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

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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.

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Project Duration: three years
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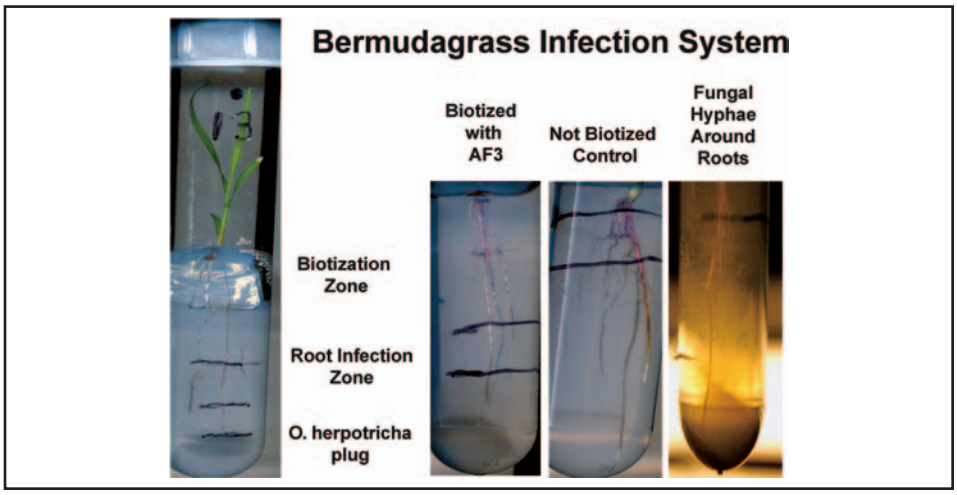
Spring dead spot (SDS) is a major turf disease that afflicts bermudagrass throughout the northern range of bermudagrass adaptation. The disease is caused by three root-rotting fungi: *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*.

Endophytes are microorganisms that are already well adapted to living within plant tissues. We plan to isolate antagonistic endophytic bacteria from bermudagrass and biotize them at high concentrations into the bermudagrass seedlings prior to planting. The combination of biotization with antagonistic endophytes may produce an effective strategy to supplement improvements in plant resistance to SDS.

This research constitutes the first attempt to identify and characterize endophytes in bermudagrass species. Bermudagrass endophytes were classified into over 21 genera within four phyla. Over 96% of the endophytes belonged to two phyla, namely: the actinobacteria and proteobacteria with a few isolates associated with the firmicutes and bacteroidetes.

Over 400 endophytes were screened for antifungal activity against *O. herpotricha*. Thirty-one isolates showing moderate to high levels of activity were identified. Results from our assay revealed that the highly active bermudagrass endophytes were predominately from the genus *Pseudomonas*, with more moderate activities in *Stenotrophomonas* and *Enterobacter*. These results indicate that the *Psuedomonas spp*., which are known to possess biocontrol capability, are the most potent endophytes found in bermudagrass in this study.



At Oklahmoma State University, a rapid laboratory screening procedure was developed to examine root infection under controlled environmental conditions.

Bermudgrass endophytic isolates were obtained from both infected and non-infected resistant 'Midlawn' and susceptible 'Tifgreen varieties'. Results showed that of the 31 antifungal bermudagrass endophytes, 14 came from resistant 'Midlawn' and 17 came from susceptible 'Tifgreen'. The resistant variety did not show greater numbers of antifungal isolates as originally hypothesized when compared to susceptible, suggesting that total numbers of antifungal endophytic isolates may not be a contributing factor in the natural resistance mechanism.

A rapid laboratory screening procedure was developed to examine root infection under controlled environmental conditions. Bermudagrass seedlings were grown and infected in a clear agar-based medium inoculated with *O. herpotricha*. This system provides both reliable infection and visualization of the infection progress. Once infection occurred, the roots characteristically turned brown to black reflecting the ectotrophic growth of the fungus and defense reaction by the roots. Control reactions without fungus show no browning indicating that the response is not due to the growth conditions. High powered image analysis software was used to measure the degree of root browning as an indicator of infection.

Summary Points

- Isolated 1200 and identified a subset of 229 bermudagrass endophytes from roots.
- Screened 225 endophytes for antifungal activity against *O. herpotricha*.
- The number of endophytic isolates was reduced in infected bermudagrass roots compared to non-infected roots.
- Resistant bermudagrass did not contain greater numbers of antifungal isolates compared to susceptible bermudagrasses.
- Discovered an additional 16 very potent antifungal endophytes.
- Developed an agar-based tube biotization and infection system for screening of biocontrol potential under controlled environmental conditions.
- Application of biocontrol agent AF1 to the field did not reduce disease symptoms in the following year.
- *In vitro* screen used to identify SDS-resistant types within seeded populations.