A Biocontrol Strategy for Increasing Resistance to Spring Dead Spot in Bermudagrass

Michael P. Anderson
Oklahoma State University

Objectives:

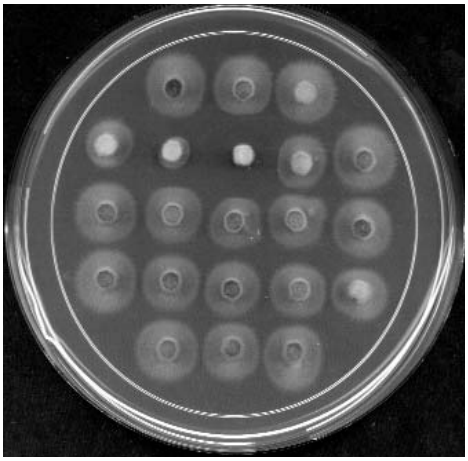
1. Isolate and identify additional antagonistic endophytes or rhizosphere competent bacteria with antagonistic properties against *Ophiosphaerella herpotricha*.
2. Develop a successful biotization strategy to introduce stable colonizing endophytes into bermudagrass tissues.
3. Screen the biotized endophyte-plant associations selected in objective 2 in the field for effectiveness in inhibiting the development of SDS.

Start Date: 2003
Project Duration: three years
Total Funding: $60,000

Spring dead spot (SDS) is a major disease that afflicts bermudagrass throughout the northern range of its adaptation. The disease is caused by three root-rotting fungi: *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari* all of which are found within the United States.

As the name suggests, symptoms appear in the spring as unsightly patches of dead and dying turf anywhere from and six inches to three feet in diameter. Modified cultural practices and improved plant resistance help, but do not prevent disease occurrence. Here we proposed the development of a novel biocontrol strategy against SDS.

We found a *Bacillus subtilis* strain that strongly inhibited the growth of *O. herpotricha* in the laboratory. We have isolated, identified, and characterized some of the active ingredients, which includes several antifungal peptides. We plan to use this agent to try to improve control measures.



Oklahoma State University research found a Bacillus subtilis *strain that strongly inhibited the growth of* O. herpotricha *in the laboratory. They have isolated, identified and characterized some of the active ingredients, which includes several antifungal peptides.*

One problem with conventional biocontrol is that the introduced agent must compete with an already established microflora. Biotization may increase the competitive ability of the introduced agents by giving it a leg up on the competition. This is done by inoculating the seedling with the biocontrol agent prior to planting in a soil medium. The theory behind the biotization strategy is that the first microorganisms to colonize the rhizosphere will have the competitive advantage over the latecomers.

Another improvement in biocontrol may involve antagonistic endophytes. Endophytes are microorganisms that are already well adapted to living within plant tissues. In this project, we plan to isolate antagonistic bermudagrass endophytic bacteria and biotize them at high concentrations into the bermudagrass seedlings prior to planting. We think the combination of biotization with antagonistic endophytes will produce an effective strategy to supplement improvements in plant resistance and cultural practices.

Over 1200 endophytes from both resistant and susceptible varieties of bermudagrass were isolated. Identification of 223 bermudagrass endophytes through the sequencing of the 16S ribosomal gene showed that bermudagrass endophytes are represented by over 21 genera of bacteria. Only four phyla were represented with over 96% of the endophytes belonging to two phyla: the Actinobacteria and the Proteobacteria. The 223 were screened for antifungal activity which resulted in 35 isolates showing moderate to high levels of activity. The most highly active bermudagrass endophytes were predominately from the genus of *Pseudomonas*, while the more moderate activity were from *Stenotrophomonas* and *Enterobacter*. Only one *Microbacteria* and a *Bacillus* species showed significant activity.

Researchers believe that by inoculating bermudagrass seedlings with Bacilus subtilis, *this may make the bermudagrass host resistant to infection by the SDS pathogens.*

In addition to the work on endophyte isolation, we are also developing a detection strategy including RSGP arrays: RT-PCR and TGGE. These detection strategies will be essential to quantitatively evaluate the results of the biotization procedure. In addtion we have reinoculated field plots at the OSU Turfgrass facility in anticipation of field screening of biotized seedlings as outlined in objective 3.

Summary Points

- Isolated 1200 and identified a subset of 229 bermudagrass endophytes from root tissues.
- Screened 223 endophytes for antifungal activity against *O. herpotricha*.
- Developing the biotization strategy to introduce the antifungal endophytes into bermudagrass seedlings.
- Infected field plots for screening of bermudagrass seedlings for biocontrol purposes .