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Rethinking how volatiles are released from plant cells

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For plant volatile organic compounds (VOCs) to be emitted, they must cross membrane(s), the aqueous cell wall, and sometimes the cuticle, before moving into the gas phase. It is presumed that VOC movement through each barrier occurs via passive diffusion. However, VOCs, which are primarily nonpolar compounds, will preferentially partition into membranes, making diffusion into aqueous compartments slow. Using Fick's first law, we calculated that to achieve observed VOC emission rates by diffusion alone would necessitate toxic VOC levels in membranes. Here, we propose that biological mechanisms, such as those involved in trafficking other hydrophobic compounds, must contribute to VOC emission. Such parallel biological pathways would lower barrier resistances and, thus, steady-state emission rates could be maintained with significantly reduced intramembrane VOC concentrations.

VOCs must cross multiple cellular compartments to reach the environment

Plants emit up to 10% of photosynthetically fixed carbon in the form of VOCs [1]. These metabolites, when released from leaves, flowers, and fruits into the atmosphere, and from roots into the soil, have key roles in the attraction of pollinators [2] and seed dispersers [3,4], above- and belowground defense against herbivores [5–8], protection against pathogens [9,10], and plant–plant signaling [11,12]. Certain VOCs are also able to protect plants against abiotic stresses, such as high light, temperature, or oxidative stress [13–15]. While VOC functions have long been studied, the past decade has also resulted in substantial progress in the understanding of VOC biosynthesis and regulation [16]. It has been shown that VOC production and release are spatially, developmentally, and/or temporally regulated and depend on biotic (e.g., pollination status and herbivore infestation) and abiotic (e.g., light intensity, atmospheric CO_2 concentration, temperature, relative humidity, and nutrient status) factors [13,16,17].

Based on their biosynthetic origin, plant VOCs can be divided into several classes, including terpenoids (monoterpenes and sesquiterpenes), phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives [13,16,18]. Despite such chemodiversity, plant VOCs are generally lipophilic low-molecular-weight (~100–200 Da) compounds with high vapor pressures at ambient temperature. These physicochemical properties enable VOCs to be emitted into the environment, although the site from which this occurs varies. The biosynthesis of VOCs in nongreen tissues (e.g., flowers or roots) occurs predominantly in epidermal cells, which are closest in proximity to the atmosphere [19–22] or rhizosphere [23] for immediate release. In vegetative organs, VOCs are often synthesized in the secretory cells of glandular trichomes located on the leaf surface [24–27] and then secreted to a sac created by an extension of the cuticle, where they are stored until mechanical disruption [28–30]. When trichomes are not involved in vegetative VOC production, these compounds are often synthesized in mesophyll cells [31,32], mainly in the palisade parenchyma [33], and released through stomata [34–37], mechanical disruption, or emission through cuticle [36]. Regardless of the tissue and whether they exit via stomata, at the subcellular level, VOCs must move from their site of biosynthesis through the cytosol to the plasma membrane, and then subsequently traverse the plasma membrane, hydrophilic cell wall, and, in some cases, the cuticle to exit the cell. While mechanical disruption provides direct access for VOCs inside the cell to the atmosphere, it remains unclear how VOCs cross these barriers for release from intact cells directly to the environment or to the intercellular air spaces connected to stomata.

It is largely presumed that, upon synthesis, VOCs passively diffuse across cellular barriers into the environment. However, many examples exist where VOC emission rates cannot be explained by a concentration-dependent diffusion mechanism [38–41]. Assuming that VOC emission is solely driven by diffusion, we have calculated the concentrations of VOCs at each cellular barrier interface needed to attain published emission rates. Based on the extremely high concentrations of VOCs predicted to accumulate in membranes, we deduced that this would be detrimental to membrane integrity and function. Thus, we propose here that active biological mechanisms are additionally required to lower VOC concentrations in membranes to achieve the observed emission rates. Such mechanisms become even more important under stresses such as

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herbivore attack, where VOC emission is drastically increased [42] and membrane integrity is compromised systemically throughout the plant [43]. Finally, based on the cellular trafficking of other hydrophobic compounds (i.e., waxes and diterpenes) in plants, we discuss possible biologically controlled routes for VOC export out of cells.

Diffusion-based models for VOC emission

Over the past couple of decades, VOC emission has been modeled as a diffusion process based on Fick's first law, which states that the steady-state flux of any molecule is dictated by the concentration gradient and the resistance of the diffusion path (Box 1). These models have primarily been formulated at the tissue level [44–48], taking into consideration the effects of biosynthesis and environmental factors [49–53]. However, few studies have looked at VOC movement at the microscopic scale. In their work to investigate the effect of stomatal opening on VOC emission, Niinemets and Reichstein developed a meticulously detailed model taking into account the resistance of individual cellular barriers [51,52]. Barrier resistances were evaluated based on the physicochemical properties of VOCs and structural parameters of each layer. The calculated resistances were then combined to simulate VOC emission and the total internal VOC pools inside the cell.

One concern with diffusion-based models, which has not yet been addressed, is that VOCs may accumulate to cytotoxic levels in membranes. Due to their high octanol-water partition coefficients (Box 1, $K_{o/w}$), VOCs favorably partition into hydrophobic environments. For small VOCs such as isoprene, the diffusivity may be high enough that a build up of high concentrations does not occur in membranes. However, for most VOCs to passively diffuse at a physiologically relevant rate into subsequent hydrophilic layers, such as the cell wall, they would accumulate to very high levels in membranes. To get an idea of whether such levels would be biologically tolerable, we calculated the barrier concentrations required to match experimentally measured VOC emission fluxes from snapdragon (*Antirrhinum majus*) flowers (Box 1) using a similar diffusion-based model as Niinemets and Reichstein [51,52]. Our calculations revealed that the concentration of a given phenylpropanoid/benzenoid or monoterpenoid VOC in the plasma membrane would have to be approximately 50–120 mM to sustain its reported emission flux (see Table III in Box 1). This extremely high concentration is primarily due to favorable partitioning of VOCs into the lipid phase and the high resistance imparted by the cuticle (see Figure IB in Box 1). Accumulation of lipophilic molecules in membranes increases permeability, which leads to the disruption of proton and ion gradients that are responsible for intracellular pH homeostasis and the driving forces for import and export of compounds out of the cell [54]. Therefore, it is our opinion that biological mechanisms must exist to lower the barrier resistance, thereby reducing the VOC concentration in membranes (Box 1). This concept logically extends to interfaces between internal membranes and the cytosol. Despite lower VOC emission fluxes, similar mechanisms might also occur in other plant organs, although alternative emission paths must be considered. In leaves, for example, VOCs will readily partition from cell walls into the intercellular gas spaces directly connected to substomatal air spaces, which provide a pathway of lower resistance to diffusion compared with the cuticle.

Possible biological mechanisms involved in VOC emission

Based on analogy to the intracellular trafficking of other hydrophobic compounds, such as waxes and diterpenes, there are several biological mechanisms that could be involved in shuttling VOCs out of undamaged cells [55]. VOCs synthesized in the cytosol will favorably partition into both plasma membranes and subcellular membranes (Figure 1). VOCs in internal membranes could be trafficked to the plasma membrane via endoplasmic reticulum (ER)–plasma membrane contact sites or via vesicle trafficking processes associated with the ER and Golgi, the *trans*-Golgi network (TGN), and/or vacuole (Figure 1). These processes would also effectively reduce the accumulation of VOCs in internal membranes. Although vesicular transport of metabolites is relatively unexplored in plants, it is thought to have a role in the movement of cytotoxic phytochemicals [56,57], hormones [58], antimicrobial compounds [59,60], pigments [61], and cuticular wax components [62,63]. VOCs synthesized in other organelles, such as monoterpenes in plastids, could also be delivered to the ER via interorganellar membrane hemifusion (Figure 1), a mechanism recently proposed for the transfer of lipophilic metabolites between plastids and the ER [64,65]. Alternatively, VOC trafficking to the plasma membrane could be mediated by soluble carrier proteins with hydrophobic pockets capable of escorting lipophilic compounds across aqueous environments [66] (Figure 1).

The subsequent translocation of VOCs across the plasma membrane to the apoplast entails the movement of hydrophobic molecules across a lipophilic layer into an aqueous compartment. One possibility is that plasma membrane-localized transporters are involved in the directional export of VOCs out of the cell. Recently, it has been proposed that vesicles carrying cuticle wax components deliver their cargo to plasma membrane ATP binding cassette (ABC) transporters for export to the cell exterior [63,67]. Moreover, an *Arabidopsis thaliana* plasma membrane ABC transporter, AtABCG29, has been shown to transport the monolignol *p*-coumaryl alcohol [68], which is structurally similar and shares the same core biosynthetic pathway as the VOCs isoeugenol, eugenol, and vanillin emitted from *Petunia hybrida* flowers [69]. Remarkably, expression of *PhABCG1*, a petunia *AtABCG29* homolog encoding a plasma membrane-localized transporter with unknown function, is regulated by the ODORANT1 (ODO1) transcription factor [70]. Given that the ODO1 transcription factor is known to control several biosynthetic genes encoding enzymes involved in synthesizing precursors for phenylpropanoid/benzenoid VOCs, it will be important to determine whether PhABCG1 exports VOCs to the cell wall. Once VOCs cross the lipophilic plasma membrane, they must traverse the hydrophilic cell wall. Although our diffusion model (Box 1) does not indicate the cell wall exerts a large resistance *per se*, small carrier proteins, such as extracellular lipid transfer proteins (LTPs),