Research *You Can Use*

Infection and Colonization of Bermudagrass by a Spring Dead Spot Pathogen

Work continues at Oklahoma State University to understand the infection process of spring dead spot.

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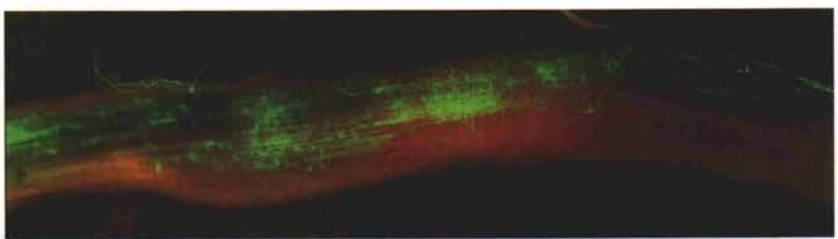
OBJECTIVES

- To incorporate fluorescent protein genes into *Ophiosphaerella herpotricha*, one of the pathogens causing spring dead spot of bermudagrass.
- Evaluate infection and colonization of bermudagrass cultivars by fluorescent *O. herpotricha* at different temperatures.
- Evaluate differences in infection and colonization among bermudagrass cultivars that vary in disease susceptibility.

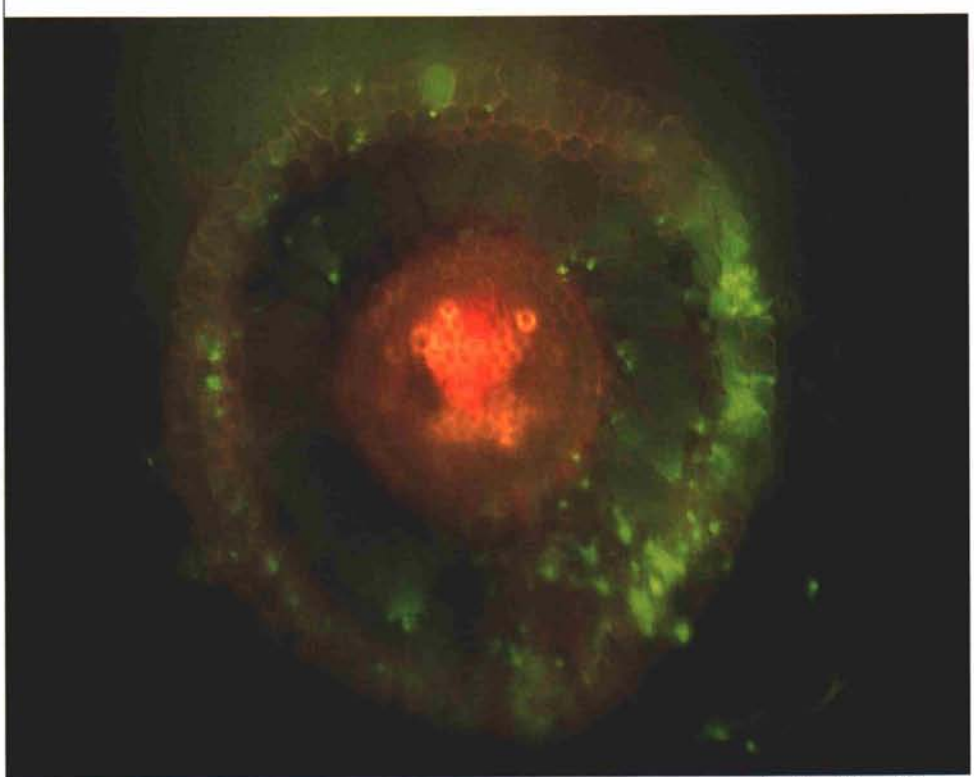
Start Date: 2006
Project Duration: Three Years
Total Funding: $59,684

Spring dead spot (SDS) is the most devastating and important disease of bermudagrass that undergoes winter dormancy. The disease is caused by one or more of three fungal species in the genus *Ophiosphaerella* (*O. herpotricha*, *O. korrae*, or *O. narmari*). The disease causes unsightly dead patches on fairways, tee boxes, and bermudagrass greens, resulting in increased management inputs to eliminate weeds and encourage regrowth of bermudagrass into the dead areas.

Despite the identification of the causal agents of the disease in the 1980s, the underlying factors that ultimately lead to death of the plants remain poorly understood. A critical



O. herpotricha transformant expressing green fluorescent protein (GFP, a visualization gene) is currently being used to follow root infection and colonization of various bermudagrass cultivars at different temperatures. The transformed fungus fluoresces green (40x).



Transverse section of infected Tifway root reveals extensive internal necrosis and cell wall breakdown of cortical cells corresponding with colonization by *O. herpotricha* expressing GFP. The transformed fungus fluoresces green and the vascular bundle autofluoresces red (200x).

Spring dead spot is caused by one or more of three fungal species (*O. herpotricha, O. korrae*, or *O. narmari*) and is the most devastating disease of bermudagrass.

limitation to the study of turfgrass root diseases is the inability of researchers to rapidly and easily study the plant-fungus interactions because they occur below ground and often inside of roots. The overall goal of this study is to enhance our understanding of the interaction between *O. herpotricha* and its bermudagrass host and how environmental factors influence this interaction for the development of strategies for more effective disease control.

Through the insertion of genes into the fungus, transgenic isolates of *O. herpotricha* expressing fluorescent protein genes (visualization genes) have been generated and are currently being used to follow root infection and colonization of various bermudagrass cultivars at different temperatures (conducive and non-conducive). Root necrosis surrounding fungal hyphae was observed for the susceptible cultivars Tifway and Jackpot 10 days after inoculation. Only minor root discoloration was observed around hyphae of the more resistant Midlawn cultivar. Transverse sections revealed extensive internal necrosis and infection of Jackpot and Tifway root cortices. In contrast, infection of Midlawn appeared limited to the outermost cortical cells, and these cells did not appear necrotic. No vascular infection by *O. herpotricha* was observed in any of the cultivars examined.

Future studies will utilize a confocal scanning laser microscope that can optically "section" infected roots, producing three-dimensional images of the fungus as it moves on and into bermudagrass roots. We expect to further observe cellular differences in the infection and colonization of bermudagrass cultivars that differ in susceptibility to *O. herpotricha*. This basic information on how the cultivars react to the causal fungus will improve our ability to enhance and deploy host-plant resistance through traditional breeding efforts at Oklahoma State University.

SUMMARY POINTS

- Fluorescent transgenic fungi have been generated.
- These fluorescent fungi are being used to study the progression of disease in bermudagrass varieties that differ in susceptibility to the disease. Susceptible varieties display more extensive root cortical cell necrosis associated with fungal invasion than that observed in a resistant variety.
- These fluorescent fungi also are being used to study the progression of disease under conducive and non-conducive temperatures regimes.
- This information will be used to enhance host-plant resistance through traditional breeding efforts at Oklahoma State University.

CONNECTING THE DOTS

An interview with the authors regarding their investigations into the infection and colonization of bermudagrass by a spring dead spot pathogen.

Q: *Using fluorescent protein genes expressed in the pathogen to visualize infection seems like a very ingenious way to track the infection and colonization process. Where did you learn about this approach and has it been used in other pathogen/host systems?*

A: This approach has been used to study fungi, bacteria, and viruses that cause diseases of many important crop plants such as rice, wheat, and vegetables. Early on, this approach was not widely used, but now it has become very common and is being applied to a large range of plant pathogens.

Q: *Where did these fluorescent protein genes come from and how difficult was it for you to transform* O. herpotricha *with them?*

A: The green fluorescent protein was originally obtained from a jellyfish and the red proteins from a sea coral. Now these genes can be purchased from commercial sources, obtained from colleagues, or directly synthesized, which is how we produced the red protein gene, tdTomato. To express these genes in fungi, they must be engineered with a fungal gene promoter and then introduced into the fungus's genome by transformation. The transformation of *O. herpotricha* was very difficult at first. We tried several different approaches that were successful for other fungi, but not for *O. herpotricha*. In time, we overcame the difficulties, and now we can do the transformation on a fairly regular basis and have expanded our efforts to other similar fungi.

Q: *Spring dead spot is a devastating disease of bermudagrass and one that still holds mysteries regarding its management. How do you envision that your work in establishing the infection and colonization process of the pathogen(s) will help in the overall understanding of this disease with regard to managing and controlling it?*

A: Our efforts are aimed at shedding light on the disease system. So far, we have learned how the fungus penetrates root cells and how the fungus moves through the root, causing cortical cell death as it progresses. We have seen how the plant reacts directly and indirectly to the pathogen. If we better understand how the fungus is interacting with the plant, this may give us greater insight into mechanisms of plant resistance or tolerance to the pathogen.

Q: *From previous work to date, it appears that more cold-tolerant bermudagrass cultivars are more resistant to infection and colonization by the spring dead spot pathogen. Is this your view, and what does that tell us about the interaction of infection by these pathogens and cold hardiness of bermudagrass?*

A: We knew very little about the direct infection and colonization of bermudagrasses in the past. Often we were limited to seeing dead patches and the necrotic roots, crowns, and rhizomes. Now we can directly visualize infection and colonization of cultivars, including the more cold-tolerant cultivars. Yes, based on our studies, the more cold-tolerant cultivar Midlawn is colonized to a lesser extent than Tifway 419, which is less cold tolerant. So it appears that there is a correlation between reduced colonization and greater cold tolerance.

Q: *You mention that this work will help Oklahoma State University's traditional breeding efforts to produce greater host-plant resistance in future bermudagrass releases. Please explain how that will work. Does that mean each potential new bermudagrass release will be screened for SDS host-plant resistance using this visualization-gene method?*

A: Previously, to evaluate a new prospective bermudagrass, it was established in the field, inoculated with the fungus, and, after several years, disease symptoms could be evaluated. In addition to the time required to conduct the assay, much has been invested in the prospective line only to find out late in the selection process that it may not be very resistant. With some of the new approaches we are attempting to develop, we may be able to screen germplasm many years in advance of field release, and we can pre-screen accessions for resistance and incorporate those lines into the breeding program for selection of the resistance trait. These new approaches take weeks, not years.

Q: *How important is this technique in understanding host-pathogen interactions, and do you feel other turfgrass diseases can be studied this way? If so, what other turfgrass disease complexes do you feel would benefit from this approach?*

A: The use of transgenic pathogens that express fluorescent proteins or visualization genes has been extremely important in the study of many different disease systems. Many investigators have been able to document the infection of plants by pathogens, movement of pathogens in the host, and reproduction of the pathogens using this technique. There are many other turf diseases that could be studied using this technique and not just the soil-borne diseases. I expect that there would be new information gained about other important diseases of turfgrass such as dollar spot and those caused by *Rhizoctonia* pathogens if transgenic isolates of those pathogens were produced.

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