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Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates

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Abstract. Isoprene plays an important role in regulating the atmospheric trace gas composition, in particular the tropospheric ozone concentrations. Therefore realistic estimates of the seasonal variation of isoprene emission source strengths of strong isoprene-emitting deciduous trees such as pedunculate oak (*Quercus robur* L.) are required in temperate regions of Europe. In 1995 to 1997 a study was conducted to survey the annual fluctuations of oak isoprene synthase activity and photosynthetic pigment contents, the latter as a parameter for the development of the photosynthetic apparatus of oak leaves. Depending on annual temperature and light profiles (photosynthetic photon flux densities (PPFD)), different seasonal patterns of isoprene synthase activity were observed with maximum activities of 18.4 ± 10.6 nmol m^{-2} s^{-1}, 14.1 ± 5.8 nmol m^{-2} s^{-1}, and 19.9 ± 7.9 nmol m^{-2} s^{-1} in 1995, 1996, and 1997, respectively. On the basis of isoprene synthase activity, chlorophyll a measurements, and phenological data collected from pedunculate oaks of 89 ecological regions covering all of Germany a model was developed for the calculation of the seasonal variation of oak isoprene synthase activity in relation to annual fluctuation of temperature and PPFD. By coupling this model to a numeric process-based isoprene emission model it was possible to predict isoprene emission rates of individual pedunculate oak trees with a deviation of 55%.

1. Introduction

Volatile naturally emitted organic compounds (VOC) such as isoprene (2-methyl-1,3-butadiene) from forests are important components for atmospheric trace gas composition. Because of its reactivity with OH radicals, isoprene influences atmospheric chemistry as a direct sink of oxidants [*Thompson*, 1992], and thus affects the degradation and distribution of several tropospheric trace gases, including methane and carbon monoxide. On regional scales, in populated areas under anthropogenic NO_x emissions isoprene or its oxidation products, for example, ketones, aldehydes influence ozone chemistry by competing with ozone as a sink for NO [*Fehsenfeld et al.*, 1992]. These oxidation products force tropospheric ozone formation [*Biesenthal et al.*, 1997; *Derwent et al.*, 1998]. The annual global flux of isoprene from vegetation has been estimated in the range of 175 and 450 Tg carbon yr^{-1} [*Guenther et al.*, 1995]. The high uncertainty of estimates of natural isoprene emission is mainly due to insufficient knowledge about the heterogeneity of biological sources, the processes controlling isoprene formation, and the seasonal variations of basal isoprene emission capacity of the leaves.

Isoprene emission rates are controlled at two different levels. Daily short-term variations of isoprene emission rates can be explained by the temperature dependence of isoprene synthase activity and other related enzymes of the isoprenoid pathway, for example, IDP isomerase [*Zimmer et al.*, 2000] and reflects the response of leaf isoprene emission to temperature [*Monson et al.*, 1992]. In a previous study with pedunculate oak (*Quercus robur* L.) [*Lehning et al.*, 1999], isoprene synthase activity correlated with the isoprene emission rate of the leaves. Under optimal conditions in the stroma of illuminated chloroplasts, enzymatic isoprene production from oak leaves accounts for the observed leaf isoprene emission rates. The correlation with photosynthetic photon flux densities (PPFD) is consistent with the light saturation response of photosynthetic processes in the leaves [*Sharkey and Loreto*, 1993], while other factors such as soil moisture fluctuations, CO_2 mixing ratio, and stomatal conductance have minor influence on the short-term variations of leaf isoprene emission rates [*Guenther et al.*, 1991]. Although regulatory mechanisms controlling isoprene emission are not fully elucidated, there is good evidence that long-term and seasonal variations in basal isoprene emission capacity [*Monson et al.*, 1994, 1995] can be explained by long-term variations in the amount of active isoprene synthase [*Monson et al.*, 1992; *Kuzma and Fall*, 1993; *Schnitzler et al.*, 1997]. This basal emission capacity depends on physiological adaptations, for example, leaf expansion and development, or the position of the leaf in the forest canopy as Sun or shade leaf, which seem to be triggered by growth temperatures in spring [*Monson et al.*, 1994], availability of nutrients, UV-B radiation [*Harley et al.*, 1996], or genetic disposition [*Isebrands et al.*, 1999]. In general, isoprene emission rates increase during springtime, peak in summer, and then decline until leaf shedding in autumn [*Monson et al.*, 1994; *Kempf et al.*, 1996; *Geron et al.*, 1997].

In the present study we examined whether the seasonal variation of isoprene synthase activity in *Q. robur* leaves represents an acclimation to prevailing environmental conditions, for example, seasonal variations of temperature and/or light. For this purpose, the variation of isoprene synthase activities among different genotypes and years was quantified, and a model was developed in order to predict isoprene synthase activity in oak leaves in dependency of annual changes of temperature and light (PPFD).

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2. Materials and Methods

2.1. Plant Material

Two-year-old seedlings of pedunculate oak (*Quercus robur* L.) grown from seeds collected at a natural oak stand near Freising, Germany, were obtained in April 1995 from the nursery of the Staatliche Samenklenge Laufen, Germany. The saplings were planted in pots containing 10 kg calciferous mineral soil originating from a spruce stand close to the Fraunhofer-Institute. The plants were cultivated in Garmisch-Partenkirchen (47.50°N, 11.10°E) during three vegetation periods (1995–1997) at 730 m above sea level. They were irrigated regularly and were treated, if necessary, with Compo Rosenschutz (0.125% (vol/vol) in H_2O) (BASF, Mannheim, Germany) against pathogens, especially oak mildew (*Microsphaera alphitoides*). Herbs and grasses growing in the pots were weeded out twice during the growing season.

In May 1997 each plant was fertilized with 1 L Ingestad solution [*Ingestad*, 1959] applied in 100 mL portions within 10 days. In this year, single branches of seven trees were covered with shading gauze to lower PPFD by 50% after the emergence of the leaves. Independent leaf samples (three branch samples per tree with three leaves per sample from similar unshaded branch locations) of each tree were collected during each growing season at time intervals of 3–4 weeks between 1400 and 1600 LT. In 1997, additional leaves from shaded branches were taken. Immediately after sampling, leaf area was determined with a Li-3100 area meter (Li-Cor Inc., Lincoln, Nebraska, United States), and subsequently leaves were shock-frozen in liquid N_2. Half-hour means of air temperature and PPFD (μmol m^{-2} s^{-1}) were measured during the entire experimental periods and were further processed to get daily mean temperature and PPFD during the light phase.

2.2. Protein Extraction and Isoprene Synthase Assay

Leaves were homogenized with a mortar and pestle in liquid N_2. All further steps were performed at 4°C. The fine leaf powder (100 mg fresh weight) was resuspended in 4.5 mL extraction buffer (100 mM Tris/HCl pH 7.0, 20 mM $MgCl_2$, 5% (vol/vol) glycerol, 2% (vol/vol) Triton-X-100; 2 mM DTT and 100 mg PVPP were added prior to use of the buffer). The homogenate was centrifuged at 20,000 *g* for 20 min, and the clear supernatant was used for further analyses. Protein was precipitated in three steps with $(NH_4)_2SO_4$ (0–30%, 30–60%, and 60–100% saturation). The pellets were collected by centrifugation at 40,000 *g* for 20 min and resuspended in 1 mL isoprene synthase buffer (ISB: 50 mM Tris/HCl, pH 8.5, 20 mM $MgCl_2$, 5% glycerol and 2 mM DTT). Isoprene synthase activity was completely recovered in the 30–60% $(NH_4)_2SO_4$ fraction. The protein solution was stored at −80°C. For enzyme assays, dimethylallyl diphosphate (DMADP) was synthesized according to *Keller and Thompson* [1993]. Isoprene synthase activity was assayed according to *Lehning et al.* [1999]. Protein concentrations were determined by the Bradford assay [*Bradford*, 1976] with BSA as a standard. Photosynthetic pigment contents were quantified according to *Lichtenthaler and Wellburn* [1983]. C/N analysis was carried out with an element analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany) with lyophilized and homogenized plant material.

2.3. Measurement of Isoprene Emission Rates

Isoprene emission rates were measured with a leaf cuvette system according to *Lehning et al.* [1999]. The trees were transferred to a greenhouse, and a leaf cuvette was mounted on the respective branch the evening before measurements were started. The following day, isoprene emission rates were determined hourly by gas chromatography, starting from 0900 to 1800 LT. The isoprene concentration of the air at the outlet port of the cuvette was analyzed with a Chrompack CP 9000 Air Analyser System (Chrompack, Middelburg, Netherlands). Air samples were collected for 15 min by focusing on a cryotrap (fused-silica capillary column), and GC analysis was performed on a combination of two capillary columns of different polarity (column 1: 25 m Al_2O_3/KCl, ID 0.53 μm, film thickness 10 μm; column 2: 25 m CP-SIL 13 CB, ID 0.53 μm, film thickness 2 μm; Chrompack, Middelburg, Netherlands). Best resolution was achieved with a temperature program from 50°C up to 200°C at a rate of 5°C min^{-1}. Volatile organic compounds were detected by flame ionization, and isoprene was identified by cochromatography with authentic standard (10 ppm, Messer Griesheim, Germany).

2.4. Statistical Analysis

Statistical analysis was performed with SPSS Version 3.0 for Windows. Differences between mean values of the samples were tested on significance using one-way analysis of variance (ANOVA) (Waller-Duncan test).

3. Experimental Results

3.1. Seasonal Fluctuations of Isoprene Synthase Activity

Measurements of isoprene synthase activity of pedunculate oak leaves (Figure 1d) in the present study revealed strong seasonal variations with maximum activities of 18.4 ± 10.6 nmol m^{-2} s^{-1}, 14.1 ± 5.8 nmol m^{-2} s^{-1}, and 19.9 ± 7.9 nmol m^{-2} s^{-1} in 1995, 1996, and 1997, respectively. Significant isoprene synthase activity could be detected 34 days (1995), 25 days (1996), and 19 (Sun leaves, 1997) and 25 (shaded leaves, 1997) days after leaf emergence (Julian days: 140, 123, and 127 in 1995, 1996, and 1997, respectively). In the experiments, isoprene synthase activity had maximum values after the contents of photosynthetic pigments (Figure 1b) reached their summer plateau. In autumn 1996 and 1997 (Figure 1b; in 1995, pigment analysis was not carried out because the trees were too small to be sampled too extensively) the decline of photosynthetic pigments coincided with a strong reduction of isoprene synthase activity in oak leaves. In 1996 and 1997 a plateau of approximately 1.4 mg g fw^{-1} of chlorophyll a, 0.35 mg g fw^{-1} of chlorophyll b, and 0.38 mg g fw^{-1} of carotenoids was reached in July and kept till September (Julian days 180–270). Shading of leaves in 1997 caused a significant increase ($p \leq 0.05$) in chlorophyll a and b content of approximately 28 and 38%, respectively, reflecting low light acclimation of photosystems. This is consistent with a shift of the chl_a/chl_b ratio of 5.65 in Sun-exposed to 3.82 in shade-exposed leaves.

In 1995, isoprene synthase activity peaked about the Julian day 185 during a period with high PPFD and high temperatures (Figure 1a), while in 1997 maximum enzyme activity was measured at Julian day 240 following a period with high temperatures and high light intensity. The effects of shading on PPFD and air temperature in 1997 are illustrated in Figure 1a on the right panel. Under the shading gauze, mean PPFD

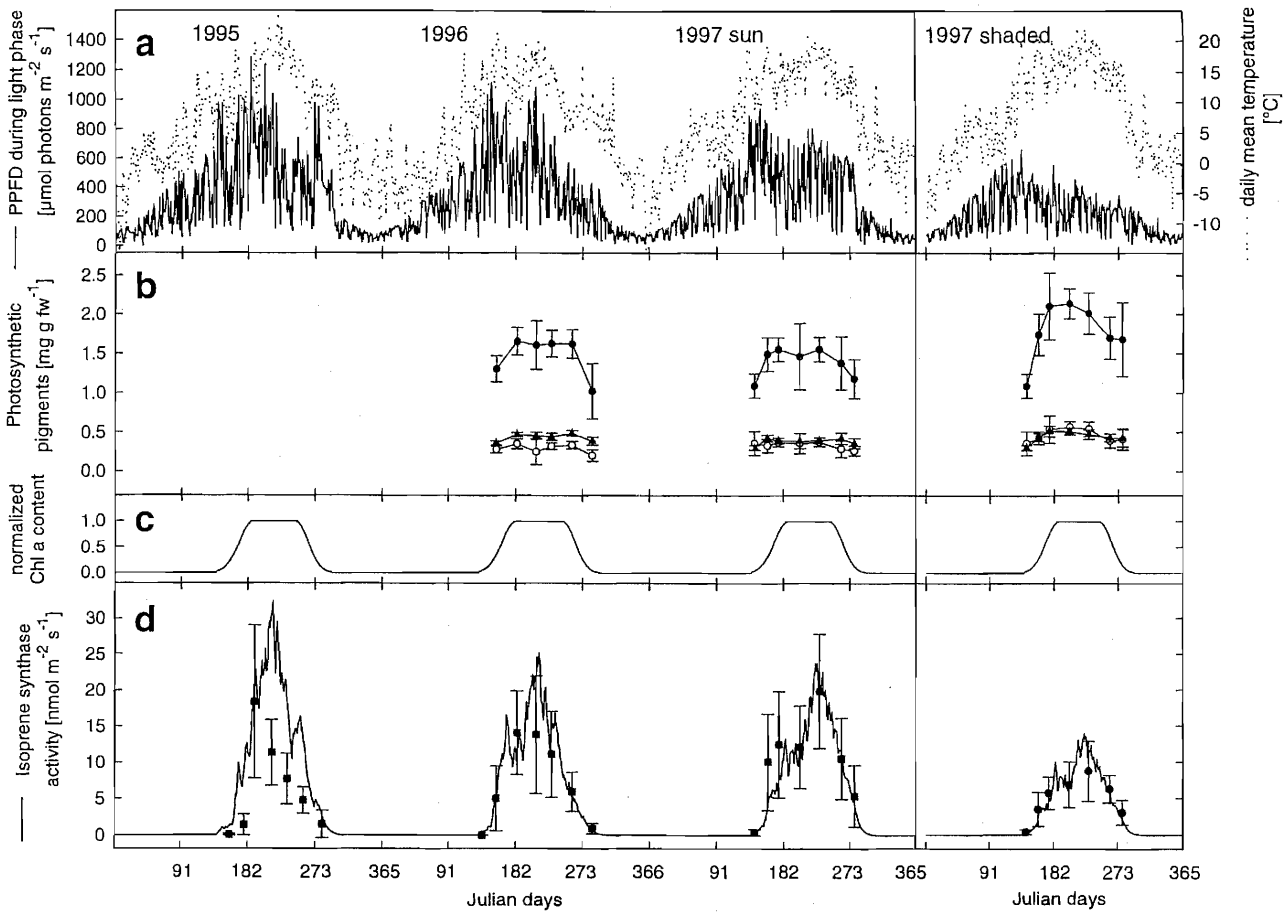

Figure 1. Annual variations (1995–1997) of (a) daily mean temperature (dotted curve) and PPFD during the light phase (solid curve); (b) photosynthetic pigments (chlorophyll a, solid circles; chlorophyll b, solid triangles; carotenoids, open circles; n = 8, ±s.d.); (c) normalized (maximum value equal to 1.0) chlorophyll a content calculated from the years 1996 and 1997 (Sun and shaded leaves); (d) isoprene synthase activity, solid squares; n = 8; ±s.d.. In addition, in Figure 1d the solid curve shows the calculated isoprene synthase activity (nmol m^{-2} s^{-1}) obtained by the model. Leaves emerged (±1 week) on May 20, 1995 (Julian day 140), May 2, 1996 (Julian day 123), and May 7, 1997 (Julian day 127). The right panel of the figure shows the data set for shaded branches of trees (n = 7; ±s.d.) in 1997. Start of shading was May 27, 1997 (Julian day 147).

during the light phase was reduced by 50%, while mean air temperatures were slightly enhanced (approximately 1°C), probably due to reduced wind speed under the gauze. As a consequence of this treatment, isoprene synthase activity (Figure 1d, right panel) in these leaves was approximately 40% lower than the corresponding enzyme activities of Sun-exposed leaves (Figure 1d). Despite the high variability of enzyme activity (see also Table 1) between trees, the response of the single tree to shading was similar. A ~50% reduction of PPFD caused a significant reduction of enzyme activity in shaded leaves by 49.8 ± 9.7% compared to leaves of light-exposed branches of the same tree. Still, the seasonal variation of isoprene synthase activity in Sun-exposed and shaded leaves was similar.

3.2. Phenotypic Variation of Isoprene Synthase Activity

In 1995, 1996, and 1997, eight pedunculate oak saplings originating from seeds from a natural oak stand were used, representing a natural range of phenotypes. Table 1 summarizes the variability of active isoprene synthase (percent of maximum activity at the different sampling times) between single trees over the 3 years of investigation. In general, isoprene synthase activity between trees varied by a factor up to 4, but as an extreme (e.g., tree 3 compared to tree 6; Table 1) isoprene synthase activity differed by a factor up to 10. Within the set of trees, trees with significantly low (e.g., 3 and 4; Table 1) and significantly high (5, 6, and 7) isoprene synthase activity were identified. However, the position of a particular tree within these groups varied strongly from year to year: Tree 8, for example, was the tree with the highest and second highest isoprene synthase activity in 1995 and 1996, respectively, and in 1997 it was the second lowest one. The leaf nitrogen contents and the C/N ratios were determined during the vegetation period in 1996 and 1997 to reveal whether observed tree-specific differences in isoprene synthase activity are related to these parameters. A mean nitrogen content of 2.8% ± 0.4% (% N of leaf dry weight; n = 96) and a mean C/N ratio of 47.7 ± 1.6 (n = 96) were measured, and statistical analysis showed no correlation of these parameters with isoprene synthase activities at the different sampling dates in 1996 and 1997.

Table 1. Variation of Isoprene Synthase Activity Between Oak Trees in the Years 1995, 1996, and 1997 (Only From Sun-Exposed Leaves)[a]

Julian Days	Isoprene Synthase Activity as Percent of Maximum Activity at the Different Sampling Dates								Maximum Activity, nmol m^{-2} s^{-1}
	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6	Tree 7	Tree 8	
178 (95)	15	22	13	18	55	26	33	100	4.2
192 (95)	52	40	17	34	100	36	33	86	28.7
215 (95)	100	45	41	53	40	...	42	75	15.6
236 (95)	100	60	26	25	...	63	70	56	10.4
258 (95)	50	56	24	51	80	74	100	49	6.0
Mean 95 ± s.d.	63 ± 36	45 ± 15	24 ± 11	36 ± 15	69 ± 26	50 ± 23	55 ± 29	73 ± 21	
Significance ($p > 0.05$)[b]	a	ab	b	ab	a	ab	ab	a	
157 (96)	39	59	23	57	83	21	100	49	10.4
185 (96)	50	88	16	48	100	66	85	87	16.2
211 (96)	23	55	15	35	63	33	84	100	21.0
232 (96)	41	67	14	22	76	82	100	99	13.8
260 (96)	52	61	12	37	59	100	72	81	7.7
Mean 96 ± s.d.	41 ± 11	66 ± 13	16 ± 4	40 ± 13	76 ± 16	61 ± 33	88 ± 12	83 ± 21	
Significance ($p > 0.05$)[b]	d	bc	e	d	abc	cd	a	ab	
161 (97)	28	72	18	39	78	100	12	31	19.6
176 (97)	34	62	10	31	67	100	52	30	24.1
205 (97)	47	49	24	31	84	86	100	47	19.2
232 (97)	59	84	36	53	93	78	100	27	28.0
262 (97)	56	58	23	23	71	82	100	32.14	17.6
Mean 97 ± s.d.	45 ± 13	65 ± 14	22 ± 9	35 ± 11	79 ± 10	89 ± 10	73 ± 40	34 ± 8	
Significance ($p > 0.05$)[b]	cd	bc	e	d	a	ab	ab	abc	

[a]Enzyme activities were expressed as percent of maximum isoprene synthase activity (nmol m^{-2} s^{-1}) measured at the different sampling dates (see right column of the table). According to the annual or overall mean of the percentages the trees were ranked and analyzed by one-way ANOVA (Waller-Duncan test) for statistical differences. Different letters indicate significant differences at $p \leq 0.05$.
[b]Waller-Duncan test.

4. Model Development

The model was developed in the programming language C and runs on a computer with a Linux platform or equivalent.

4.1. Modeling of Bud Break

As the leaves are the responsible plant organs for the release of isoprene, a seasonal model has to calculate first the Julian date of the beginning of leaf development. Several authors [*Monson et al.*, 1994; *Fabian and Menzel*, 1998] stated that the beginning of bud break can be determined by the sum of average temperatures during a time period before leaf emergence. However, it is known that temperature dependencies of developmental processes are not linear [*O'Neill*, 1968; *Logan et al.*, 1976]. Therefore the beginning of leaf development was determined in the current model by adding up the product of average daily temperature and relative daylength (setting the daylength of 12 hours to 1.0), leading to an enforcement of the bud break pressure with increasing daylength. The statistical analysis showed that this product predicted the onset of leaf development more precisely than a temperature sum alone (data not shown). When the daily average temperature declines below zero, adding up is started again as soon as this frost episode is over. The relation is described by

$$\sum_{n=d-t_{\text{int}}}^{d} T_n L_n \geq \text{TL}_{\text{sum}} \qquad (1)$$

where

d first Julian day for which the relation is valid, equal to day of leaf emergence;
t_{int} chosen time period;
n number of the Julian day after the beginning of t_{int} with average daily temperatures remaining above zero;
L_n relative daylength (normalized to a daylength of 12 hours = 1.0) of the Julian day n;
TL_{sum} calculated sum of temperature daylength products;
T_n averaged daily temperature of the Julian day n with $T_n > 0°\text{C}$.

In order to obtain statistically sound data for the length of the time period t_{int} and for the temperature-daylength sum TL_{sum}, necessary for the beginning of leaf development in pedunculate oak, temperature and leaf emergence data of the years of 1951 to 1998 of 89 ecological areas distributed all over Germany were used (Deutscher Wetterdienst (DWD), Offenbach, Germany). On the basis of these data the leaf emergence date was calculated by varying the sum of temperature times daylength between 10 and 500°C and the time period between 1 and 180 days. For each of these combinations the deviation to the observed date of leaf emergence was calculated. The deviations of all 89 areas from 1951 to 1998 were averaged (Figure 2). The resulting area of data points exhibits the smallest deviation from the observed leaf emergence for a temperature times daylength sum of 370°C and a time period of 41 days. These values

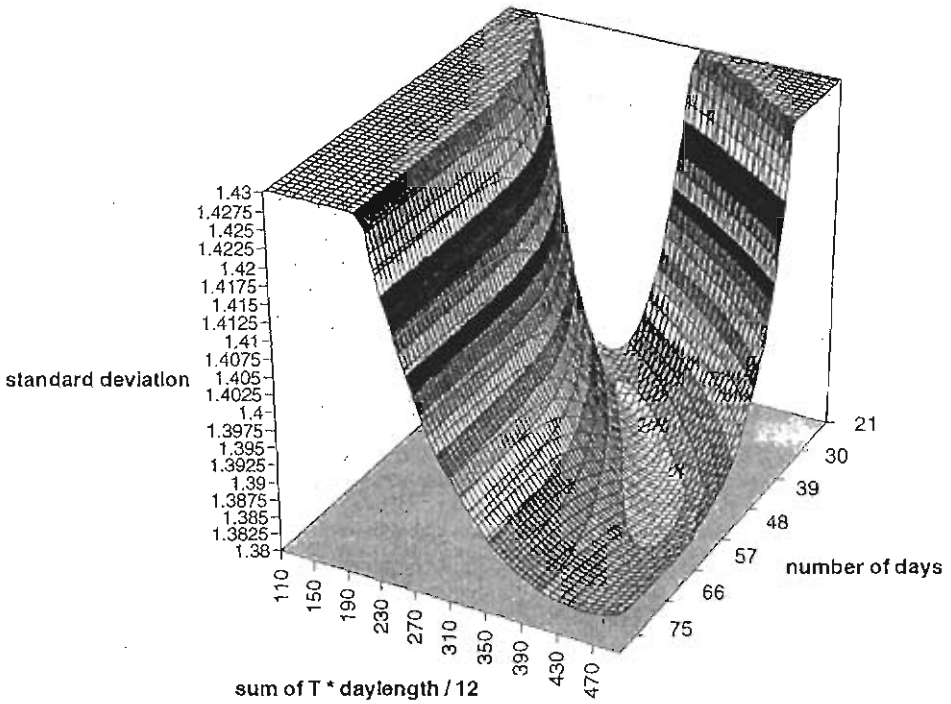

Figure 2. Estimation of the optimal daily temperature times daylength sum and induetion time period (t_{int}) for the ealculation of the bud break of pedunculate oak leaves in Germany. The resulting area of data points exhibits the smallest standard deviation (1.38) to thc observed date of bud break at a temperature-daylength sum of 370°C and an induction time period of 41 days. For the calculation, temperature and phenological data of 89 natural areas distributed all over Germany from 1951 to 1998 were used. The optimal values were ehosen as input data to calculate the oak leaf emergence for the model.

were chosen as input data to calculate the onset of leaf emergence of pedunculate oak in the model (Table 2).

4.2. Modeling of the Physiological State of the Leaves

Isoprene emission is described to be developmentally delayed by several days relative to photosynthesis in kudzu (*Pueraria lobata* (Willd) Ohwi.) [*Grinspoon et al.*, 1991; *Sharkey and Loreto*, 1993] and aspen [*Monson et al.*, 1994]. In these experiments the time courses of net photosynthesis and isoprene emission rates indicated that isoprene emission capacity reached their maximum when the leaves had their full photosynthetic competence. In senescent leaves, isoprene emission rates declined parallel to net photosynthesis [*Grinspoon et al.*, 1991; *Monson et al.*, 1994]. Therefore the leaf chlorophyll a content was chosen as a measure for the physiological state of the leaves. This value reaehed a maximum after leaf expansion and declines again during senescence (Figure 1b). The physiological state of the leaf was included into the model by a Gauss function with an inserted plateau (equation (2)) where the maximum was set by the previously calculated leaf expansion fitted to the chlorophyll a data of 1996 and 1997 and normalized to a maximal value of 1.0 (Figure 1c). The start of the decline of chlorophyll a content was observed approximately 40 days before leaf fall. Therefore the start of the decline of the function (equation (2)) was set also 40 days before leaf shedding. The day of leaf shedding (287 ± 4.8; see Table 2) was determined by averaging the observed leaf shedding days, as the date of leaf shedding for the 89 areas in Germany did not correlate with the geographical latitude (data not shown).

Table 2. Source of Parameters for the Annual Model

Parameter	Value	Unit	Source
Temperature times daylength sum for bud break, TL_{sum}	370	°C	this work
Time iuterval relevant for bud break, t_{int}	41	days	this work
Day of leaf shedding of pedunculate oaks	287 ± 4.8	Julian day	Deutscher Wetterdienst, Offenbach, Germany
Activation energy E of the Arrhenius term for a doubling of the reaction velocity from 20°C to 30°C	51164.8	J mol^{-1}	this work
Factor A to normalize the Arrhenius term to 1 for 30°C	660.1×10^6		this work
Factor for the isoprene synthase formation term, α_0	0.014	s^{-1}	this work
Factor for the isoprene synthase decay term, μ	0.175	s^{-1}	this work
Delay of enzyme induction	1	day	this work
Average interval for enzyme induction	1	day	this work

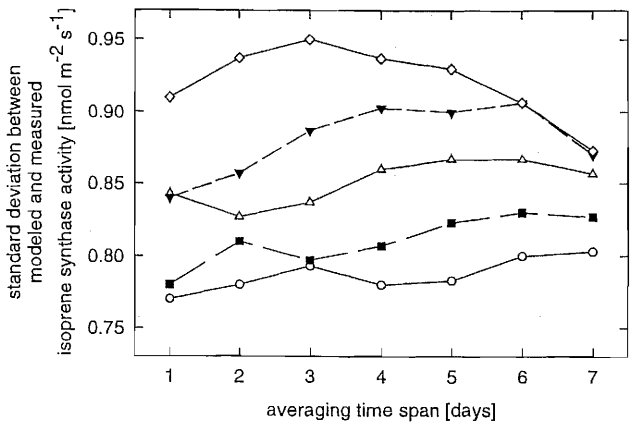

Figure 3. Standard deviation between measured and modeled isoprene synthase activity in dependence of time delay and averaging time span for the temperature and light value of the growth term of isoprene synthase activity. The standard deviation between measured and modeled isoprene synthase activity was calculated for 1 day, open circles; 2 days, solid squares; 3 days, open triangles; 4 days, solid triangles; and 5 days delay, open diamonds.

For $t < t_{\text{leaf emergence}} + t_{\text{max start}}$

$$f(\text{physiological state, date of leaf emergence, date of leaf shedding}) = \exp\left[-(t - t_{\text{leaf emergence}} - t_{\text{max start}})^2/(t_{1/2\ \text{max start}} - t_{\text{max start}})^2 \ln(2)\right] \quad (2)$$

For $t_{\text{leaf emergence}} + t_{\text{max start}} \leq t \leq t_{\text{max senescence}}$

$$f(\text{physiological state, date of leaf emergence, date of leaf shedding}) = 1.0$$

For $t > t_{\text{max senescence}}$

$$f(\text{physiological state, date of leaf emergence, date of leaf shedding}) = \exp\left[-(t - t_{\text{max senescence}})^2/(t_{1/2\ \text{max senescence}} - t_{\text{max senescence}})^2 \ln(2)\right]$$

where

t Julian day;
$t_{\text{leaf emergence}}$ Julian day of leaf emergence;
$t_{\text{max start}}$ day after leaf emergence where maximum chl a content is reached;
$t_{1/2\ \text{max start}}$ day after leaf emergence where half maximum chl a content is reached;
$t_{\text{max senescence}}$ Julian day at which chl a content starts to decline;
$t_{1/2\ \text{max senescence}}$ Julian day at which chl a content has declined to the half maximal value.

4.3. Modeling the Formation of Isoprene Synthase Activity

Biosynthesis of isoprene synthase formation is calculated, taking into account the product of PPFD during the light phase and the temperature dependence of the enzyme formation according to the Arrhenius equation with an assumed doubling of the formation speed in the interval from 20°C to 30°C [*Atkins*, 1997; *Bisswanger*, 1994]. For the decay of the isoprene synthase activity an exponential function was assumed. Taking both processes together, the difference in enzyme activity from one day to the next can be numerically described by the following differential equation:

$$\Delta V_{\text{max}}/dt = \alpha L A e^{-E/[R(T+273.1)]} - \mu V_{\text{max}} \quad (3)$$

where

V_{max} isoprene synthase activity;
α formation coefficient;
μ decay coefficient;
A factor to normalize the Arrhenius term $e^{-E/[R(T+273.1)]}$ to 1 for 30°C;
E activation energy chosen to yield a doubling of the result of the Arrhenius term from 20°C to 30°C.

Isoprene emissions of plants were described to react on a change of light and temperature with a delay of approximately 2 days [*Sharkey et al.*, 1999]. It is further published that plants can memorize the temperature and light values of a proceeding period of about 7 days [*Sharkey and Loreto*, 1993]. In order to estimate the optimal values for the delay and the time span for an averaging of the light and temperature values for the model, the model was run varying the delay from 1 to 5 days and the time span from 1 to 7, and the standard deviation to the measured activities during the 3 years was determined (Figure 3). The lowest deviation at the optimal α_0 and μ (Table 1) was observed, when only 1 day delay and only 1 day time span was considered for the calculation of the growth term of the isoprene synthase activity. Therefore T and L in the equation (equation (3)) are the values of 1 day before the actual day, for which the activity shall be calculated. The value of α depends on the physiological state of the leaf described by (2) and was calculated by

$$\alpha = \alpha_0 f(\text{physiological state, date of leaf emergence, date of leaf shedding}). \quad (4)$$

On the basis of (2) and (3) the V_{max} of a Julian day of the year was calculated by integrating the V_{max} values after leaf emergence using the PPFD and temperature data.

In order to obtain α_0 and μ in (3) and (4) the model itself and the experimentally determined isoprene synthase V_{max} values of the years 1996 and 1997 (Sun and shaded leaves) were used (Figure 1d). The value of α_0 was varied between 0.7 and 1.3 nmol m^{-2} s^{-1}, and μ was varied between 0.05 and 0.2 nmol m^{-2} s^{-1}. With each combination, isoprene synthase activity was calculated by the model using the PPFD and temperature data of 1996, 1997_{light}, and 1997_{shadow}, respectively. For each of the resulting activity time courses the deviation from the experimentally determined V_{max} values was determined. The resulting deviation fields were averaged. The α_0 and μ values were chosen at a minimum deviation between calculated and experimental V_{max} value ($\alpha_0 = 0.014$ s^{-1}, $\mu = 0.175$ s^{-1}; see Table 2). These values were used for all further calculations of the model.

4.4. Seasonal Modeling of Isoprene Synthase Activity

A comparison between measured and modeled mean isoprene synthase activities for the period of 1995 to 1997 was performed (Figure 1d). In 1995 the calculated isoprene synthase activities were higher than the measured values, and the onset of enzyme activity was a little earlier than could be expected from the measurements. Nevertheless, the shape of the calculated enzyme activity corresponded well to the exper-

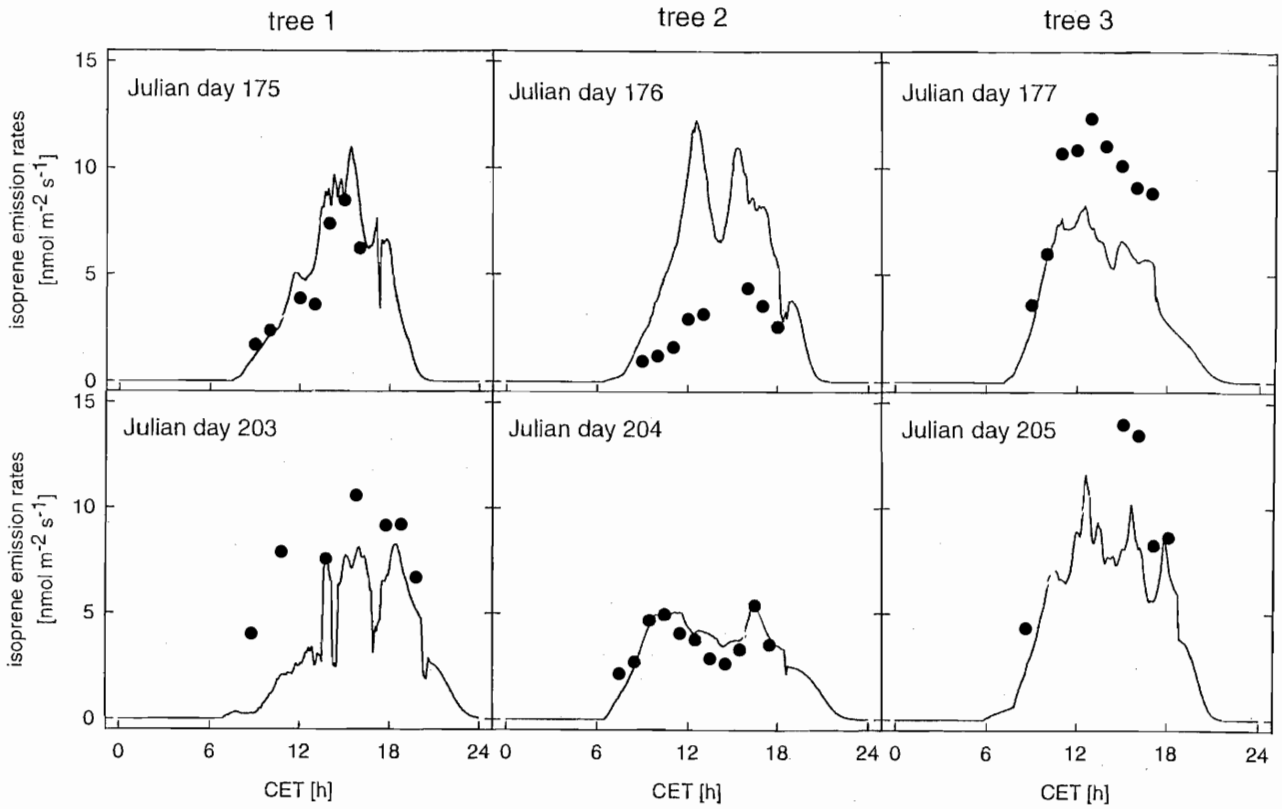

Figure 4. Comparison of measured isoprene emission rates of pedunculate oak (*Quercus robur* L.) leaves with modeled values of three trees measured from Julian days 175–177, 1997, and Julian days 203–205, 1997 (for details, see *Lehning et al.* [1999]). Daily course of experimentally determined isoprene emission rates (solid circles) compared with the emission calculated by the seasonal model coupled to the biochemical isoprene emission model (BIM) (solid curve) (for details, see *Zimmer et al.* [2000]). All data are displayed as 15 min averages.

imental data. In the following years, the model data matched the experimental data with a standard deviation of 0.95 nmol m^{-2} s^{-1} in 1996, and of 1.01 nmol m^{-2} s^{-1} in 1997 in Sun leaves, and of 0.35 nmol m^{-2} s^{-1} in shaded leaves. In all years the model calculated a correct onset of the increase in isoprene synthase activity during leaf expansion and development, as well as the decline of enzyme activity in autumn.

4.5. Coupling of the Seasonal Isoprene Synthase Model to a Numerical Process-Based Emission Model

To predict isoprene emission rates from oak leaves, a numerical process-based model (biochemical isoprene emission model (BIM)) was developed [*Zimmer et al.*, 2000] taking into account enzymatic reactions of isoprenoid enzymes in leaf chloroplasts coupled to a light-sensitive photosynthesis model [*Kirschbaum et al.*, 1998]. Since isoprene synthase activity reflects the general capacity of isoprene biosynthesis of oak leaves [*Lehning et al.*, 1999], the coupling of the seasonal model of isoprene synthase activity to the numerical emission model enables the calculation of diurnal fluctuations of isoprene emission rates in relation to seasonal leaf development. In order to test this coupled model an independent experiment was performed during the summer of 1997: On 3 days in June (Julian days 175–177) and July (Julian days 203–205) the diurnal variations in leaf temperature, PPFD, and isoprene emission rates of three separate oak trees were monitored, providing six data sets (Figure 4) [see also *Lehning et al.*, 1999]. On the basis of the temperature and PPFD climate in 1997, isoprene synthase activities were calculated by the seasonal model for the respective Julian day and used in BIM as default data to calculate the isoprene emission rates in relation to the diurnal records of temperature and PPFD. The values for isoprene emission rates calculated by the coupled models agree reasonably well with the tree-specific experimentally determined emission rates (Figure 4), for example, on the Julian days 175 and 204 for trees 1 and 2, respectively. An overestimation of approximately 181% in the calculated emission rate compared with the measured tree-specific values occurred on Julian day 176, while on Julian days 177, 203, and 205 the model underestimated the measured values by approximately 32, 38, and 35%, respectively. Taking all data sets together, the coupled model predicted the measured isoprene emission rates with a mean deviation of 55%.

5. Discussion

Isoprene emission rates can be influenced by the growth environment (e.g., light, temperature, CO_2 mixing ratio), the leaf developmental stage, the position of the leaf in the canopy, the nitrogen supply, as well as by physical and biotic stresses (for review, see *Guenther et al.* [1995]). All these processes are partially important for the determination of short-term (day-to-day) and long-term variations of isoprene emission. Despite all efforts, the investigations have not yet resulted in an reliable

numerical model that can be used to describe the seasonal variation of isoprene emission. Several studies [*Monson et al.*, 1994; *Fuentes et al.*, 1996; *Kempf et al.*, 1996] have observed a strong seasonal variation of isoprene emission rates and canopy fluxes with a rapid increase in springtime and a rapid decline in autumn. Therefore the central task in the present work was to study whether the seasonal variation of isoprene synthase activity in pedunculate oak leaves is related to annual differences in temperature, light, and leaf development and use this information for the development of a phenological model describing and predicting seasonal fluctuations of active isoprene synthase in Sun and shaded leaves.

The data set from 3 subsequent years (1995, 1996, and 1997) as well as from Sun- and shade-exposed pedunculate oak leaves clearly showed that the seasonal variation of the isoprene emission potential is dependent on the level of isoprene synthase activity, since apparent isoprene synthase activity and standard isoprene emission factors in oak are closely related to each other [*Lehning et al.*, 1999]. In addition, the different levels of isoprene synthase activity in Sun-exposed and shaded leaves are consistent with previous studies [*Harley et al.*, 1996, 1997; *Sharkey et al.*, 1996] showing that Sun leaves near the top of the canopy emit twice as much isoprene as shaded leaves within the canopy. The data also showed that the phenotypic variability of isoprene synthase activity, and hence the isoprene emission potential, in pedunculate oak leaves is very large and therefore has to be considered as an important factor responsible for the large uncertainties of standard emission factors currently used in modeling [*Simpson et al.*, 1995, 1999; *Guenther*, 1997]. *Monson et al.* [1994] and *Litvak et al.* [1996] showed that nitrogen supply can influence isoprene emission rates in aspen (*P. tremuloides*) and white oak (*Q. alba*). To make sure that proposed phenotypic variability is not simply the result of different leaf nitrogen contents, we examined the C/N ratio and leaf nitrogen content in 1996 and 1997. The measurements showed that the deviation between the samples was very low and that the leaf nitrogen content and C/N ratio were in the proposed optimal range for oaks [*Bergmann*, 1988]. In addition, statistical analysis of the data showed that factors other than leaf nitrogen content must be responsible for the observed variability of isoprene synthase activity.

Central questions which had to be answered during the development of the phenological model were as follows: (1) How can the onset of leaf development and the senescence of the leaves be described? (2) How can the observed delay of the increase in isoprene synthase activity compared to the onset of leaf development in spring be described? and (3) How can the formation and decay of isoprene synthase and a possible delay and memory integration interval be modeled in dependence of the history of temperature and light signals?

1. On the basis of the phenological data set of 89 ecological areas totally covering Germany, the bud break could accurately be described as the day at which the sum of the product of the relative daylength and the daily mean temperature raises above 370°C during a time interval of 41 days.

2. Previous investigations on kudzu (*Pueraria lobata* (Willd) Ohwi.) [*Grinspoon et al.*, 1991; *Sharkey and Loreto*, 1993] and aspen [*Monson et al.*, 1994] demonstrated that isoprene emission is developmentally dependent on photosynthesis. In senescent leaves of both species, isoprene emission rates declined parallel to net photosynthesis [*Grinspoon et al.*, 1991; *Monson et al.*, 1994]. In the present work with pedunculate oak, isoprene synthase activity slowly increased in spring after the onset of leaf development and was fully developed at a time point when the photosynthetic pigment content of the leaves reached a more or less constant concentration during the midsummer period. In autumn, isoprene synthase activity and photosynthetic pigment content declined in parallel. Since the observed seasonal courses of photosynthetic pigments for oak leaves are similar in shape to the seasonal variation of net photosynthesis of aspen leaves [*Monson et al.*, 1994], pigment content is proposed to be a reliable estimate for the development of the photosynthetic apparatus [*Lichtenthaler*, 1996]. The observations of (1) rapid rise of isoprene synthase activity when photosynthetic contents are at the summer plateau and (2) decline of pigments in autumn were used in the phenological model as averaged and normalized time courses of chlorophyll a to trigger the synthesis rate of isoprene synthase. This model approach that isoprene synthase formation is in its optimum after the photosynthetic apparatus of the leaves is maximal developed or maintained, allowed the correct modeling of the slow increase of isoprene synthase activity in spring and its decline during leaf senescence.

3. The question during which time period plants do integrate temperature and light signals used to set the basal isoprene emission potential is an important topic of debate. To date, little information is available how variations in weather patterns over hours to days affect the isoprene emission potential although it is assumed by *Geron et al.* [1994] that temperature changes over longer periods than several hours may account for ±50% uncertainty of biogenic emission factors. As the developed model processes a daily calculated formation and decay term by which the history of light and temperature values during the previous days is considered, it was not necessary to take into account a delay time of isoprene formation of more than 1 day. Because of this concept of the model, no additional averaging of temperature and light data during the days before the actual day was found to be necessary. Our results are in full agreement with the observation of *Sharkey et al.* [1999]. In their experiments on white oak (*Q. alba*), 95% of the variation of the isoprene basal emission rate could be explained by temperature and PPFD over the 2 days before measurement. In laboratory experiments with kudzu plants [*Sharkey and Loreto*, 1993] even a shorter time interval (6 hour treatment) was sufficient to induce the isoprene emission. Since it is also possible that the temperature and light effects have different time constants, further laboratory experiments and sensitivity studies with the model are required to better understand the regulation of isoprene synthase formation as well as to improve the accuracy of the model predictions.

The model constructed in the recent work describes the annual trends of isoprene synthase activity in oak leaves fairly and in a more realistic manner than the existing seasonal algorithm [*Guenther*, 1997]. The coupling of the seasonal model to the biochemical isoprene emission model (BIM) improves the calculation of the diurnal fluctuations of isoprene emission rates in Sun as well as shade leaves of forest canopies since annual data of temperature and light are the only driving variables of the model. In contrast to the existing seasonal model [*Guenther*, 1997] where the standard emission factors follow a fixed annual Gauss function, the new model considers the historical variations in temperature and light during the vegetation. In particular, this dynamic adaptation of the model enables the calculation of isoprene synthase activity for Sun and shaded leaves without any further corrections.

The application of the present model to other deciduous

isoprene-emitting trees, however, awaits further details of the temperature and light activation of isoprene synthase in these tree species. In particular, the factors α_0 and μ, describing synthesis and decline of isoprene synthase activity, have to be calculated from experimental data, and species-specific phenological data have to be implemented in the model. The present model can also be extended to the use of remote sensing data. In this case, seasonal variations of chlorophyll a content may be replaced by satellite-based chlorophyll fluorescence measurements that describe the seasonal variation of the leaf area index (LAI). In light of changing global temperatures the new model might be of considerable predictive value when used for sensitivity studies that simulate isoprene emission rates under elevated temperatures during different annual seasons and atmospheric CO_2 levels.

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