Plant-Microbe Interfaces: Metabolic consequences of the introduction of a *Populus trichocarpa* lectin receptor-like kinase into *Arabidopsis thaliana*, a non-ectomycorrhizal host species

Timothy Tschaplinski[1*](tschaplinstj@ornl.gov), Zhihao Zhang,[1] Jessy Labbé,[1] Wellington Muchero,[1] Aurélie Deveau[2], Claire Veneault-Fourrey[2], Yongil Yang,[1] Priya Ranjan,[2] Sara Jawdy,[1] Daniel Jacobson,[1] Jin-Gui Chen,[1] Francis Martin[2], **Jerry Tuskan,[1]** and **Mitchel Doktycz[1]**

[1]Oak Ridge National Laboratory, Oak Ridge, TN; and [2]University of Tennessee-Knoxville, Knoxville, TN; [2]INRA, UMR 1136 INRA-University of Lorraine, Interactions Arbres/Microorganismes, Laboratory of Excellence ARBRE, INRA-Nancy, 54280, France

http://pmi.ornl.gov

Project Goals: The goal of the PMI SFA is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serves as the experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

A lectin receptor-like kinase (RLK) was identified in black cottonwood (*Populus trichocarpa*) that facilitates the initiation of a symbiotic ectomycorrhizal association with *Laccaria bicolor*. This *Populus* RLK was introduced via genetic transformation into *Arabidopsis thaliana*, a non-ectomycorrhizal host species, generating transgenic lines; LK8, LK100. The metabolomic profiles of mature transgenic *Arabidopsis* plants were determined by gas chromatography-mass spectrometry (GC-MS) and contrasted with wild-type 'Col' plants. The effects of transgene insertion on the metabolomic profiles were determined + and – *L. bicolor*, a *Populus* fungal symbiont. Additionally, given that this *Populus* RLK is predicted to bind mannose, the metabolomic profiles of plants with and without the RLK gene were grown + and – a brief (4 h) exposure to mannose vs glucose.

L. bicolor generates an intense defense response in *Arabidopsis*. Metabolites up-regulated in response included defense metabolites histidine, sinapoyl malate, kaempferol, quercetin, sinapic acid-4-O-glucoside, other sinapoyl conjugates, but sterols, such as cholesterol and campesterol, and monolignol glucosides, coniferin and syringin, were also elevated. Many of the highest up-regulated metabolomic fold changes associated with exposure to *L. bicolor* were suppressed by

the RLK gene insertion. Exposure to *L. bicolor* alone also induces large declines nitrogenous compounds and organic acids, metabolites, previously assumed to be consumed by the host's symbiont. Also of note are reductions in defense signaling and related metabolites, including salicylic acid and the defense priming metabolite, azelaic acid. These declines are likely indicative of altered C partitioning to defense, with RLK, again, lessening the degree of these responses. Furthermore, mannose addition tended to reduce the fundamental transgene up-regulated responses (especially in the weaker expressing LK100), whereas glucose had less of an effect.

In summary, *L. bicolor* induces an intense defense response in non-host, wildtype *Arabidopsis* 'Col', but these defense responses were suppressed by the presence of RLK gene. Interestingly, many of the highest metabolite fold changes associated with exposure to *L. bicolor* are the same as those driven solely by RLK gene, indicating that gene insertion, itself, triggers a defense response. The presence of mannose, a putative ligand, reduced the magnitude of the gene-induced defense response. The concomitant declines in a number of fatty acids and amino acids by both *L. bicolor* and RLK gene insertion that are typically attributed to utilization by the fungal symbiont, are likely the result of the altered C partitioning to defense. The RLK gene primary effect is to suppress the defense response induced by the potential fungal symbiont, thereby, facilitating the symbiosis.

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.