Title: Volatile Emissions and Cell Wall Ester Signatures of Abiotic Stress in Poplar

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Project Goals:

Cell wall polysaccharides can be heavily decorated with methyl and acetyl esters, which can impact fermentation yields of poplar biomass. The hydrolysis of these ester groups results in the production of volatile methanol and acetic acid, which were generally considered waste products of cell wall metabolism but have recently been shown to be tightly coupled to plant growth, stress and senescence processes. However, methanol and acetic acid are not captured by traditional metabolomics analysis and thus represents an important knowledge gap within cell wall metabolism and its interaction with the environment. The 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 goal of this research is to modify the expression of key genes involved in cell wall metabolism in order to modify the amount of methyl and acetyl groups present on cell walls. These genetic modifications will be evaluated for potential impacts on plant 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:
A common thread among many of the biochemical and physiological processes that determine plant responses to climate change variables are alterations in plant cell wall chemical composition, structure, and function. A large proportion of the plant cell wall can be modified with methyl and *O*-acetyl ester groups which may play important roles in cell growth, tissue development, proper xylem and stomatal functioning, central carbon and energy metabolism, and stress communication and signaling. While the hydrolysis of these esters leads to rapid physiological changes in the cell wall and the emission of methanol (meOH) and acetic acid (AA) to the atmosphere, little is known about their changes in response to abiotic stress. Here we provide evidence that drought and high

temperature stress induce a coordinated change in plant cell wall esters composition, central energy metabolism, and leaf–atmosphere fluxes of methanol, acetic acid, carbon dioxide (CO_2), and water (H_2O). $^{13}C_2$-acetate feeding of poplar branches (*Populus trichocarpa*) resulted in emissions of $^{13}CO_2$ from illuminated leaves, suggesting the utilization of free acetate as a respiratory substrate in the light via activation to acetyl-CoA. Moreover, ^{1}H-NMR analysis of leaf cell walls from branches exposed to $^{13}C_2$-acetate suggests that unlike previously assumed, *O*-acetylation of cell wall polysaccharides is reversible, potentially allowing plants to rapidly modify cell wall acetylation patterns. Continuous branch gas exchange observations demonstrated diurnal patterns of methanol and acetic acid emissions that were dominated by methanol in physiologically active control plants (AA/meOH < 10 %). Branch feeding with a 50:50 solution of $^{13}C_2$-acetate:^{13}C-methanol also revealed an AA/meOH ratio dominated by methanol (AA/meOH < 1 %). In contrast, experimental drought treatments resulted in a suppression of methanol emissions and a strong enhancement in acetic acid emissions together with metabolites of the acetate fermentation pathway, acetaldehyde and ethanol. These drought-induced changes in emission patterns lasted > 6 days with their initiation coinciding with a reduction in leaf water potential, stomatal conductance, transpiration, and photosynthesis. The strong enhancement in AA/meOH emission ratios during drought (up to 500 %) was associated with an increase in leaf cell wall *O*-acetylation. Moreover, AA/meOH emission ratios were found to increase with temperature in physiologically active poplar branches and detached leaves and stems. The temperature dependence of AA/meOH emissions ratio was also observed at the ecosystem scale using eddy covariance above a managed poplar plantation in Belgium, a citrus orchard in California, and a diverse forested ecosystem in Alabama. The results are consistent with the activation of the acetate fermentation pathway and acetate photoassimilation as an evolutionarily conserved drought and high temperature survival strategy with important implications for understanding acetate-mediated drought responses to cell wall *O*-acetylation patterns and plant hydraulics, transcription, cellular metabolism, and hormone signaling and its associated changes in carbon cycling and water use from individual plants to whole ecosystems. We suggested that AA/meOH emission ratios could be used as a new quantitative ecosystem sensor to discriminate between plant growth (enriched in meOH) and stress responses including reductions in conductance, transpiration and gross primary productivity (enriched in acetic acid).

References/Publications

1. Dewhirst RA, Afseth CA, Castanha C, Mortimer JC, Jardine KJ (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
2. Dewhirst RA, Mortimer JC, Jardine KJ (2020) Do cell wall esters facilitate forest response to climate? Trends in Plant Science, 25(8), 729-732

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