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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Dollar spot is one of the most problematic diseases for many golf courses, particularly on creeping bentgrass, often requiring repeated applications of fungicides. In addition to creeping bentgrass, dollar spot can also be a problem on strong creeping red fescue. However, when strong creeping red fescue is infected with the symbiotic fungal endophyte *Epichloë festucae*, the plants exhibit resistance to dollar spot (Clarke et al., 2006). How infection by the endophytic fungus confers disease resistance to the host red fescue is not known. Resistance to fungal pathogens is not an established effect of endophyte infection of other grass species, and may therefore be unique to the fine fescues.

We recently carried out a large scale transcriptome study comparing endophyte-free and endophyte-infected red fescue plants, with the goal of identifying plant or fungal genes that may be involved in the observed disease resistance (Ambrose and Belanger, 2012). Analysis of the plant genes whose transcript levels were affected by the presence of the fungal endophyte did not reveal any strong candidates for genes directly related to enhanced disease resistance. However, one of the fungal abundant secreted proteins is of particular interest regarding the disease resistance observed in endophyte infected fine fescues. This protein is similar to characterized antifungal proteins from *Penicillium* and *Aspergillus* (Marx, 2004). We have therefore begun referring to the *E. festucae* protein as an antifungal protein. As a secreted protein, the *E. festucae* antifungal protein could come into direct contact with invading pathogens. Surprisingly, the gene for this antifungal protein is not present in most *Epichloë* species. The limited presence of the gene among the *Epichloë* species and its high level of expression in *E. festucae* suggest it may be involved in the disease resistance seen in *E. festucae* infected red fescue.

Understanding the mechanism behind the endophyte-mediated disease resistance in the fine fescues may lead to new approaches for dollar spot management in other grass species, such as creeping bentgrass. The objective of this project is therefore to characterize the endophyte antifungal protein and determine if it does play a role in the disease resistance.

We used protein sequencing to confirm the presence of the antifungal protein as a component of the secreted proteins extracted from endophyte-infected plants. The secreted proteins were extracted from the leaves of endophyte-free and endophyte-infected plants and compared on a SDS-polyacrylamide gel. One protein band was more prominent in the endophyte-infected samples and protein sequence analysis of the band indicated the antifungal protein was the major component in the band (Figure 1, E- and E+ lanes). We have partially purified the antifungal protein from the secreted proteins (Figure 1, lane 2). The partially purified protein did have antifungal activity against the dollar spot fungus in a plate assay (Figure 2). We are

currently working on expressing the protein in yeast in order to produce larger quantities of the protein for additional tests of antifungal activity. In summary, the data we have obtained so far are promising regarding the possibility that the *E. festucae* antifungal protein may be a component of the disease resistance seen in endophyte-infected strong creeping red fescue.

References

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Bullet Points

1. The fungal endophyte *Epichloë festucae* secretes an abundant protein into the plant apoplast (the space between the plant cells) that is similar to proteins from *Penicillium* and *Aspergillus* that have antifungal activity.

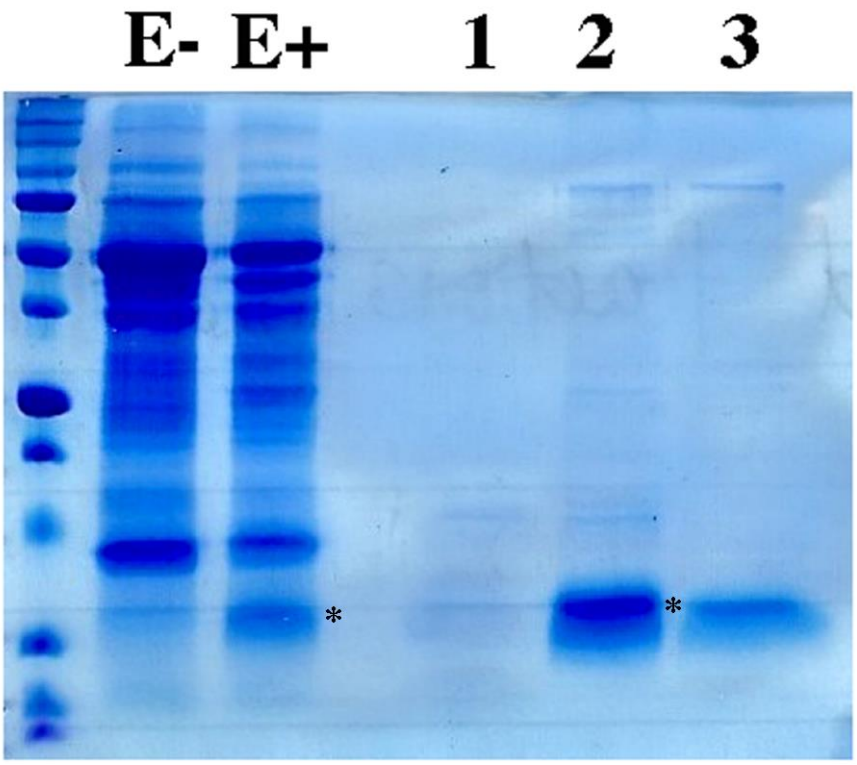
2. We have partially purified the *E. festucae* protein from the secreted proteins.

3. The partially purified *E. festucae* protein had antifungal activity against the dollar spot fungus in a plate assay. It may therefore be a component of the well-established endophyte-mediated disease resistance seen in strong creeping red fescue.

Figure Legends

Fig. 1. SDS-polyacrylamide gel of secreted proteins from endophyte-free (E-) and endophyte-infected (E+) strong creeping red fescue leaves and column fractions from the purification of the antifungal protein (lanes 1, 2, and 3). The asterisks indicate the protein bands containing the antifungal protein. The antifungal protein was most concentrated in column fraction 2.

Fig. 2. Plate assay of activity of the partially purified antifungal protein against the dollar spot fungus. A small piece of the dollar spot fungus was placed in the center of an agar plate and the partially purified antifungal protein and a buffer control were spotted at opposite ends of the plate. The growth of the dollar spot fungus was inhibited by the antifungal protein (top, black arrow) but not by the buffer control (bottom, white arrow).



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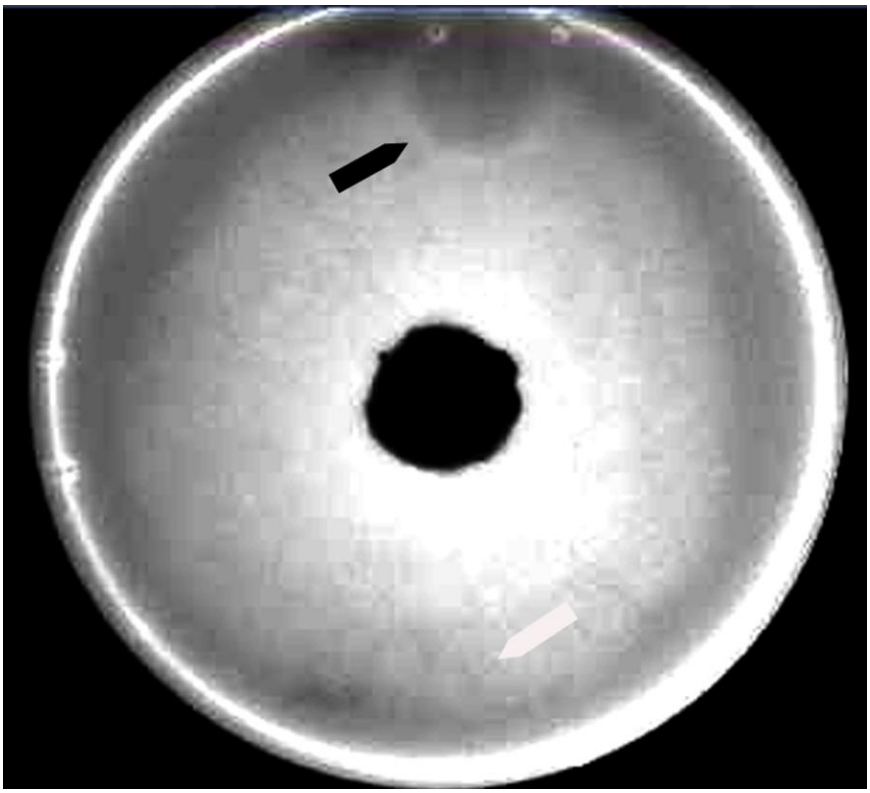

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