ABSTRACT

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Title: Analysis of Mass Transfer in the Emission of Floral Volatile Organic Compounds
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Plants synthesize and release a variety of volatile organic compounds (VOCs) that are important for their reproduction, defense, and communication. These low-molecular-weight, lipophilic molecules also serve as practical products in industries such as food additives, fragrances, colorants, nutraceuticals, and pharmaceuticals. In addition, they have agricultural applications such as sustainable methods for pest control. Therefore, identifying the biological mechanisms involved in volatile emission could help researchers develop new ways to control the timing and release of volatiles, defend against pests, and engineer the production of these valuable chemicals.

While progress has been made in understanding plant volatile biosynthesis, their release from the cell remains incomplete. For plant VOCs to be emitted into the environment, they must move from their site of biosynthesis through the cytosol, transverse the plasma membrane, hydrophilic cell wall, and sometimes cuticle to exit the cell. It was previously shown by mathematical modeling that to achieve observed emission rates solely by diffusion, VOCs would accumulate in the cellular membranes to levels that are likely detrimental to the membrane integrity and function. Hence, it was proposed that there are biological mechanisms involved to lower VOC concentrations in membranes. In this work, we focus on the aqueous cell wall, the thickest layer among the three subcellular barriers that should act as a barrier for the diffusion of VOCs. We hypothesize that the transport of VOCs across the cell wall is facilitated by lipid transfer proteins (LTPs) which enhance the solubility of hydrophobic volatiles in the aqueous environment, prevent their back partition into the plasma membrane after entering the cell wall, and hence enhance their net diffusion. To investigate if the presence of LTPs has influence on the total VOC efflux, we use reverse-genetic, biochemical, and mathematical modeling approaches. Out of three highly expressed LTPs identified in the petunia petal, only downregulation of *PhLTP3* expression led to a decrease in VOC emission in the corresponding transgenic plants. A facilitated diffusion model was built to quantify the VOC flux difference with the presence of LTPs in the cell wall. Modeling of the steady state system revealed the facilitation of VOC flux by LTPs is greatest when the VOC concentration

gradient across the cell wall is shallow, which is a physiologically relevant condition. In addition, there exists an optimal protein dissociation constant value for maximal facilitated flux, indicating the balance between the binding and the unloading of VOC is critical. With the in vitro displacement assay, the binding constants of candidate PhLTPs with VOCs were obtained and were all found to be in the μM range, which is close to our model predicted optimal value. The results revealed that LTPs, specifically PhLTP3, play a role in the export of VOCs from the plasma membrane, across the cell wall, to the cuticle.

In our earlier mathematical model, the emission of VOCs from the petunia flowers was modeled assuming negligible mass transfer resistance on the surface of the cuticle because of their high volatility. However, the resistance imposed by the surface boundary layer was not considered. To examine if surface convection influences VOC emission, a model system which utilized a model cuticular wax film containing 2-phenylethanol (2-PE) was built to imitate the VOC emission from plant cuticle. The convection mass transfer coefficient of 2-PE emission from a model cuticular wax film was obtained by experimental data fitting and calculated from the correlation that involves Sherwood number. The obtained $Bi_m/K_{cut/air}$ values that were smaller than unity indicates that the surface boundary layer imposes a higher mass transfer resistance than the model cuticle for the emission of 2-PE in the range of wind velocities investigated. The examination of petunia flowers under air flow showed increases in total emission but no significant differences in total internal pools, which indicates an increase in biosynthesis. The emission changes of individual compounds were different and does not clearly correlate to any molecular properties of the compounds.