ABSTRACT

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Title: Modeling Biosynthesis and Transport of Volatile Organic Compounds in Plants
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To compensate for their sessile existence, plants synthesize and emit a wide diversity of volatile organic compounds (VOCs) that serve important biological functions pertaining to defense, reproduction, and plant-plant signaling. In addition to their importance in plant secondary metabolism, VOCs are used as fragrances, flavoring agents, and therapeutics. Plant metabolic engineering has successfully been implemented towards the design of value-added plants with enhanced defense, improved aroma and flavor, and increased production of specialty chemicals. However, rational design requires rigorous characterization of the mechanisms controlling metabolic fluxes in a network. Thus, the major aims of this dissertation are to study biological and physical mechanisms controlling the synthesis and emission of plant VOCs. This dissertation focuses on (*i*) modeling 2-phenylethanol biosynthesis in *Arabidopsis* and (*ii*) characterization of the biophysical properties of flower cuticles with respect to the emission of VOCs.

2-Phenylethanol (2-PE) is a naturally-occurring aromatic volatile with properties that make it a candidate oxygenate for petroleum-derived gasoline. In plants, 2-PE biosynthesis competes with the phenylpropanoid pathway for the common precursor L-phenylalanine (Phe). The phenylpropanoid pathway directs up to 30% of fixed carbon towards the production of lignin, a major constituent of plant cell walls that renders biomass recalcitrant to pretreatment techniques impeding the economical production of biofuels. An initial genetic engineering approach was proposed, whereby a portion of the carbon flux towards lignin production is diverted towards the biosynthesis 2-PE. Transgenic *Arabidopsis thaliana* expressing enzymes catalyzing the biosynthetic steps from Phe to 2-PE were generated. Excised stems from transgenic Arabidopsis were supplied $^{13}C_6$-ring labeled Phe, and isotopic enrichment of downstream metabolites were quantified to calculate fluxes. By combining flux measurements with predictions from a kinetic model of the Phe metabolic network, we hypothesized that 2-PE biosynthesis in transgenic Arabidopsis was limited by endogenous pools of cytosolic Phe. Multiple independent genetic

strategies were proposed based on model-guided predictions, such as inducing Phe hyper-accumulation, reduction of the activity of the competing phenylpropanoid pathway, and sequestering the 2-PE biosynthesis pathway in plastids. Combining kinetic modeling with time-course *in vivo* metabolomics led to successful rational engineering of 2-PE accumulating plants.

The plant cuticle is the physical interface between the flower and its surrounding environment. Passage of VOCs through the cuticle is driven solely by diffusion and is thus dependent on the cuticle physicochemical properties. Wax compounds in the cuticular matrix self-assemble into a multiphase system of crystalline and amorphous regions, where their relative amounts and arrangements govern VOC diffusion. To investigate the effect of wax composition on the crystallinity and permeability of the cuticle, we characterized the cuticular waxes of *Petunia hybrida* petals using GC-MS, FTIR, DSC, and XRD. Petal waxes were found to be enriched with long-chain hydrocarbons forming semi-crystalline waxes localized on petal surfaces. A ternary system of wax compounds was proposed as a model for petal cuticles to investigate the effect of wax composition on cuticle crystallinity and permeability. Atomistic simulations of VOC displacement in waxes of varying chemical composition were performed at 298 K and 1 bar under NPT conditions to estimate diffusivities. Wax anisotropy was found to be highly dependent on the elongation of methylene chains, restricting the molecular diffusion path. Changes in crystalline symmetry were found to have measurable effects on VOC diffusion. Simulations of compositional variants of the model cuticle shows that changes in relative crystallinity exert differential control on the dynamics of VOC emissions.

To directly determine the effect of the cuticle on VOC emissions in petunia flowers, the wax exporter PhABCG12 was silenced using RNA interference, resulting in flowers with thinner cuticles. However, VOC emissions were found to have significantly decreased in transgenic flowers relative to the wild-type control. Dewaxing wild-type and transgenic petunia revealed that the cuticle serves as a site of VOC build-up during emission, and deficient coverage limits the extent to which compounds can accumulate. In addition, the cuticle was found to impart differing levels of mass transfer resistance for certain VOCs, suggesting that the cuticle controls the dynamics of VOC emissions. Taken together, petal cuticles provide an additional layer of regulation in emission of VOCs from plants.