Infection and Colonization of Bermudagrass by Ophiosphaerella *Species, the Causal Agents of Spring Dead Spot*

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Objectives:

1. Transform *Ophiosphaerella korrae* to express green (GFP) and red fluorescent (tdTom) proteins.
2. Compare and contrast infection and colonization of roots and stolons/rhizomes of resistant and susceptible interspecific hybrid, common, and African bermudagrasses by *O. herpotricha* and *O. korrae* that express fluorescent proteins.

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Bermudagrasses in the transition region will undergo cool-temperature induced dormancy during winter months. In this region, spring dead spot is the most devastating and important disease of bermudagrass. The disease is caused by any one of three fungal species in the genus *Ophiosphaerella* (*O. herpotricha, O. korrae, or O. narmari*). The disease results in unsightly dead patches in the spring on bermudagrass fairways, tees, and greens and the patches can persist for months.

A critical limitation to the study of turfgrass diseases that occur on roots, stolons, and crowns in the soil, such as spring dead spot, is the inability of researchers to rapidly and easily study the plant-fungus disease interaction. This is a result of the interaction occurring below ground and often inside of plant organs. The overall goal of this study is to enhance our understanding of the interaction between *Ophiosphaerella* species and different bermudagrass hosts and to reduce the impact of this disease to bermudagrass in the transition zone.

Spring dead spot is the most devastating and important disease of bermudagrass in the transition zone.

The green or red fluorescing O. korrae *transformants are currently being used to study growth and colonization on several different bermudagrass varieties that differ in their tolerance to the disease.*

To genetically transform *O. korrae* to express fluorescent protein genes, an *Agrobacterium tumefaciens*-mediated transformation system that we previously optimized for *O. herpotricha* was utilized. A gene for green fluorescent protein (GFP) under the control of the ToxA constitutive promoter with a hygromycin-selectable marker gene was moved into the genome of the fungus. A second cassette encoding the red fluorescent protein gene, tdTomato also driven by the ToxA promoter, was used to generate red transformants.

Colonization of bermudagrass roots by the transformant fungi was compared to the wild-type to verify comparable phenotypes. The green or red fluorescing *O. korrae* transformants are currently being used to study growth and colonization on several different bermudagrass varieties that differ in their tolerance to the disease. Studies are also being conducted to determine if there are differences in these events between *O. herpotricha* and *O. korrae* and their interactions with the various bermudagrasses.

The studies with *O. korrae* are in their early stages and ongoing. However, to date no significant differences have been observed for root colonization between the two fungal species. We will also examine the interaction using the confocal microscopy which permits examination of the fungal colonization not possible through traditional microscopy. With the fungal transformants we have also begun examining the release of reactive oxygen by the plants in response to fungal colonization. This response is part of the plants' defense against fungal invasion.

This basic information on how the cultivars react to the fungus will improve our ability to enhance and deploy host-plant resistance through traditional breeding efforts at Oklahoma State University.

Summary Points

- Transformants of *O. korrae* have been generated and are being use to study growth and colonization on several different bermudagrass varieties.
- Confocal microscopy has revealed details of plant-fungal interactions not obtainable through tradition microscopy approaches.
- This information will be used to enhance host-plant resistance through traditional breeding efforts at Oklahoma State University.