Title: The Scent of Senescence: Cell Wall Ester Modifications and Volatile Emission Signatures of Plant Responses to Abiotic Stress

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Project Goals: The Poplar Esterified Cell Wall Transformations and metabolic INtegration (PECTIN) project aims to study the metabolism of cell wall ester modifications and volatile intermediates, and their role in central physiological processes in the emerging biofuel species California poplar (*Populus trichocarpa*). A key goal of this research is to evaluate abiotic stress responses in plants with modified expression patterns of key genes involved in cell wall metabolism with altered amounts of methyl and acetyl groups present on cell walls. These genetic modifications will be evaluated for potential impacts on plant hydraulics, physiology, and stress responses. Understanding and manipulating the metabolism of cell wall modifications will not only provide important knowledge on the physiology and ecology of plants but will also allow the generation of engineered bioenergy crops such as poplar for sustainable production of biofuels and bioproducts, addressing BER's goal of developing renewable bioenergy resources.

Abstract Text: Growth suppression and defense signaling are simultaneous strategies that plants invoke to respond to abiotic stress. Here, we show that the drought stress response of poplar trees (*Populus trichocarpa*) is initiated by a suppression in cell wall derived methanol (meOH) emissions and activation of acetic acid (AA) fermentation defenses. Temperature sensitive emissions dominated by meOH (AA/meOH < 30%) were observed from physiologically active branches, detached stems, leaf cell wall isolations, and whole ecosystems. In contrast, drought treatment resulted in a suppression of meOH emissions and strong enhancement in AA emissions together with fermentation volatiles acetaldehyde, ethanol, and acetone. These drought-induced changes coincided with a reduction in stomatal conductance, photosynthesis, transpiration, and leaf water potential. The strong enhancement in AA/meOH emission ratios during drought (400-3,500%) was associated with an increase in acetate content of whole leaf cell walls, which became significantly $^{13}C_{1,2}$-labeled following the delivery of $^{13}C_{1,2}$-acetate via the transpiration stream. The results are consistent with central roles of acetate fermentation in regulating plant defense and metabolic responses to drought, and suggest that cell wall *O*-acetylation may be reversible

allowing plants to rapidly respond to drought stresses by down regulating methyl ester hydrolysis and growth processes while enhancing *O*-acetylation. We suggest that AA/meOH emission ratios could be used as a new highly sensitive non-destructive sensor to discriminate between thresholds of rapid plant growth and biomass accumulation and negative drought stress impacts on carbon metabolism, water use, and net primary productivity.

References/Publications

- Dewhirst R, Handakumbura P, Clendinen C, Arm E, Wang W, Washton N, Young R, Mortimer J, McDowell N, Jardine K (2021) Temperature acclimation of leaf gas exchange and photochemistry in *Populus trichocarpa*, ***ACS Earth and Space Chemistry*,** 5, 8, 1813–1828.
- Dewhirst R, Lei J, Afseth C, Castanha C, Wistrom C, Mortimer J, Jardine K (2021) Are Methanol-Derived Foliar Methyl Acetate Emissions a Tracer of Acetate-Mediated Drought Survival in Plants? ***Plants***, 10, 411.
- Dewhirst R, Mortimer J, Jardine K (2020) Do cell wall esters facilitate forest response to climate? **Trends in Plant Science**. https:/doi.org/10.1016/j.tplants.2020.05.011
- Dewhirst R, Afseth C, Castanha C, Mortimer J, and Jardine K (2020) Cell wall *O*-acetyl and methyl esterification patterns of leaves reflected in atmospheric emission signatures of acetic acid and methanol, ***PLoS ONE*** 15(5), e0227591.

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