Identifying Plant Genes Associated With Pathogen Antagonism in *Populus trichocarpa*

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Project Goals: Within the leaves of *P. trichocarpa*, antagonistic interactions among non-pathogenic endophytes and pathogens can result in reduced plant disease severity. The overarching goal of our project is to use genome-wide-association-studies (GWAS) to identify and validate plant SNPs/genes associated with the abundance of pathogen antagonists and effective disease suppression in the *P. trichocarpa* leaf microbiome. **Aim 1:** Identify plant SNPs/genes associated with *P. trichocarpa* leaf microbiome composition and the abundance of putative pathogen antagonists in contrasting common garden environments. **Aim 2:** Identify plant SNPs/genes associated with the abundance of known fungal antagonists of *Melampsora* leaf rust in *P. trichocarpa,* and with fungal antagonism of *Melampsora* leaf rust, in a controlled greenhouse environment. **Aim 3:** Validate the effect of selected genes associated with fungal antagonism of *Melampsora* leaf rust using the CRISPR/cas9 system to introduce loss-of-function mutations.

Abstract: Managing plant microbiomes to facilitate pathogen antagonism could complement traditional methods of combating disease (e.g., breeding for resistance and fungicide), enhancing the productivity and sustainability of *Populus* feedstock production for biofuels. Because plants play an active role in shaping the composition of their microbiome, breeding or genetic modification aimed at promoting pathogen antagonism could provide one approach to microbiome management for disease protection. However, before the promise of enhanced pathogen antagonism through these methods can be realized, many basic questions must be answered regarding the genomic basis of host control over endophyte colonization. Toward this end, our research seeks to identify plant genes that influence the species composition of the *P. trichocarpa* leaf microbiome across contrasting environments, with a focus on known and putative antagonists of *Melampsora* leaf rust.

This abstract focuses on the results of our first study aim. We used ITS metabarcoding to characterize fungal communities in the leaves of > 500 *P. trichocarpa* genotypes planted in two common garden environments (Corvallis OR, Boardman OR), in both early (June) and late (September) season. These gardens represent a dramatic contrast in the abiotic environment – approx. 110 cm rain/yr in Corvallis vs. 20 cm rain/yr in Boardman – and dissimilar regional propagule pools of plant pathogens and endophytes. Greater moisture availability is known to positively impact fungal growth and survival. We found large differences in fungal species composition between sites (Fig 1), and between the early and late season samples within each garden (Fig 1). Species richness and diversity also varied across space and time. Observed richness was greater in the wet site, though differed minimally between early and late season

sampling points in both gardens. However, evenness increased over time in Corvallis and decrease over time in Boardman.
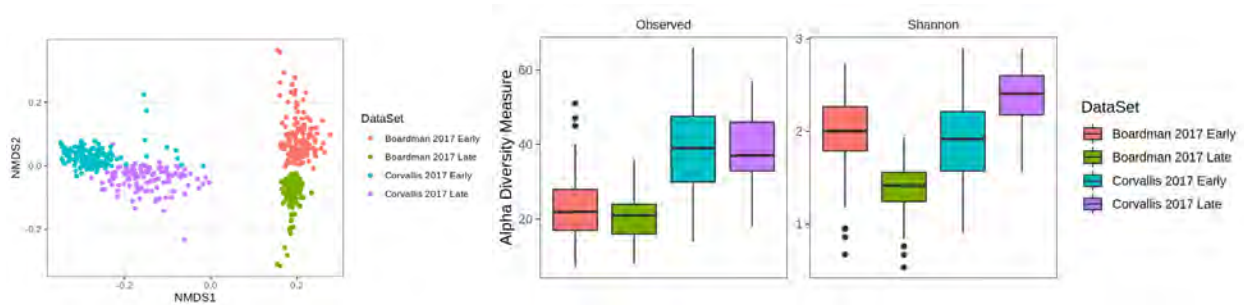

Figure 1. Species composition and diversity of foliar fungi sampled from identical *Populus trichocarpa* genotypes in two contrasting common gardens (Boardman and Corvallis, Oregon) and at two time points (June and September). Left: Non-metric multidimensional scaling (NMDS) reveals large differences in species composition between gardens and at early and late season time points. Right: Observed species richness was consistently higher in the wetter environment (Corvallis). Shannon diversity in the two gardens was similar early in the growing season but diverged over time.

In our preliminary GWAS analysis of the four datasets, the majority of plant SNPs/genes associating with differential relative abundances of individual fungi varied among the datasets. However, across all datasets we identified fungi significantly associated with genes involved in phytohormone action, cell wall modification, lipid metabolism, and protein modification (among others). Boardman shows a larger number of genes associated with vesicle trafficking, while Corvallis has a higher prevalence of solute transport genes. In comparing early versus late season samples within gardens we see modest differences in the prevalence of genes associated with protein homeostasis, protein modification, and solute transport. Some fungi were consistently associated with SNPs/genes across all four datasets, including putative pathogen antagonist species in the genus *Cladosporium*. Though about half the fungi for which we found significant associations with SNPs/genes were identified in just one dataset.