

Integrating Modern “Caller ID” with Disease Control



Lee Miller, Ph.D.

University of Missouri

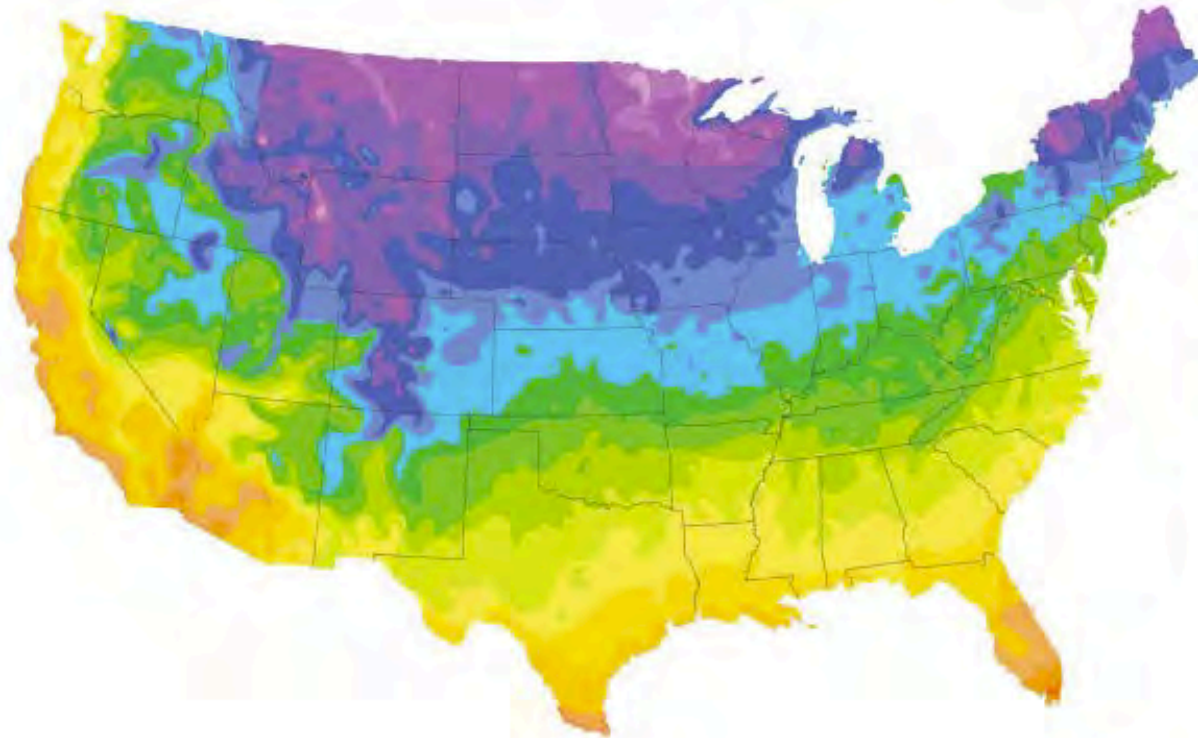


Source: Library of Congress

A New North?

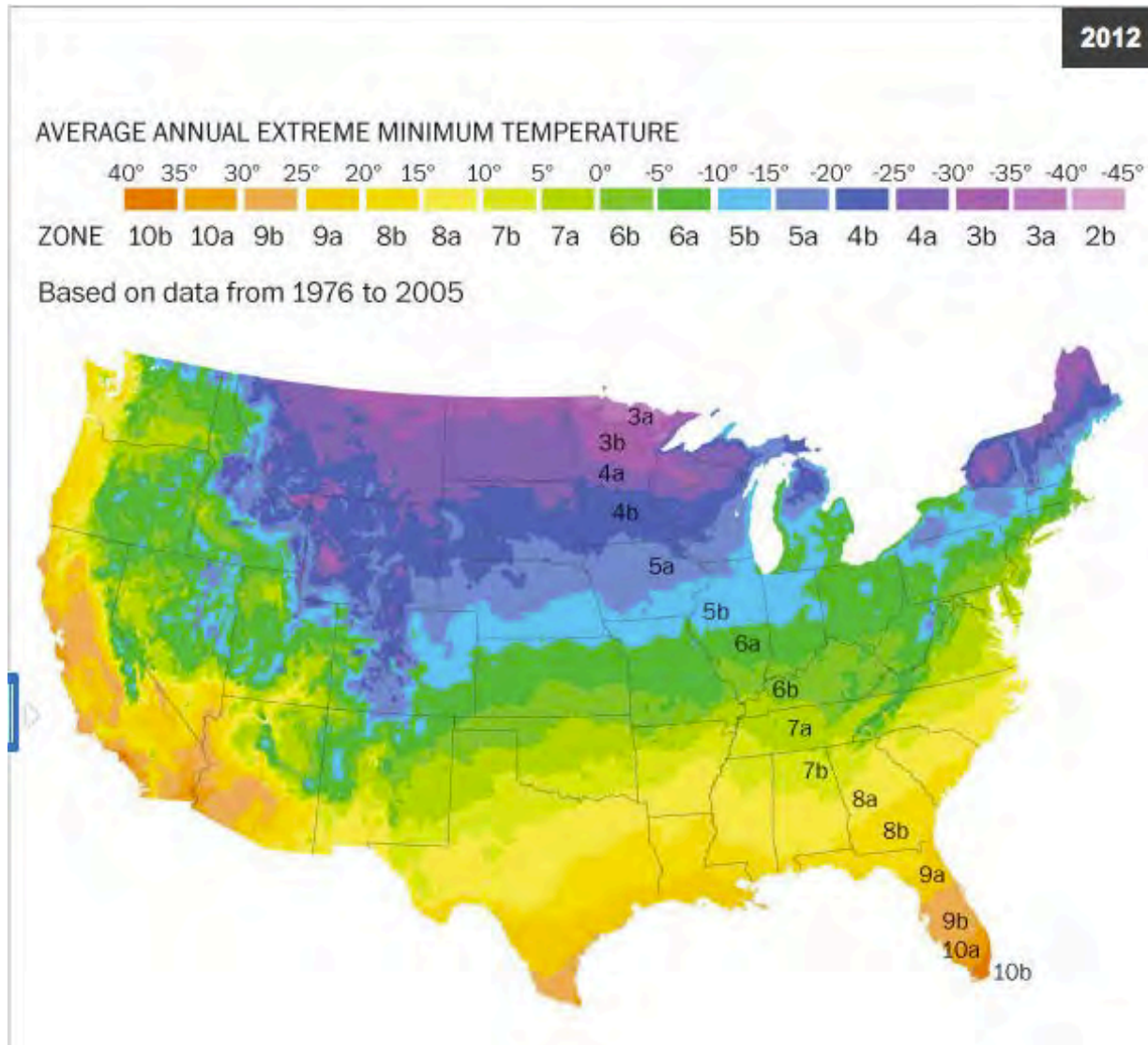
1990

AVERAGE ANNUAL EXTREME MINIMUM TEMPERATURE



Source: Washington Post

A New North?



Source: Washington Post

Case Study

- Mid July – Hot & Dry
- ‘Penn A1/A4’ – 4 year old, sand-based greens
- Soil pH: 7.7
- 1 – 1.5 ft + diameter patches, several with a distinct frog-eye appearance



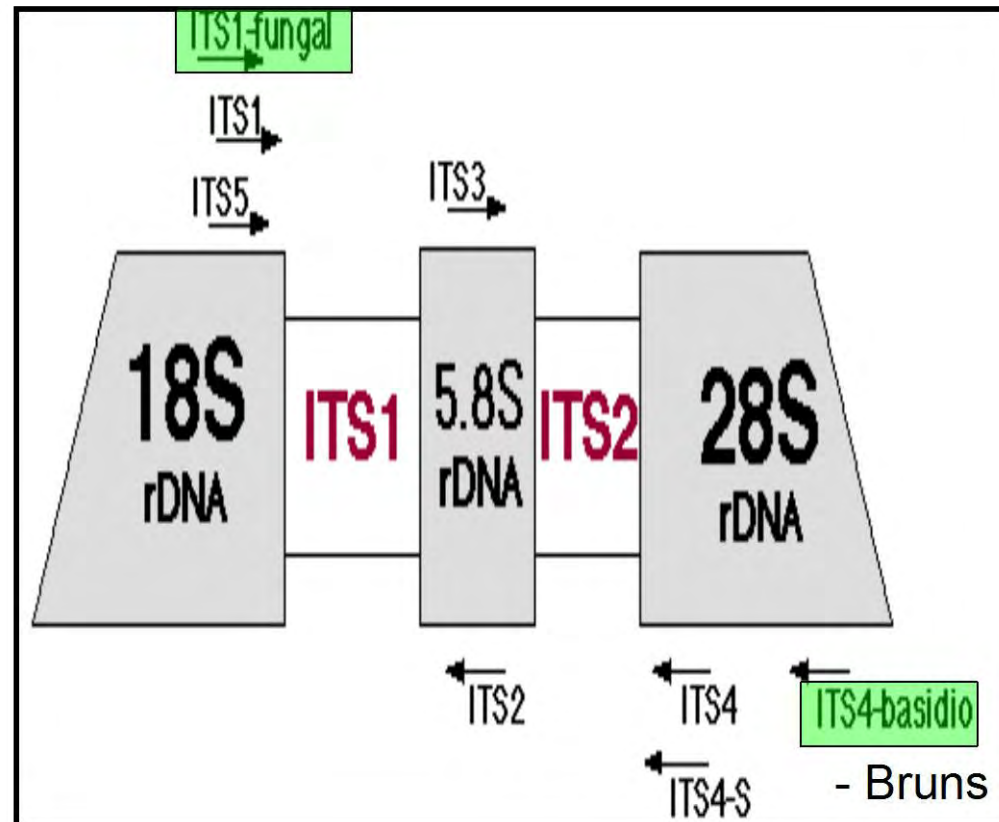


Summer Patch Control on Putting Greens

1. Get it diagnosed.
2. Curative:
 - Avoid use of nitrate-based fertilizers
 - At symptom onset, drench in ~0.1 – 0.2 lb ammonium sulfate in 20 gallons of water to encourage recovery
 - Fungicides*: High rate of QoI fungicides, potentially a DMI/QoI combination like Briskway
3. Preventive
 - Manganese sulfate application in spring? Lean on ammonium based nitrogen sources
 - Fungicides*: Initiate QoI, DMI, or combinations when soil temperatures average 65 F. 2-3 applications necessary.

* All fungicides should be watered in with $\geq \frac{1}{8}$ " of irrigation

Molecular Identification



What is ITS?

- Stands for Internal Transcribed Spacer region, a portion of ribosomal DNA
- Most widely sequenced DNA region in fungi
- Advantages
 - Easy to amplify due to high number of ribosomal DNA genes
 - High degree of variation
- Currently used as evidence for speciation and also as a site for species specific primer development.
- Proposed as the primary fungal Barcode of Life

NCBI Blast:Nucleotide Sequence (984 letters)

blast.ncbi.nlm.nih.gov/Blast.cgi

Descriptions [Provide feedback on the new report](#)

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments [Download](#) [Send to](#) [Graphics](#) [Detailed list of results](#)

Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/> Leptosphaeria korrae 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1251	1251	99%	0.0	89%	AF486626.1
<input type="checkbox"/> Ophiophaerella korrae ribosomal DNA ITS region	944	944	55%	0.0	98%	U04862.1
<input type="checkbox"/> Ophiophaerella herpotricha ribosomal DNA ITS region	883	883	55%	0.0	96%	U04861.1
<input type="checkbox"/> Ophiophaerella korrae 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	880	880	50%	0.0	99%	AF250827.1
<input type="checkbox"/> Uncultured fungus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: IU-FSC Fun09d_FuD022	715	715	54%	0.0	90%	AB520278.1
<input type="checkbox"/> Uncultured fungus clone IIN3-13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	713	713	47%	0.0	94%	EU518981.1
<input type="checkbox"/> Fungal endophyte 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), isolate 3364	669	669	52%	0.0	90%	FN394694.1
<input type="checkbox"/> Fungal endophyte 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), isolate 3284	669	669	52%	0.0	90%	FN392311.1
<input type="checkbox"/> Ophiophaerella sp. 00W11 5S rRNA and partial 18S rRNA genes and ITS1 and 2, isolate OOW11	669	669	53%	0.0	90%	AJ246157.1
<input type="checkbox"/> Uncultured fungus clone LX039935-122-022-C11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	664	664	55%	0.0	89%	GU053811.1
<input type="checkbox"/> Pleosporales sp. JP35 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	658	658	54%	0.0	88%	AB255255.1
<input type="checkbox"/> Ophiophaerella narmari strain CA3 18S ribosomal RNA gene, complete intron sequence	658	658	41%	0.0	95%	AF102181.1
<input type="checkbox"/> Ophiophaerella narmari strain CA1 18S ribosomal RNA gene, complete intron sequence	658	658	41%	0.0	95%	AF102180.1
<input type="checkbox"/> Ophiophaerella narmari strain DAR 20806 18S ribosomal RNA gene, complete intron sequence	658	658	41%	0.0	95%	AF102178.1
<input type="checkbox"/> Phaeosphaeriaceae sp. LM160 18S ribosomal RNA gene, partial sequence	654	654	55%	0.0	88%	EF060518.1
<input type="checkbox"/> Uncultured fungus clone LX039935-122-022-D10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	652	652	55%	0.0	88%	GU053819.1
<input type="checkbox"/> Ophiophaerella narmari strain A16-57A 18S ribosomal RNA gene, complete intron sequence	652	652	41%	0.0	95%	AF102186.1
<input type="checkbox"/> Ophiophaerella narmari strain A16-28A 18S ribosomal RNA gene, complete intron sequence	652	652	41%	0.0	95%	AF102185.1
<input type="checkbox"/> Ophiophaerella narmari strain A11-96A 18S ribosomal RNA gene, complete intron sequence	652	652	41%	0.0	95%	AF102184.1

At last count over 889,000 ITS sequences (!!!) were entered into a Genbank, a searchable online database

First report of *Magnaporthe poae*, cause of summer patch disease on annual bluegrass in Canada

**M.M.I. Bassoriello, M.S., and
K.S. Jordan, Ph.D.**



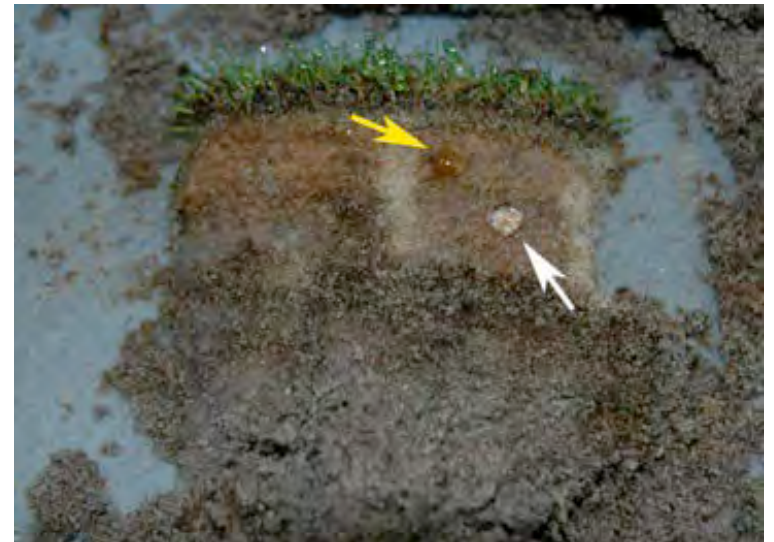
A *Poa annua* root infected by

The ectotrophic, root-infecting fungus *Magnaporthe poae* Landschoot & Jackson, the causal agent of summer patch disease in the United States, is implicated in the damage and loss of annual bluegrass (*Poa annua* L.) on golf course greens. This pathogenic fungus, one of the important root pathogens of turfgrass, attacks and colonizes susceptible turfgrass roots suffering from environmental or cultural stresses.

More than 100 turf samples that exhibited symptoms (chlorotic, circular or irregular patches ≥ 6 inches [≥ 15 centimeters] in diameter with necrotic crowns and discolored roots) reminiscent

Fairy Ring

- Cause a variety of ring types
 - Type III: Basidiocarp formation
 - Type II: Rings of luxuriant growth
 - Type I: Rings of necrotic turf
- Present at various depths in soil profile
- Cause Type I symptoms in a variety of ways
 - Hydrophobicity
 - Direct penetration
 - Ammonium toxicity
 - Insufficient nutrients
 - Toxin production (hydrogen cyanide, agrocybin)
- Associated with a reported 60 + different basidiomycete species



Pathogen Identification



Soil DNA Extraction

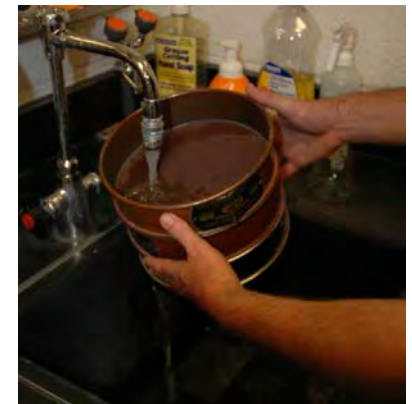
1



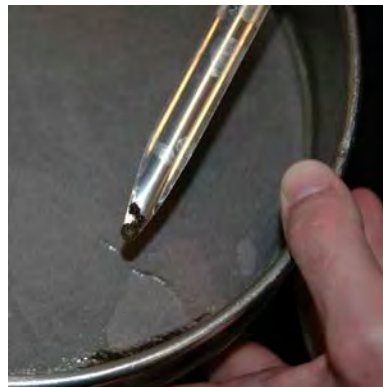
2



3



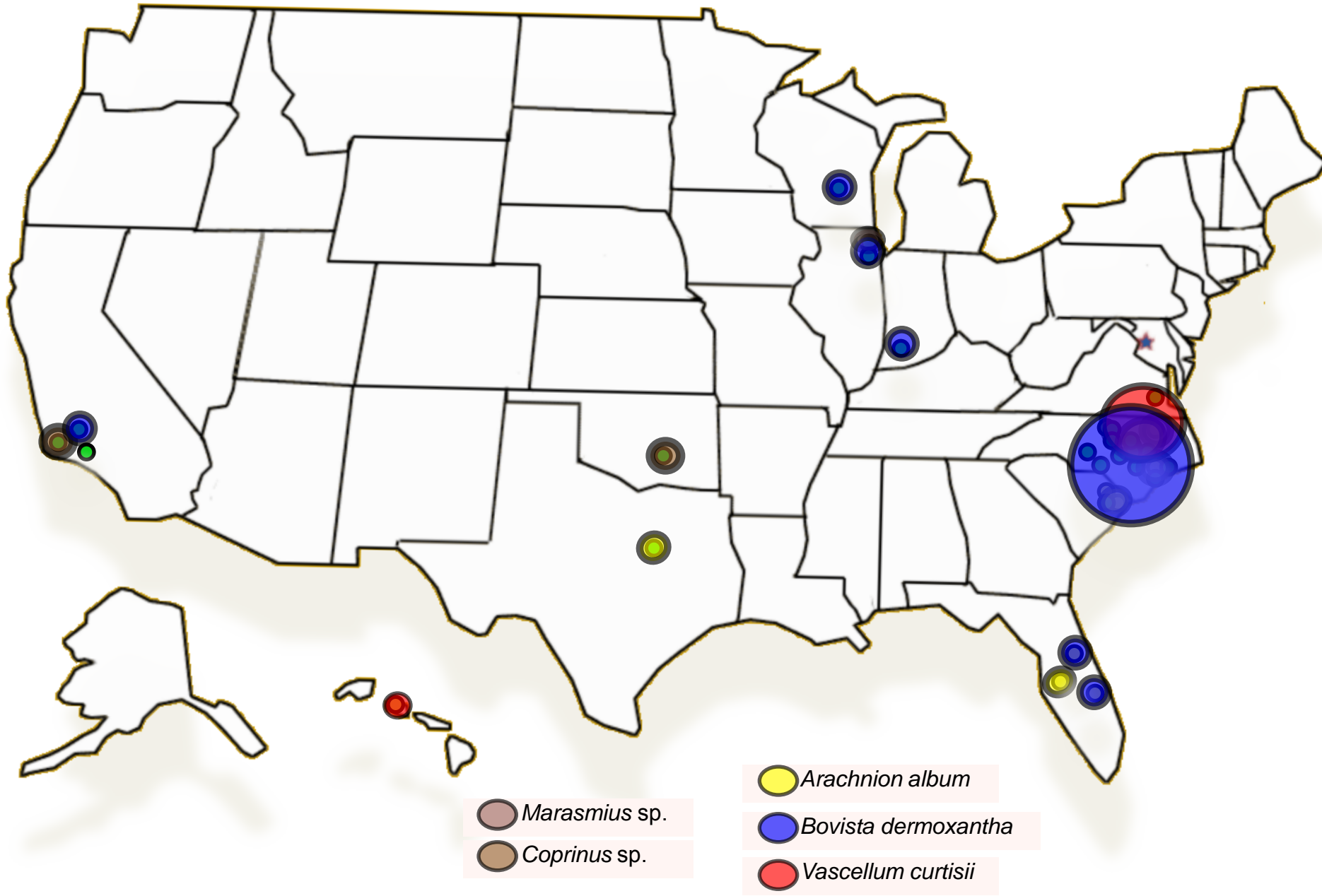
4



5



Distribution Map



Preventive fairy ring control on putting greens

Several DMI fungicides can be effective for preventive control of fairy ring on creeping bentgrass greens.



This research was funded in part by the Environmental Institute for Golf.

Gerald L. Miller, Ph.D.
Michael D. Soika
Lane P. Tredway, Ph.D.

Fairy rings are a severe disease problem on golf courses and other highly maintained turf areas. Symptoms appear along the outer margin of a developing subsurface fungal colony, where the density of mycelium is greatest. Rings can exhibit three types of symptoms, all of which can be observed in an affected area at the same time (7). The most severe symptom (type I) leaves necrotic bands of turf from 4 to 12 inches (10 to 30 centimeters) wide and up to 15 to 30 feet (4.5 to 9 meters) in diameter. These necrotic bands are most commonly an artifact of drought-stressed turf, caused by a combination of dense fungal mycelium and the production of organic acids that coat sand particles and render the underlying soil hydrophobic.

A second symptom of fairy ring is the stimulation of lush green turf growth in rings or arcs (type II) caused by release of plant-available nitrogen. Ammonia levels may reach toxic levels, contributing to plant mortality (2,8). Fairy rings may also produce circles of basidiocarps (type III), which have no direct effect on turf health but can negatively affect appearance and playability.

Nearly 60 different basidiomycete fungi have been implicated in fairy ring occurrence (1). However, the fungus responsible for a fairy ring outbreak on a green is usually unknown because routine mowing inhibits formation of the mature basidiocarp (mushroom or puffball) required for traditional identification. *Marasmius oreades* is the most researched and most commonly named causal agent of fairy ring damage. However, in a recent study, *Arachnion album* Schwein., *Bovista dermosanthes* (Vittad.) De Toni, (= *Lycoperdon dermosanthes* Vittad.), and *Vasellum curtisii* (Berk.) Kreisel (= *L. curtisii* Berk.) were more commonly observed in association with type I and type II fairy ring symptoms on sand-based greens in the southeastern United States (5). The different fungi involved could differ in their sensitivity to fungicides, resulting in inconsistencies in prod-

uct performance across locations.

Fairy ring control with fungicides

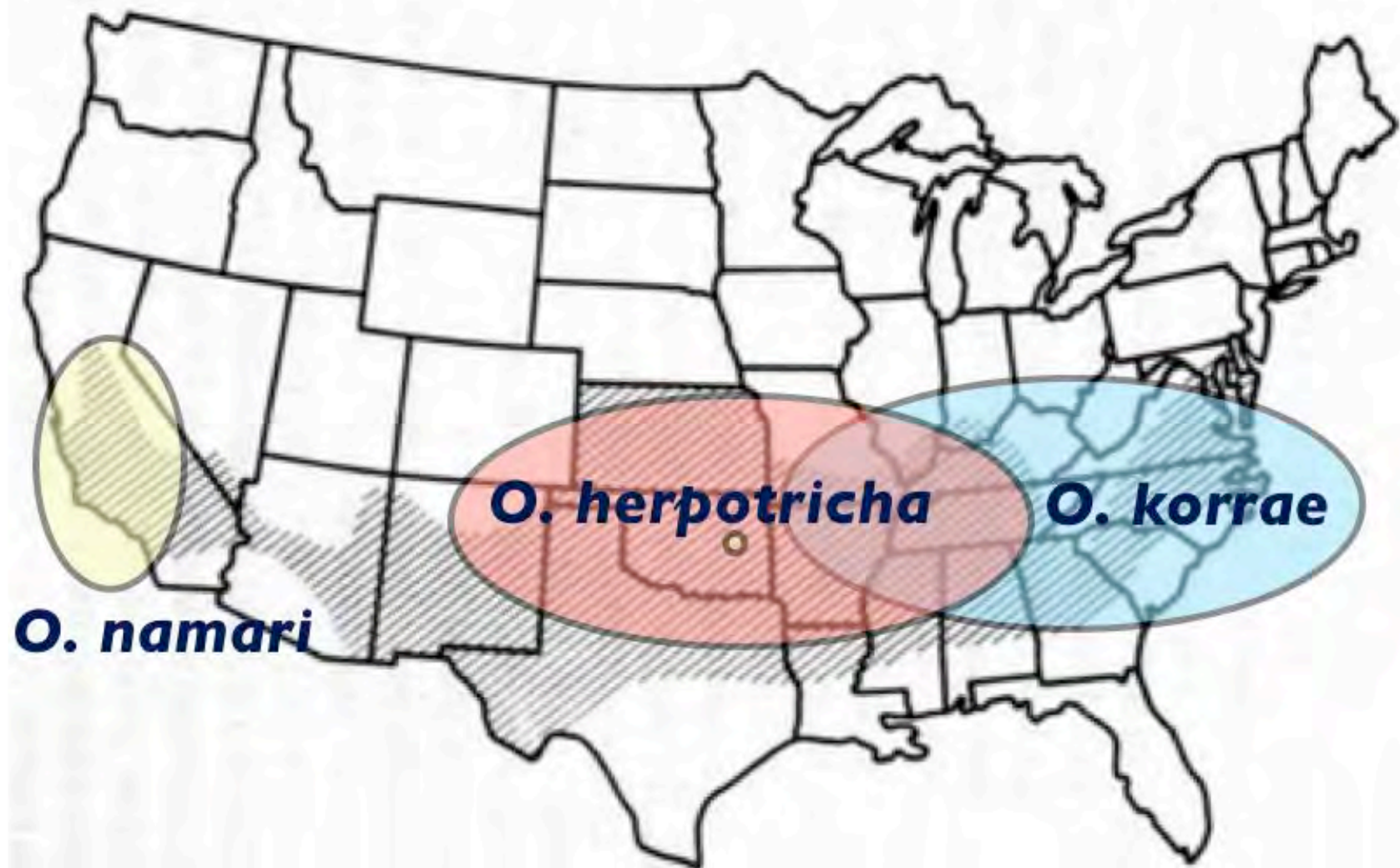
Fungicides that target basidiomycetes, such as



Three symptom types of fairy rings (top to bottom): necrotic rings (type I) most often caused by water repellency imparted on the soil; green rings of luxuriant growth (type II) caused by the release of plant-available nitrogen; and puffballs (basidiocarps associated with a type II fairy ring) on a TifEagle bermudagrass green. Photos by G.L. Miller

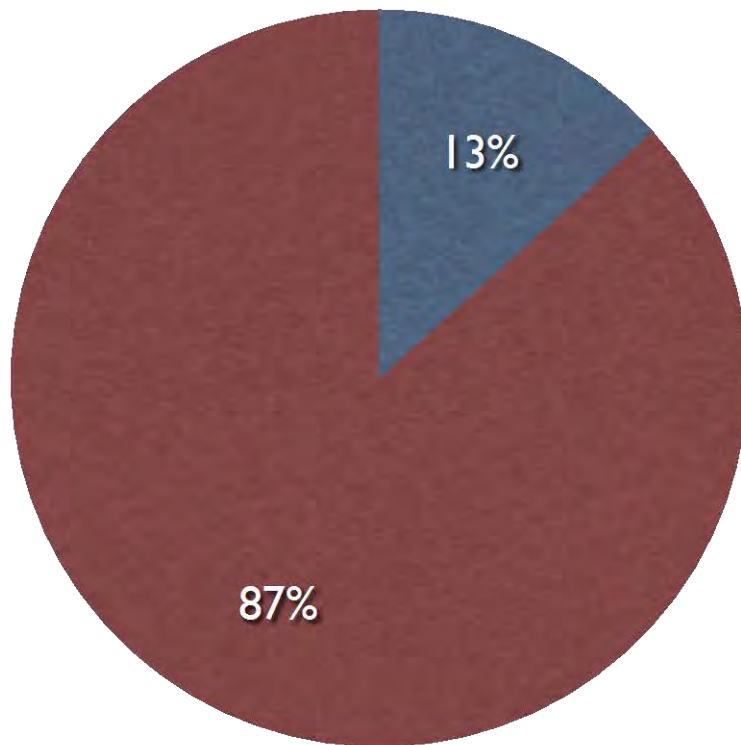
Ultradwarf Bermuda Greens






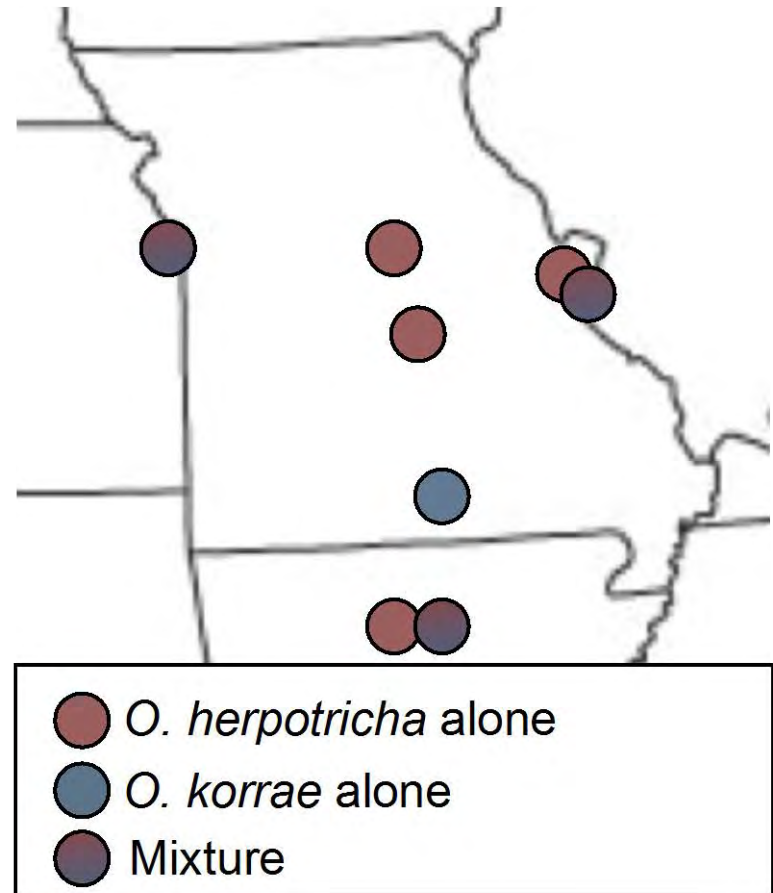


SDS Pathogen in Missouri?

Spring Dead Spot – ITS Use for Distribution



-  *O. korrae*
-  *O. herpotricha*
-  *O. namari*



Root infecting *Pythium* spp.

Funding provided by GCSAA -EIFG Chapter Cooperative Research Program
Heart of America, Ozark, & Wisconsin GCSA Turf Chapters

OBJECTIVES

- Distribution study of *Pythium* spp. in the Midwest on bentgrass putting greens
- Develop a rapid diagnostic assay to determine *Pythium* spp.
- Inoculate bentgrass in ***field*** microplots with most prevalent *Pythium* spp. and develop control measures.

Research initiated on June 1, 2012. Based in Missouri, John (JB) Workman dissertation project.

Thank You for Your Attention & Any Questions??

