Dynamics of monoterpene emissions in *Pinus sylvestris* during early spring

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The seasonal dynamics of biogenic volatile organic compound (BVOC) emissions, which can be related to the formation and growth of secondary organic aerosols, represent an important but at the present poorly understood linkage between vegetation activity and climate. Although a close relationship between photosynthesis and terpenoid emissions has been proposed, high monoterpene emission rates for Scots pine shoots (*Pinus sylvestris*) are frequently recorded during spring, in times when photosynthetic activity is strongly inhibited due to inherent seasonal restrictions. We suggest that terpenoid emissions are related to either photosynthesis or photorespiration for precursors for terpenoid biosynthesis. We developed two dynamic models describing temporal fluctuations in Scots pine monoterpene emissions, calculating the emissions by using CO₂ exchange and ambient climate data. The models accurately predicted the measured monoterpene flux, and especially in March–April, during the time when photosynthesis was negligible and ambient temperatures were between –5 and +15 °C, a good agreement was found with measured emissions and the model involving photorespiration.

Introduction

Significant feedback linkages exist between BVOC emissions and climate, which at the moment are not properly accounted for in regional or global atmospheric models (IPCC 2001). For example, forest ecosystems are a considerable source of organic compounds such as terpenoids, which may condense to form secondary organic aerosols in gas phase photochemical reactions and eventually lead to cloud development (e.g. Kulmala et al. 2001, 2004). This is especially important during the growing season since abundant evidence exists for the metabolic links between BVOC emissions and photosynthetic activity (e.g. Sharkey et al. 1991, Delwiche and Sharkey 1993). However, in boreal evergreen forests BVOC emission rates can be high already during late winter—early spring, and they may even be comparable to summertime emission rates (Janson et al. 2001, Tarvainen et al. 2005). This coincides with the maximum occurrence in the events of new particle formation (Kulmala et al. 2001), but also with the minimum photosynthetic activity (Ensminger et al. 2004). From where do these significant biogenic emissions originate if there is close to zero photosynthetic activity?
Terpenoid biosynthesis in higher plants occurs by two spatially distinguishable pathways, located either in the cytoplasm or in plastids (for details see Lichtenthaler 1999). Carbon skeletons for the plastidial biosynthesis of terpenoids (responsible for most monoterpene biosynthesis) are provided by glycerolaldehyde-3-phosphate (GA-3-P), which is a Calvin cycle intermediate, and phospho-enolpyruvate (PEP), mainly produced cytoplasmically in glycolysis from sucrose and transported into the chloroplast via a specific translocator (Flügge 1999). An additional, cytoplasmic substrate pool was recently shown to be metabolites that are formed from glucose imported from other organelles (Kreuzwieser et al. 2002, Affek and Yakir 2003, Funk et al. 2004).

In a sequence of enzymatic reactions in the chloroplast stroma, cytoplasm-derived PEP is converted into pyruvate, which then reacts with plastidic GA-3-P to produce DOXP (1-deoxy-D-xylulose-5-phosphate), and finally IPP and DMAPP (isopentenyl pyrophosphate and dimethylallyl pyrophosphate, respectively) (Lichtenthaler 1999). These are the common precursors for a plethora of terpenoids, including isoprene, monoterpenes, and sesquiterpenes, xanthophylls and the phyto side-chain of chlorophyll. Terpenoid biosynthesis can be regulated either by the supply of substrates and availability of energy, or by enzyme activities in the metabolic branching points of the DOXP pathway (e.g. deoxyxylulose-5-P synthase or monoterpen synthase) (Bohlmann et al. 1998, Fischbach et al. 2002, Dudareva et al. 2004).

In addition to the complex regulation of the biosynthetic pathway, another factor influencing the terpenoid emissions is that many BVOC-emitting species, including conifers, also possess two different types of monoterpene storage pools. A dynamic, fast-responding temporary pool of monoterpenes is located in chloroplasts and in the intercellular spaces of the mesophyll tissue (Loreto et al. 1996b, Shao et al. 2001, Niinemets and Reichstein 2003). On the other hand, terpenoids forming the constitutive defense of conifer needles are mainly synthesized in leucoplasts of the resin duct epithelial cells already during development of the new needle cohort, and are stored in the lumen of the thick-walled resin canals (Lerdau and Gray 2003). This storage pool is probably much larger than the temporary pool, yet it changes in size significantly only if there is foliar damage, which induces both de novo synthesis and increased emissions (Holopainen 2004).

Although recent research has been successful in revealing the biochemical details of the biosynthetic pathway, the regulatory mechanisms controlling terpenoid formation are still far from being fully elucidated (Dudareva et al. 2004). The supply of primary substrates in vivo may constitute an equally important regulating factor as the activation/deactivation of the enzymes in determining actual terpenoid formation and emissions from mature leaves (Fischbach et al. 2002, Wolfertz et al. 2003, 2004, Funk et al. 2004). In many tree species there is a close relationship between isoprene and some monoterpene emissions and irradiance, suggesting that the regulation of the emissions is provided by photosynthesis (Schuh et al. 1997, Niinemets et al. 2002b). The fast labeling kinetics of emissions, when $^{13}$CO$_2$ is introduced, further suggests a dependence on recently fixed carbon under normal conditions (Sharkey et al. 1991, Loreto et al. 1996b, Wolfertz et al. 2003). Some process-based models, describing terpenoid synthesis and emissions therefore use photosynthesis as a starting point for the synthesis, and assume direct coupling of the processes (e.g. Niinemets et al. 1999, 2002a, Martin et al. 2000, Zimmer et al. 2000). However, due to inconsistencies frequently observed in responses of emissions to CO$_2$ and O$_2$ concentrations and temperature, and due to incomplete labeling after feeding the plant with $^{13}$CO$_2$, the contribution of other processes in providing substrates for terpenoid biosynthesis, especially during periods of stress has been a matter of debate (e.g. Kreuzwieser et al. 2002, Affek and Yakir 2003, Funk et al. 2004). These responses suggest that monoterpene emissions may not always be linearly linked to CO$_2$ assimilation, but are rather depending on the Calvin cycle activity which can be kept operational either by photosynthesis or photorespiration.

Under natural conditions, the relative rate of photorespiration compared with photosynthesis may change due to many factors. With
increasing temperature, increasing mesophyll O₂, or decreasing mesophyll CO₂ concentration, the reaction mediated by Rubisco favors oxygenation (e.g. Peterson 1983, Jordan and Ogren 1984, Wingler et al. 2000). These conditions may prevail in nature for instance during periods of drought stress or high temperatures, both of which promote stomatal closure and thus decrease the CO₂ concentration inside mesophyll, but do not influence the photosynthetic electron transport rates to the same extent (Niinemets et al. 2002b), or barely affect Rubisco activity, which is only reduced under severe water stress (Bota et al. 2004). Furthermore, the changes in membrane permeability, especially those of the chloroplast envelope, during the cold hardening process may result in slower CO₂ diffusion into the chloroplast, leading to lower than expected CO₂ concentration at the site of carboxylation (Zhang et al. 2002). A transient increase in the ratio between photorespiration and photosynthesis is supposed to be an important protective mechanism by which boreal and alpine plants are able to maintain photosynthetic electron flow in springtime, when high light levels and low temperatures coincide (Heber et al. 1996, Wingler et al. 2000). Even though the air temperatures can be close to zero, instantaneous needle surface temperatures can rise several degrees higher than the surrounding air due to strong irradiance (Hari and Mäkelä 2003), which may sustain significant photorespiratory activity due to low CO₂ concentrations in mesophyll even in relatively low temperatures.

Our objective was therefore to analyze whether BVOC emissions in a boreal coniferous forest during spring can be better explained by either photosynthesis or photorespiration as a source for substrates. To address this issue, we developed two dynamic models, with which we attempted to portray the seasonal and diurnal variations in Scots pine (Pinus sylvestris) shoot monoterpene emissions. To investigate the dynamic emission behaviour existing under field conditions we measured shoot gas exchange, monoterpene emissions, and the environmental factors influencing them, during a period in late winter–early spring in a boreal coniferous forest in southern Finland, and used the data as an input for testing the monoterpene emission model.

Materials and methods

Site description

The experimental Scots pine (Pinus sylvestris) stand is located in southern Finland, at Hyytiälä Forestry Field Station (61°51’N, 24°17’E, 181 m a.s.l.). The SMEAR II site (Station for Measuring Forest Ecosystem–Atmosphere Relations) was planned and constructed to monitor material and energy fluxes between forests and the atmosphere within and above the stand, and to measure the environmental factors affecting the fluxes. Intensive measurements of canopy–atmosphere interactions have been performed at SMEAR II since 1996 (see Vesala et al. 1998, for a general description). The forest is dominated by 40-yr-old Scots pines of 14 m average canopy height. An automated measuring system monitors the CO₂ exchange of four to six pine shoots of two pine trees continuously throughout the year, also during the winter period. Data from a single clap-type gas exchange chamber (quartz-glass and acryl-plastic, vol 3.5 litres), situated in an upper canopy shoot, were used to calculate the photosynthetic parameters of the sample tree. The cuvette is closed for 60 seconds for measurements of CO₂ and H₂O concentrations at five-second intervals, giving two to three measurements per hour. Photosynthetic rate is determined from the CO₂ mass balance equation of the chamber (Hari et al. 1999). PAR and air temperatures are measured using a Li-Cor 190 SB sensor and a shielded CuKo thermocouple, respectively.

Scots pine monoterpene emission characterization

Measurements of the shoot monoterpene emission rates were performed in the same tree where the shoot photosynthetic rate was measured between March and May 2003. The shoot for VOC measurement was situated mid-canopy (at about 11-meter height), but received direct sunlight only for a couple of hours a day. The emission samples were collected using a dynamic, flow-through technique. The shoot was enclosed in a transparent Teflon® bag (20 litres), equipped
with air inlet and outlet ports and a thermometer. PAR was measured with a Li-Cor 190 SB sensor outside of the cuvette. Ozone was removed from the inlet air with a fresh, MnO$_2$-coated copper net. The flow of air through the cuvette was eight litres per minute and no additional mixing or homogenization was applied.

About 100 ml min$^{-1}$ flow of air was trapped in Tenax TA and Carbopack adsorbent tubes simultaneously from both the inlet and outlet ports. The shoot enclosure and thus the VOC sampling period ranged between 30 and 120 minutes. The measured emission rate ($E$; g g$^{-1}$ h$^{-1}$) was determined as the mass of compound per needle dry mass and time according to

$$E = (C_2 - C_1)qm^{-1}. \quad (1)$$

$C_1$ and $C_2$ are the monoterpane concentrations (g m$^{-3}$) in the inlet air and in the outgoing air, respectively, $q$ is the flow rate into the cuvette (m$^3$ h$^{-1}$) and $m$ is the needle dry mass (g). The corresponding needle area (m$^2$) was calculated to yield emission rates in g m$^{-2}$ s$^{-1}$.

The adsorbent tubes were analyzed using a thermodesorption instrument (Perkin-Elmer ATD-400) connected to a gas chromatograph (HP 5890) with HP-1 column (60 m, i.d 0.25 mm) and a mass-selective detector (HP 5972). The sample tubes were thermodesorbed at 300 °C for 5 minutes. The temperature programme of the GC was as follows: initial temperature 50 °C, increase to 150 °C at a rate of 4 °C min$^{-1}$, the temperature remained at 150 °C for 5 minutes and was then increased to 250 °C at a rate of 8 °C min$^{-1}$. The carrier gas was helium (purity 6.0) and the flow rate 0.9 ml min$^{-1}$. Quantification was achieved with five-point calibration using liquid standards in methanol solutions. The detection limits were about 10 pptv for the monoterpenes. Detailed results of the branch bag enclosure studies were reported elsewhere (Tarvainen et al. 2005), and thus only the data relevant for the model development are given here.

The data presented are non-normalized midday monoterpane emission rates from whole shoots, or when several measurements were made, daily average emission rate values between 08:00–16:00 ($n = 2–13$). We also measured the diurnal variations in shoot monoter-
flows of CO$_2$ and H$_2$O between the atmosphere and stomatal cavity are proportional to the corresponding concentration difference; CO$_2$ concentration in the stomatal cavity is stable; water vapor concentration inside the mesophyll is saturated at the prevailing temperature; there is a cost of transpiration expressed in carbon units (g[CO$_2$] g[H$_2$O]$^{-1}$); and stomata open and close to maximize the long-term photosynthetic production, while simultaneously minimizing the cost of transpiration. Six of the model’s nine parameters are assumed to be constant over the growing season. The three variable parameters are the initial slope and saturation value of the light-response curve of carboxylation efficiency, and the cost of transpiration, in carbon units, regulating the degree of stomatal opening. For a detailed description of the model, we refer to Hari and Mäkelä (2003).

The model calculates the photosynthetic rate, $A$ (g[CO$_2$] m$^{-2}$ s$^{-1}$), and the mesophyll CO$_2$ concentration, $C_{m}^{CO_2}$ (g[CO$_2$] m$^{-3}$), as a function of irradiance, $I$ (mol m$^{-2}$ s$^{-1}$), ambient temperature, $T$ (°C), and water vapor pressure deficit, VPD.

Fig. 1. (a) Schematic structure of the CASE I model including photosynthesis. (b) Schematic structure of the CASE II model including photorespiration. Boxes indicate the amounts of substances within the needle, and clouds the source and sink of the model. Arrows indicate flows of substances between the processes (double circles). External or internal factors affecting the processes are illustrated by dashed arrows. Abbreviations and symbols as presented in the text, and $\Gamma'$ = photorespiration.
(g[H$_2$O] m$^{-3}$), as follows:

$$A = A(I,T,VPD) \quad (2)$$

$$C_{m}^{cO_2} = C_{m}^{cO_2}(I,T,VPD). \quad (3)$$

The measured and modeled $A$ are illustrated in Fig. 2 for two days in May 2003.

**Terpene biosynthesis**

The plastidial pathway involved in synthesis of monoterpenes is strongly coupled with environmental variables such as light intensity, CO$_2$ concentration, and temperature (Staudt and Bertin 1998, Lerdau and Gray 2003). The sub-model for monoterpene formation is based on simplified assumptions on the biosynthetic pathway. We consider two cases:

**CASE I:** the availability of GA-3-P for terpene biosynthesis is regulated by the rate of CO$_2$ assimilation (Fig. 1a).

**CASE II:** the availability of GA-3-P for terpene biosynthesis is regulated by the difference between the atmospheric and mesophyll CO$_2$ concentrations (Fig. 1b). Photorespiration is favoured when the concentration difference is large.

In both cases, the energy for monoterpene synthesis is dependent on photochemistry and mediated into an active Calvin cycle through ATP and NADPH. Energy availability does not restrict the synthesis. RuBP is carboxylated by Rubisco when the CO$_2$ concentration in the mesophyll is high (CASE I) and oxygenated when the availability of CO$_2$ is limited, giving rise to photorespiration (CASE II). The availability of GA-3-P is the regulatory factor, but the availability of pyruvate is considered to be non-limiting for terpene biosynthesis. Let $S_p$ and $S_r$ denote the terpene synthesis rate (g m$^{-2}$ s$^{-1}$), dependent on photosynthesis and photorespiration, respectively.

**CASE I:**

$$S_p = \alpha_1 A(I,T,VPD) \quad (4)$$

**CASE II:**

$$S_r = \alpha_2 [C_a - C_{m}^{cO_2}(I,T,VPD)] \quad (5)$$

where $\alpha_1$ equals 0.5 mg[monoterpenes] g[CO$_2$]$^{-1}$ and $\alpha_2$ 140 ng[monoterpenes] m[CO$_2$]^{-1} s$^{-1}$), describing the efficiency of monoterpene biosynthesis, and $C_a$ is the CO$_2$ concentration of air (g m$^{-3}$).

**Storage pools**

The monoterpene leakage from the large, permanent storage pool, located in resin ducts, to the mesophyll pool is considered to be stable under normal conditions, and thus the changes in the instantaneous monoterpene emissions originate from the temporary mesophyll storage pool. For our approach, we consider the temporary monoterpene storage to consist of two spatially separated sub-pools: a mesophyll pool and a surface pool, both of which are physically located in the liquid phase in apoplast. Separating these pools in the model is necessary in order to create a gradient of monoterpenes, which then diffuse from a higher concentration in the mesophyll pool to a lower concentration at the surface pool. The presence and physico-chemical characteristics of these multiple temporary pools produce delays in the emission responses to external stimuli.

The synthesis and transport of monoterpenes change their mass in the mesophyll pool, thus

$$\frac{dM_m}{dt} = S_m - i \quad (6)$$

where $i$ (g m$^{-2}$ s$^{-1}$) is the flux of monoterpenes inside the leaf, $M_m$ (g m$^{-2}$) is the monoterpene mass in the mesophyll pool in a square meter area of mesophyll tissue, and $S_m$ is either $S_p$ or $S_r$.

If there is some damage-induced leakage of monoterpenes from the permanent storage pool to the mesophyll pool, then the synthesis term $S_m$ increases by the amount coming from storage. It will consequently be erroneously large. However, the dynamics of the concentration change from mesophyll to surface pool and thus the patterns of emissions are not affected by the leakage.
Transport within leaf

The transport of monoterpenes from the mesophyll pool (i.e. the site of synthesis) out to the surface pool (i.e. the site of emission) is based on diffusion, which is proportional to the concentration differences between the components of the pathway. Emissions of highly volatile compounds especially exhibit only limited stomatal control as shown in many studies for e.g. isoprene and α-pinene (Monson and Fall 1989, Loreto et al. 1996a, Shao et al. 2001). Also the lipophilic nature of monoterpenes suggests possible liberation routes directly through the cuticle (Guenther et al. 1991, Fall and Monson 1992). This implies that the emissions are practically independent of stomatal regulation, and thus we chose to omit the stomatal conductance from the model, and suggest that monoterpene emission may occur homogeneously from the leaf surface.

The concentrations of monoterpenes in mesophyll and surface pools \(C_m, C_s\) (g m\(^{-3}\)) are derived from the masses of monoterpenes \(M_m, M_s\) and volumes of the pools \(V_m, V_s\) (m\(^3\)), which were calculated from experimentally determined anatomical dimensions for Scots pine needles (average mesophyll thickness 150 µm, average epidermal thickness 30 µm; Bäck et al. 1994). The flux \(i\) (g m\(^{-2}\) s\(^{-1}\)) from mesophyll pool to surface pool is expressed as

\[
i = g_m (C_m^i - C_s^i)
\]

where \(g_m\) describes the monoterpene conductance in the mesophyll liquid phase (m s\(^{-1}\)), and was calculated using the concept presented by Niinemets and Reichstein (2002). We assumed the synthesized monoterpenes possessed equal conductances in the mesophyll, which is justified due to their equal molecular weights.

Emission

Monoterpenes are freely emitted into the atmosphere from the leaf surface. Let \(f_e\) (g m\(^{-2}\) s\(^{-1}\)) denote the temperature dependence of monoterpene emission from the liquid phase. We assume that the temperature dependence of emission can be approximated with

\[
f_e(T_a) = \exp[a + b/(T_a + 273)]
\]

where \(T_a\) is the air temperature inside the photosynthesis cuvette (°C) and the empirical parameter values \(a = 24.5\) (dimensionless) and \(b = -5474\) K (for α-pinene; CRC 2003).

Let \(M_s\) (g m\(^{-2}\)) denote the monoterpene mass in 1 m\(^2\) area of leaf surface (surface pool size). The emission rate, \(e\) (g m\(^{-2}\) s\(^{-1}\)), is determined by the mass of monoterpenes in the leaf surface pool and temperature, thus

Fig. 2. Measured and modeled daily course of photosynthesis of a Scots pine shoot in 20–21 May 2003.
\[ e = \beta M_s f_e(T_a) \]  
(9)

where $\beta$ equals $0.005 \text{ m}^2 \text{ g}^{-1}$.

The fluxes, i.e. flow inside the leaf ($i$) and emission ($e$), change the mass of monoterpenes in the surface pool as

\[ \frac{dM_s}{dt} = i - e. \]  
(10)

The values of the parameters $\alpha_1$, $\alpha_2$ and $\beta$ in the synthesis and emission submodels were estimated using the data on $\Delta^3$-carene emissions (dominant component in emissions) measured between 20 and 21 May 2003 (Fig. 3). The estimation was done by minimizing the residual sum of squares. Initial values for $M_m$ and $M_s$ were set as 100 and 50 ng m$^{-3}$, respectively.

The constructed monoterpene emission models are simple descriptions of the most...
important processes involved in monoterpene synthesis and emissions, and they were used for calculating the emissions, after the prevailing environmental conditions in the field and incident photosynthetic rate were known. Correlation coefficients (Pearson correlation, r) were calculated to compare the modeled and measured emissions and to study the variations in emission composition.

**Results and discussion**

**Characteristics of monoterpene emission**

The dominant monoterpene in emissions from the experimental Scots pine shoot was Δ3-carene (50%–80% of the total mass), and the remainder of the emission was mainly composed of α-pinene (5%–30%), camphene, sabinene, β-phellandrene and β-pinene. This is in line with results of other studies on Scots pine monoterpene emissions (e.g. Janson 1993, Shao et al. 2001). Limonene, terpinolene and 1.8-cineol were recorded in minor, variable amounts. The Δ3-carene emissions varied by an order of a magnitude (3.5–24 ng m⁻² s⁻¹) during the observation period, which was initiated in conditions with daily maximum temperatures of about 10–15 °C, continued under a cold spell with maxima below zero and ended with a daytime maximum of 20 °C (Fig. 4a). Other emitted compounds showed parallel variations in emission rates (correlations with Δ3-carene: α-pinene 0.69, β-pinene 0.94, sabinene 0.77, camphene 0.67).

The emission rates of all major emitted compounds were highest during the afternoon hours, and at night the emissions declined to 30%–50% of the afternoon maxima (Fig. 3). The standardized (+25 °C) monoterpene emission rate here was 1.16 µg g⁻¹ h⁻¹ (details in Tarvainen et al. 2005), which is in accordance with earlier measurements from Scots pine. Komenda and Koppmann (2002) measured standardized emission rates between 0.06–0.64 µg g⁻¹ h⁻¹ for young pines and 0.24–3.7 for mature pines, and Janson (1993) also reported values quite close to our measurements (0.8 µg g⁻¹ h⁻¹).

**The diurnal dynamics of monoterpene emissions**

Temperature and PAR level are the most important driving factors for plant metabolism under field conditions, in addition to the concentration of carbon dioxide and VPD. The current models for monoterpene-storing species, such as conifers, generally use only changes in leaf temperature as driving variables for emissions, although ample evidence exists of their dependence on incident PAR levels in several species, including pines (Janson 1993, Loreto et al. 1996b, Schuh et al. 1997, Staudt and Bertin 1998). A more developed model was proposed by Shao et al. (2001), which accounted for BVOC emissions from pools as well as those from immediate biosynthesis. According to our theory, the changes in temperature and light and the gradual depletion of the mesophyll pool available for emission during the night fully explain the observed dynamics between day and night. Temperature influences the emission and thus the turnover rate of terpenoids in the surface pool. The synthesis of monoterpenes for the mesophyll pool ceases during the night due to lack of light, leading gradually to depletion of substrates.

The measured maximum daytime Δ3-carene emissions of Scots pine shoot were eight times higher than the minimum nocturnal emissions (Fig. 3), which leveled off during early morning hours due to depletion of the mesophyll pool. Both models managed to reproduce the measured (20–21 May 2003) diurnal fluctuations of monoterpene emissions with reasonable accuracy (Fig. 3, only shown for the CASE II model including photorespiration, correlation coefficient 0.79). This corresponds well with the model assumption of the substrate-limitation, which will in the beginning gradually decrease the emissions and eventually lead to nearly complete cessation of emissions when light levels are reduced for a longer period.

**The springtime dynamics of monoterpene emissions**

Using the estimated parameter values and the available environmental and photosynthesis data,
we simulated the temporal changes in Scots pine monoterpene emissions during spring 2003 (Fig. 4b and c). Both monoterpene emission models could reproduce the average springtime emission rates. The Pearson correlation coefficients between the measured and modeled emission rates were 0.61 and 0.66 (Δ3-carene) and 0.86 and 0.89 (α-pinene) for CASE I and CASE II, respectively. However, during early spring (March–April) when the temperatures ranged from –5 °C to +15 °C, the emission model where photorespiration was involved in processing the precursors (CASE II) proved to be more precise in producing the observed monoterpene emis-
sions (both Δ⁴-carene and α-pinene). The correlation coefficients for the early spring measurements were 0.34 and 0.43 (Δ⁴-carene) and 0.71 and 0.82 (α-pinene) for CASE I and CASE II, respectively.

Based on these model results, we propose an important role for the regulation of Rubisco reactions in monoterpene emissions. In order to maintain the continuous substrate (GA-3-P, pyruvate) and energy (ATP, NADPH) flow to the terpenoid biosynthetic pathway, a close interaction between the photochemistry and operational Calvin cycle is required (Sharkey and Yeh 2001). The cytoplasmic PEP (phosphoenolpyruvate), which is abundant and, in the short term, independent of photosynthetic activity (Kreuzwieser et al. 2002, Affek and Yakir 2003) is probably the main source of the pyruvate necessary for terpenoid biosynthesis. The GA-3-P availability is, however, mostly dependent on the plastidial carbon metabolism (Poolman et al. 2000). Therefore it is evident that an active Calvin cycle is a prerequisite for monoterpene biosynthesis in plastids also at suboptimal CO₂ concentrations or in CO₂-free air. This is possible to achieve only through photorespiration, which will keep the cycle in progress, thus maintaining the carbon flux and enzymatic substrate turnover (Sharkey and Yeh 2001, Funk et al. 2004), provided that enough energy is available as ATP and NADPH. It was shown that a cytoplasmic protein synthesis inhibitor, affecting plastidial triose-phosphate utilization, inhibits photosynthesis but the Calvin cycle runs through photorespiration, and terpenoid emissions are sustained (Sharkey and Yeh 2001). This indicates that carbon assimilation and terpenoid synthesis can be decoupled at the first metabolic stage, providing substrates for the DOXP pathway.

Some previous studies suggested that the source for substrates in isoprene biosynthesis would be photorespiration (e.g. Jones and Rasmussen 1975, Tingey et al. 1981), and attempted to prove this by feeding plants intermediates of the photorespiratory pathway. However, this only provided an external carbon source for isoprene biosynthesis, thus demonstrating that an active Calvin cycle is essential to the isoprenoid pathway. Monson and Fall (1989) and Hewitt et al. (1990) did not establish a direct relationship between photorespiration and isoprene biosynthesis. A potential, indirect contribution of photorespiration, which is favoured under conditions when mesophyll CO₂ concentration is low, to terpenoid synthesis can however be hypothesized from the variable responses of terpenoid emissions following changes in ambient CO₂ concentrations and temperature, some of which are listed below.

i Low Cₘ (mesophyll CO₂ concentration), which strongly favours photorespiration instead of photosynthesis, has only minor effect on terpenoid emissions, although photosynthesis is critically dependent on Cₘ. In many species emissions do not cease when Cₘ is decreased due to stomatal closure or to CO₂ removal from the surrounding air (i.e. there seems to be no CO₂ compensation point for emissions; Hewitt et al. 1990, Fall and Monson 1992, Singsaas et al. 1997, Affek and Yakir 2003). Furthermore, the emissions are totally inhibited only in atmospheres free of both CO₂ and O₂ i.e. when both photosynthesis and photorespiration are blocked (Loreto et al. 1996a, Singsaas et al. 1997, Woffertz et al. 2003). The optimum CO₂ concentration for terpenoid emissions often has a broad maximum between 50–500 µmol mol⁻¹, and the decline in emissions at high CO₂ is more pronounced under low oxygen concentrations (Loreto and Sharkey 1990). The average proportion of assimilated carbon lost as terpenoid emissions is greater at low Cₘ as compared with when mesophyll CO₂ concentration is at a higher level (Niinemets et al. 2002a, Funk et al. 2004, Pegoraro et al. 2004).

ii The short-term temperature response of terpenoid emissions also differs from that of photosynthesis: emissions have often been noted to increase significantly in response to temperature increase (maximum between 30–55 °C), concurrently when measurable photosynthesis is unaffected or declines and photorespiration is preferred due to increase in the Rubisco affinity for oxygen with increasing temperature (e.g. Loreto et al. 1996a, Singsaas et al. 1997, Woffertz et al. 2003, Funk et al. 2004). Moreover, the sea-
sonal variations in emissions do not seem to follow changes in photosynthetic rates either (Llusiá and Peñuelas 2000).

And finally, monoterpenic production in *Quercus* leaves is higher under photorespiratory conditions as compared with that under anoxic conditions, especially at high temperatures (Peñuelas and Llusiá 2002). The thermostropic effect of exogenously added monoterpenes and isoprene is enhanced under conditions that inhibit their endogenous formation, such as in a non-photorespiratory atmosphere (Sharkey and Singsaas 1995, Peñuelas and Llusiá 2002). The generally low BVOC emissions in C4 plants may be related to their reduced photorespiratory activity (Peñuelas and Llusiá 2003).

Understanding the contribution of alternative pathways for substrate production for terpenoid biosynthesis during periods of suppressed photosynthetic activity may considerably change the estimates of regional emission patterns. In the standard empirical emission models, the biosynthesis and thereafter the emissions of BVOC, such as isoprene and some monoterpenes, are generally considered to depend on either temperature alone, or on both temperature and irradiance, normalized with the basal emission rate specific for a set of defined environmental conditions and for a vegetation type (Tingey et al. 1980, Guenther et al. 1993, Schuh et al. 1997). However, diurnal or seasonal variations of several orders of magnitude have been observed for many species both in the basal monoterpane emission rates (Lehning et al. 2001), and in the actual emissions (Janson 1993, Llusiá and Peñuelas 2000, Hakola et al. 2003, Tarvainen et al. 2005). Such large variations in monoterpane emissions imply that the pronounced seasonal changes in plant metabolism (e.g. the dormancy period and the onset of photosynthesis and growth after dormancy), influencing the biosynthetic activity, are not easily taken into account in the current monoterpane emission algorithms (but see Guenther 1997, Lehning et al. 2001). This may lead to significant underestimation of emission rates.

The photosynthetic capacity of boreal coniferous forest trees exhibits a clear annual cycle related to plant activity, which dynamically follows the change of ambient temperature (Mäkelä et al. 2004). During cold hardening in autumn the photosynthetic activity is down-regulated due to several biochemical and structural adjustments. The lowest photochemical efficiency occurs before the period of spring recovery (Öquist and Huner 2003, Ensminger et al. 2004). Very low photosynthetic rates were measured from the Scots pine shoot at the beginning of our measuring period in mid-March, and they coincided with significant monoterpane emissions (Fig. 4). It seems therefore evident that photosynthesis alone cannot be responsible for providing substrates for terpenoid synthesis at all times when emissions are measured. We suggest that the regulating factors for terpenoid production can change during the course of the year correspondingly, as do the parameters involved in the developmental stage of the plants in the optimum stomatal control model (Hari and Mäkelä 2003). This could at least partially explain the results obtained by Tarvainen et al. (2005), where the regression with temperature alone explained most of the Scots pine monoterpane emission rates during summer, but failed to do so during spring.

The fit of the models was better for α-pinene than for Δ^3-carene, which may be caused by compound-specific factors controlling either biosynthesis or emissions. This is in line with Shao et al. (2001), who found a clear PAR-dependence for α-pinene and camphene emissions but less clear dependence for e.g. Δ^3-carene, and hypothesized that this was due to larger proportion of emissions of Δ^3-carene originating from the storage pools. Thus, our limited data suggests that the present model structure is more adequate for α-pinene than Δ^3-carene. In the future development of the model the compound-specific factors, both dealing with biosynthesis and the physicochemical properties, need to be taken into account. The information content of the presently available measurements, especially concerning nighttime emissions, is too limited for proper elaboration of the models, and we urgently need continuous, simultaneous measurements of shoot monoterpane emissions and photosynthesis (Ruuskanen et al. 2005).

An evident question concerns why plants use
their carbon resources to produce the relatively high-cost VOC compounds during springtime — at times when their net carbon uptake is close to zero. It has generally been suggested that the inducible BVOCs are associated with defence against herbivores and pathogens, but recently it has also been proposed that they are involved in protection against reactive oxygen species (ROS) created by several abiotic stresses such as high temperatures or ozone exposure (e.g. Singsaas et al. 1997, Peñuelas and Llusiá 2002, Loreto et al. 2004). Also with the exposure to high light intensities e.g. during springtime in boreal areas, removal of the excess excitation absorbed by pigments is vitally important, in order to avoid permanent damage to the photosynthetic apparatus from triplet chlorophyll and ROS formation (Öquist and Huner 2003). Excess absorbed energy leads to increased non-photochemical quenching by the xanthophyll cycle pigments (Demmig-Adams and Adams 1996). The maximum concentrations of xanthophylls in Scots pine needles occur during the spring in March–April, before onset of photosynthetic activity (Ensminger et al. 2004). As xanthophylls and monoterpenes both belong to the diverse products of terpenoid metabolism, it is plausible that active terpenoid biosynthesis occurs during that time of low photosynthesis. Indeed, a parallel function of monoterpenes and xanthophyll pigments for protection from photoinhibition has already been suggested (Peñuelas and Llusiá 2002).

The dynamic linkage between atmospheric processes and forest ecosystems is likely to exhibit similar seasonality to plant metabolic activity. Recently it has been reported that in the boreal forested areas the growth rate of atmospheric particles able to form secondary aerosols correlates well with photosynthesis, and with the concentration of the oxidation products of BVOCs (Kulmala et al. 2004). However, the number of events when this type of new particle formation takes place in boreal areas is at its maximum during late winter and spring (Kulmala et al. 2001), coinciding with the lowest levels of photosynthetic activity. This has raised a question about the origin of the participating VOCs, which we now propose to be related to photorespiratory metabolism, feeding substrates for essential, protective terpenoid biosynthesis during the otherwise inactive metabolic stage. The seasonal dynamics of terpenoid biosynthesis in the boreal evergreen foliage, and dependence on the prevailing environmental factors, need to be studied in more detail by using, for example, metabolic markers such as $^{13}$C and fast-response analytical methods.

## Conclusions

The Scots pine shoot monoterpane emissions in spring occur at about the same level as the summertime emissions, and may easily be substantial enough to contribute significantly to new atmospheric particle formation, which is frequently recorded in boreal forests during springtime (e.g. Kulmala et al. 2004). Modeling the emissions with a dynamic, process-based model, capable of producing real measured seasonal trends, is therefore a valuable tool to estimate regional and global emission capacities during the past and in the future. Furthermore, the model available currently can be utilized to improve the understanding of the complex interactions between environmental factors and terpene emissions from plants in different vegetation types.

Our dynamic model was able to predict monoterpane emission dynamics in a natural forest environment when data on needle gas exchange, ambient temperature and solar irradiance were available. In earlier models where the foliar photosynthetic activity was linked with terpenoid emissions, fast response times and many variables dealing with the physico-chemical characteristics of emitted compounds, or with the biosynthetic pathway, were necessary to formulate the dynamics of emissions (e.g. Niinemets et al. 1999, 2002a, Martin et al. 2000, Zimmer et al. 2000). We suggest here that the relationship between photosynthetic activity and BVOC emissions may be dynamic such that, under conditions where limitations for maximum efficiency of photosynthesis exist, photorespiration may also contribute to sustain the emissions. Our model is based on strong, idealized assumptions about the biochemical pathway and physical factors involved, yet it succeeds in reproducing the diurnal and seasonal emission trends.
The principal strength of our approach is the use of a well parameterized and verified optimal stomatal control model, which enables us to use the measured diurnal and seasonal patterns of gas exchange parameters under field conditions (Ruuuskainen et al. 2005). Further development of our model includes formulating a more realistic description of the effects of the annual cycle on both photosynthesis and photorespiration, and applying compound-specific parameters to follow specifically the dynamics of the various components of monoterpane emission.

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