2015-06-521

Title: Understanding endophyte-mediated dollar spot resistance in red fescue as a new approach to improving management of dollar spot in creeping bentgrass

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Objectives of the project: The goal of this project is to understand the mechanism behind the well-established phenomenon of endophyte-mediated dollar spot resistance in strong creeping red fescue. This mechanism could possibly be developed as a method for control of dollar spot on creeping bentgrass.
Start Date: 2015
Project Duration: 3 years
Total Funding: $90,000

Summary Text:

Control of dollar spot disease on creeping bentgrass is a major problem for golf course managers and currently relies heavily on fungicide applications. Ongoing efforts to address this problem have focused on breeding tolerant cultivars and on improving management protocols. We are pursuing a different and complementary approach, which is to understand the mechanism of dollar spot resistance in fungal endophyte (*Epichloë festucae*) infected strong creeping red fescue. Endophyte-mediated disease resistance is well established in fine fescues (Clarke et al., 2006), but is not a general feature of other endophyte-infected grasses such as perennial ryegrass or tall fescue. If we can uncover the mechanism of the endophyte-mediated disease resistance in fine fescues, it may be possible to adapt it for use in other turfgrasses such as creeping bentgrass, which are not infected with *Epichloë* endophytes.

Previously we identified an abundant endophyte transcript for an antifungal protein. The antifungal protein gene found in *E. festucae* infecting strong creeping red fescue is not present in most *Epichloë* genomes for which whole genome sequence is available (Ambrose and Belanger, 2012). The transcript abundance and the limited existence of the antifungal protein gene among *Epichloë* spp. suggested the *E. festucae* antifungal protein may be a component of the unique endophyte-mediated disease resistance observed in strong creeping red fescue.

We partially purified the antifungal protein from the strong creeping red fescue apoplastic proteins and showed it did have activity against the dollar spot fungus in a plate assay. The abundance of the antifungal protein among the secreted proteins in the endophyte-infected strong creeping red fescue plants suggests it could come into contact with invading fungal pathogens and may therefore be an important factor in the fungal disease resistance observed in endophyte-infected strong creeping red fescue. We also expressed the antifungal protein in the yeast *Pichia pastoris* and partially purified it from the yeast culture filtrate. The partially purified recombinant antifungal protein also had activity against the dollar spot fungus in a plate assay.

As a first step in uncovering the mechanism of action of the antifungal protein we used the viability stains SYTOX Green and Evans blue, which are dyes that can only enter cells that have damaged membranes. Treatment of the dollar spot fungus with the antifungal protein resulted in uptake of the SYTOX Green (Fig. 1) and Evans blue (Fig. 2) dyes, visualized by the green fluorescence and deep blue color, respectively. In contrast, treatment with the empty vector control proteins had no effect. These results

suggested that the antifungal protein inhibited growth of the pathogen by causing damage to the plasma membranes.

The results described above have been published (Tian et al., 2017a,b). The results we have obtained from the *E. festucae* antifungal protein support the hypothesis that it may be a component of the disease resistance seen in endophyte-infected strong creeping red fescue.

The next important question is whether the endophyte antifungal protein can protect the host plant from dollar spot infection. Now that we have confirmed that the endophyte antifungal protein by itself can inhibit growth of the dollar spot fungus we will test its activity on creeping bentgrass and endophyte-free strong creeping red fescue inoculated with the dollar spot fungus. If the antifungal protein is effective in protecting creeping bentgrass from dollar spot, then it could be developed as an alternative method to reduce synthetic fungicide use.

References

Ambrose, K.V., Belanger, F.C. (2012) SOLiD-SAGE of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. PLoS ONE 7(12):e53214

Clarke, B.B., White, J.F. Jr., Hurley, R.H., Torres, M.S., Sun, S., Huff, D.R. (2006) Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant Disease 90:994-998

Tian, Z., Wang, R., Ambrose, K.V., Clarke, B.B., and Belanger, F.C. (2017a) Isolation of a potential antifungal protein produced by *Epichloë festucae*, a fungal endophyte of strong creeping red fescue. International Turfgrass Society Research Journal 13: 233-235

Tian, Z., Wang, R., Ambrose, K.V., Clarke, B.B., Belanger, F.C. (2017b) The *Epichloë festucae* antifungal protein has activity against the plant pathogen *Sclerotinia homoeocarpa*, the causal agent of dollar spot disease. Scientific Reports 7:5643

Summary Points:

1. The fungal endophyte (*Epichloë festucae*) that infects strong creeping red fescue produces an abundant antifungal protein that is not found in most *Epichloë* species. It may be involved in the disease resistance observed in endophyte-infected strong creeping red fescue.

2. We have partially purified the antifungal protein from the infected plant and from expression in the yeast *Pichia pastoris*. The partially purified protein had inhibitory activity against the dollar spot fungus.

3. The antifungal protein may act by damaging the membranes of the dollar spot fungus as evidenced by the uptake of dyes that only enter damaged cells.

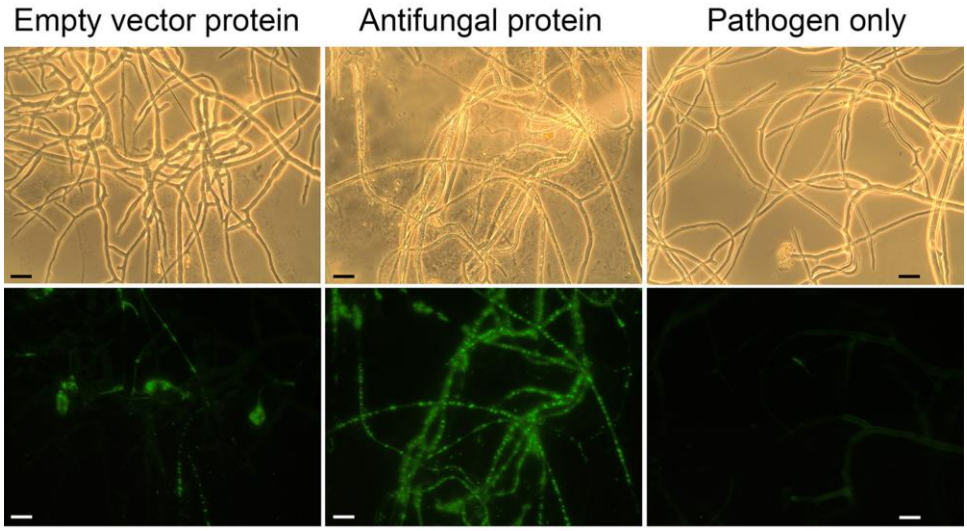

Fig. 1. Microscopy of dollar spot mycelium treated with the purified antifungal protein and empty vector control proteins purified from the yeast culture filtrates. The dollar spot fungus was grown in potato dextrose broth for 2 days and then 17 μg of the antifungal protein or empty vector control proteins were added. After 2 hours of incubation the sample was examined by fluorescence microscopy in the presence of 12 μM SYTOX Green. The upper panels are bright-field images and lower panels are fluorescence images of the same fields. The scale bar is 20 μm.

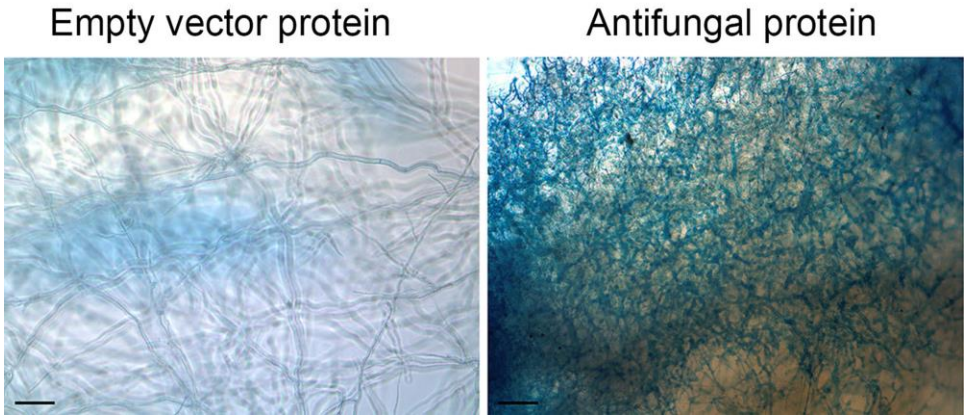

Fig. 2. Microscopy of dollar spot mycelium treated with the purified antifungal protein and empty vector control proteins purified from the yeast culture filtrates. The dollar spot fungus was grown in the center of a microscope slide and was treated with 35 μg of the purified antifungal protein or empty vector control proteins for 1 day and then stained with Evans blue. The scale bar is 100 μm.