Physiological and physicochemical controls on foliar volatile organic compound emissions

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Plant leaves emit a broad spectrum of organic compounds that typically play multiple roles in plant protection. Furthermore, most of these compounds actively participate in tropospheric chemistry. There has been rapid progress in understanding how the emission of volatiles is regulated, mostly focusing on the biochemical controls over compound production. However, physicochemical characteristics such as low volatility or diffusion can also control the emissions and interact with physiological limitations. In particular, non-specific leaf storage of less volatile compounds smooths the emission responses to fluctuating environmental conditions, and diffusion through stomata leads to conspicuous emission bursts after stomatal opening and modifications of diurnal emission time courses. Because natural conditions always fluctuate, both physiological and physicochemical controls exert a major influence over plant volatile emissions.

All plants emit a plethora of chemically divergent volatile hydrocarbons (VOCs). At a global scale, the production of plant-generated VOC species greatly exceeds the emission from anthropogenic sources [1]. Plant emissions play a central role in atmospheric reactivity, particularly in ozone-forming reactions and as condensation nuclei in aerosol formation and aerosol growth [2]. Therefore, a mechanistic understanding and the reliable prediction of biogenic emissions (see Glossary) are crucial if we are to predict global changes in atmospheric ozone concentration and radiation transmission – modifications that have long-term impacts on human health and the global carbon balance [3,4]. Furthermore, because the capacity for volatile compound emission modulates plant tolerance to heat [4,5], pollutant [6,7] and water [8] stresses, and affects plant–plant and plant–insect interactions [9], understanding the long-term effects of global change on vegetation requires a full understanding of the feedbacks between volatile emissions and environmental perturbations.

Emission rates of all plant-emitted volatile compounds depend on temperature and, for many compounds (particularly isoprenoids), on light [1,10]. Such environmental responses were included in simple empirical emission algorithms that predicted plant emissions with high predictive power in some, but not in all, situations [1]. As the biochemical reaction pathways of formation and controls of synthesis of various plant volatiles started to be unravelled [11,12], mechanistic models were also generated [13]. However, a series of key physiological observations are not incorporated in the current models. Specifically, there are important study-to-study discrepancies between VOC emission responses

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Glossary

**Biogenic emissions:** release of volatile compounds caused by biochemical reactions. Emitted compounds can either be organic (containing carbon such as hydrocarbons) or inorganic (such as nitric oxide). Plant leaves constitute a major source of non-methane hydrocarbons (VOCs) in the atmosphere.

**Henry’s law constant:** equilibrium gas–aqueous phase partition coefficient (H: measured in Pa m3 mol-1). The compounds with a larger H partition primarily to the gas phase, whereas those with a low H partition mainly to the aqueous phase. Diluted aqueous solutions with an equal compound concentration support a larger gas-phase partial pressure for compounds with larger H and are accordingly more volatile.

**Physicochemical controls:** control of organic compound emissions by limited volatility and diffusion. Physiological factors determine the rate of compound synthesis through intermediate availability and enzyme activity, whereas physicochemical factors affect the emission of synthesized compounds from the site of synthesis to the ambient air. Volatility is determined by compound equilibrium partitioning among the leaves and atmosphere (within-leaf storage) and primarily affects the diffusion gradient. Diffusion is mainly controlled by compound molar size and leaf internal structure and stomatal aperture.

**Stomatal conductance:** a measure of the limits on gaseous transport between the leaf and ambient atmosphere caused by the small pores (stomata) on the leaf surface. At a common partial pressure gradient between the atmosphere and leaf (ΔP), compound flux rate (F) increases linearly with increasing stomatal conductance (G): F = GΔP. Leaf stomatal apertures vary strongly as plants track the ambient light, temperature and humidity conditions. In particular, water-stressed plants strongly reduce G to limit water loss. Gaseous transport also occurs through a waxy layer covering the rest of the leaf surface (cuticle), but the contribution of the cuticle to the passage of gases is small relative to stomatal transport.

**Tropospheric chemistry:** overall series of chemical reactions occurring in the troposphere (the bottom 7–17 km layer of the atmosphere) that modifies the quality of ambient air, particularly the balance between ozone generation and ozone destruction. Biogenic emissions affect the chemistry of the troposphere because of the high reactivities of these compounds with hydroxyl radicals and chemical species of anthropogenic origin.
to elevated CO₂ [14–16], heat stress [17,18], stomatal closure because of water or salinity stress [19–22] and modifications in VOC product profiles caused by changes in environmental variables [19,23,24] and mechanical damage [25,26]. In addition, leaves can emit strongly in conditions that partly or entirely suppress the synthesis of volatile compounds [13,27,28]. Given that, in natural communities, plants always experience stress, we argue that the state-of-the-art volatile emission scenarios based on current models might not reflect current and future emissions.

Here, we outline the limitations in the mechanistic understanding of plant volatile emissions and argue that biochemical, physiological and physicochemical controls should be included in the simulation models to predict the biogenic volatile emissions reliably. We further demonstrate that unique responses of some specific volatile emissions to changes in environmental conditions can be entirely determined by the principal physicochemical characteristics of emitted compounds rather than by physiological mechanisms.

Physiological versus physicochemical controls on VOC emissions

Environmental controls on the emission of VOC species are due to temperature- and light-dependent changes in the compound synthesis rates (Figure 1). Temperature regulates isoprene [29] and monoterpene [30] synthase activities, thus controlling the synthesis of most isoprenoids. Temperature also affects respiration and can indirectly regulate the amount of pyruvate available to form isoprenoid intermediates in the chloroplast in a similar way to postulated pyruvate changes under elevated CO₂ [15]. Light availability determines the amount of the isoprenoid precursor glyceraldehyde-3-phosphate produced by photosynthesis, and also controls the availability of ATP and NADPH, which, for highly reduced volatile compounds, are required to a greater extent than for sugar synthesis [31] and can limit the formation of many volatiles [17,32]. For isoprenoid emissions [13,17], such environmental effects have been successfully simulated by combining the rates of intermediate production with the kinetics of several key enzymes made available by advances in biochemistry and molecular biology [12,14,30,33]. Process-based emission algorithms can also be used to explain partly the apparent emission anomalies that are observed under stress conditions or in response to global change factors [13,17].

Perhaps the best-documented example of the puzzling controls on isoprenoid emission is the effect of increasing atmospheric CO₂ concentrations (CO₂). Elevated [CO₂] uncouples isoprenoid emission and photosynthesis [14,15] by reducing the availability of isoprenoid precursors [15] or the activity of monoterpene synthases [14]. Modification of isoprene emission in response to changing [CO₂] is rapid in plants grown under either ambient or elevated [CO₂] [34]. These rapid emission changes are consistent with post-translational modification of isoprene synthase or with alterations in the availability of the isoprene precursors. At current CO₂ levels, the emission seems to be predominantly limited by enzyme activity because species emitting isoprenoids in low quantities accumulate the immediate isoprenoid precursor dimethylallylphosphate in their chloroplasts [35]. Although it is still unclear which is the first step to be affected by [CO₂], a process-based algorithm [17] successfully predicts the emission rate of isoprenoids under changing [CO₂] [36].

Environmental effects on the emission responses can also be caused by temperature-dependent increases in the volatility and diffusion rates of specific compounds or by the pool size of specific leaf volatiles (Figure 1). The relative importance of limitations on leaf VOC emissions because of synthesis and physicochemical constraints varies with the extent to which specific volatile compounds are stored within the leaves. For instance, there are specialized storage structures for terpenes, such as resin ducts in conifers and oil glands in several angiosperms, and it was believed that terpenoid emission from the foliage of these species was primarily under volatility control [37]. By contrast, in species lacking specialized storage structures, VOC emissions were thought to be primarily under synthesis control.

However, it has now been shown that this dichotomy of synthesis versus volatility-controlled emissions only partly corresponds to the actual leaf emissions. In particular, even in species with specialized storage compartments for terpenes, the emissions also depend on light, indicating that the immediate synthesis can significantly affect the overall emission rates [38,39] or that two independent pathways of terpene synthesis are simultaneously but independently active. For instance, in Italian stone pine (Pinus pinea), the monoterpenes trans-β-ocimene and linalool are emitted in a light-dependent manner and are formed directly from the intermediates of photosynthetic metabolism only during certain periods of the year [25]. By contrast, the other monoterpenes (such as limonene) are emitted at a low level year-round from the resin ducts [25]. There are also significant time lags between the synthesis and emission rates in plant species lacking specialized VOC storage compartments, suggesting that the volatile compounds are non-specifically stored within the leaves and that physicochemical limitations always interact with the synthesis rates of compounds [24].

All VOCs are stored within the leaves

Lack of specific anatomical structures for VOC storage does not imply negligible internal VOC concentrations. Lipophilic compounds with a high octanol-to-water partition coefficient (Kₒₒ/W), such as non-oxygenated terpenes (Kₒₒ/W = 10 000–30 000) can be stored in significant quantities in the lipid phase of the leaf’, which generally comprises 1–3% of total leaf dry mass. Hydrophilic compounds such as oxygenated monoterpene linalool, by contrast, are found in high concentrations in the aqueous phase of the leaf [28]. Direct measurements of intraleaf isoprenoid pools demonstrate that emissions of some monoterpenes can continue with a maximal rate at the expense of the non-specific storage for at least 10–15 min without de novo synthesis [28], whereas isoprene internal pools can support emissions for a few seconds after cessation of synthesis [28,40]. These data conclusively
demonstrate the existence of a VOC storage pool in species lacking specific anatomical storage compartments [24,28].

Non-specific storage explains several apparently anomalous emission responses to environmental fluctuations. First, biochemical regulation of VOC synthesis has been assumed to be fast, but monoterpene emissions do not respond immediately after the illumination of darkened leaves (Figure 1): maximum values of emission are reached after several hours of light exposure, and terpene emission does not stop immediately after switching off the light [23,27,41]. Furthermore, there are often significant nocturnal emissions from the leaves of broad-leaved emitting species [13,27,41]. Slow monoterpene labelling and unlabelling kinetics in 13C-feeding experiments [23,27] and incomplete labelling of emitted compounds [27] corroborate the idea that these time lags can be explained by the presence of conspicuous internal pools of VOC. Second, there are important modifications in profiles of emitted monoterpene species after alteration of environmental variables [19,23,24] and in response to mechanical damage [25,26]. Because every monoterpene species has a unique set of physicochemical variables, temporal increases in the emission of some monoterpene species and decreases in the emission of other species result from varying kinetics of emptying and filling of non-specific monoterpene storage pools [24].

Single plant species can emit more than 20 different monoterpene species. Because the rate constants for gas-phase reaction with ozone and hydroxyl radicals differ by several orders of magnitude for various terpenes, such modifications in product profiles might importantly alter atmospheric reactivity. Furthermore, in leaf-to-leaf, plant-to-plant and plant-to-animal signalling studies, there is a tendency to associate any change in plant-generated VOC emission rate and product profile (e.g. after insect feeding) with physiological plant response [9], but it is also important to consider physicochemical arguments in understanding such emissions.

How can stomata affect VOC emissions?
Apart from the volatility, gas-, aqueous- and lipid-phase diffusion within the leaves, and gas-phase diffusion at the leaf–air interface can interact significantly with the VOC synthesis of certain volatile compounds (Figure 1). Rapid decreases in stomatal conductance (G) do not alter the emission rates (F) of isoprene [22] (Figure 2a) and α-pinene [19] from plant leaves. The insensitivity of the emission of these compounds to changes in stomatal openness has
been explained by the rapid build-up of isoprenoid partial pressure within the leaf gas-phase, such that the decrease in the diffusion conductance is exactly balanced by increases in the diffusion gradient from the internal air space to the ambient air ($\Delta P$) \[22\].

The lack of stomatal control has been generalized to all volatile compounds \[10,42\]. However, there are strong short-term limitations in foliar volatile efflux rates after decreases in $G$ in other studies (Figure 2a) \[20,21,43\]. In addition to the condition that isoprenoid synthesis must remain constant after stomatal closure, insensitivity of isoprenoid emission fluxes to stomata is only possible if the internal isoprenoid partial pressure rises fast enough to balance $G$. In a non-steady-state situation, stomata can control the emission until the increase in the diffusion gradient $\Delta P$ balances the decrease of $G$.

The speed of the rise in $\Delta P$ depends on how fast the VOC concentration builds up in the leaf intercellular air space, suggesting that the question of stomatal sensitivity or insensitivity is a matter of kinetics of VOC internal gaseous and liquid pool sizes. The response time of the $\Delta P$ rise is different for various VOC species and depends mainly on its Henry’s Law Constant ($H$, gas–aqueous-phase partition coefficient). Compounds with a low $H$ value, such as acetic acid, formic acid, formaldehyde and methanol, primarily partition to the aqueous phase, whereas compounds with a large $H$, such as isoprene and non-oxygenated terpenes, partition mainly to the gas phase (Figure 2b). For compounds with a low $H$, larger changes in liquid pool size are necessary to obtain a similar change in $\Delta P$ than for less water soluble compounds, explaining the strong stomatal sensitivity of some compounds, such as methanol and organic acids, and the stomatal insensitivity of others, such as isoprene and non-oxygenated monoterpenes (Figure 2).

**Physicochemical limitations significantly modify in vivo VOC emissions**

Previous discussion underscores the argument that the overall extent to which physicochemical constraints affect the emission rates in species lacking specialized storage compartments depends on whether the system is in a steady state or not. Under the steady-state conditions often maintained in laboratory measurements, the VOC efflux is exactly the same as the rate of VOC synthesis. However, under strongly fluctuating conditions in the field, the emissions are rarely in a steady state, suggesting that physicochemical effects always modify the emission responses. In particular, non-specific volatile compound storage ‘smooths’ the responses of isoprenoid synthesis rates to rapid changes in light and temperature (Figure 3a), thereby significantly modifying the diurnal time courses of monoterpene emissions.

As the sensitivity analysis of gas-phase diffusion limitations (Figure 2b) and actual measurements \[20,21,43\] demonstrate, reaching the steady state can take hours for some volatile compounds, indicating that the emission of these compounds often deviates from a steady state in natural conditions. Therefore, the daily time courses of compounds that preferentially partition to the aqueous phase (such as methanol) are strongly modified by stomatal openness (Figure 3b). A conspicuous feature of
stomatal controls on VOC emissions is the bursts of emission following stomatal opening [21,44]. These bursts result as the size of the aqueous VOC pool rapidly adjusts to a new equilibrium pool size to correspond with the new value of stomatal aperture [44]. The bursts of emission are often observed for water-soluble volatile compounds [21,45] but were poorly understood until the development of dynamic emission models. Owing to the emission bursts following moderate changes in stomatal opening and temporal changes in liquid pool size, the diurnal time courses of water-soluble compounds are complex and are weakly related to the actual synthesis rates (Figure 3b). This complex behaviour driven by physicochemical characteristics is also the primary reason why recent empirical emission models using only the environmental controls on VOC synthesis rates have provided particularly poor fits to daily time courses of water-soluble compound emissions, particularly in water-stressed leaves [46].

Conclusions

Plants emit >30 000 divergent compounds, including alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters and carboxylic acids. The water solubility of this broad spectrum of compounds varies by more than six orders of magnitude and membrane solubility by four orders of magnitude [44]. Our analysis demonstrates that this vast heterogeneity among emitted volatiles often explains compound-specific responses of VOC emissions to changes in environmental drivers that cannot be predicted on the basis of physiological limitations alone. In particular, physicochemical differences explain the damping or amplification of emission rates and the large modifications in product profiles after alterations in environmental variables, without any changes in enzyme activation or gene expression [24,44]. Furthermore, in stressed leaves, gas-phase diffusion limitations can lead to complex diurnal patterns of water-soluble volatile compound emissions that occasionally overshadow the synthesis-level emission controls. Thus, we argue that physicochemical limitations
driven by the huge variability in physicochemical characteristics among volatile compounds need to be included in the physiological models to simulate the emissions realistically and that plant-generated emissions should be corrected for the physicochemical effects. Although the inclusion of physicochemical effects in existing process-based algorithms might first seem overly complex, physicochemical characteristics, once available, can be taken as constants. However, in certain cases, there are also important interactions between physiological and physicochemical controls [20] that complicate the implementation of these suggestions and call for further research into the regulation of enzymes controlling the pathway flux.

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