the majority of protein coding genes (~70%) (where three circles overlap). Fewer genes are shared between two species (where two circles overlap). Each species has a lower number of unique genes (where circles do not overlap). Figure areas are not to scale.