

Cell Wall *O*-Acetyl and Methyl Esterification Patterns of Leaves Reflected in Atmospheric Emission Signatures of Acetic Acid and Methanol

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Project Goals: The main goal of the poplar esterified cell wall transformations and metabolic integration study (**PECTIN**) is to quantify *O*-acetyl and methyl ester cell wall metabolism in poplar trees and its interactions with central metabolism and volatile metabolite exchange with the atmosphere. We aim to 1) **Characterize** leaf and stem cell wall esterification reservoirs and sources/sink dynamics during plant development, 2) **Quantify** integration of cell wall esters with central metabolism and the atmosphere during abiotic stress, 3) **Modify** *O*-acetylation and methyl esterification of cell walls through the identification of natural genetic variants, and 4) **Evaluate** impacts of cell wall ester metabolism on plant physiology and stress responses.

Abstract

Polysaccharides are major components of plant cell walls that can be converted into fuels by microbial fermentation, making plant biomass an important bioenergy resource (Mortimer 2019). However, a substantial fraction of plant cell wall polysaccharides are chemically modified with methyl and acetyl groups that impact the yield of microbial fermentation. Although little is known about the biochemical and physiological functions of those cell wall modifications in trees, evidence suggests that they may be highly dynamic and play central roles in the control of cell growth, tissue development, and function (e.g. proper development and function of xylem vessels and leaf stomata), facilitate within and between plant signaling in response to abiotic and biotic stress, and integrate into primary C₁ and C₂ metabolism. While deesterification reactions result in the formation of volatile intermediates (methanol: meOH and acetic acid: AA) these central metabolites are not captured by traditional metabolomics analysis, representing an important gap in our knowledge of cell wall ester metabolism. Plants emit high rates of meOH, generally assumed to derive from pectin demethylation, and this increases during growth and abiotic stress. In contrast, less is known about the emission and source of AA. In this study (Dewhirst *et al.*, 2019), we connect leaf volatile emissions of meOH and AA to patterns of plant cell wall *O*-acetyl- and methyl-esters for the first time. We present a new concept of leaf cell wall *O*-acetyl/methyl ester ratios and demonstrate that they are quantitatively reflected in the AA/meOH emission ratios.

Populus trichocarpa leaves in different developmental stages were desiccated and quantified for total meOH and AA emissions together with bulk cell wall acetylation and methylation content. While young leaves showed high emissions of meOH (140 $\mu\text{mol m}^{-2}$) and AA (42 $\mu\text{mol m}^{-2}$), emissions were reduced in mature (meOH: 69%, AA: 60%) and old (meOH: 83%, AA: 76%) leaves. In contrast, the ratio of AA/meOH emissions increased with leaf development (young: 35%, mature: 43%, old: 82%), mimicking the pattern of *O*-acetyl/methyl ester ratios of leaf bulk cell walls (young: 35%, mature: 38%, old: 51%), which is driven by an increase in *O*-acetyl and decrease in methyl ester content with age. The results are consistent with meOH and AA emission sources from cell wall de-esterification, with young expanding tissues producing highly methylated pectin that is progressively demethyl-esterified. In the near future, it will be necessary to grow dedicated bioenergy crops as a feedstock for the production of liquid transport fuels and bioproducts. The presented methods will help advance rapid non-invasive phenotype screening and genetic manipulation of the cell wall ester content, with the goal of increasing biofuel yields. Moreover, the methods could be used in future studies at a wide range of spatial and temporal scales to help understand the impacts of cell wall esterification on cell wall structure and function, and numerous physiological and biochemical process including growth, stress responses, signaling, plant hydraulics, and central carbon metabolism.

References

1. Cell wall *O*-acetyl and methyl esterification patterns of leaves reflected in atmospheric emission signatures of acetic acid and methanol (2019) Dewhirst R, Afseth C, Castanha C, Mortimer J, and Jardine K, *Plant Cell and Environment* (in review).
2. Plant synthetic biology could drive a revolution in biofuels and medicine. Mortimer J, *Experimental Biology and Medicine* 244.4 (2019): 323-331.

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