Genomics-Assisted Breeding for Leaf Rust (*Melampsora*) Resistance in Shrub Willow (*Salix*) Bioenergy Crops

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Project Goals: We will leverage recent significant investments in genomics and genetics resources for *Salix* and *Melampsora* by the DOE and USDA and also develop new systems genetics tools to characterize willow leaf rust diversity and map genes for resistance in willow. We will use ITS-LSU sequencing and genotyping-by-sequencing to characterize 200 single uredinial isolates of *Melampsora* infecting *Salix purpurea* cultivars across the Northeast. The pathogenicity of those isolates will also be tested. We will accurately map QTL for resistance to *Melampsora* in *S. purpurea* in both a segregating F_2 linkage mapping population and association mapping populations. We will map eQTL controlling gene-level and coordinated gene network transcriptomic responses to *Melampsora* colonization by performing RNA-Seq on leaves of F_2 *S. purpurea* progeny inoculated with *Melampsora.* We will map and characterize major genes for resistance to *Melampsora* in different *Salix* species hybrids using a common parent mapping approach.

Abstract: Shrub willow (*Salix* spp.) is a proven, high-yielding perennial woody crop that can be grown on underutilized or marginal agricultural land, but which faces a major long-term threat of yield losses due to leaf rust, caused by *Melampsora* spp. Although *Melampsora* has been described taxonomically, species diversity within a single site and regionally is unknown. Approximately 200 single pustule isolates were collected from MI, NY, PA, VT, and WV, and are being characterized by rDNA sequencing. Preliminary data show that both *M. americana* and *M. paradoxa* are present in the shrub willow growing region. Further investigation of *Melampsora* spp. diversity throughout the Northeast will be conducted using genotyping-by-sequencing, a genome wide high-throughput SNP identification method, providing thousands of markers for population studies targeting un-methylated coding sequences. Additionally, we explored disease resistance by surveying association and F_2 linkage mapping populations (Fig. 1) of *S. purpurea* for rust severity in September 2015 with the aim of identifying QTL and tightly-linked SNPs for marker-assisted selection. Preliminary data show a significant QTL for rust severity that maps to a common locus on Chr01 in both the linkage and association populations. GWAS identified a significant QTL on Chr02, while linkage mapping identified QTL on Chr05 and Chr10. There is also good evidence that major, qualitative resistance genes to *Melampsora* can be introgressed from diverse *Salix* species through hybridization. In order to map rust resistance genes across diverse species, we have developed eight species hybrid populations

produced by crossing reference female (94006) and male (94001) *S. purpurea* genotypes with six different *Salix* species. These have been established in a replicated field trial, will be genotyped by GBS, and phenotyped for rust incidence to map and characterize resistance loci. The parents of these mapping populations have been challenged with three isolates of *M. americana* and one isolate of *M. paradoxa* and display a range of resistance, including some progeny that are more resistant than the *S. purpurea* parents. This project will generate markers tightly linked to both major resistance genes and to QTL involved in quantitative resistance that will be developed into tools for early marker-assisted selection in the introgression of major resistance genes, while also selecting long-term for durable resistance in improved cultivars of this long-lived perennial bioenergy crop.
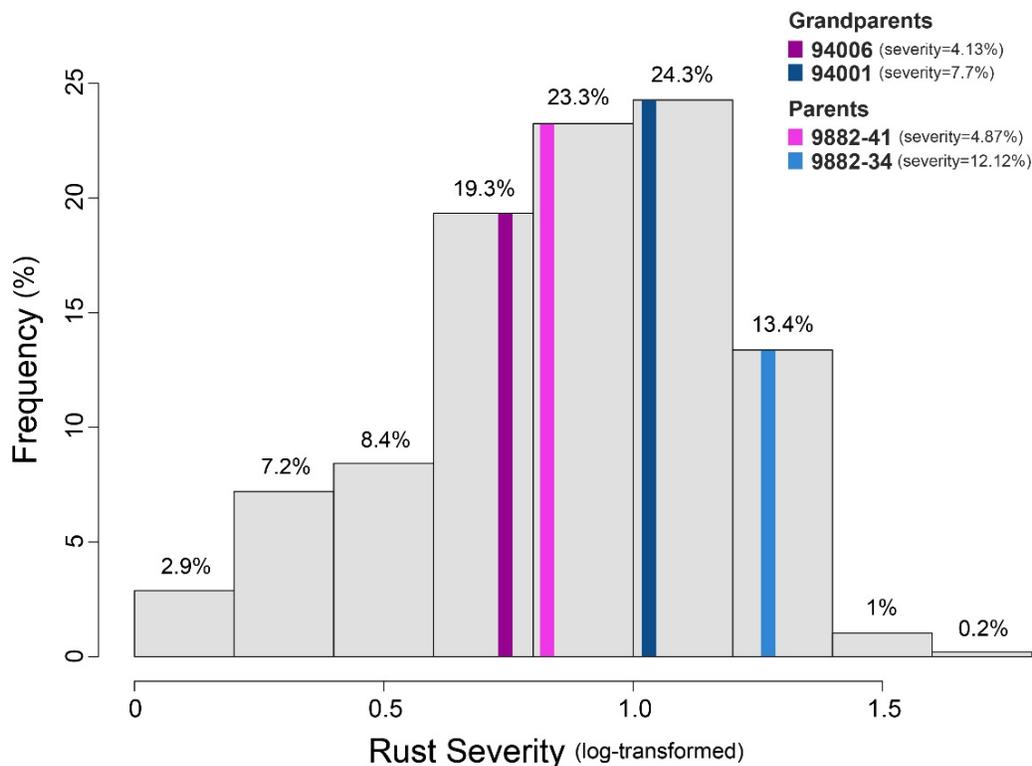

Figure 1. Frequency distribution of rust severity (log-transformed) in the F_2 mapping population. Parents and grandparents of the F_2 progeny are highlighted within bins along the frequency distribution. Log-transformed rust severity (%) was subsequently used for mapping.