**Plant-Microbe Interfaces: From gene discoveries to molecular, genetic and biochemical validations in *Populus***

**Jay Chen**[1*] (chenj@ornl.gov), Wellington Muchero,[1] David J. Weston,[1] Xiaohan Yang,[1] Udaya C. Kalluri,[1] Sara S. Jawdy,[1] Lee E. Gunter,[1] Jessy L. Labbé,[1] Gerald A. Tuskan,[1] Dale A. Pelletier,[1] Timothy J. Tschaplinski,[1] and **Mitchel J. Doktycz**[1]

[1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

http://PMI.ornl.gov

**Project Goals: The goal of the Plant-Microbe Interfaces (PMI) SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. *Populus* and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic *Populus*-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.**

One of the primary objectives of PMI research is to identify genetic loci regulating *Populus*-microbial interactions and to dissect the signals and pathways responsible for initiating and maintaining beneficial relationships between the host and its associated microbes. In order to accomplish these goals, we have developed and leveraged two key genomic and genetic resources including ~1,000 *P. trichocarpa* natural variants and ~400 *P. trichocarpa* × *P. deltoides* pseudo-backcross pedigree, which have enabled Genome-Wide Association Studies (GWAS) and Quantitative Trait Locus (QTL) mapping, respectively. By taking these approaches, we have identified a number of genetic loci associated with *Populus*-microbial interactions. Validating the function of these genetic loci represents a rate-limiting step in elucidating the signaling cascades from the perception of microbial signal to the manifestation of biological responses. In order to address these challenges, we have developed two key systems including the *Populus* protoplast transient expression system and the *Populus* hairy root transient expression system to enable robust molecular, cellular and biochemical validation. To further validate the function of targeted genes at the organismal level, we apply both a heterologous expression system using the model plant *Arabidopsis* for rapid generation of transgenic plants and a *Populus* transformation system for generating stable transgenic lines over- or under-expressing the gene of interest. These genetically modified materials and *P. trichocarpa* natural variants with high-impact single nucleotide polymorphisms (SNPs) (*i.e.*, resulting in opening read frame shift, deletion or insertion) are subject to microbial inoculation, microscopic examination and colonization analysis to validate the function of selected genes at the physiological level. Furthermore, transcriptomics, metabolomics and proteomics are used to reveal global changes at the transcript, metabolite and protein levels, respectively. Taken together, this is a general strategy to identify genetic loci regulating *Populus*-microbial interactions and to functionally validate these loci. We continue to explore other efficient means to accelerate our discoveries and functional validations.

*The Plant Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S. Department of Energy, Office of Science, Biological and Environmental Research.*