Understanding Endophyte Interactions in a Native Wild Barley Species (*Hordeum jubatum*)

Background:

Fusarium Head Blight (FHB) is among the most devastating diseases of barley and wheat in the world. The disease, caused chiefly by the fungus *Fusarium graminearum*, leads to yield losses as well as accumulations of toxins in harvested grain. However, for many native grasses, it appears that *F. graminearum* and other closely related *Fusarium* species reside inside the plant without causing any symptoms of disease (Lofgren et al., 2018). Although the innocuous nature of *Fusarium spp.* in wild grasses is not completely understood, it is hypothesized that *F. graminearum* could have originated from North America and co-adapted with native grasses as an endophyte (Lofgren et al., 2018). The term endophyte describes fungi, bacteria, and unicellular eukaryotes that live inside of plants, often granting the plant stress resistance in return for extracting nutrients (Murphy et al., 2015b). The reason for why *F. graminearum* behaves as a pathogen in cultivated grains and a benign endophyte in native grains needs further exploration.

This study will focus on the endophyte community in the native grass species, foxtail barley (*Hordeum jubatum*), which is a distant relative of cultivated barley (*Hordeum vulgare*). If other endophytes in *H. jubatum* control *F. graminearum*, whether through competition or a more direct form of antagonism, they could be responsible for making *F. graminearum* non-virulent. Experiments with *Hordeum* species show that certain endophytes can enable the plant to more successfully endure abiotic and biotic stressors, including FHB (Murphy et al., 2014; Song et al., 2014; Murphy et al., 2015a). This research project seeks to better understand how *Fusarium* species and other endophytes interact within *H. jubatum* in an effort to gain insights on how the native grass can successfully resist symptoms of FHB while infected with *F. graminearum*.

Related Research:

A recent study from the Dr. H. C. Kistler project at the USDA-ARS Cereal Disease Laboratory isolated *Fusarium* species in wild grasses native to Minnesota, including *H. jubatum* (Lofgren et al., 2018). By plating the seeds on Nash-Snyder medium, which prevents the growth of bacteria and most other fungi other than *Fusarium*, the researchers found the frequent presence of *Fusarium* species in *H. jubatum* (Lofgren et al., 2018). The research methods of Lofgren et al. (2018) for isolating *Fusarium* will be used as a model for this project.

Materials and Methods:

Specimens of *H. jubatum* will be analyzed for their endophyte communities. This project has access to more than 140 mature spike specimens of *H. jubatum* from nearly 100 different collection sites in Minnesota, Wisconsin, North Dakota, and Canada. Habitats where *H. jubatum* specimens were collected include agricultural land, urban areas, forested areas, and waterfront, all of which could contain different endophyte niches due to the site-specific nature of endophytes. In order to capitalize on potential diversity of endophytes, multiple spikes will be selected from approximately 20 collection sites, which will consist of about five collection sites from each of the four main habitat types. Each sample, comprised of seeds from a *H. jubatum* spike, will be divided in half. One half of every sample will be placed in Nash-Snyder medium to selectively promote the growth of *Fusarium*. The other portion of the sample will be plated on acidified potato dextrose agar (PDA). The acidified PDA medium will be non-selective toward fungi while reducing the distraction of bacteria by restricting bacterial growth.

Endophyte communities from each sample on the two different media will be compared for differences in the growth of *Fusarium*. The next step involves recognizing if there are

cultures in the non-selective medium that foster growth of certain endophytes while suppressing *Fusarium*, especially if the culture grown on Nash-Snyder medium from the same sample indicates the presence of *Fusarium*. If there are disparities of *Fusarium* between media, the hypothesis would be that other endophytes restrict the growth or exclude the colonization of *Fusarium*, which can be tested by pairing a selected endophyte with *Fusarium* in a petri plate and analyzing possible inhibitory interactions. Any endophytes that appear to suppress *Fusarium* will be identified through morphological keys and DNA sequencing following procedures in the Kistler and Steffenson laboratories.

Project Scope:

This research project will take place in the Steffenson and Kistler labs during the 2019 spring semester. The goal of the UROP project is to identify endophytes that interact with *Fusarium* in *H. jubatum*, which is an important step in understanding why *Fusarium* is presumably non-pathogenic to *H. jubatum* but pathogenic to cultivated barley. In addition, this project could reveal endophytes that potentially could be used to control FHB in cultivated barley. After completing and presenting the UROP project, I plan to continue this research as an Honors Thesis during the 2019-2020 academic year to further test the pathogenicity of *Fusarium* on *H. jubatum* and *H. vulgare* when it is paired with other endophytes identified in this project.

Bibliography:

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