

Plant-Microbe Interfaces: The nature of the progression of drought stress drives differential metabolomic responses in *Populus deltoides*

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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.

Use of woody crops for Quad-level (10^{15} BTU) energy production will require use of marginal agricultural lands, where periods of water stress are frequent, especially given the predictions of increased frequency and severity of droughts associated with the predicted global climate change. Our previous research demonstrated that some *Populus* sp. genotypes have the capacity to increase dehydration tolerance by lowering the osmotic potential via osmotic adjustment, the active accumulation of solutes under stress, which allows turgor and growth maintenance under mild to moderate stress and facilitates growth recovery after stress relief. Despite the large number of drought stress studies that have been published, few studies have contrasted the degree and nature of solute accumulation if the nature of drought stress progression is varied (e.g., cyclic vs acute, short-term vs long term, moderate vs severe stress). The aim of the study was to determine how the inherent genetic potential of a given clone interacts with the nature of the stress experienced to determine the degree of the biochemical response.

A drought stress study on *Populus deltoides* 'WV94' was conducted in a greenhouse and the resulting metabolomic profiles of leaves were determined for plants subjected to cyclic mild (-0.5 MPa predawn leaf water potential) drought vs cyclic severe (-1.26 MPa) drought after 2 or 4 drought cycles in contrast to well-watered controls (-0.1 MPa), and in contrast with plants subjected to acute drought, where plants were not rewatered, but were allowed to desiccate for up to 8 days. Leaves were rapidly sampled, fast frozen on dry ice, ground with liquid N and then twice extracted with aqueous ethanol (80%). Dried aliquots of extracts were analyzed for metabolites by gas chromatography-mass spectrometry (GCMS) with electron impact ionization (70 eV) following trimethylsilylation.

The nature of drought onset (cyclic vs acute), frequency of drought (number of cycles), and the severity of drought (mild vs severe), all interact to dictate the degree of osmotic adjustment and the nature of the

organic solutes that accumulate. Acute onset of prolonged, severe drought induced the greatest osmotic adjustment after withholding water for 7 days (1.42x) with the greatest accumulations in the large, complex higher-order salicylate conjugates. Organic solute accumulation under cyclic stress relative to well-watered controls was moderate (1.20x) and was largely constituted by soluble sugars, organic acids, and amino acids. In contrast, acute onset of prolonged drought induced the greatest osmotic adjustment and the largest responses in secondary metabolism, with hydroxycinnamic acid conjugates of salicin; the populosides, playing a key role in drought tolerance of *P. deltoides* by lowering osmotic potential and increasing the likelihood of recovery following stress alleviation. Current studies are attempting to integrate these metabolomic responses with proteomic and transcriptomic responses measured on the same plants to obtain a more complete understanding of drought tolerance in *P. deltoides* and how these responses affect plant-microbe interactions.

Publications

Tschaplinski, T.J., S.S. Jawdy, L.E. Gunter, M.Z. Martin, N.L. Engle, X. Yang, and G.A. Tuskan. 2019. The nature of the progression of drought stress drives differential metabolomic responses in *Populus deltoides*. *Ann. Bot.* (in press) <https://doi.org/10.1093/aob/mcz002>

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