Process-based modelling of isoprene emission by oak leaves

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ABSTRACT

The emission rate of the volatile reactive compound isoprene, emitted predominantly by trees, must be known before the level of photo-oxidants produced during summer smog can be predicted reliably. The emission is dependent on plant species and local conditions, and these dependencies must be quantified to be included in any empirical algorithm for the calculation of isoprene production. Experimental measurements of isoprene emission rates are expensive, however, and existing data are scarce and fragmentary. To overcome these difficulties, it is promising to develop a numerical model capable of precisely calculating the isoprene emission by trees for diverse ecosystems, even under changing environmental conditions. A basic process-based biochemical isoprene emission model (BIM) has therefore been developed, which describes the enzymatic reactions in leaf chloroplasts leading to the formation of isoprene under varying environmental conditions (e.g. light intensity, temperature). Concentrations of the precursors of isoprene formation, 3-phosphoglyceric acid and glyceraldehyde 3-phosphate, are provided by a published light fleck photosynthesis model. Specific leaf and enzyme parameters were determined for the pedunculate oak (*Quercus robur* L.), so that the BIM is capable of calculating oak-specific isoprene emission rates as influenced by the leaf temperature and light intensity. High correlation was observed between isoprene emission rates calculated by the BIM and the diurnal isoprene emission rates of leaves measured under controlled environmental conditions. The BIM was even capable of describing changes in isoprene emission caused by midday depression of net photosynthesis.

Key-words: *Quercus robur*; isoprene emission; numerical model; oak; process-based model.

INTRODUCTION

Isoprene (2-methyl-1,3-butadiene) is one of the most dominant volatile hydrocarbons emitted by plants, especially by trees (Kreuzwieser *et al*. 1999). Because the decomposition of this biogenic volatile organic compound (BVOC)

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reduces the concentration of atmospheric OH radicals required for example for degradation of the greenhouse gas methane (Thompson 1992), it plays an important role in atmospheric chemistry. Moreover, degradation of BVOC in the presence of NO_x can lead to the formation of ozone and other aggressive gases in the lower atmosphere (Paulson & Seinfeld 1992). Algorithms based on experimental emission data with incorporation of the effects of light and temperature have been developed for calculating isoprene emission using tree-specific standard emission rates (Guenther *et al*. 1993). Correction factors or terms were included to account for the seasonal variation of the isoprene emission rate (Monson *et al*. 1994; Guenther 1997; Schnitzler *et al*. 1997). Isoprene emission by vegetation, however, is species-dependent and strongly influenced by the environmental conditions, and it is time-consuming and expensive to obtain emission data. For these reasons, the available data are too fragmentary to allow calculation of the emission rates by empirical algorithms on a regional or global scale. It is not possible therefore to predict isoprene emission rates as required for the calculation of photo-oxidant formation during summer smog in diverse biotopes. Consideration of probable future climatic conditions such as elevated CO_2 concentration and higher temperatures is beyond the scope of the present empirical models.

It may be assumed that a precise description of isoprene emission rates can be obtained even under changed climatic conditions by considering the biosynthetic processes in the leaves leading to the formation of the volatile compound. The aim of the current work is to demonstrate that realistic isoprene emission rates can be calculated by a numerical biochemical isoprene emission model (BIM) using a mathematical description of the reactions of the chloroplastic isoprene biosynthesis. In addition to modelling the supply of the carbon substrates for isoprenoid biosynthesis, described by a published photosynthesis model (Kirschbaum *et al*. 1998), such a model requires a species-specific set of parameters for the biosynthetic pathway of isoprene production, such as the kinetic parameters of the enzymes involved. It is likely that such a plant species-specific model could subsequently be adapted to other plant species by changing only some of its parameters or key variables.

Recently, it has been shown that isoprene is synthesized in the chloroplasts, and that chloroplasts possess a

cytoplasm-independent additional biosynthetic pathway for isoprenoids (Lichtenthaler *et al.* 1997; Wildermuth & Fall 1998), which does not include the intermediate mevalonate. The first enzyme in this pathway catalyses the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) from the photosynthetic intermediates glyceraldehyde 3-phosphate and pyruvate (Sprenger *et al.* 1997; Lange *et al.* 1998; Lois *et al.* 1998). The next reaction, leading to 2-methyl-erythritol 4-phosphate (MEP), is catalysed by the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (Takahashi *et al.* 1998; Lange & Croteau 1999). The product of this reaction is further converted to isopentenyl diphosphate (IDP) by several reduction and dehydration steps which also involve a phosphorylation step. These steps have not been characterized up to now. The produced IDP is converted by the IDP isomerase (E.C. 5.3.3.2) to dimethylallyl diphosphate (DMADP), which serves as the substrate for the isoprene synthase (Silver & Fall 1995; Schnitzler *et al.* 1996). To include these enzymatic reactions in the model, species-specific enzyme properties had to be determined either experimentally or by inverse modelling. Data for calculation of the metabolite concentrations (e.g. the volume of the chloroplasts per leaf volume) had also to be determined. On the basis of this data set, and using a photosynthesis model that reacts rapidly to changes in light (Kirschbaum *et al.* 1998), a numerical model for the calculation of isoprene emission rates was constructed. The model was validated against an experimental set of diurnal isoprene emission data for pedunculate oak leaves and also compared with the algorithm proposed by Guenther (1997).

Materials and methods

Plant material

Shoot cultures of *Quercus robur* L. were obtained from the Austrian Research Center Seibersdorf and cultivated at the institute from November 1994. Cultivation was carried out in a phytochamber according to Meier-Dinkel (1987) under a 16/8 h light/dark regime at 24/15 °C day/night temperature, and under photosynthetic photon flux densities (PPFD) between 80 and 100 μmol m^{-2} s^{-1}. Trees derived from shoot cultures were cultivated further under greenhouse conditions in plastic pipes (50 cm in length and 7·5 cm in diameter), filled with an autoclaved mixture of silica sand (0·1–0·5 mm and 0·6–1·2 mm grain size 1:1) and peat 1:1, enriched with a slow-releasing fertilizer (45 g Osmocote per litre of soil mixture). To ensure mycorrhizal development, the substrate was inoculated with *Laccaria laccata* according to Seegmüller *et al.* (1996). For the solar dome experiments, 1-year-old trees were used.

Solar dome experiment

Oak trees were cultivated during an entire growing season from May until October 1998 in solar domes under controlled environmental conditions at Mt. Wank (1780 m above mean sea level), Garmisch-Partenkirchen. To ensure

irradiation close to the natural level, the solar domes were covered with UV-B transparent plexiglass (4 mm, GS 2458, Röhm, Darmstadt, Germany) and with a 25 μm Teflon film inlay (ET 6235, Nowofol, Siegsdorf, Germany; near-ambient UV-B radiation). The chambers were vented by fans installed in the base of the domes, providing varying wind conditions (exchange of the cabinet volume 2–3 times per minute) depending on the temperature difference between the inside and outside of the solar domes. Characterization of the light climate inside the domes revealed photosynthetic photon flux densities (PPFD, 400–700 nm) of 75–85%, UV-A radiation (320–400 nm) of 70–75% and UV-B radiation (< 320 nm) of approximately 70% compared with the values outside.

Monitoring photosynthetic gas exchange, temperature and isoprene emission rate

Isoprene emission rate was measured with a leaf cuvette system. The leaf cuvettes were mounted on the branches the evening before the measurements were started. The cuvettes were made of an aluminium frame with plexiglas (Polymethylmethylacylate, Sahlberg, Feld Kirchen, Germany) top and bottom covers. Two fans ensured homogeneous mixing of the air inside the cuvette. The cuvette was flushed continuously with air. The sensors for cuvette air and leaf temperatures (NiCr–Ni temperature transmitters GNTP, Greisinger Electronic GmbH, Regenstauf, Germany) as well as for PPFD (Li-Cor sensor, Li-Cor Inc., Lincoln, Nebraska, USA) were installed inside the cuvette. The absolute CO_2 concentration of the air at the inlet port of the cuvette was measured by an infra-red absorption analyser (BINOS 1000, Rosemount, Hanau, Germany), and the water vapour content by dew point mirrors (Walz, Effeltrich, Germany). The photosynthetic gas exchange was monitored with a differential infra-red absorption analyser (BINOS 4b, Rosemount, Hanau, Germany). The isoprene concentration of the air at the outlet port of the cuvette was analysed with a Fast Isoprene Sensor (FIS, Hills Scientific, Boulder, Colorado, USA). The FIS operates on the basis of the chemiluminescent reaction of isoprene with ozone (Monson *et al.* 1991). It makes use of a photo-multiplier tube in its reaction cell where the sample air is combined with an oxygen–ozone mixture supplied by an external ozone generator. The response time of the FIS is 0·5 s, that of the whole system (cuvette, tubing, analyser) is less than 60 s. The data were recorded as 1 min means. For comparison with modelled data, 3 min averages were calculated. At the end of the measurements, all leaves that had been inside the cuvettes were harvested and shock-frozen in liquid nitrogen for subsequent determination of enzyme activities.

Calculation of standard emission factors

Standard isoprene emission factors (30 °C; PPFD 1000 μmol m^{-2} s^{-1}) for the different oak trees were calculated as 3 min averages during the light phase according to Guenther (1997). For the calculation of tree-specific stan-

dard isoprene emission factors, all values between the 25th and 75th percentiles were used. These data were used as input for the empirical model according to Guenther (1997) to allow a comparison with the BIM.

Enzyme assays

For the enzyme assays, dimethylallyl diphosphate (DMADP) and its isomeric form isopentenyl diphosphate (IDP) were synthesized according to Keller & Thompson (1993). Protein extraction from leaves and the assay for isoprene synthase were performed as described by Lehning *et al.* (1999). Isopentenyl diphosphate (IDP) isomerase activity was determined by acidic hydrolysis of the dimethylallyl diphosphate (DMADP) formed from IDP by this enzyme, and subsequent GC analysis of the resulting isoprene in the head space of the vial. For this purpose, 88 μl of protein extract, 2 μL of 1 M MgCl$_2$ in H$_2$O and 10 μL of 2·5 mM IDP in an assay buffer (50 mM MOPS, 20 mM MgCl$_2$, 5% glycerol (v/v), pH 7·5) were filled into a 2 ml gas-tight, clear crimp seal vial (Supelco, Bellefonte, PA, USA). The vial was sealed immediately afterwards and incubated at 30 °C for 90 min. To determine the temperature dependence of the IDP isomerase, the vials were heated for 90 min to different temperatures (15–50 °C). To measure the DMADP concentration, the reaction mixture was acidified by addition of 10 μL of 85% H$_3$PO$_4$ with a 10 μL syringe. The vial was then heated to 70 °C for 90 min, and the isoprene released from DMADP was assayed as described (Lehning *et al.* 1999).

Determination of chloroplast volume

It has been reported that isoprene synthesis is localized to the chloroplasts (Wildermuth & Fall 1998). Model calculations of isoprene biosynthesis as well as enzyme activities must therefore be based on chloroplast concentrations of isoprene precursors. Counting of chloroplasts and determination of the ratio of chloroplast volume to the whole leaf volume was performed by confocal laser scanning microscopy (CLSM, Zeiss Axiovert 100 microscope, Zeiss, Oberkochen, Germany) allowing the generation of 3D images (Hutzler *et al.* 1998). For this analysis, oak leaves were cut into 5–10 mm wide slices with a razor blade, infiltrated with 10 mL freshly prepared fixative solution (4% formaldehyde (w/v) and 0·2% glutaraldehyde (w/v) in 0·1 M sodium phosphate buffer pH 7·0) and incubated at 0 °C for 2 h. In the present work, optical sections were taken from upper and lower sides of oak leaves as well as from leaf cross-sections. For determination of chloroplast abundance and volume, a single-line excitation (at 633 nm, long-pass filter > 665 nm) was found to be most suitable, as chlorophyll fluorescence emission occurs almost exclusively at such wavelengths. Chloroplasts were counted manually in 100×100 μm slices. The chloroplast volume was calculated by approximating the chloroplast shape to a flattened cylinder. The average ratio of chloroplast volume to whole-leaf volume was found to be 0·076 + 0·02 ($n = 10$ leaves).

Numerical program for the calculation of isoprene emission

The program contains the complete light fleck photosynthesis model (Kirschbaum *et al.* 1998) combined with the numerical model for calculating the isoprene biosynthesis (BIM) to be described here. The model is written in the programming language C and can be run on computers with a Linux platform or equivalent. A configuration file which is initially loaded by the program allows modification of the parameters for the calculation (Table 1). Details of the model including the sources of the parameter values are given in Results and Discussion.

RESULTS AND DISCUSSION

The biochemical isoprene emission model (BIM) described here was developed on the basis of the biochemistry of the recently discovered 2-methyl-erythritol 4-phosphate (MEP) pathway of isoprenoid biosynthesis (see Lichtenthaler 1999). Isoprene biosynthesis is known to be strongly correlated with formation of the first stable product of photosynthetic CO$_2$ fixation, 3-phosphoglyceric acid (Delwiche & Sharkey 1993), and so the BIM is based on the photosynthetic supply of carbon. The work presented here is divided into three sections: (i) formulation of the biochemical isoprene emission model, (ii) acquisition of the data set required by the BIM, and (iii) validation of the model and a comparison with the empirical model of Guenther (1997).

Formulation of the biochemical isoprene emission model (BIM)

Differential equations for the numerical calculation of isoprene emission rates

To construct a model capable of predicting isoprene emission, the metabolism in the leaf must be described by biochemical reaction equations. For most of the reactions, Michaelis–Menten kinetics were employed according to

$$v = v_{max} [S]/(K_M + [S]) \tag{0}$$

where v is the reaction velocity, S is the substrate, K_M is the Michaelis–Menten constant and v_{max} is the maximum reaction velocity. For most of the reactions, the kinetics could be shown experimentally to be of Michaelis–Menten type (see 'Acquisition of the data set required by the BIM'). The changes in the pool sizes for compounds of the pathway leading from the photosynthesis products 3-phosphoglycerate (PGA) and glyceraldehyde 3-phosphate (GAP) to isoprene (Fig. 1a) can be described by the following differential equations with the reaction rates v_1–v_5 given in Fig. 1(b):

$$\mathrm{d[DXP]}/\mathrm{d}t = v_1 - v_2 \tag{1}$$

$$\mathrm{d[IDP]}/\mathrm{d}t = v_2 - v_3 - v_5 \tag{2}$$

$$\mathrm{d[DMADP]}/\mathrm{d}t = v_3 - v_4 - v_5 \tag{3}$$

$$\mathrm{d[Isoprene]}/\mathrm{d}t = v_4 \tag{4}$$

Table 1. Source of parameters for the model and Abbreviations and numbering of the reactions are as for Fig. 1

Parameter	Value	Unit	Source
Time step for photosynthesis	0·1	s	Kirschbaum *et al.* 1998
Time step for the isoprene synthesis calculation	7·2	s	This work
DXP synthesis (v_1)			
$v_{\max \text{DXPs}}$, $v_{\max}$ value at 30 °C	0.38×10^{-6}	mol L^{-1} s^{-1}	[a]*Q. robur*, this work
$K_{\text{M DXPs GAP}}$, K_{M} for GAP	0.33×10^{-3}	mol L^{-1}	[b]Literature
$K_{\text{M DXPs PGA}}$, K_{M} for PGA	0.33×10^{-3}	mol L^{-1}	[b]Literature
IDP synthesis (v_2)			
$v_{\max \text{IDPs}}$, $v_{\max}$-value at 30 °C	0.34×10^{-6}	mol L^{-1} s^{-1}	[c]*Q. robur*, this work
$K_{\text{M IDPs}}$, K_{M} for DXP	42.0×10^{-6}	mol L^{-1}	[d]Literature
IDP isomerase (v_3)			
$v_{\max \text{IDPi}}$, $v_{\max}$ value at 30 °C	0.64×10^{-6}	mol L^{-1} s^{-1}	[e]*Q. robur*, this work
$K_{\text{M IDPi IDP}}$, K_{M} for IDP	7.0×10^{-6}	mol L^{-1}	[e]*Q. robur*, this work
$K_{\text{M IDPi DMDP}}$, K_{M} for DMADP	7.0×10^{-6}	mol L^{-1}	[e]*Q. robur*, this work
$k_{\text{eq IDPi}}$, equation constant for [DMADP]/[IDP]	3·1		[f]Literature
Isoprene synthase (v_4)			
$v_{\max \text{Is}}$, $v_{\max}$ value at 30 °C	0.53×10^{-6}	mol L^{-1} s^{-1}	[e]*Q. robur*, this work
$K_{\text{M Is}}$, K_{M} for DMADP	0.5×10^{-3}	mol L^{-1}	[g]*Q. robur*, literature
GDP synthase (v_5)			
$v_{\max \text{GDPs}}$, $v_{\max}$ value at 30 °C	4.37×10^{-9}	mol L^{-1} s^{-1}	[h]Literature
$K_{\text{M GDPs DMADP}}$, K_{M} for DMADP	8.5×10^{-6}	mol L^{-1}	[h]Literature
$K_{\text{M GDPs IDP}}$, K_{M} for IDP	56.0×10^{-6}	mol L^{-1}	[h]Literature
Ratio of chloroplast volume to whole leaf volume	0·076	L L^{-1}	*Q. robur*, this work
Ratio of chloroplast volume to total leaf area	0·009	L m^{-2}	*Q. robur*, this work
$V_{\max}$ factors by which all $v_{\max \text{30°C}}$-values were multiplied			
Q. robur, 24 June 1997	2·1		[i]This work
Q. robur, 25 June 1997	1·0		[i]This work
Q. robur, 26 June 1997	3·7		[i]This work
Q. robur, 11 July 1998	4·6		[i]This work
Q. robur, 30 July 1998	4·3		[i]This work
Q. robur, 10 September 1998	1·9		[i]This work

[a]The $v_{\max \text{DXPs}}$ for the DXP synthesis was determined by inverse modelling on the basis of three independently performed greenhouse experiments (see Fig. 3). [b]Value taken from the transketolase reaction (E.C. 2.2.1.1) of yeast as the most closely functionally related enzyme (Schomburg & Stephan 1996). [c]The $v_{\max \text{IDPs}}$ value for the IDP synthesis was determined by inverse modelling on the basis of three independently performed experiments (see Fig. 3). [d]Value of the phosphomevalonate kinase as the functionally most closely related enzyme (Skilleter & Kekwick 1971). [e]Experimentally determined for the forward reaction of the isomerase of *Q. robur* in this work. [f]Determined for *Narcissus pseudonarcissus* L. (Lützow & Beyer 1988). [g]*Q. robur*, Lehning *et al.* (1999). [h]*Vitis vinifera* L., Clastre *et al.* (1993). [i]The tree-specific values of the $v_{\max}$ factor were calculated by dividing the experimentally determined $v_{\max}$ of the isoprene synthase of the actual tree by the $v_{\max}$ value of the isoprene synthase given in the table. Thus the maximum temperature-dependent rate of the specific enzyme activity used in the model is given by $v_{\max \text{table}} \times v_{\max}$ factor $\times$ f(T).

$$\text{d[GDP]}/\text{d}t = v_5 \qquad (5)$$

As the production of IDP and isoprene (Fig. 1a) are irreversible reactions, the description of v_2 and v_4 according to Eqn 0 is adequate (Fig. 1b). However, the production of DXP and GDP are bimolecular reactions (Fig. 1a). In the absence of detailed information about the bimolecular reaction type, the formation of products from two substrates (DXP, GDP, Fig. 1a) has been represented by the product of two independent Michaelis–Menten terms, [S]/(K_{M} + [S]) (v_1, v_5, Fig. 1b), corresponding to a random mechanism without substrate interaction

(Bisswanger 1994). The equilibrium reaction v_3 (IDP → DMADP), catalysed by the IDP isomerase (Fig. 1a), is described according to Segel (1993). Its constant k_{eq} (Fig. 1b) has been determined as [DMADP]/[IDP] at the equilibrium (Lützow & Beyer 1988). Whereas the K_{M} values were assumed not to change with the temperature, the $v_{\max}$ values (Fig. 1b) were included as temperature-dependent functions (see below, Fig. 2). Thus, recording of the diurnal changes in the concentrations of GAP and PGA and in leaf temperature is required to calculate the isoprene emission rates during the day by means of the BIM.
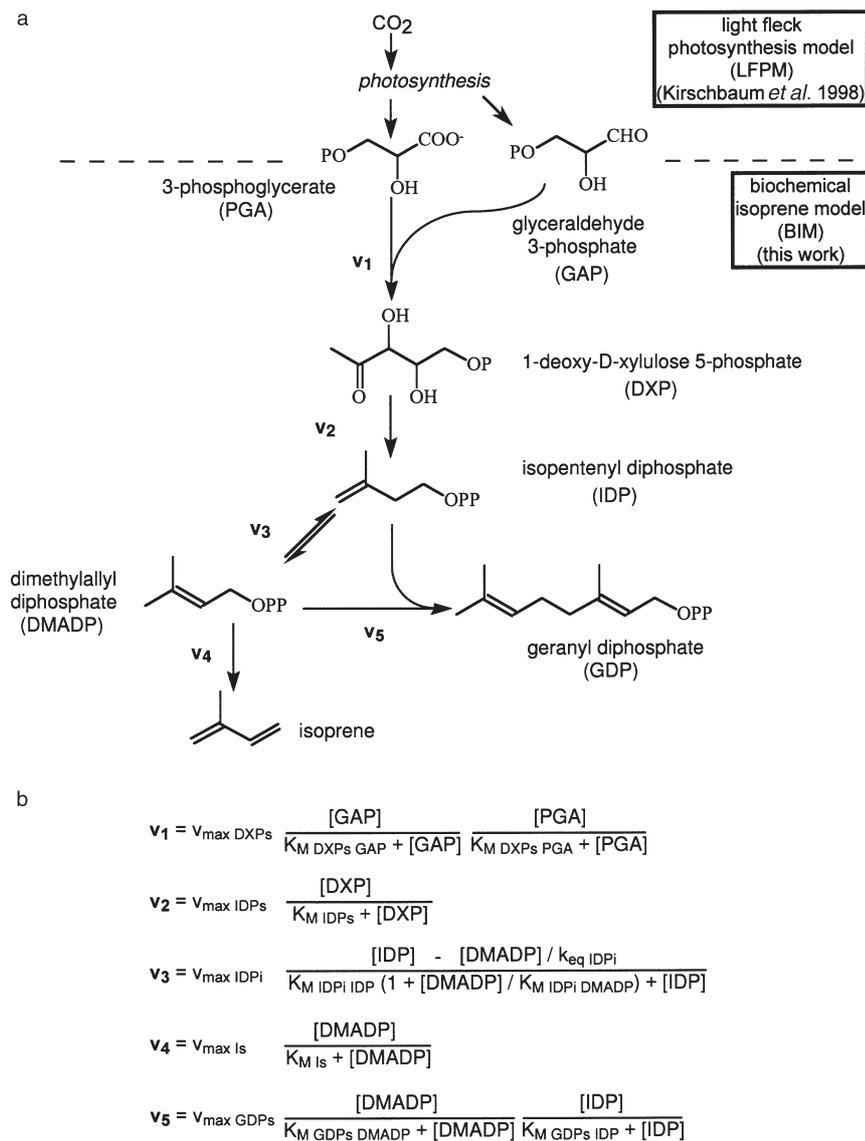
Figure 1. Scheme of the numerical model. (a) The reactions contributing to the production and consumption of the intermediates of the isoprene synthesis in the chloroplasts are described by (temperature-dependent, see below) Michaelis–Menten kinetics (see (b)). Enzymes and their reaction velocities are (v_1) 1-deoxy-D-xylulose 5-phosphate synthesis, (v_2) isopentenyl diphosphate synthesis, (v_3) isopentenyl diphosphate isomerase (E.C. 5.3.3.2), (v_4) isoprene synthase, and (v_5) geranyl diphosphate synthase (E.C. 2.5.1.1). To account for the temperature dependence of the individual v_i, the v_{max} values measured at 30 °C were multiplied by the factors given by the corresponding spline function (see Fig. 2 and text). Light- and temperature-dependent production of the photosynthesis products was calculated by a light fleck photosynthesis model (Kirschbaum *et al.* 1998).

Numerical solution of the differential equations

The system of equations (Eqns 1–5) is a coupled non-linear system of ordinary differential equations. As K_M and [S] are of the same order of magnitude for most of the reactions (Fig. 1b), a simple analytical solution to the linearized system is of little relevance to the dynamic behaviour of the model. Therefore, the equations were solved numerically by means of the Euler algorithm. The concentrations of the two input substrates GAP and PGA were calculated by the light fleck photosynthesis model (Kirschbaum *et al.* 1998). While the photosynthesis module was run with a time step of 0·1 s, a considerably longer time step of 7·2 s was suffi-cient for the BIM. Larger time steps (e.g. 36 s) produced oscillations in the diurnal concentrations of intermediates (data not shown) which are the first indication of the onset of numerical instabilities.

Acquisition of the data set required by the BIM

Source of data for the equation system

In order to obtain the parameter values for the reaction rates (v_1–v_5, Fig. 1b), enzyme activities of isoprene synthase and IDP isomerase were determined in leaf extracts of *Q. robur*. The reaction kinetics of the IDP isomerase (this work, data not shown) and of isoprene synthase (Lehning *et al.* 1999) followed Michaelis–Menten kinetics. K_M values were 7 mM and 0·5 μM, and $v_{max\ 30°C}$ values were 0·64 and 0·53 μM s^{-1}, for the IDP synthase and the isoprene synthase, respectively (see Table 1). The v_{max} values were found to have quite different temperature dependencies (Fig. 2). Below 35 °C, the activity of IDP isomerase increased expo-nentially with temperature and reached its maximum at about 40 °C (Fig. 2). The optimum IDP isomerase activity was about double the activity at 30 °C. In contrast, the tem-
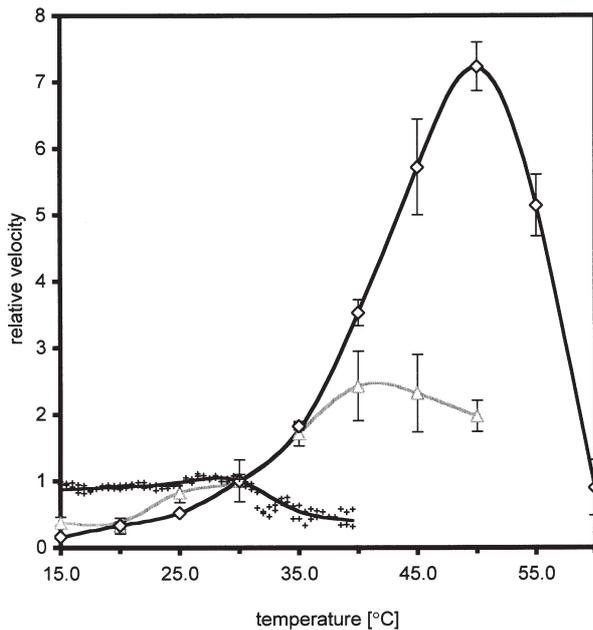
Figure 2. Experimentally determined temperature profiles of the IDP isomerase ($\triangle$) and isoprene synthase activity ($\diamond$), and the net assimilation rate of oak (*Q. robur*) (+). The relative velocity at 30 °C was normalized to 1·0 for all reactions.

perature-dependent increase of the activity of isoprene synthase reached its maximum at approximately 50 °C. This value is sevenfold higher than the 30 °C value, indicating that isoprene synthase tolerates higher temperatures and is preferentially active at higher temperatures (Fig. 2). In contrast to the high temperature maxima of IDP isomerase and isoprene synthase, the maximum of the photosynthetic net CO_2 assimilation rate of the oak leaves was reached at 28 °C. Higher temperatures up to 37 °C led to a decrease in net photosynthesis of approximately 40% compared to the maximum value (Fig. 2). Consequently, the temperature dependence of the net photosynthesis of oak leaves was included in the photosynthesic module of the model and approximated by temperature-dependent spline functions which were used to correct the corresponding v_{max} values given in Table 1 for the actual temperature. Because the temperature dependencies of other enzymes of the isoprenoid biosynthesis are not yet available, the temperature dependence of the IDP isomerase was also used for the other enzymatic steps in the model. Apart from some non-kinetic information on DXP reductoisomerase in *E. coli* and peppermint (Takahashi *et al.* 1998; Lange & Croteau 1999), no enzymatic data are available for the enzymes responsible for the conversion of DXP into IDP. The complete reaction from DXP to IDP (Table 1), probably involving 4–5 enzyme reactions, was therefore described as one single step. The K_M value of 42 μM (Table 1, Skilleter &

Kekwick 1971) was taken from the phosphomevalonate kinase (E.C. 2.7.4.2) of *Hevea brasilensis* Müll. Arg. (Table 1). The enzyme catalyses the transfer of the second phosphate group to the molecule, necessary both in the mevalonate pathway and the MEP pathway of IDP formation. Although DXP synthase could be detected in various organisms (Lange *et al.* 1998; Lois *et al.* 1998), no K_M value has yet been reported for this enzyme. The K_M value of 0·33 mM for the comparable transketolase (E.C. 2.2.1.1) reaction has therefore been used, representing an average value of known transketolases (Schomburg & Stephan 1996). No oak-specific v_{max} or K_M values are available for the GDP synthase (E.C. 2.5.1.1), so *Vitis vinifera* L.-specific values of 8·5 μM, 56 μM and 4·47 nM s^{-1} (Clastre *et al.* 1993) were used for $K_{M\ DMADP}$, $K_{M\ IDP}$ and $v_{max\ 30°C}$, respectively, since *V. vinifera* is also a woody plant (Table 1).

Estimation of v_{max} values by inverse modelling

Oak-specific $v_{max\ 30°C}$ values for the DXP synthesis and the IDP synthesis are still lacking. Three independently performed emission experiments (24, 25 and 26 June 1997) with three individual oak trees were used as a data source (Lehning *et al.* 1999). Each of these trees had different enzyme activities for the isoprene biosynthesis pathway. However, whereas the v_{max} of the isoprene synthase and of the IDP isomerase differed by a factor of 5 from tree to tree, the relation between isoprene synthase and IDP isomerase activity ($v_{max\ Is\ 30°C}/v_{max\ IDPi\ 30°C}$) was constant at a value of 0·83 ± 0·10 ($n = 3$). It was therefore assumed that the $v_{max\ 30°C}$ values of the other enzymatic reactions of the pathway also have constant ratios. Consequently, the $v_{max\ 30°C}$ of isoprene synthase was estimated for each of the trees, and all $v_{max\ 30°C}$ values given in Table 1 were multiplied by a v_{max} factor, which is the ratio of the $v_{max\ Is\ 30°C}$ of the actual tree and the $v_{max\ Is\ 30°C}$ value given in Table 1. To estimate the missing $v_{max\ 30°C}$ values of reactions v_1 and v_2 (Fig. 1b), the model was fed with the data of the actual leaf temperature, the photosynthetic photon flux densities and isoprene emission rates of the three experiments. The values for $v_{max\ DPXs\ 30°C}$ and $v_{max\ IDPs\ 30°C}$ were varied between 0·1 and 0·7 μmol L^{-1} s^{-1}. For each of the three experiments, the course of isoprene emission was calculated over the assumed range of $v_{max\ 30°C}$ values for DXPs and IDPs, and the averages of the deviation of the predicted emission from the observed emission (eight data points per experiment) were calculated. The resulting data fields of the three experiments were averaged (Fig. 3). The $v_{max\ 30°C}$ values that yielded the best fit to the measured emission data (0·38 μM s^{-1} for DXP synthesis and 0·34 μM s^{-1} for IDP synthesis) were taken as 30 °C values (Table 1) for all subsequent calculations. In order to account for differences in the isoprene synthesis activities, all $v_{max\ 30°C}$ values (Table 1) were multiplied by the tree-specific v_{max} factor (Table 1) for subsequent calculations.
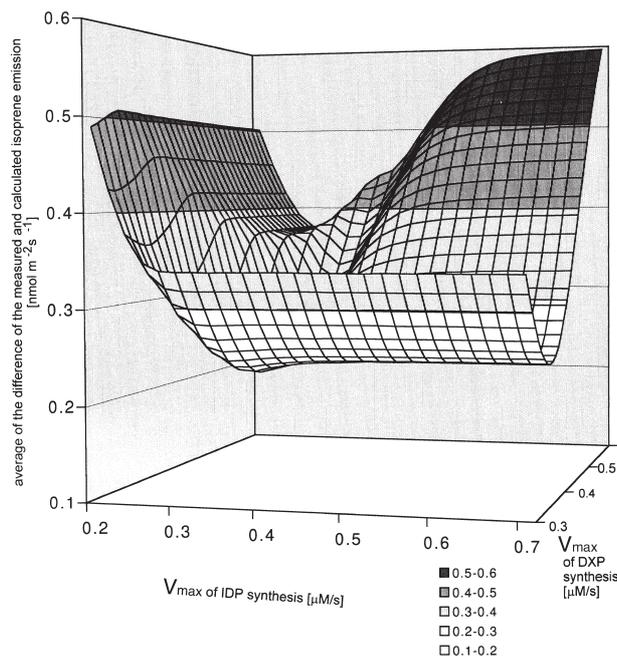
Figure 3. Estimation of v_{max} for DXP synthesis and IDP synthesis. For three independent oak trees, the enzyme data and the isoprene emission rates were determined (experiments were performed on 24 June, 25 June and 26 June 1997). For each of the trees, a daily course of isoprene emission was calculated by the numerical model with varying maximal velocities for the DXP synthesis and the IDP synthesis, and the deviations between the calculated and experimentally determined data were plotted. The minimum deviation between calculated and measured emission rates occurred at reaction velocities of 0·38 and 0·34 μ_M s^{-1} for DXP and IDP synthesis, respectively.

Validation of the model, and comparison with an empirical model

Comparison of experimental and modelled isoprene emission rates as a function of light intensity (PPFD) and temperature

To validate the model results of the BIM under conditions of increasing light intensities, the isoprene emission of two independent twigs was monitored in cuvettes, keeping the temperature constant at 30 °C and raising the PPFD from 20 to 1000 μmol m^{-2} s^{-1} (Fig. 4a). The isoprene emission rates rose slightly sigmoidally with increasing light intensity, and reached their highest levels of 18 and 23 nmol m^{-2} s^{-1} for trees 1 and 2, respectively, at the relative low PPFD of about 360 μmol m^{-2} s^{-1}. Further light increase to the maximal PPFD of 1000 μmol m^{-2} s^{-1} did not significantly change the rate of isoprene emission (Fig. 4a). Compared to the experimental data, the sigmoid initial phase is less pronounced and the plateau is reached at slightly higher light intensities in the simulation. Nevertheless, there is a good correlation between the calculated isoprene emission rate and the experimental data. This shows that the numerical isoprene emission model including the photosynthesis module responds to increasing light inten-

sities in a realistic way, and that there is no need to include other processes such as the hypothetical light activation of the isoprene synthase discussed by Fall & Wildermuth (1998).

To compare the leaf temperature dependencies of the modelled isoprene emission rates with the experimental data, isoprene emission rates of two oak twigs were monitored in cuvettes. The leaf temperature increased from 10 to 42 °C (Fig. 4b) and the PPFD was kept constant at 1000 μmol m^{-2} s^{-1}. The isoprene emission rates were also calculated by the model. The experimentally determined isoprene emission rates increased with rising temperature until maxima of 38 and 40 °C for trees 1 and 2, respectively, were reached. At still higher temperatures, the isoprene emission rates declined. The maximum of the isoprene emission rate is similar to the observed maximum at 40 °C of the IDP isomerase (Fig. 2). Therefore, at temperatures exceeding 40 °C, the substrate supply for the isoprene synthase reaction with its higher maximum of reaction velocity seems to be limited by one of the preceding enzymatic reactions. The calculated temperature dependencies of the isoprene emission rates are comparable to the experimental data. However, the calculated emission maxima were already reached at lower temperatures of about 36 °C. One probable reason for this could be the assumption that the isoprene synthesis rate in the model is exclusively driven by photosynthesis products, so that the flow of carbon intermediates is dependent on the activity of CO_2 fixation which is reduced in the model above 28 °C (see Fig. 2). As the following enzymes have higher temperature optima (Fig. 2), the calculated amount of substrate for isoprene biosynthesis obviously becomes limiting above 36 °C. In reality, the situation under these extreme conditions is more complicated than described in the current model version. The storage pools of carbohydrates such as starch, which can be mobilized by respiration, as well as carbon influxes from transport processes within the plant have to be considered. Both sources might be capable of additionally supporting isoprene biosynthesis under these conditions. In order to overcome this discrepancy, further improvements to the model should take into account storage pools and carbon exchange between leaf, shoot and root.

Validation of the BIM by observed diurnal isoprene emission rates

In order to validate the numerical model, an independent experiment closely resembling natural environmental conditions was performed during the summer of 1998 using the solar domes at 1780 m above mean sea level at Mt. Wank, Garmisch-Partenkirchen, Germany. On 3 days during the vegetation period (11 July, 30 July, 10 September), the diurnal variations in leaf temperature, PPFD and isoprene emission rates were monitored for twigs of two independent oak trees, giving six data sets. For each of these trees, enzyme activities of the isoprene synthase and IDP isomerase were determined after the experiment. Based on these enzyme activities, the v_{max} factor for all other
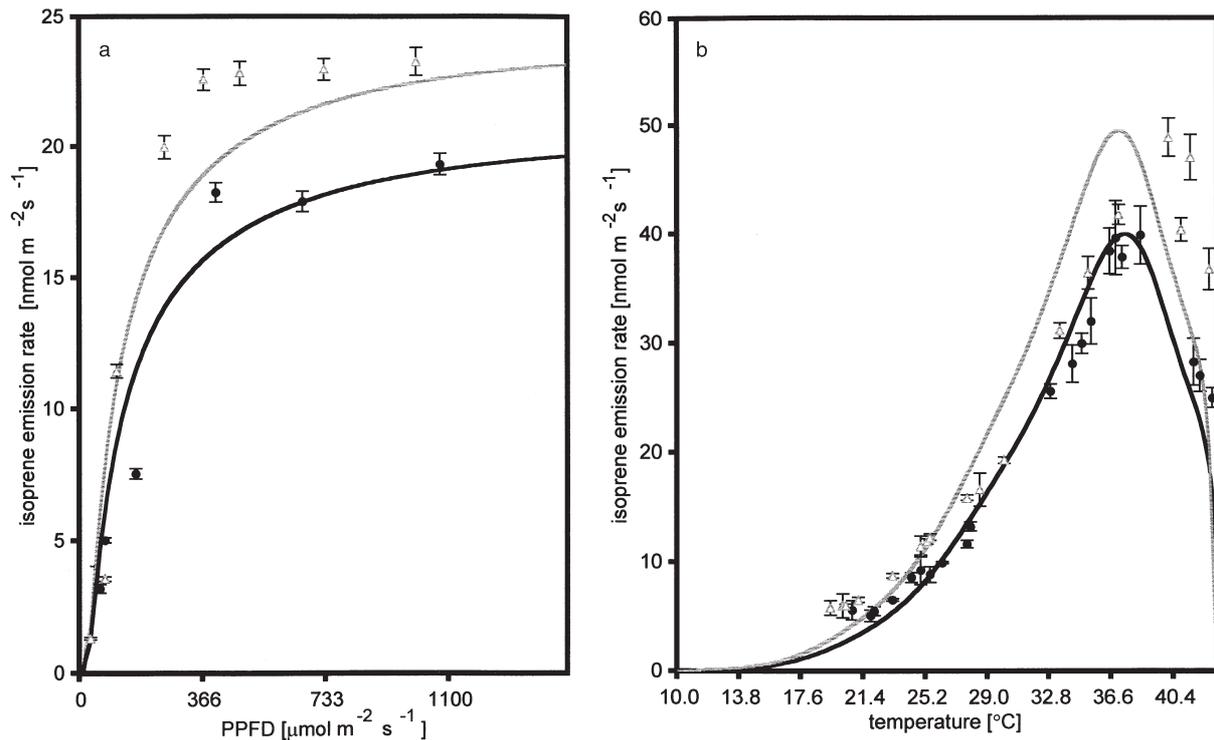
Figure 4. Experimental and modelled light- and temperature-dependent isoprene emission. Isoprene emission rates were determined with a Fast Isoprene Sensor (Monson *et al*. 1991) from twigs of two oak trees which were inserted in cuvettes (cuvette 1: ●; cuvette 2: △). For the simulations, the specific v_{max} factor for these trees was used (tree 1: black line, tree 2: grey line). The light-dependent and temperature-dependent isoprene emission rates were measured 5 min after reaching a steady state following the stepwise change. For the modelled values, either the PPFD (a) was increased steadily over 2 h from 0 to 1440 μmol m^{-2} s^{-1} while keeping the temperature at 30 °C, or the temperature was increased from 10 to 42 °C while keeping the PPFD constant at 1000 μmol m^{-2} s^{-1}.

enzymes was calculated and isoprene emission rates were calculated by the numerical model with the default data (Table 1) for the recorded diurnal PPFD and temperature variations (for examples see Fig. 5). While on 11 and 30 July temperature maxima of approximately 35 °C were observed, the temperature maximum on 10 September was approximately 40 °C (Fig. 5). The average of the PPFD during the light phase from 06:00 to 18:00 h was highest on 30 July (510 μmol m^{-2} s^{-1}), followed by 11 July (432 μmol m^{-2} s^{-1}) and lowest on 10 September (261 μmol m^{-2} s^{-1}). Thus, owing to the different PPFD and leaf temperature profiles, very different diurnal variations of isoprene emission rates were observed for the 3 days. Nevertheless, values for isoprene emission rates calculated by the numerical model correlated well during longer time periods with the experimentally determined emission rates, e.g. on 11 July from 06:00 to 10:00 h and from 12:00 to 18:00 h, on 30 July from 06:00 to 07:30 h and from 12:30 to 18:00 h as well as on 10 September from 08:00 to 12:00 h and from 16:00 to 18:00 h (Fig. 5). An over-estimate of up to 40% in the calculated emission rate compared with the measured values occurred on 11 July, from 10:00 to 12:00 h and 30 July from 07:30 to 12:30 h as well as at the afternoon of the 10 September from 12:00 to 16:30 h (Fig. 5). One reason for this discrepancy might be that the stomata reg-

ulation in the photosynthesis module is parameterized for the tropical Araceae *Alocasia macrorrhiza* (L.) G. Don. and not adapted yet for *Q. robur*. The closure of the stomata of the *Q. robur* leaves at high light intensities and high temperature is therefore not adequately described by the current version of the model. Another reason for the deviation might be the fact that the temperature during the experiment was determined by thermo-elements on the lower surface of the leaves. At high temperatures, however, the closed stomata prevent cooling of the leaf mesophyll by evaporation, which can lead to a higher temperature inside the leaves than at the surface. In preliminary experiments, it was observed that the temperature inside the leaf was about 1–2 °C higher than the surface temperature when the stomata were closed (data not shown). As the temperature dependence of v_{max} of the isoprene synthase rises by a factor of about 1·15 per °C at temperatures higher than 35 °C (Fig. 2), a temperature difference of 1–2 °C can cause 15–30% higher emission rates. Therefore, the leaf temperature used for the modelling might have been too low for high temperature periods when the stomata were closed. Consequently, the modelled isoprene emission rates were lower than the measured ones. In further experiments, the temperature should be monitored inside the leaf (e.g. by an infra-red sensor) rather than at its lower surface.
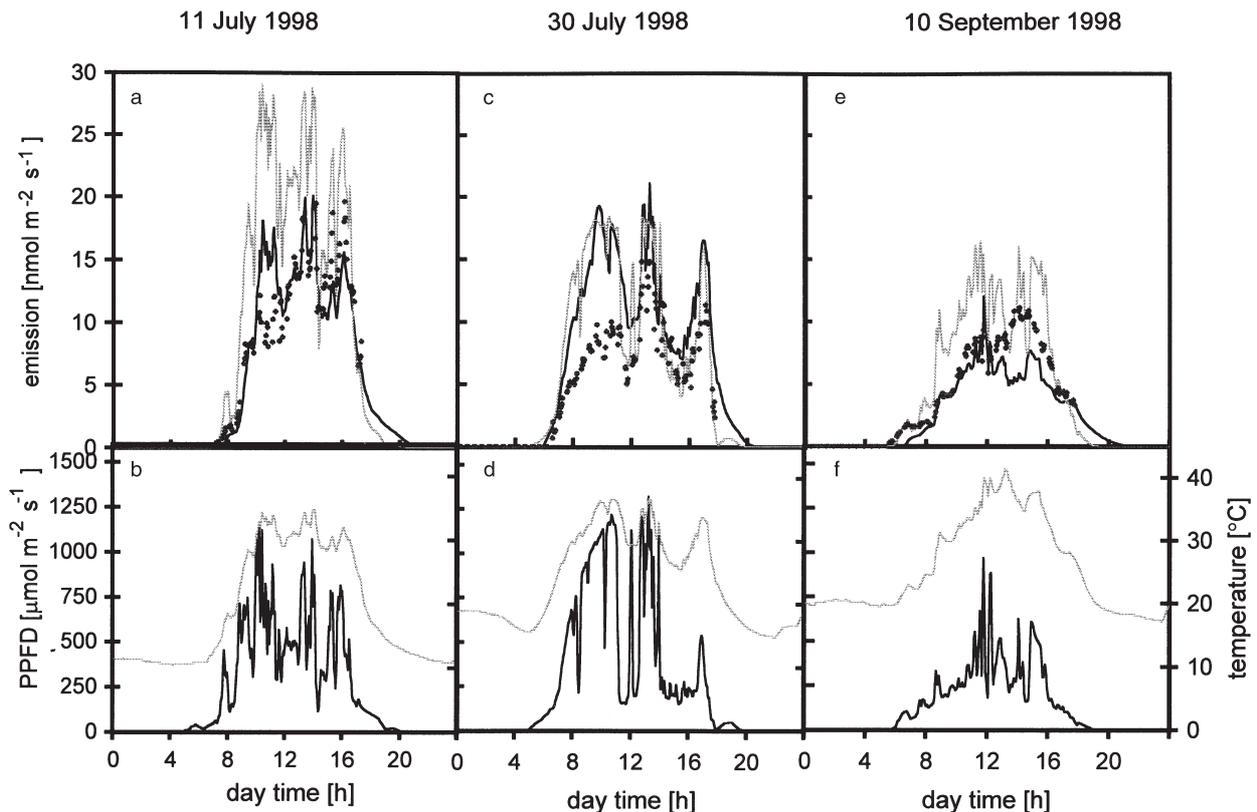
Figure 5. Comparison of calculated isoprene emission rates of oak leaves with modelled values. (b, d, f) Leaf temperature (grey line) and photosynthetic photon flux density (PPFD) (black line) for 11 July, 30 July and 10 September 1998 in the solar domes at 1780 m above mean sea level. (a, c, e) Daily course of experimentally determined isoprene emission rate (●) compared with the emission calculated by the numerical model (black line), and the values calculated by the empirical algorithm of Guenther (1997) (grey line). The latter are based on the individual emission factors determined for each of the trees considered (a: $14.66 + 1.72$ nmol m^{-2} s^{-1}, $n = 53$; c: $9.24 + 2.29$ nmol m^{-2} s^{-1}, $n = 57$; e: $9.37 + 2.22$ nmol m^{-2} s^{-1}, $n = 63$). All data are displayed as 3 min averages during the light phase.

Isoprene emission rates calculated by the empirical algorithm (Guenther 1997) had the tendency to over-estimate isoprene emission rates (Fig. 5). This is pronounced on 11 July and 10 September, where over-estimates of up to 50% occurred. Moreover, emission rates calculated by the empirical algorithm (Guenther 1997) are very sensitive to changes in temperature and light intensity (Fig. 5). The real plant leaf and also the 'oak leaf' modelled by the BIM responded much less dramatically to these changes, because production of isoprene includes a series of metabolite pools so that the sum of turnover times led to a smoothing of the light and temperature dependence. The average of the deviation between the measured and the modelled isoprene emission for the six data sets was 0.24 ± 0.03 nmol m^{-2} s^{-1}, whereas the difference between the measured values and the isoprene emission rates calculated by the Guenther (1997) algorithm was about 50% higher (0.36 ± 0.06 nmol m^{-2} s^{-1}). One reason for the better correlation of the BIM compared with the empirical algorithm is the fact that the BIM considers process-specific temperature dependencies such as reduced net photosynthesis during periods of very high temperatures (see Fig 5, 10 September

1998) which are reached at noon, and the fact that the BIM does not react too sensitively to changes in light intensities and temperature as discussed above.

CONCLUDING REMARKS

The current work demonstrates that a numerical model like the BIM can describe the isoprene emission by oaks in a realistic way. It reacts to rapid changes in light and temperature by accounting for plant physiological processes such as the turnover of intermediate pools so that realistic diurnal isoprene emission rates can be calculated. Modelling of isoprene formation on a biochemical basis is therefore a powerful tool for predicting isoprene emission rates under diverse conditions. Neither the empirical model of Guenther (1997) nor the BIM in the present version are, however, capable of describing the observed decline of isoprene emission at high CO_2 concentrations (Loreto & Sharkey 1990). Under these conditions, the redox charge and energy charge in the chloroplasts are low owing to increased CO_2 fixation and formation of glyceraldehyde 3-phosphate in the Calvin cycle. By including NADPH- and

ATP-consuming reactions in a future version of the model, the decreased redox charge and energy charge caused by elevated CO_2 concentrations will result in a decreased isoprene emission. The corresponding reaction equations and their dependencies on photosynthetically produced redox (NADPH) and energy (ATP) equivalents will therefore be included into the BIM as soon as they are biochemically characterized. The importance of including redox equivalents is emphasized in a recent report showing that a model based on the availability of NADPH was capable of describing realistically the diurnal variations of field measurements of isoprene emission (Niinemets *et al.* 1999). Including plant developmental processes in the BIM will probably allow the prediction of isoprene emission even under changed plant growth conditions such as the higher CO_2 concentrations and higher average temperatures which are expected in the near future.

The activity of isoprene synthase was found to correlate well with the emission factor used in the Guenther (1997) algorithm (Lehning *et al.* 1999). Thus there is the possibility of calculating the isoprene synthase activity using this correlation. To involve a model such as the BIM in cataster calculations of isoprene emission which are commonly used, for example for regional ozone forecasts, the existing emission factors can be simply converted into isoprene synthase activity values and hence also into the corresponding v_{max} factors for the BIM (Table 1) on the basis of the statistically averaged correlation of the two values (Lehning *et al.* 1999).

To apply the model to other isoprene-emitting species, it is likely that only the species-specific isoprene synthase activity has to be measured (to determine the new v_{max} factor) and that the temperature dependencies of the enzyme activities must be checked.

The model can also be extended to calculate monoterpene emission. In this case, the reaction velocity of the geranyl diphosphate synthase specific to monoterpene-emitting trees must be taken into account, and the enzyme parameters of monoterpene cyclases/synthases must be included. This will allow the numerical model to be used for predictions of monoterpene emissions from Mediterranean evergreen oaks (e.g. *Quercus ilex* L. and *Quercus coccifera* L.) and other monoterpene-emitting woody angiosperms. In the cases of conifer trees, the stored monoterpenes in the resin system have also to be taken into account in order to calculate the apparent monoterpene emission.

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