Controls on the emission of plant volatiles through stomata: Differential sensitivity of emission rates to stomatal closure explained

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[1] Volatile (VOC) flux from leaves may be expressed as $G_S \Delta P$, where G_S is stomatal conductance to specific compound and ΔP partial pressure gradient between the atmosphere and substomatal cavities. It has been suggested that decreases in G_S are balanced by increases in ΔP such that stomata cannot control VOC emission. Yet, responses of emission rates of various volatiles to experimental manipulations of stomatal aperture are contrasting. To explain these controversies, a dynamic emission model was developed considering VOC distribution between gas and liquid phases using Henry's law constant (H, Pa m^3 mol^{-1}). Our analysis demonstrates that highly volatile compounds such as isoprene and monoterpenes with H values on the order of 10^3 have gas and liquid pool half-times of a few seconds, and thus cannot be controlled by stomata. More soluble compounds such as alcohols and carboxylic acids with H values of $10^{-2}-10^1$ are controlled by stomata with the degree of stomatal sensitivity varying with H. Inability of compounds with high solubility to support a high partial pressure, and thus to balance ΔP in response to a decrease in G_S is the primary explanation for different stomatal sensitivities. For compounds with low H, the analysis predicts bursts of emission after stomatal opening that accord with experimental observations, but that cannot be currently explained. Large within-leaf VOC pool sizes in compounds with low H also increase the system inertia to environmental fluctuations. In conclusion, dynamic models are necessary to simulate diurnal variability of the emissions of compounds that preferably partition to aqueous phase. *INDEX TERMS:* 0315 Atmospheric Composition and Structure: Biosphere/atmosphere interactions; *KEYWORDS:* emission dynamics, Henry's law constant, isoprene, methanol, monoterpene, gas-phase conductance

1. Introduction

[2] Volatile organic compounds (VOC) emitted by plant leaves make up a major source of reactive hydrocarbons in the atmosphere [*Fuentes et al.*, 2000], and their atmospheric concentrations may drive in a large extent the tropospheric ozone forming reactions [*Chameides et al.*, 1988; *Guenther et al.*, 1994; *Simpson*, 1995; *Benjamin and Winer*, 1998], as well as generation of aerosols through photo-oxidation of emitted VOC species [*Vesala et al.*, 1998; *Yu*, 2000]. Plants produce a large number of volatile compounds, emission of which is under strong environmental control. For prediction of foliar VOC emission rates in dependence on incident quantum flux density and leaf temperature, empirical models have been developed that track the diurnal dynamics of VOC emission with a varying degree of success [*Guenther et al.*, 1994, 1995, 2000; *Simpson et al.*, 1995].

[3] A key assumption of these emission algorithms is that stomata do not constrain the flux, such that the rate of volatile emission always equals the rate of the compound production. This allows to use only the meteorological drivers - light and/or temperature, that control the rate of volatile production - to simulate the rates of emission. Missing stomatal controls have been explained by low foliar VOC air-phase partial pressures, which readily increase in response to decreasing stomatal conductance, and thereby balance the decrease in conductance by an enhanced diffusion gradient from the leaf intercellular air-space to ambient air [*Sharkey*, 1991; *Fall and Monson*, 1992; *Kesselmeier and Staudt*, 1999]. This opinion is supported by experimental observations of the lack of significant stomatal control over the emission rates of isoprene [*Monson and Fall*, 1989; *Fall and Monson*, 1992] and α-pinene [*Loreto et al.*, 1996c]. However, experimental evidence indicates that stomatal closure may constrain methanol [*Nemecek-Marshall et al.*, 1995] and carboxylic acid [*Kesselmeier et al.*, 1998; *Gabriel et al.*,

1999] emission rates, but a mechanistic explanation of these contrasting responses is missing.

[4] The complete lack of stomatal control is further called into question by field measurements, in which VOC emission rates exhibit a midday inhibition that is paralleled by a suppression of stomatal conductances. Such effects have been observed for carboxylic acids [*Gabriel et al.*, 1999], 2-methyl-3-buten-2-ol [*Schade et al.*, 2000], aldehydes [*Kesselmeier et al.*, 1997], and monoterpenes [*Valentini et al.*, 1997; *Niinemets et al.*, 2002b]. These phenomena cannot be described by the current empirical emission models that predict largest emission rates in midday because of highest leaf temperatures and incident quantum flux densities. Because of a large energy requirement for VOC synthesis, such midday decreases in VOC emissions may be partly explained by decreased compound synthesis rates [*Niinemets et al.*, 1999, 2002c]. Alternatively, *Niinemets et al.* [2002b] hypothesized that slow rise of oxygenated monoterpene gas-phase concentrations after stomatal closure may partly explain the stomatal sensitivity of emissions of VOC species that preferentially partition to aqueous phase. Although this hypothesis was partly supported by the data, there was also evidence of biochemical limitations of emission, underscoring the importance to develop models that can discriminate between the gas-phase diffusion limitations and the biochemical feedbacks arising from stomatal closure.

[5] Occasionally, bursts of VOC emission during stomatal opening or after rapid changes in environmental conditions have been demonstrated [*Nemecek-Marshall et al.*, 1995; *Holzinger et al.*, 2000]. These emission bursts do not relate to the immediate leaf light or temperature environment, and cannot be explained within the current mechanistic understanding of the emission dynamics. Moreover, emission of several VOC species exhibits an anomalous response to changes in leaf temperature and light environment [*Kreuzwieser et al.*, 2000; *Staudt et al.*, 2000; *Sabillón and Cremades*, 2001] leading to a lack of fit in parameterization of daily time courses of emissions. For atmospheric reactivity estimations, it is crucial to correctly simulate diurnal dynamics of the emission rates. Consequently, a theoretical framework is needed to remedy the inconsistencies between the observations and theoretical predictions, and estimate the potential significance of changes in volatile emission dynamics that arise from modifications in gas-phase diffusion conductance and meteorological conditions.

[6] We reassessed the available experimental information of the stomatal responses of VOC emission with the aim to reconcile the apparent discrepancies between experimental observations of stomatal controls on the emission of volatile compounds. We demonstrate that, provided the VOC synthesis is unaffected by the gas-phase diffusion conductance, changes in conductance may affect the VOC efflux only in a nonsteady state situation, at which the intercellular volatile partial pressure differs from the equilibrium pressure. Implicit in our analysis is that stomatal closure leads to increases in gas- and liquid-phase VOC concentrations, but the velocity with which leaf VOC concentrations increase to balance the decrease in stomatal conductance differs between plant volatiles. We argue that the compound partitioning between gas- and liquid-phases is the primary determinant governing the emission dynamics, and thus, the

results of the current study quantitatively test the hypothesis of *Niinemets et al.* [2002b].

[7] Although the model requires estimates for a large number of physico-chemical parameters of volatile compounds, and leaf anatomical and structural characteristics, we demonstrate in the accompanying paper [*Niinemets and Reichstein*, 2003] that the model is relatively insensitive to various leaf structures. Given that the physico-chemical characteristics of volatiles may be taken as constant, the modeling scheme outlined here has a large potential for simulation of stomatal limitations of VOC emissions in the field.

2. Theory: A Model Describing VOC Emissions in Dependence on Stomatal Conductance

2.1. Description of Steady State VOC Emission Rates

[8] Although there is evidence that nonpolar volatiles may be emitted through the leaf cuticle [*Guenther et al.*, 1991; *Fall and Monson*, 1992], recent studies suggest that these observations may be associated with damage of foliar surface during enclosure the leaves in the gas-exchange cuvette [*Loreto et al.*, 1996c, 2000]. For nonpolar compounds such as monoterpenes, the experimental cuticular diffusion coefficients of 1.18×10^{-14} m^2 s^{-1} for α-pinene and 4.65×10^{-15} m^2 s^{-1} for limonene [*Schmid*, 1991; *Schmid et al.*, 1992] are on average more than eight orders of magnitude smaller than the diffusion coefficients in the air, and more than four orders of magnitude lower than the diffusion coefficients in the water (Table 1), indicating that cuticula provides an efficient barrier for the plant volatiles.

[9] Thus, we assume that the cuticular VOC emissions are nil, and relate VOC flux (F, mol m^{-2} s^{-1}) from the leaves to stomatal aperture [see also *Tingey et al.*, 1991] by an equation analogous to that previously employed for CO_2 diffusion into the leaf [*Farquhar and Sharkey*, 1982; *Ball*, 1987; *Field et al.*, 1989]:

$$F = \frac{G_S(P_s - P_a)}{P} + E\left(\frac{P_s - P_a}{2P}\right), \quad (1)$$

where G_S (mol m^{-2} s^{-1}) is the stomatal conductance to emitted compound vapor, P_s (Pa) is the compound partial pressure in substomatal cavities and P_a is the compound partial pressure in the leaf boundary layer, P is the total air pressure and E the leaf transpiration rate (mol m^{-2} s^{-1}). The first part of the equation describes the control of VOC flux by stomata, the second part of the flux is attributable to mass flow resulting from net water efflux through the stomata. Although the second part of equation (1) is conceptually important and has been previously included in the first biophysical models of monoterpene emission [*Tingey et al.*, 1991], it is generally very small, and will be neglected in the following calculations. The stomatal conductance to specific volatile, G_S (mol m^{-2} s^{-1}), can be determined from measurements of stomatal conductance to H$_2$O, G_V (mol m^{-2} s^{-1}):

$$G_S = \frac{D_A G_V}{D_V}, \quad (2)$$

where D_A (m^2 s^{-1}) is the binary diffusion coefficient for a specific compound in the air, and D_V that for water vapor

(m^2 s^{-1}). Because experimental data are lacking for many important plant volatiles, and the available values are generally reported for a single temperature only, we use a set of predictive equations to derive the physico-chemical characteristics of plant volatiles for any leaf temperature (Appendix A, Table 1).

[10] In addition to stomatal conductance, the gas-phase volatile flux also depends on the compound diffusion from the outer surface of cell walls to the substomatal cavities. This part of the diffusion pathway is characterized by the intercellular gas-phase conductance, G_{ias} that depends on internal leaf architecture [*Niinemets and Reichstein*, 2003]. For the two conductances in series, the total gas-phase diffusion conductance is given as:

$$G_G = \frac{1}{1/G_S + 1/G_{ias}}, \quad (3)$$

and the flux from the outer surface of the cell walls to the ambient air as:

$$F = \frac{G_G(P_i - P_a)}{P}, \quad (4)$$

where P_i is the steady state intercellular partial pressure of the volatile. From equation (4), the P_i is expressed as:

$$P_i = \frac{F \cdot P}{G_G} + P_a. \quad (5)$$

Because no volatile build-up generally occurs in the leaf boundary layer or the ambient air, P_a is practically zero under natural conditions. The monoterpenoid gas-phase concentration in the intercellular air space, C_g (mol m^{-3}), is equal to $P_i/(RT_k)$, where R is the gas constant (8.314 J mol^{-1} K^{-1}), and T_k the leaf temperature (K).

[11] Using the analogy with CO_2 diffusion [*Laisk and Oja*, 1998], we express the VOC flux from the site of synthesis to substomatal cavities, F_m, as:

$$F_m = G_L(C_w - P_i/H), \quad (6)$$

where C_w is the water-phase volatile concentration at the site of synthesis (mol m^{-3}), G_L is the liquid phase diffusion conductance (m s^{-1}) of specific volatile from the site of synthesis to the outer surface of cell walls, and H, the Henry's law constant (Pa m^3 mol^{-1}), is the equilibrium air-water partition coefficient, which for dilute aqueous solutions may be defined as [*Mackay and Shiu*, 1981; *Staudinger and Roberts*, 1996]:

$$H = \frac{P_i}{C_a}, \quad (7)$$

where C_a (mol m^{-3}) is the water-phase volatile concentration at a volatile partial pressure of P_i. For environmental applications, aqueous solutions with less than 0.001 to 0.01 mole fraction of solute are considered dilute [*Staudinger and Roberts*, 1996]. Although the solubility of some of the plant volatiles such as ethanol or methanol, which are miscible, may exceed this solubility limit, the cellular

concentrations of even very soluble compounds are at most in the millimolar range in physiological conditions [*Nemecek-Marshall et al.*, 1995; *Joseph and Kelsey*, 1997; *Kreuzwieser et al.*, 2000].

[12] All plant volatiles share the same gas-phase pathway. However, the liquid phase diffusion conductance, G_L, is a composite conductance consisting of several conductances in series. For the compounds synthesized in chloroplasts, such as isoprene, the liquid-phase diffusion pathway includes chloroplast stroma, chloroplast envelope, cytosol, plasmalemma and cell wall [*Niinemets and Reichstein*, 2003]. However, other leaf compartments may be the primary source for other compounds, and therefore we make several further assumptions of the potential components of the diffusion pathway for various volatiles. For our analysis, it is relevant that the site of compound synthesis primarily affects the G_L calculations when both the diffusion flux densities out of the leaf as well as the gradients along the diffusion pathway are high. The equilibrium concentration gradients between the cell compartments are considerably smaller when the diffusion flux out of the leaf is restricted, for example, because of stomatal closure. Thus, the longest diffusion pathway may be more appropriate in such situations.

2.2. Dynamic Model of VOC Diffusion Through the Stomata

[13] Given that the synthesis rate of the volatile, I, is not affected by stomatal conductance, stomatal closure inevitably leads to a gradual increase in the intercellular volatile partial pressure. After the steady state has been reached, $F = F_m = I$ (equations (4) and (6)), the increase in P_i exactly balances the decrease in G_G, and the volatile flux out of the leaf, F, equals that before the stomatal closure. Thus, stomata cannot control the emission over the long term. However, the crucial question with respect to stomatal control on VOC emission is how fast the leaf gas (S_G, nmol m^{-2}) or liquid (S_L, nmol m^{-2}) pools of the volatile reach a steady state after a change in the leaf stomatal conductance. If they reach the steady state situation sufficiently slowly, stomata may temporarily have a large impact on VOC emission.

[14] Using the mass balance approach, we describe the dynamics of the gas and liquid pools of a volatile as:

$$\frac{dS_G}{dt} = F_m - F \quad (8a)$$

$$\frac{dS_L}{dt} = I - F_m, \quad (8b)$$

where the diffusion flux density from the site of synthesis to outer surface of cell walls, F_m, is given by equation (6), and the diffusion flux density through the stomata, F, by equation (4). The gas pool size is given as:

$$S_G = \frac{P_i}{RT} \cdot \frac{f_{ias}V}{A}, \quad (9)$$

where V (m^3) is leaf volume, A is leaf surface area, and f_{ias} is the fraction of gas volume in total leaf volume. Thus, $f_{ias}V/A$

gives the gas (intercellular) leaf volume per leaf surface area. The liquid pool size is given as:

$$S_L = C_w \frac{f_w V}{A}, \qquad (10)$$

where f_w is the aqueous fraction of total leaf volume. Combining equations (5) and (9), assuming that P_a is negligible, and solving for F leads to a first order kinetics of the gas pool:

$$F = \left(\frac{A}{f_{ias}V} \cdot \frac{RT}{P} G_G \right) S_G \overset{Def}{:=} k_G S_G, \qquad (11)$$

where k_G (s^{-1}) is the turnover rate of the gas-phase, and the half-time of the gas pool, τ_G is:

$$\tau_G = \frac{\ln(2)}{k_G}. \qquad (12)$$

2.3. The Gas Pool Dynamics After a Rapid Stomatal Closure

[15] The further analysis may be considerably simplified if the τ_G values are sufficiently small compared with the time constants of stomatal closure and opening such that S_G could be considered as essentially in a steady state. Very short values of τ_G, on the order of 0.02–0.08 s, have been inferred for isoprene [*Singsaas and Sharkey*, 1998], suggesting that at least for some compounds, the assumption of a steady state may be legitimate. Combining equations (8a), (9), and (11), and solving the resulting differential equation, the gas pool size at time t, S_G^t, is given by:

$$S_G^t = \frac{F_m}{k_G} - \left[\frac{F_m}{k_G} - S_G^{t_0} \right] \cdot e^{-k_G \cdot t}, \qquad (13)$$

where $S_G^{t_0}$ is the initial gas pool size at time t_0. Figure 1 illustrates the response of the α-pinene emission rate after an instantaneous change of stomatal conductance (G_V, equation (1)) from 230 mmol m^{-2} s^{-1} to 2 mmol m^{-2} s^{-1}. When the stomata are open, the rate constant of the gas pool of α-pinene (Table 1) is 7.5 s^{-1}, corresponding to a half-time of the gas pool ($\tau_G = \ln(2)/k_G$) of 0.09 s. The time constant of the gas pool decreases after simulated stomatal closure to a value of 0.23 ($\tau_G = 3.11$ s), and a new steady state is reached in ca. 15 s. After that, the flux is maintained, because the rises in intercellular α-pinene partial pressure increase the gradient between the intercellular air-space and atmosphere, allowing to compensate for the decreased stomatal closure (equation (4)). The situation will be analogous for other volatiles listed in Table 1, but the system would respond faster because of a higher binary diffusion coefficient (D_A). For example, for acetone ($D_A = 1.030 \times 10^{-5}$ m^2 s^{-1}), the half-times would be 0.04 s for open stomata, and 1.74 s for closed stomata, and the volatile partial pressure balancing the stomatal closure would be 0.67 Pa ($P_i = 1.1$ Pa in the case of α-pinene, Figure 1).

[16] Given that the time constants for stomatal movements are on the order of minutes [*Tinoco-Ojanguren and*
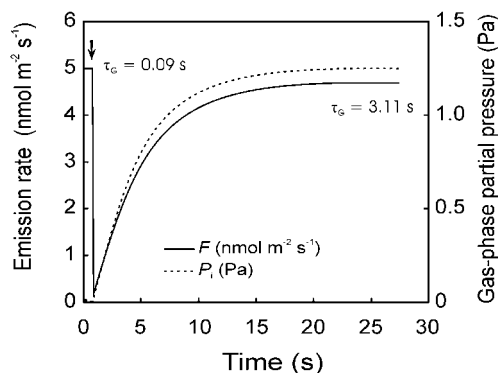

Figure 1. Modeled (equations (11)–(13)) effects of instant stomatal closure on α-pinene intercellular partial pressure (P_i) and emission rate (F) at 25°C. The steady state monoterpene flux rate was 5 nmol m^{-2} s^{-1}, and the stomatal conductance to water vapor was changed from 230 mmol m^{-2} s^{-1} to 2 mmol m^{-2} s^{-1} at time 1 s (denoted by arrow). The gas pool size (S_G) was calculated assuming that monoterpenoid efflux into the substomatal cavities (F_m, equation (6)) is independent of P_i. The physico-chemical characteristics of α-pinene are reported in Table 1, and the leaf structural variables for calculation of internal conductances were those of *Quercus ilex* [*Niinemets and Reichstein*, 2003].

Pearcy, 1993], we conclude that the gas-phase may be essentially considered as in a steady state, i.e., $F_m = F$.

2.4. Simplified Emission Model

[17] For a steady state gas-phase, P_i from equation (5) may be substituted in equation (6) giving:

$$F_m = G_L \left(C_w - \frac{F_m P}{G_G H} \right) \Leftrightarrow F_m = \frac{G_L C_w}{\left(1 + \frac{G_L P}{G_G H} \right)}. \qquad (14)$$

Substituting $C_w = S_L A / (f_w V)$ from equation (10) into equation (14), the governing differential equation (equation (8b)) becomes:

$$\frac{dS_L}{dt} = I - \frac{G_L \dfrac{A}{f_w V}}{\left(1 + \dfrac{G_L P}{G_G H} \right)} S_L \overset{Def}{:=} I - k_L S_L. \qquad (15)$$

The last term in this equation is the flux into the gas pool, and insofar as the gas pool is in a steady state, also the emission flux. Thus, the efflux from the liquid pool obeys a first order kinetics, where the rate constant k_L depends on the gas- and liquid-phase conductances (G_L, G_G), and on the Henry's law constant (H). Of course, the leaves possess an extremely complex and compartmentalized structure, and consideration of the leaf as a composite of only liquid and gas-phases is a crude simplification. Nevertheless, we argue that even such a simplified analysis provides fundamental insight into the dynamics and behavior of the system in response to rapid changes in stomatal conductance and environmental conditions. We demonstrate in the accom-

panying study [*Niinemets and Reichstein*, 2003] that leaf structural differences may affect the system dynamics, but also that these effects are superimposed by volatile liquid/gas-phase partitioning analyzed in the current paper.

[18] The analytical solution of equation (15) is given as:

$$S_L(t) = \frac{I}{k_L} - \left(\frac{I}{k_L} - S_L^0 \right) \cdot e^{-k_L \cdot t}, \qquad (16)$$

where S_L^0 is the pool size at $t = 0$. The analytical solution was used in simulations with a constant k_L, and I. In all other cases, equation (15) was solved numerically. Consideration of the gas pool may be important for gaining detailed insight into the emission of very volatile compounds with a high value of H, because in such cases most of the within-leaf compound pool is in the gas-phase. Yet, the exact effects also depend on the share of internal resistance between liquid and gas-phase pathways. Such potential effects of gas-phase on emission kinetics were analyzed by another model version solving the system of both differential equations (equations (8a) and (8b)) numerically for a nonsteady state gas-phase. The differences in the rate constants of the system response with the more complex model were only minor in comparison with the predictions by equations (14)–(16), and did not alter qualitatively the conclusions with respect to the stomatal control on emissions. Therefore, only simulations with the simplified model are presented.

3. Model Parameterization

3.1. Physico-Chemical Characteristics of the Volatiles

[19] Simulation of the dynamics of the VOC emission rates requires information of compound gas- and liquid-phase diffusion coefficients as well as the compound's distribution coefficients between water, air and lipid phases. These data were obtained from literature, and using a variety of estimation methods to determine the missing physical constants as fully described in Appendix A. The equations employed to scale the compound characteristics experimentally determined at one temperature to another temperature are also provided in Appendix A.

3.2. Calculation of Internal Conductances

[20] Internal gas-phase (G_{ias}) and liquid-phase (G_L) conductances for various plant volatiles were calculated as described in *Niinemets and Reichstein* [2003] using detailed anatomical and morphological data for each species. To roughly estimate the reliability of our calculations, the same anatomical data were employed to determine liquid- and gas-phase internal diffusion conductances for CO_2. Our estimates for various species agreed with the experimentally determined values [*Syvertsen et al.*, 1995; *Evans and Loreto*, 2000], indicating that our calculations provide a realistic description of the internal diffusion pathway [*Niinemets and Reichstein*, 2003].

3.3. Compounds for Detailed Analyses and the Appropriate Diffusion Pathways

[21] For detailed analyses, we chose nine representative compounds of contrasting Henry's law constant, that is, formaldehyde, methanol, 2-methyl-3-buten-2-ol, linalool, acetaldehyde, bornyl-acetate, *p*-cymene, isoprene, and α-pinene. We argue that all other uncharged compounds can be simulated following analogous logic. Plant cells are highly compartmentalized, and specific metabolic pathways and formation of certain volatiles can reliably be circumscribed to particular cell locations. However, a plethora of pathways may be operative for other compounds. Because the length of diffusion pathway and possible diffusion barriers may critically depend on the location of the compound production, assumptions regarding the site of synthesis are necessary to determine the appropriate diffusion gradient.

[22] Formaldehyde ($H = 0.0305$ Pa m^3 mol^{-1}) is emitted in many species in significant quantities [*Kesselmeier et al.*, 1997; *Martin et al.*, 1999; *Kesselmeier*, 2001], and several metabolic pathways operative in various cell organelles may lead to its release [*Hourton-Cabassa et al.*, 1998; *Igamberdiev et al.*, 1999]. For calculation of its liquid-phase conductance, we assumed that formaldehyde originates from conversion of methanol in the cytosol [*Hourton-Cabassa et al.*, 1998]. Methanol ($H = 0.461$ Pa m^3 mol^{-1}), the assumed precursor of formaldehyde, is likely released via de-methylation of cell walls as the result of pectin formation [*Fall and Benson*, 1996], and particularly high rates of its emission are observed in young leaves [*Nemecek-Marshall et al.*, 1995]. Thus, for methanol, we used only the cell wall conductance.

[23] 2-Methyl-3-buten-2-ol ($H = 1.56$ Pa m^3 mol^{-1}) is an important volatile in pines [*Harley et al.*, 1998; *Schade et al.*, 2000], and is formed in chloroplasts [*Zeidler and Lichtenthaler*, 2001], whereas linalool ($H = 2.07$ Pa m^3 mol^{-1}) is an essential component in emissions in many monoterpene emitting species [*Staudt et al.*, 1997; *Ciccioli et al.*, 1999; *Hansen and Seufert*, 1999; *Sabillón and Cremades*, 2001]. We assume that linalool as well as all other monoterpenes, their derivatives and isoprene are synthesized in chloroplasts [*Chappell*, 1995]. This assumption is likely valid for broad-leaved terpene-emitting species [*Loreto et al.*, 1996b; *Schuh et al.*, 1997], but not necessarily for conifers, in which the emission rates may also rely on the monoterpenes stored in resin ducts [*Shao et al.*, 2001].

[24] Acetaldehyde ($H = 7.00$ Pa m^3 mol^{-1}) is commonly released from leaves in response to root flooding [*Kreuzwieser et al.*, 1999, 2000; *Holzinger et al.*, 2000], but acetaldehyde emission may occasionally be observed in nonflooded plants as well [*Kesselmeier et al.*, 1997]. The proposed mechanism of acetaldehyde emission involves ethanol transport from the roots to the leaves with the transpiration stream, and further enzymatic conversion to acetaldehyde [*MacDonald and Kimmerer*, 1991; *Kreuzwieser et al.*, 1999], likely in the cytosol [*Kimmerer and MacDonald*, 1987; *MacDonald and Kimmerer*, 1993]. In the current study, the liquid-phase diffusion conductance calculations assumed that acetaldehyde is synthesized in the cytosol.

[25] Bornyl-acetate ($H = 44.3$ Pa m^3 mol^{-1}), and *p*-cymene ($H = 947$ Pa m^3 mol^{-1}) are repeatedly found in the emission patterns in monoterpene-emitting species [*Steinbrecher*, 1989; *Loreto et al.*, 1996a; *Kesselmeier et al.*, 1997; *Niinemets et al.*, 2002a], but generally in only trace quantities. Isoprene ($H = 7780$ Pa m^3 mol^{-1}) is emitted

Table 1a. Physico-Chemical Properties of Selected Plant Volatiles at 25°C: Aliphatic Compounds With Short Chain Length[a, b]

Compound	Molecular Mass, g mol^{-1}	H, Pa m^3 mol^{-1}	P_V, Pa	δ, mol m^{-3}	$K_{o/w}$, mol mol^{-1}	D_A, m^2 s^{-1}	D_W, m^2 s^{-1}	G_{ias}, m s^{-1}	G_L, m s^{-1}
Acetic acid[c]	60.1	0.0133	2070	∞[d]	1.74	1.075×10^{-5}	1.364×10^{-9}	1.016×10^{-2}	3.876×10^{-5}
Formic acid[c]	46.0	0.0176	5701	∞	0.288	1.373×10^{-5}	1.799×10^{-9}	1.297×10^{-2}	1.579×10^{-5}
Formaldehyde	30.0	0.0305	516400	∞	0.457	2.801×10^{-5}	2.431×10^{-9}	2.647×10^{-2}	2.801×10^{-5}
Methanol	32.0	0.461	16980	∞	0.170	1.570×10^{-5}	1.799×10^{-9}	1.483×10^{-2}	1.055×10^{-5}
Ethanol	46.1	0.507	7926	∞	0.490	1.175×10^{-5}	1.364×10^{-9}	1.111×10^{-2}	1.306×10^{-5}
2-methyl-3-buten-2-ol	86.1	1.56	474	1170[e]	17.8[e]	7.949×10^{-5}	9.501×10^{-10}	7.512×10^{-3}	1.057×10^{-4}
Acetone	58.1	3.88	30950	∞	0.575	1.030×10^{-5}	1.196×10^{-9}	9.735×10^{-3}	9.986×10^{-6}
Acetaldehyde	44.0	7.00	121610	∞	0.457	1.293×10^{-5}	1.476×10^{-9}	1.222×10^{-2}	1.380×10^{-5}
Isoprene	68.1	7780	73410	11.7	263	8.826×10^{-6}	9.811×10^{-10}	8.341×10^{-3}	9.796×10^{-4}

[a]The compounds were ordered according to their Henry's law constant values. Averages were calculated whenever multiple estimates for a specific parameter were available.
[b]H - Henry's law constant [*Mackay and Shiu*, 1981; *Howard and Meylan*, 1997; *Anonymous*, 2001; *Sander*, 2001]; P_V - saturated vapor pressure [*Josephy and Radt*, 1948; *Jordan*, 1954; *Lide and Kehiaian*, 1994; *Simon et al.*, 1995; *Howard and Meylan*, 1997; *Bauer et al.*, 1998; *Daubert et al.*, 1998; *Fichan et al.*, 1999; *Anonymous*, 2001; *Brown and Stein*, 2001]; δ - water solubility [*Josephy and Radt*, 1948; *Mackay and Shiu*, 1981; *Schmid et al.*, 1992; *Weidenhamer et al.*, 1993; *Loreto et al.*, 1996a; *Howard and Meylan*, 1997; *Eggersdorfer*, 1998; *Fichan et al.*, 1999]; $K_{o/w}$ - octanol-water partition coefficient [*Gimmler et al.*, 1981; *Schmid et al.*, 1992; *Howard and Meylan*, 1997; *Anonymous*, 2001]; D_A - diffusion coefficient in air (equation (A1)); D_W - diffusion coefficient in water (equation (A2)); G_{ias} - intercellular gas-phase conductance from outer surface of cell walls to substomatal cavities [*Niinemets and Reichstein*, 2003]; G_L - liquid-phase diffusion conductance from the site of synthesis to outer surface of cell walls [*Niinemets and Reichstein*, 2003]. Leaf anatomical variables for *Q. ilex* were used in calculations of G_{ias} and G_L, and chloroplast was assumed to be the site of synthesis for all compounds.
[c]All characteristics for the undissociated form only.
[d]Miscible with water.
[e]An average value for 3-methyl-3-buten-1-ol.

in a vast number of species [*Sharkey and Yeh*, 2001], whereas α-pinene (H = 10840 Pa m^3 mol^{-1}) comprises often a major fraction of emitted monoterpenes [*Loreto et al.*, 1996a; *Staudt et al.*, 1997; *Niinemets et al.*, 2002a].

4. Results

4.1. Variation in Volatile Physico-Chemical Properties

[26] Physico-chemical information for a large number of plant volatiles was compiled, of which a small subset of compounds with differing Henry's law constant (H) is presented in Table 1. Across important plant volatiles, the value of the Henry's law constant varies more than seven orders of magnitude, indicating large differences in the compound distribution between gas and liquid phases. Both in short-chained aliphatic compounds (Table 1a), and in cyclic plant compounds and aliphatic substances with longer chain-length such as monoterpene derivatives (Table 1b) the corresponding alcohols, aldehydes, ketones and carboxylic acids have low H values, indicating preferential partitioning to aqueous phase. In contrast, alkanes, alkenes and unsubstituted aromatics are preferentially partitioned to gas-phase. Although the saturated vapor pressures and aqueous solubilities are generally larger for compounds with a smaller

molecule size, compounds of contrasting size may have similar volatility (H, e.g., compare methanol and α-terpineol in Table 1). However, the volatiles with a larger molecular mass are generally more permeable to membranes because of a larger octanol to water partition coefficient ($K_{o/w}$, Table 1) - the ratio of the compound concentrations in water-saturated octanol to that in octanol-saturated water. Thus, the liquid-phase conductances calculated as described in the accompanying study [*Niinemets and Reichstein*, 2003] were generally larger for monoterpenes and their derivatives (cf. Tables 1a and 1b) implying potentially greater diffusion flux densities from the sites of synthesis to the outer surface of the cell walls [*Niinemets and Reichstein*, 2003].

4.2. Simulated Stomatal Closure Effects on Volatiles of Differing Henry's Law Constant

[27] We selected isoprene as a representative compound with a high H, and methanol as a representative compound with a low H (Table 1) for detailed simulation analyses of the emission responses to stomatal closure. Previous studies have observed no stomatal effects on isoprene emission [*Fall and Monson*, 1992], but a strong stomatal control over the emission rates of methanol [*Nemecek-Marshall et al.*, 1995].

Table 1b. Physico-Chemical Properties of Selected Plant Volatiles at 25°C: Monoterpenes and Their Derivatives[a]

Compound	Molecular Mass, g mol^{-1}	H, Pa m^3 mol^{-1}	P_V, Pa	δ, mol m^{-3}	$K_{o/w}$, mol mol^{-1}	D_A, m^2 s^{-1}	D_W, m^2 s^{-1}	G_{ias}, m s^{-1}	G_L, m s^{-1}
Thymol	150.2	0.122	8.66	6.0[b]	1995	5.443×10^{-6}	6.817×10^{-10}	5.143×10^{-3}	1.381×10^{-3}
α-Terpineol	154.3	0.239	5.07	12.6	955	5.290×10^{-6}	6.526×10^{-10}	4.999×10^{-3}	8.851×10^{-4}
Menthol	156.3	1.54	5.83	2.05	2399	5.334×10^{-6}	6.603×10^{-10}	5.041×10^{-3}	1.313×10^{-3}
Linalool	154.3	2.09	21.3	10.2	933	5.175×10^{-6}	6.262×10^{-10}	4.890×10^{-3}	8.594×10^{-4}
Bornyl-acetate	196.3	44.3	17.7	0.118	7244	4.781×10^{-6}	5.910×10^{-10}	4.518×10^{-3}	1.510×10^{-3}
p-Cymene	134.2	947	197	0.179	31620[c]	5.750×10^{-6}	6.977×10^{-10}	5.434×10^{-3}	2.030×10^{-3}
β-Pinene	136.2	9190	404	0.0592	24270	5.812×10^{-6}	7.001×10^{-10}	5.468×10^{-3}	2.106×10^{-3}
α-Pinene	136.2	10840	558	0.0411	45710	5.812×10^{-6}	7.001×10^{-10}	5.493×10^{-3}	2.171×10^{-3}

[a]Symbols and data sources as in Table 1a.
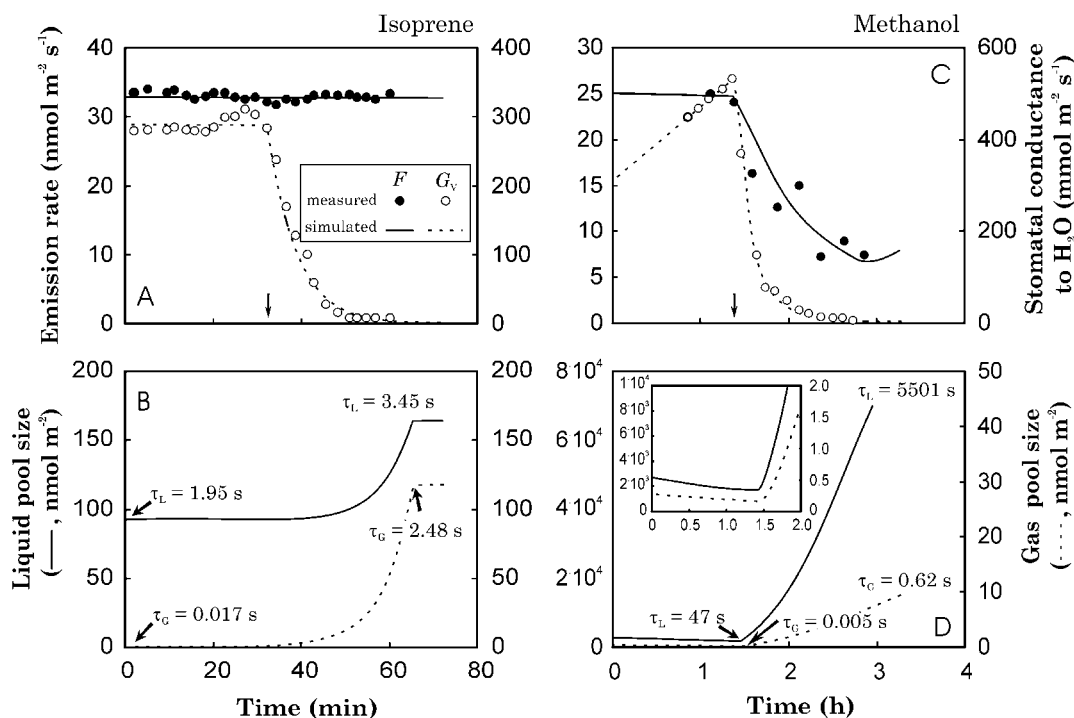[b]T = 20°C.
[c]An estimate for *m*-cymene.

Figure 2. Measured [*Fall and Monson*, 1992; *Nemecek-Marshall et al.*, 1995] and predicted rates (equations (14)–(16)) of isoprene (Figure 2a) and methanol (Figure 2c) emission, and isoprene (Figure 2b) and methanol (Figure 2d) gas and liquid pool sizes together with the half-times of the pools (τ_G for the gas, and τ_L for the liquid pool) before and after changes in stomatal conductance to H_2O (G_V) in response to added abscisic acid (ABA) solution in *Populus tremuloides* (Figures 2a and 2b; data of [*Fall and Monson*, 1992]) and *Phaseolus vulgaris* (Figures 2c and 2d; data of [*Nemecek-Marshall et al.*, 1995]). In simulations, the volatile pools were allowed to reach a steady state (e.g., inset in Figure 2d), and the stomatal conductance was decreased after ABA addition (denoted by an arrow in Figure 2a and Figure 2c) according to exponential relationships fitted to the data, whereas the isoprene and methanol synthesis rates were kept constant. The physico-chemical characteristics of isoprene and methanol (Table 1) were converted to the experimental temperature of 30°C as described in Appendix A. The leaf structural variables for *P. vulgaris* [*Niinemets and Reichstein*, 2003] were used to determine the liquid- and gas-phase intercellular diffusion conductances for simulations with methanol (only cell wall resistance considered for liquid-phase). For simulations with isoprene in *Populus*, leaf structural characteristics of *Quercus ilex* and *P. vulgaris* were averaged [*Niinemets and Reichstein*, 2003].

[28] The model analysis (equations (14)–(16)) of the isoprene (Figures 2a and 2b) emission rates in response to stomatal closure after addition of abscisic acid (ABA), which is the phytohormone regulating stomatal openness [*Tardieu et al.*, 1996; *Hartung et al.*, 1998], also suggested that the emission rates should not respond to changes in stomatal conductance, as long as the isoprene synthesis rate remains constant. The rate constant of stomatal closure of ca. 0.0025 s^{-1} was considerably lower than that of either gas- (0.28 s^{-1} for closed stomata) or liquid-phase (0.20 s^{-1}) pools of isoprene (Figure 2b). This indicates that changes in the gas-phase conductance were exactly balanced by an isoprene build-up in the leaf intercellular space, leading to an increase in the diffusion gradient between the outer surface of the cell walls and the atmosphere (equation (4)), and maintenance of the flux through the stomata. Thus, our simulations corroborate the mechanism proposed by *Fall and Monson* [1992].

[29] There were also significant increases in the liquid-phase methanol pool in response to a decrease in G_V after

ABA addition (Figure 2d). However, the increase in liquid-phase pool size supported only a small increase in the intercellular gas-phase pool of methanol such that the increases in the intercellular methanol partial pressure did not fully compensate for stomatal closure, leading to decreases in the diffusion flux rates (Figure 2c). Our model not only qualitatively described the changes in the emission rates of methanol after a decrease in G_V, but also provided a striking correspondence with experimental observations ($r^2 = 0.90$) validating the modeling scheme and estimation of the methanol physico-chemical, and leaf structural characteristics. Given that the internal leaf methanol contents on the order of 10–90 μmol m^{-2} have been observed in the leaves of *P. vulgaris* [*Nemecek-Marshall et al.*, 1995], our modeled pool sizes (Figure 2d) are realistic and correspond to the experimental values.

[30] Nevertheless, provided the synthesis rate of the compound remains constant, the increase in liquid and gas pool sizes will finally override the effect of stomatal closure, and in the steady state, stomata cannot control the emission.
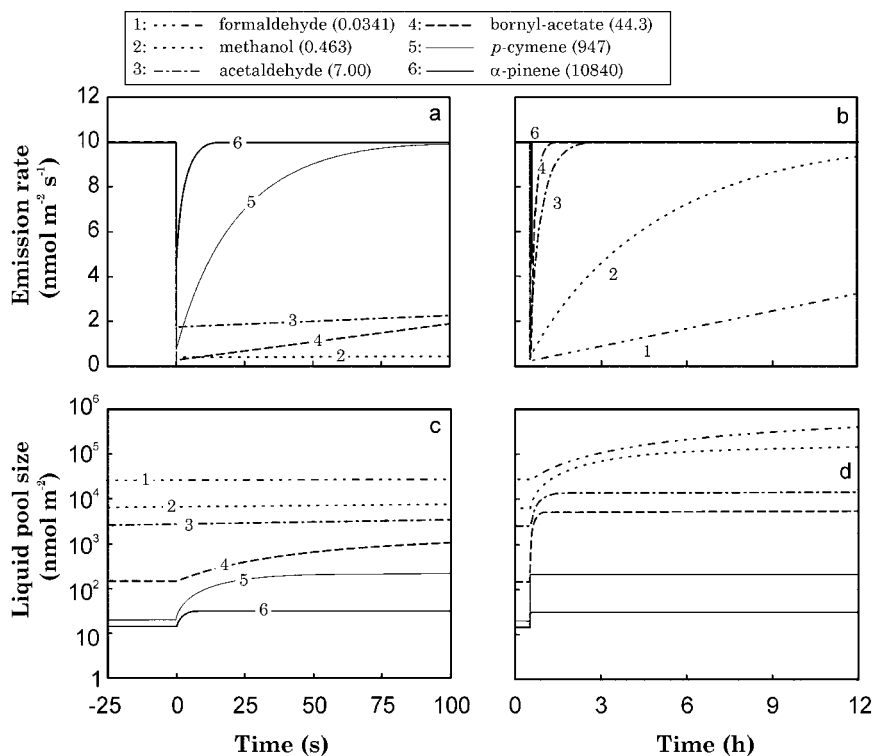
Figure 3. Simulated response (equations (14)−(16)) of the emission dynamics of volatiles with different values of Henry's law constant (H, equation (7), the values in parenthesis in Pa m^3 mol^{-1}) to an instantaneous stomatal closure from 150 mmol m^{-2} s^{-1} to 3 mmol m^{-2} s^{-1} at time $t = 0$. The data are presented in both a high (Figure 3a and 3c) and a low (Figure 3b and 3d) time-resolution. The simulation was conducted at 25°C, and all internal conductances were calculated using the values for *Q. ilex* [*Niinemets and Reichstein*, 2003]. In Figure 3a, lines 1 (formaldehyde) and 2 (methanol) are overlapping.

The primary difference between the compounds is the time needed to reach the steady state. For methanol, which has a low H and which therefore supports a low intercellular partial pressure, the predicted half-time of the liquid pool is 5500 s for closed stomata, whereas the half-time of isoprene liquid-phase pool is only 3.45 s (cf. Figures 2b and 2d). This difference corresponds to the higher H of isoprene, due to which the same water-phase concentration of isoprene supports more than 1.5×10^4 times higher partial pressure than that of methanol.

[31] Comparison of the dynamics of emission after a simulated stomatal closure in compounds of varying H (Figure 3) demonstrates that the response kinetics of different plant volatile compounds may widely vary. While formaldehyde may theoretically accumulate for days before the leaf liquid pool reaches a steady state, acetaldehyde emission rate is likely suppressed maximally for a few hours, whereas the stomatal closure will slow down the emission rates for minutes in the case of bornyl-acetate or seconds for *p*-cymene and α-pinene (Figure 3a).

4.3. Interaction Between Stomatal Control and Compound Synthesis Rates in VOC Species With Intermediate Values of Henry's Law Constant

[32] While stomata may control effectively the emission rates of compounds with the lowest H such as methanol and formaldehyde, and cannot control the emission rates of compounds with the Henry's law constant exceeding ca. 100 Pa m^3 mol^{-1} (Figure 3), the physiological significance of potential stomatal effects is less clear for compounds with intermediate H values. In particular, if the stomatal closure is accompanied by modification in the compound synthesis rates, an interpretation based on shifts in liquid/gas phase-equilibria alone may lead to wrong conclusions. In these compounds, such as acetaldehyde (Figure 4) [*Kesselmeier et al.*, 1997; *Kreuzwieser et al.*, 2000], the correlation between the emission rate and stomatal conductance is inconclusive.

[33] We analyzed the day-to-night dynamics of acetaldehyde emission from *Populus tremula* x *P. alba* [*Kreuzwieser et al.*, 2000] using four different scenarios to simulate the variation of acetaldehyde synthesis rate, I, with the time from the start of flooding ($t = 0$). In *Populus tremula* x *P. alba*, it has been demonstrated that the emitted acetaldehyde originates from the ethanol synthesized in roots in response to soil flooding and thereafter transported to the leaves by transpiration flow [*Kreuzwieser et al.*, 1999]. Although the acetaldehyde emission rates were weakly correlated with stomatal conductance (Figure 4), there was a time-lag of 2−3 h between the closure of stomata in response to switching off the light, and decreases in the acetaldehyde emission rates (Figure 5b), hinting at possible within-leaf acetaldehyde storage effects. Experimental data demonstrated a continuous increase in xylem-sap ethanol concentration rates with the time from flooding in a continuous light regime
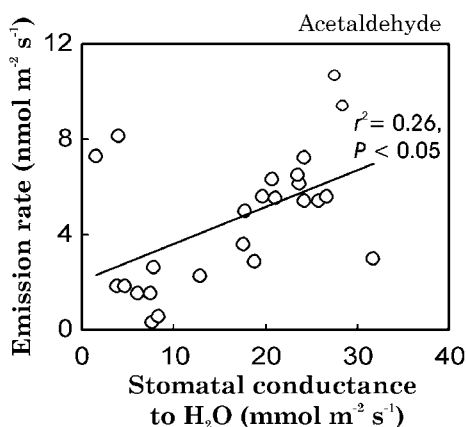
Figure 4. Correlation between the emission rate of acetaldehyde and stomatal conductance to H_2O (G_V) in *Populus tremula* x *P. alba* [*Kreuzwieser et al.*, 2000]. Decreases in G_V were achieved by switching off the light. Linear correlation analysis was employed to test the statistical significance of the trend.

[*Kreuzwieser et al.*, 1999] (inset in Figure 5a), and delayed decrease in xylem ethanol concentrations in response to plant darkening [*Kreuzwieser et al.*, 2000] (inset in Figure 5g). We used scenario analyses to get insight into the dynamic control of acetaldehyde emission rates.

[34] In scenario I, the acetaldehyde synthesis rate, I, was held constant (Figure 5a), while it was varied according to a saturating curve determined from the time-dependent changes in xylem-sap ethanol concentration for a continuous light regime (inset in Figure 5a) in scenario II. In scenario III (Figure 5d), I, the potential rate of which was given by the curve in Figure 5a, was varied in proportion to stomatal conductance. In scenario IV (Figure 5g), I was adjusted to track the observed changes in xylem-sap ethanol concentration (inset in Figure 5g) after the light/dark/light cycles. All scenarios indicated that acetaldehyde emission may be controlled by stomata to a certain degree (Figures 5b, 5e, and 5h) For example, the continuous acetaldehyde production scenario (I) and increasing emission rate scenario (II, Figure 5b) suggested that stomatal closure leads to a reduction in the emission rate which lasts for ca. 2 hrs. However, after the steady state in the liquid pool was reached at ca. $t = 13$ h, the increases in the predicted emission rates overrode the stomatal limitations, indicating that stomata cannot control acetaldehyde emission over a long term. Scenario III (Figure 5e), where I was set proportional to stomatal conductance realistically described the patterns. Yet, it failed to describe the delay in the decrease in the acetaldehyde emission rates after stomata closed at $t = 11:20$ h (Figure 5e). This effect was described by the fourth scenario (Figure 5h), where the observed light/dark changes in xylem-sap ethanol concentrations were employed. Because the leaf water potential decreases during the day, nontranspiring plants maintain the water flow from the roots in the dark until the leaf water potential reaches the equilibrium with soil water status [*Boyer*, 1985].

[35] We conclude that stomatal control of acetaldehyde emission involves a complex interplay between the limitations exerted on the gas-phase diffusion conductance and the stomatal effects on the ethanol delivery rates, whereas the latter effects may be superimposed by water movement from the roots to the leaves in nontranspiring plants.

4.4. Responses of VOC Emission Rate to Stomatal Opening

[36] Simulations with acetaldehyde (Figures 5b and 5h) predicted that stomatal opening, after a certain period of closure, may lead to a burst of emission of the accumulated compound. We analyzed this effect for methanol emission in *Phaseolus vulgaris* (Figure 6), where morning emission burst of methanol has been experimentally observed [*Nemecek-Marshall et al.*, 1995]. Assuming that the methanol synthesis rate is unaffected by stomata, the steady state rate of methanol emission in the darkness is established in ca. 5 h (Figure 6a). This is accompanied by a build-up of a large liquid-phase pool of methanol (Figure 6b). Simulating the kinetics of stomatal opening after switching on the light using actual measurements of stomatal conductance (dotted line in Figure 6a), the model predicts that the liquid pool supports an enhanced rate of methanol emission exceeding four-fold the steady state rate after the onset of stomatal opening (Figure 6a).

[37] The potential of different compounds for the "morning" emission burst is directly associated with the compound ability to build up a large liquid pool size (Figure 7), because the amount of the VOC emitted during the peak is proportional to the pool size at the time of stomatal opening. In addition, in compounds with smaller pool sizes, the maximum peak emission rate during the burst is attained faster, in agreement with lower stomatal control exerted on the emission of these volatile compound species.

4.5. Emission Response to Rapid Changes in Temperature

[38] Compound-specific liquid pool sizes in a steady state situation also imply that the VOC species not only respond differently to stomatal closure, but have different time kinetics for any change in environmental conditions that affect the equilibrium constants, as well as the compound synthesis rates. Ignoring for a moment the possible effects of temperature on the compound synthesis rates, the potential effects of changes in H are illustrated in Figure 8. It is apparent that modification of the gas/liquid phase partitioning during realistic temperature ramps may have large effects on the emission flux rates in VOC species with low values of H such as formaldehyde and methanol, while the importance of liquid/gas phase transfer is irrelevant for compounds with a large Henry's law constant, gas- and liquid-phase pools of which are generally in a steady state.

5. Discussion
5.1. Effects of Compound Physico-Chemical Characteristics on the Emission Responses to Stomatal Closure

[39] Stomata always constitute a finite resistance, and therefore, over an infinite time interval, stomata cannot control the flux rate when the compound synthesis rate remains constant. However, in practice, stomata may significantly curtail VOC flux over a certain time-span, and for constructing the reactive volatile compound emission
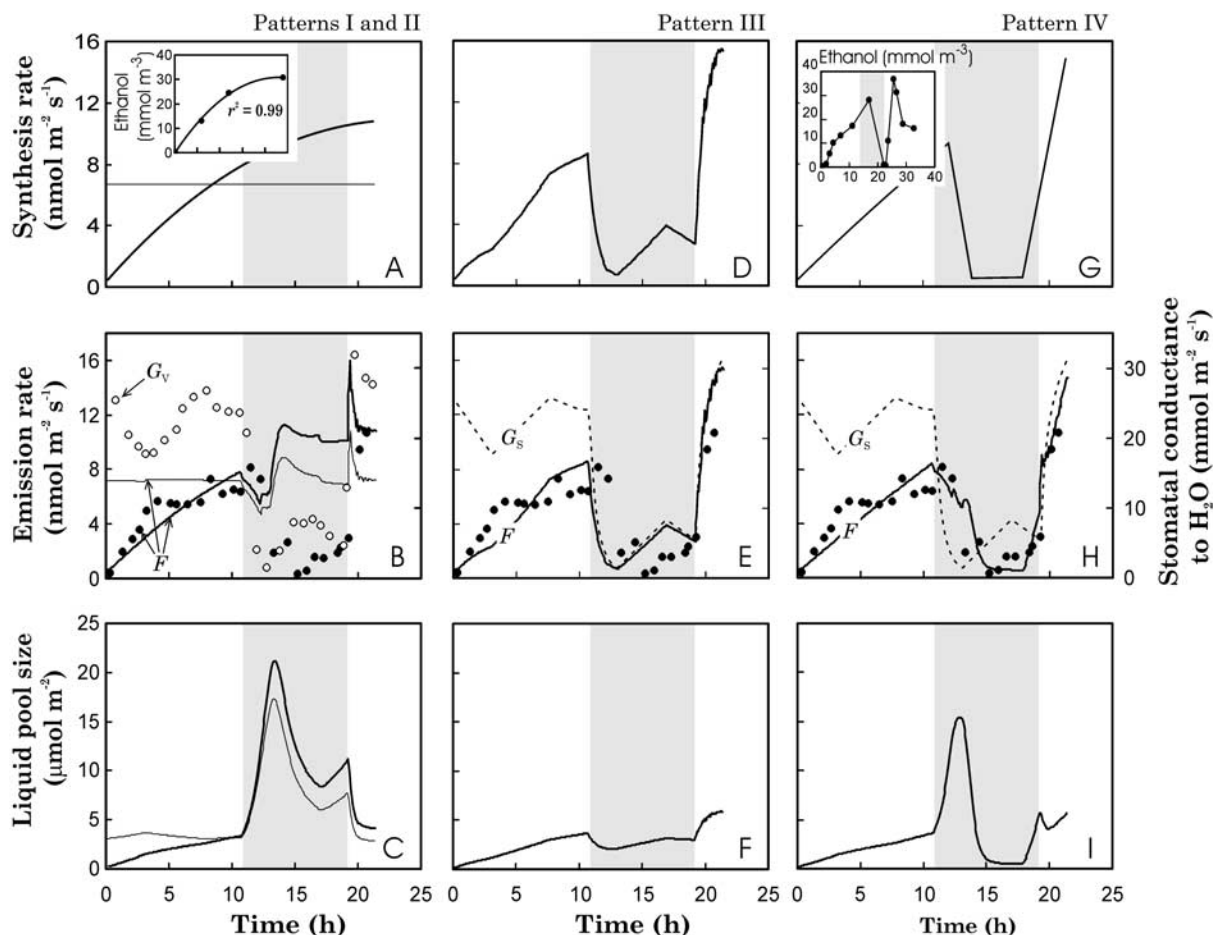
Figure 5. Measured (*Kreuzwieser et al.* [1999, 2000], Figure 4, filled and open circles) and modeled responses (continuous and dashed lines) of acetaldehyde emission rates, corresponding changes in the liquid pool size of acetaldehyde to light/dark changes and alterations in the acetaldehyde production rate in *Populus tremula* x *P. alba*. In the simulations, the acetaldehyde synthesis rate (Figures 5a, 5d, and 5g) was either held constant (Pattern I, thin line in Figures 5a–5c), changed according to a saturating curve (Pattern II, thick line in Figures 5a–5c), shape of which was adjusted to time-dependent changes in xylem-sap ethanol concentration after the start of flooding at $t = 0$ and under continuous light (inset in Figure 5a, data from *Kreuzwieser et al.* [1999]), or the potential synthesis rate in the inset of Figure 5a was varied in proportion to the maximal stomatal conductance (Pattern III, Figures 5d–5f), or the synthesis rate was changed to trace the observed temporal changes in xylem-sap ethanol concentration (inset in Figure 5g) after dark/light changes (Pattern IV, Figures 5g–5i). The physico-chemical characteristics of acetaldehyde were converted to the experimental temperature of 20°C according to Appendix A. Average values of leaf structural characteristics in *Q. ilex* and *P. vulgaris* were used to derive estimates of internal conductance[*Niinemets and Reichstein*, 2003]. The shaded area denotes dark periods.

scenarios, it is essential to determine over which time period specific volatiles may be controlled by stomata.

[40] The compound's Henry's law constant (H) determines the partition of volatile between air and liquid phases, and accordingly, the intercellular partial pressure (P_i) that can be sustained for a certain VOC liquid-phase concentration (equation (6)). Gas-phase partial pressure, in turn, is the primary determinant of the diffusion gradient between the substomatal cavities and ambient air (equations (4)–(6)). Therefore, the Henry's law constant of particular VOC species is the key characteristic of the system responsiveness to changes in gas-phase conductance (Figure 3). In compounds with a large H such as isoprene or monoterpenes (Table 1), a certain water-phase volatile concentration supports a high compound partial pressure. Thus, decreases in stomatal conductance (G_S) lead to an almost immediate elevation in gas-phase partial pressure (Figures 1 and 2a) thereby increasing the partial pressure gradient between the intercellular air-space and atmosphere and allowing the diffusion flux to be maintained at an unaltered level. This explanation is in accord with experimental observations of the insensitivity of isoprene [*Fall and Monson*, 1992] and monoterpene [*Loreto et al.*, 1996c] emission rates to experimental modification of G_S. As the sensitivity analyses
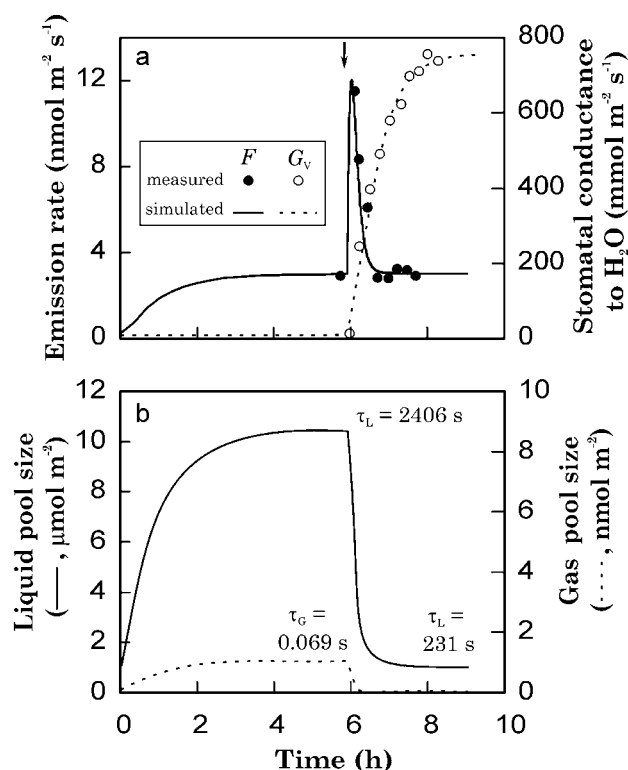
Figure 6. Measured [*Nemecek-Marshall et al.*, 1995] and predicted changes in methanol emission rate during stomatal opening in the morning (Figure 6a) and corresponding modifications in the gas and liquid pool sizes (Figure 6b) along with the estimates of half-times of both pools in *Phaseolus vulgaris*. Internal conductances were calculated using the structural data reported in *Niinemets and Reichstein* [2003] for the longest diffusion pathway, assuming that the intracellular concentrations of methanol are in equilibrium within all cell compartments. In the model analysis, the stomatal conductance was changed from 750 mmol m^{-2} s^{-1} to 10 mmol m^{-2} s^{-1} at time $t = 0$ to describe the evening stomatal closure, and the kinetics of morning stomatal opening was simulated according to data (dotted line in Figure 6a).

indicate [*Niinemets and Reichstein*, 2003], stomata cannot control the emission rates in compounds with high H over a significant time-interval even at physiologically very unrealistic situations.

[41] In compounds with low H, P_i rise is slower, because large increases in liquid pool size are necessary for a given increase in P_i. Thus, if the VOC synthesis rates remain constant, P_i cannot increase fast enough to avoid the decrease in the emission rates due to stomatal closure. Analysis of the experimental observations for methanol (Figure 2c), and simulations with H values of further important plant volatiles (Figure 3) indicated that the half-times of the liquid pool may be on the order of several hours for a number of VOC species. As a consequence of the buffering of the P_i-increases by the liquid pool in compounds with low H, a strong sensitivity of the emission rates to G_S is also expected (Figures 2c and 3). This large sensitivity is corroborated with decreases in methanol

[*Nemecek-Marshall et al.*, 1995], and acetic and formic acid [*Gabriel et al.*, 1999] emission rates after experimentally manipulated stomatal closure, but also with modification in carboxylic acid emissions after light to dark transitions [*Kesselmeier et al.*, 1998], and apparent midday inhibition of 2-methyl-3-buten-2-ol [*Schade et al.*, 2000] and carboxylic acid [*Kesselmeier et al.*, 1998] emission rates.

[42] What is the capacity of leaf liquid pools? For compounds miscible with water (Table 1), there is no theoretical limit for the upper concentration of the water-phase volatile concentration. Of course, there may be feedbacks on the volatile synthesis rates due to toxicity of high cellular VOC concentrations [*Kimmerer and Kozlowski*, 1982; *Joseph and Kelsey*, 1997; *Swan and Watson*, 1999]. However, even in poorly soluble compounds such as the monoterpene derivatives (Table 1), the system is generally far from the solubility limit because of low rates of compound synthesis. For example, using the leaf structural characteristics of *Q. ilex* [*Niinemets and Reichstein*, 2003], it is possible to calculate that for linalool with a solubility of 10.2 mol m^{-3} (Table 1), continuous synthesis of linalool with a high rate of 10 nmol m^{-2} s^{-1} fills up the liquid pool to the solubility limit in ca. 36 h.

[43] Direct measurements of internal monoterpene pools in *Q. ilex* [*Loreto et al.*, 1998] have indicated that the measured leaf monoterpene contents may occasionally exceed the actual solubility limit. For example, *Loreto et al.* [1998] observed ca. 30-fold higher α-pinene contents than our modeled liquid pool size after stomatal closure (Figure 3). However, a recent simulation study suggests that all plant leaves possess a certain capacity for nonspecific monoterpene storage, probably in the form of droplets of pure monoterpenoid in cell liquid volume or solubilized in cell membranes and cuticle [*Niinemets and Reichstein*, 2002]. Recent anatomical investigations [*Pasqua et al.*, 2002] do demonstrate that there are monoterpene droplets in the mesophyll cells of emitting species. Given that only a small part of the leaf internal monoterpenes measured by *Loreto et al.* [1998] was solubilized in water according to the recent reanalysis [*Niinemets and Reichstein*, 2002], we conclude that our simulated pools (Figure 3) are realistic and in accord with the experimental assessments.

5.2. Response of VOC Emission Rates to Stomatal Opening and the Emission Rates of Volatile Compounds in Response to Changes in Environmental Conditions

[44] As an important outcome of our analysis, we demonstrate that the dynamics of volatile emission differs for stomatal opening and closure. In particular, stomatal opening in the morning may bring about a large burst of VOC emission (Figures 5 and 6). Similarly to methanol emission from the leaves of *Phaseolus vulgaris* [*Nemecek-Marshall et al.*, 1995], and acetaldehyde emission from the leaves of *Populus tremula* x *P. alba* [*Kreuzwieser et al.*, 1999, 2000] reanalyzed in our study (Figures 5 and 6), *Holzinger et al.* [2000] have demonstrated bursts of acetaldehyde and ethanol emission from the leaves of *Q. ilex* after rapid changes in cuvette light regime. The data of *Kesselmeier et al.* [1997] also provide evidence of a large morning formaldehyde emission burst from the leaves of *Q. ilex*. None of the currently available steady state VOC emission models
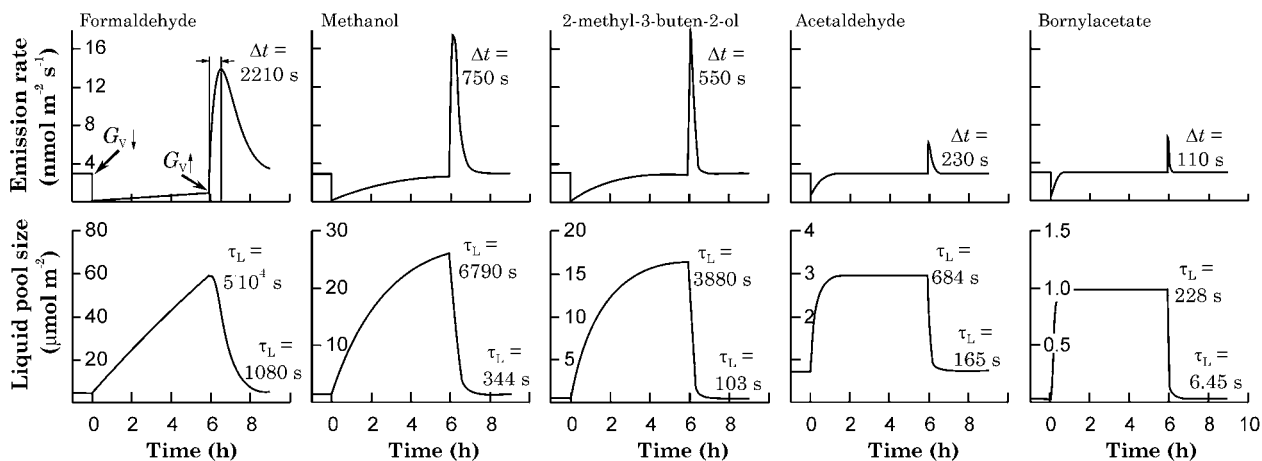
Figure 7. Simulation of VOC emission burst after a 6 h dark period for five VOC species of contrasting Henry's law constant (Figure 3, Table 1). Stomatal conductance to H_2O (G_V) was changed from 300 to 5 mmol m^{-2} s^{-1} at $t = 0$, and G_V was kept at this value for 6 hours, after which the conductance was gradually increased to 300 mmol m^{-2} s^{-1} using a saturating curve, the shape of which is depicted in Figure 6. The rate of VOC synthesis was set at 3 nmol m^{-2} s^{-1}. The maximum half-times of liquid-phase pools just after the start of stomatal opening, and the half-times after the stomata were fully opened are also given. Δt is the time span from the beginning of stomatal opening to reaching the maximum VOC emission rate. Mesophyll liquid-phase and intercellular gas-phase conductances were calculated using the anatomical parameters for *Q. ilex* [*Niinemets and Reichstein*, 2003]. The simulation was conducted at $T = 25°C$.

[*Guenther et al.*, 1993, 2000; *Ciccioli et al.*, 1997; *Fuentes et al.*, 2000] can describe these phenomena, but they are fully consistent with the mechanism of an accumulation of volatiles in the leaves during periods of stomatal closure, and release after the gas-phase conductance starts to rise (Figures 6 and 7). Various compounds sustain bursts of emission of varying time-span, whereas the peak emission rates also vary in dependence on the compound Henry's law constant (Figure 7), while the amount of volatile emitted during the burst is directly proportional to the amount of volatile accumulated during the period of low gas-phase conductance. In fact, as soon as there is an accumulation of VOC in the leaf due to low gas-phase conductance, any increase in G_S will lead to a certain burst of emission, in particular, for volatiles with low H.

[45] Not only changes in stomatal conductance, but modifications in leaf temperature may also cause bursts of emission, because of alteration of the liquid/gas phase-equilibria (Figure 8). Again, such effects are expected to be more important in compounds with low H that have large pools, emission from which is strongly limited by gas-phase conductance. *Staudt et al.* [2000] have observed experiment-to-experiment differences in changes in acetic and formic acid emissions in response to a rise in temperature in *Citrus sinensis* that could not be accommodated into the current theoretical view of VOC synthesis and release. In addition, there were large day-to-day differences in the carboxylic acid emission rates of the same leaves [*Staudt et al.*, 2000]. Our analysis suggests that such discrepancies can be explained by experiment-to-experiment variation in pool sizes, because the dynamics of the large nonequilibrium pools is likely to govern the system response to environmental changes for these compounds.

[46] *Singsaas and Sharkey* [1998] have demonstrated rapid alterations in isoprene emission rates to rapid leaf temperature changes in *Quercus alba*. However, our analysis suggests that these emission changes cannot be explained by modifications in liquid-gas phase-equilibria, because the gas and liquid pools of isoprene change very fast (Figure 2). As disputed by the authors, rapid biochemical regulation may partly be responsible for such changes [*Singsaas and Sharkey*, 1998; *Singsaas et al.*, 1999]. The possibility of a rapid regulation of VOC synthesis rates, together with associated changes in liquid-gas phase-equilibria in compounds with low H, suggests that further experimental work is necessary to gain conclusive insight into diurnal regulation of VOC fluxes.

5.3. Interaction of Stomata With the Rates of Compound Synthesis and Metabolization

[47] We analyzed solely the gas-phase diffusion effects on the emission rates, and a key assumption in our study was that the synthesis of the volatile is constant after stomatal closure. As the reanalyzed data (Figures 2 and 6), and the literature evidence outlined above indicated, this assumptions is satisfied in many instances. However, simulations with acetaldehyde (Figure 5) as well as experimental observations for linalool [*Niinemets et al.*, 2002b] show that several simultaneous processes of differing time-constant may be superimposed, significantly complicating the emission dynamics. While the synthesis rates of acetaldehyde likely varied with changes in stomatal conductance due to a modification in the ethanol delivery rate, the synthesis rates of acetaldehyde were maintained for a longer time period, allowing the acetaldehyde pool size to increase and temporarily overrule the stomatal limitations on the
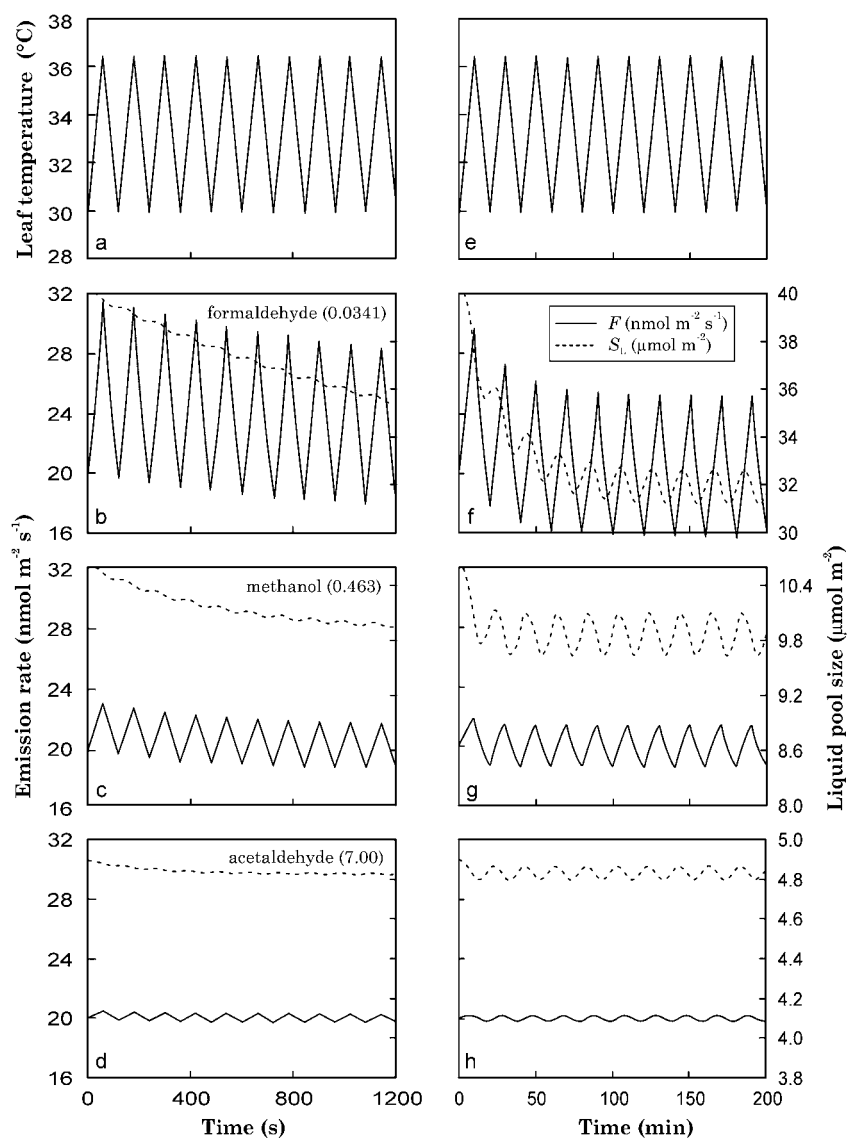
Figure 8. Modeled responses of the VOC emission rates and liquid pool sizes to fluctuations in leaf temperature (*T*) for three volatiles of contrasting Henry's law constant (Table 1). Leaf temperature was increased according to an exponential curve from 30°C to 36.5°C and symmetrically decreased thereafter to 30°C. In Figures 8a–8d, *T* was increased by 6.5°C with 60 s, corresponding to a rapid change in leaf temperature [*Singsaas and Sharkey*, 1998]. In Figures 8e–8h, the rate constants of *T* increase and decrease were divided by ten. Stomatal conductance to H_2O was set to 150 mmol m^{-2} s^{-1} and the rate of VOC production to 20 nmol m^{-2} s^{-1}. In all panels, the scale of the liquid pool axes was set proportional to the scale in Figure 8f, where the largest change in the pool size was observed. The physico-chemical characteristics in dependence on temperature were varied according to Appendix A, and all internal conductances were calculated using the *Q. ilex* leaf anatomical characteristics [*Niinemets and Reichstein*, 2003].

acetaldehyde emission (Figure 5h). Thus consideration of interacting factors of varying time-constant may be necessary to gain insight into the stomatal controls on the volatile emission rates.

[48] Currently, there is little experimental information that a build-up of the VOC pool in the leaf leads to a suppression of the compound synthesis rate, but it is fully reasonable, and would imply that gas-phase diffusion conductance effects are appended by biochemical limitations on the emission rates. There does exist evidence that

volatile accumulation leads to their increased metabolization rates. For the ethanol/acetaldehyde system, accumulation of ethanol results in a higher acetaldehyde formation rate such that the ethanol pool size and the emission rates increase much slower than when there were no ethanol metabolization [*MacDonald and Kimmerer*, 1993; *Kreuzwieser et al.*, 1999]. Within our modeling framework, the product metabolization in the cell essentially also functions as a "pool size." Thus, when the delivered or synthesized product is rapidly metabolized, the liquid concentration

increases less rapidly, implying greater stomatal effects on the emission.

[49] An important factor that may alter the stomatal sensitivity of VOC emission and emission dynamics is the species-to-species and temporal variability in the rate constants of stomata itself. The rate constants for stomatal opening and closure do vary between the species, and the rate constant for opening may decrease and the rate constant for closure may increase with developing leaf water stress [*Davies and Kozlowski*, 1975; *Aasamaa et al.*, 2002]. According to our study, this may have large potential effects on VOC emission dynamics.

5.4. Implications of Stomatal Effects for Modeling the Emission of Volatile Organics

[50] The results of the current study are of paramount importance for local and global emission inventories. Many plant species emit large quantities of short-chained alcohols, aldehydes, and carboxylic acids [*Nemecek-Marshall et al.*, 1995; *Fall and Benson*, 1996; *Kesselmeier et al.*, 1997; *Kesselmeier and Staudt*, 1999; *Martin et al.*, 1999]. In monoterpenoid-emitting species, oxygenated monoterpenoids such as linalool and 1, 8-cineole may constitute a major fraction of all monoterpenoids emitted [*König et al.*, 1995; *Baraldi et al.*, 1999; *Ciccioli et al.*, 1999; *Rohloff*, 1999; *He et al.*, 2000; *Sabillón and Cremades*, 2001]. Given that all those compounds preferably partition to liquid-phase, large stomatal sensitivity of these volatiles is expected (Figures 3 and 7).

[51] The steady state emission algorithms that assume a complete insensitivity of the emission rates to gas-phase conductance, and use only incident irradiance and leaf temperature as the drivers of the emission rates [*Guenther et al.*, 1993, 2000] provide poor fits to diurnal emission patterns of short-chained carboxylic acids and aldehydes [*Kesselmeier et al.*, 1997; *Martin et al.*, 1999; *Staudt et al.*, 2000; *Kesselmeier*, 2001] as well as to daily emission dynamics of linalool [*Sabillón and Cremades*, 2001]. We argue that in natural strongly fluctuating environments, the steady state models are inappropriate to describe the daily variation in the emission rates of compounds with a low H value, because the large foliar pools of these volatiles efficiently uncouple the emission rates from short-term to intermediate effects of environmental variables on the compound synthesis rates.

6. Conclusions

[52] Our analysis demonstrates that the emission of volatile compounds with a low Henry's law constant (H) strongly depends on the gas-phase conductance from outer surface of cell walls to ambient atmosphere. Liquid-phase has a large capacitance for the compounds with a low H, and gas-phase concentrations of these volatile substances may rise more slowly than stomatal pores close, leading to a temporal limitation of the diffusion flux through the stomata.

[53] Important dynamic phenomena such as the morning burst of VOC emission are predicted by our model, indicating that the gas-phase constraints on the emission may significantly alter the diurnal dynamics and distribution of VOC flux rates over the day. The bursts of emission are

experimentally confirmed, but cannot be predicted by the available steady state VOC emission models. We suggest that having information of physico-chemical characteristics of VOC species (Table 1), and of foliar structural characteristics [*Niinemets and Reichstein*, 2003], the dynamic model of VOC pool size (equations (14)–(16)) linked to a stomata model such as that of *Leuning* [1995] can be employed to gain mechanistic insight into the diurnal dynamics of VOC emission patterns.

[54] Given that replacement of a steady state algorithm by a dynamic one is warranted only for compounds with low H, we suggest that the approach taken here may easily be simplified to apply for simulation of field daily time courses of oxygenated isoprenoids, alcohols, aldehydes and carboxylic acids.

Appendix A: Physico-Chemical Properties of Volatile Compounds

A1. Diffusion Coefficients in Air and Water

[55] Binary diffusion coefficients of the volatiles in the air (D_A, m^2 s^{-1}) for a certain temperature (T_k, K) and air pressure (P, Pa) were calculated according to Chapman and Enskog [*Tucker and Nelken*, 1982], which empirically describes the diffusion of gases by intermolecular collision:

$$D_A = \left(1.04 + 3.66\sqrt{1/M_{air} + 1/M_M}\right) \cdot 10^{-3} \frac{\sqrt{T_k^3}\sqrt{M_R}}{P\sigma^2\Omega}, \quad (A1)$$

where Ω is the collision integral and σ is the characteristic length of the VOC molecule interacting with air molecules (Å). M_R (mol g^{-1}) is given as $(M_{air} + M_M)/(M_{air}M_M)$, where M_{air} is the molar mass of air (29 g mol^{-1}) and M_M that of the volatile [*Wilke and Lee*, 1955]. The collision integral is a function of kT_k/ε, where k is the Boltzmann's constant (1.38×10^{-23} J K^{-1}), and ε the energy of attraction (J). Boiling points (B_P, K) of specific compounds are necessary to calculate Ω, and LeBas molar volumes (V'_M, cm^3 mol^{-1}) to compute σ [*Tucker and Nelken*, 1982]. LeBas molar volumes are determined from the chemical structure of the molecules by combination of atom- and structure-specific diffusion volume increments [*Tucker and Nelken*, 1982]. Estimates of normal boiling points were obtained from organic and physical chemistry handbooks [*Fugmann et al.*, 1997; *Howard and Meylan*, 1997; *Bauer et al.*, 1998; *Eggersdorfer*, 1998]. The average absolute error of the Chapman and Enskog method is ca. 4% [*Tucker and Nelken*, 1982].

[56] Equation (A1) was also used to compute the binary diffusion coefficient for water vapor in air. Equation (A1) predicts that D_A for H$_2$O equals 2.60×10^{-5} m^2 s^{-1} at 25°C, whereas the experimental value is 2.62×10^{-5} m^2 s^{-1} at the same temperature [*Vargaftik*, 1972].

[57] The diffusion coefficients of volatiles in water (D_w, m^2 s^{-1}) were calculated according to the method of *Hayduk and Laudie* [1974] and *Tucker and Nelken* [1982]:

$$D_W = \frac{5.041 \cdot 10^{-12}}{\eta^{1.14}(V'_M)^{0.589}}, \quad (A2)$$

where η is the viscosity of water (Pa s), and V'_M the compound LeBas molar volume. The average absolute error

of equation (A2) is 5.8% [*Hayduk and Laudie*, 1974]. Temperature dependence of η was fitted to the experimental data as described by *Niinemets and Reichstein* [2002], allowing to scale D_w in dependence on temperature. Equation (A2) is strictly valid for diffusion in an infinitely dilute solution, but it provides good approximation to the experimental data if the concentration of solute is less than 50 mol m^{-3}. This condition is generally satisfied for the cellular VOC concentrations.

A2. Henry's Law Constants

[58] Henry's law constants (H) for most compounds were obtained from collections of physical chemistry reference data (Table 1). For the compounds lacking experimental observations, H was determined from volatile aqueous solubility (δ, mol m^{-3}) and the saturated vapor pressures (P_V, Pa) at a given temperature [*Staudinger and Roberts*, 1996]:

$$H = \frac{P_V}{\delta}. \tag{A3}$$

The major assumption in equation (A3) is that the solubility of water in the pure organic chemical is negligible, that is, less than 0.05 mole fraction [*Suntio et al.*, 1988], and thus, this method could only be employed for poorly soluble compounds.

[59] We use the van't Hoff equation [*Staudinger and Roberts*, 1996] to calculate the Henry's law constant for a specific temperature (T_2, K) as:

$$H_{T_2} = H_{T_1} e^{\Delta H_S / R \left(\frac{1}{T_1} - \frac{1}{T_2} \right)}, \tag{A4}$$

where H_{T_1} is the value of Henry's law constant at a reference temperature T_1 (K), ΔH_S is the enthalpy of solution (J mol^{-1}), and R the gas constant (J mol^{-1} K^{-1}). For all highly soluble short-chained organics, the values of ΔH_S were available [*Sander*, 2001], but there are extremely few experimental data of the temperature dependence of aqueous solubility of isoprenoids. However, the available evidence [*Josephy and Radt*, 1948; *Massaldi and King*, 1973] suggests that the aqueous solubilities of isoprenoids tend to be relatively constant over temperature range of 20–40°C. Given this low variability, and considering that the errors in determination of the solubilities of sparingly soluble volatiles may be potentially large, the values of H for various temperatures were determined by equation (A3) using a constant estimate of δ, and describing the saturated vapor pressure versus temperature relationships by an Antoine equation in the form of $P_V = 10^{A - B/(T_k + C)}$, where A, B and C are empirical parameters. Vapor pressures at various temperatures were obtained from the literature (Table 1). Overall, the Henry's law constants generally increase with rising temperature [*Staudinger and Roberts*, 1996] as was also predicted by our approach.

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