Infection and Colonization of Bermudagrass by Ophiosphaerella herpotricha*, a Causal Agent 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.

Start Date: 2010
Project Duration: 3 years
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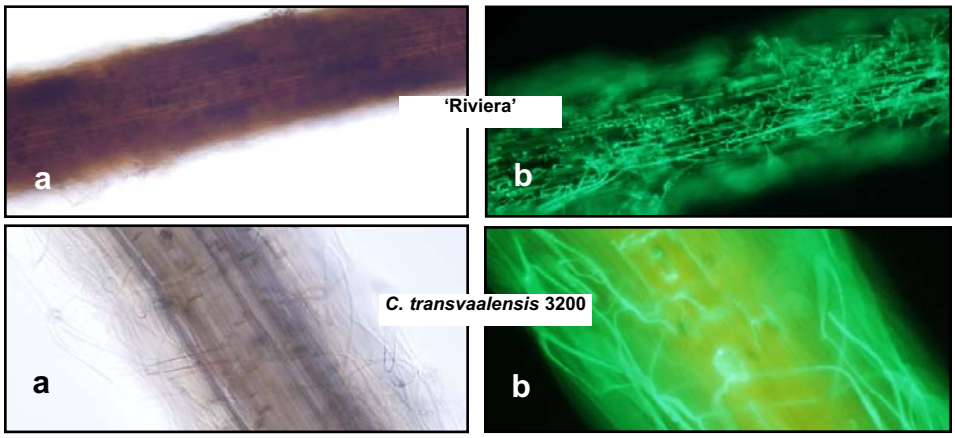

Differing plant reactions to root infection by O. herpotricha *for two species of bermudagrass.* ***Top:*** *'Riviera' illustrating a dark necrotic reaction through bright field microscopy (a), the identical fluorescent image (b) revealing presence of the fungus.* ***Bottom:*** Cynodon transvaalensis *accession 3200 illustrating much less necrosis through bright field microscopy (a), the identical fluorescent image (b) revealing presence of the fungus.*

Bermudagrasses in the transition region of the U.S. 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 root diseases, such as spring dead spot, is the inability of researchers to rapidly and easily study the plant-fungus disease interaction because it happens below ground and often inside of roots. The overall goal of this study is to enhance our understanding of the interaction between *Ophiosphaerella* species and different bermudagrass hosts.

In earlier studies, we inserted two different fluorescent reporter genes (red and green) into *O. herpotricha* and examined root and stolon infection of various bermudagrasses. Studies examined the fungal interactions with two interspecific hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) cultivars, 'Tifway' and 'Midlawn' and a *C. transvaalensis* accession. Current studies with *O. herpotricha* have been expanded to include two *C. dactylon* cultivars, 'Riviera' and 'Jackpot' and an additional *C. transvaalensis* accession (3200). Differences in infection response have been observed for these grasses. Infected 'Riviera' roots had a more extensive and dark necrotic response to the fungus in contrast to accession 3200.

To genetically transform *O. korrae* to express fluorescent protein genes, an *Agrobacterium tumefaciens*- mediated transformation system that we optimized for *O. herpotricha* is being utilized. Using this system, the genes for green fluorescent protein (GFP) under the control of the ToxA constitutive promoter with a hygromycin-selectable marker are being incorporated into the genome of the fungus.

A second cassette encoding the red fluorescent protein gene, tdTomato, driven by the ToxA constitutive promoter, is also being used to generate red transformants. We have repeatedly tried to transform *O. korrae* to express both these cassettes. While positive control fungi have been readily transformed, the several strains of *O. korrae* used have remained recalcitrant to transformation, and no fluorescent transformants have been obtained. The failure of these efforts demonstrates the inherent difficulty in transforming this group of fungi. We will adjust transformation conditions to transform this fungus with both proteins. As soon as we obtain green or red fluorescing transformants, their phenotypes will be tested and the growth and colonization of roots evaluated.

Studies using the confocal scanning laser microscope have produced numerous 2 and 3-dimentional still images and video of the fungus in and on bermudagrasses. Images are not possible through conventional microscopy techniques. For example, we can look underneath fungal colonization of bermudagrass stolons to evaluate the plant-fungus interaction. 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

- Plant responses to fungal colonization appear variable across species and hybrids of bermudagrass, which is promising for the identification of resistant germplasm.
- Efforts to transform *O. korrae* are underway; but the correct conditions to transform the fungus have yet to be identified.
- Confocal microscopy has revealed details of plant-fungus interactions not obtainable through traditional microscopy.
- This information will be used to enhance host-plant resistance through traditional breeding efforts at Oklahoma State University.