Global Change Biology (2004) **10,** 663–682, doi: 10.1111/j.1529-8817.2003.00771.x

Seasonal differences in isoprene and light-dependent monoterpene emission by Amazonian tree species

U. KUHN*, S. ROTTENBERGER*, T. BIESENTHAL*, A. WOLF*, G. SCHEBESKE*, P. CICCIOLI†, E. BRANCALEONI†, M. FRATTONI†, T. M. TAVARES‡ and J. KESSELMEIER*

**Biogeochemistry Department, Max Planck Institute for Chemistry, Joh.-J.-Becherweg 27, D-55020 Mainz, Germany, †Instituto di Metodologie Chimiche, Area della Ricerca del CNR di Montelibretti, Via Salaria km 29.300,00019 Monterotondo Scalo, Italy, ‡Universidade Federal da Bahia, Instituto de Química, Salvador, Brazil*

Abstract

Whereas for extra-tropical regions model estimates of the emission of volatile organic compounds (VOC) predict strong responses to the strong annual cycles of foliar biomass, light intensity and temperature, the tropical regions stand out as a dominant source year round, with only little variability mainly due to the annual cycle of foliar biomass of drought-deciduous trees. As part of the Large Scale Biosphere Atmosphere Experiment in Amazônia (LBA-EUSTACH), a remote secondary tropical forest site was visited in the dry-to-wet season transition campaign, and the trace gas exchange of a strong isoprene emitter and a monoterpene emitter are compared to the wet-to-dry season transition investigations reported earlier. Strong seasonal differences of the emission capacity were observed. The standard emission factor for isoprene emission of young mature leaves of *Hymenaea courbaril* was about twofold in the end of the dry season (111.5 $\mu g C g^{-1} h^{-1}$ or 41.2 $nmol m^{-2} s^{-1}$) compared to old mature leaves investigated in the end of the wet season (45.4 $\mu g C g^{-1} h^{-1}$ or 24.9 $nmol m^{-2} s^{-1}$). Standardized monoterpene emission rate of *Apeiba tibourbou* were 2.1 and 3.6 $\mu g C g^{-1} h^{-1}$ (or 0.3 and 0.8 $nmol m^{-2} s^{-1}$), respectively. This change in species-specific VOC emission capacity was mirrored by a concurrent change in the ambient mixing ratios. The growth conditions vary less in tropical areas than in temperate regions of the world, and the seasonal differences in emission strength could not be reconciled solely with meteorological data of instantaneous light intensity and temperature. Hence the inadequacy of using a single standard emission factor to represent an entire seasonal cycle is apparent. Among a host of other potential factors, including the leaf developmental stage, water and nutrient status, and abiotic stresses like the oxidative capacity of the ambient air, predominantly the long-term growth temperature may be applied to predict the seasonal variability of the isoprene emission capacity. The dry season isoprene emission rates of *H. courbaril* measured at the canopy top were also compared to isoprene emissions of the shade-adapted species *Sorocea guilleminiana* growing in the understory. Despite the difference in VOC emission composition and canopy position, one common algorithm was able to predict the diel emission pattern of all three tree species.

Keywords: biosphere–atmosphere exchange, carbon cycle, isoprene, monoterpene, seasonality, VOC

Received 16 July 2003; revised version received 27 August 2003 and accepted 16 September 2003

Introduction

About 90% of the global flux of volatile organic compounds (VOC) is emitted by vegetation with major impact on the chemistry of the atmospheric boundary layer (Guenther *et al*., 1995; Kesselmeier & Staudt, 1999; Monson & Holland, 2001). The impact of short-lived VOC extends over the entire troposphere. Due to their reactions with the hydroxyl radical (OH), ozone (O_3), and nitrate (NO_3), they form longer lived intermediates like carbon monoxide, reactive nitrogen species, aerosols and various carbonyl and carboxyl compounds (e.g. Poisson *et al*., 2000), the latter representing a large

Correspondence: Uwe Kuhn, tel. + 49(0)6131-305-486, fax + 49(0)6131-305-487, e-mail: kuhn@mpch-mainz.mpg.de

fraction of acidity in precipitation particularly in remote tropical areas (Keene *et al*., 1983). The influence on the atmospheric oxidative balance, greenhouse gas concentration, and the formation of aerosols implies a crucial role of VOC in climate forcing (Collins *et al*., 2002). Moreover, biogenic VOC emissions represent a non-trivial carbon loss for plants and significantly contribute to the carbon balance of terrestrial ecosystems with particularly strong impact by tropical areas (Guenther 2002; Kesselmeier *et al*., 2002a).

Even though tropical vegetation has a major impact on global VOC emission strength, these regions have been the least investigated to date and flux estimates have high uncertainties. Due to the insufficient data base, we still largely depend on modelling to estimate the abundance and fate of isoprenoids in the tropical atmosphere (Harley *et al*., 2003). The magnitude and relative abundance of VOC are highly dependent on landscape level plant species distribution and environmental conditions.

Among VOC, isoprene and monoterpenes play a central role in influencing the oxidative capacity of the atmosphere and were predicted to have a particularly strong impact on atmospheric chemistry of tropical regions (Jacob & Wofsy, 1988, Guenther *et al*., 1999b). Isoprene emission by vegetation is the world's largest source of VOC and about 50% of the global isoprene flux stems from tropical regions (Guenther *et al*., 1995). The primary short-term controls over isoprene production are light and temperature, whereas other factors, such as ambient CO_2 mixing ratios, soil water availability and stomatal conductance play a minor role (Guenther *et al*., 1991; Penuelas & Llusia, 2001). Studies on temperate and tropical plants have shown that isoprene emission is a function of light intensity, which approximates a rectangular hyperbola, similar to the relationship between photosynthesis and light, and depends on temperature according to enzyme activation kinetics. In contrast, monoterpenes may be partially stored prior to emission and most emission models still assume minor dependence on light but an exponentially increased emission with temperature. Especially conifers accumulate generated monoterpenes in specialized structures like resin ducts, glandular trichomes or related structures, and their emission is not directly dependent on instantaneous biosynthesis. The emission of monoterpenes from these pools is governed by leaf temperature and the effect of leaf temperature on the vapour pressures of individual monoterpenes and/or their emission pathway (Monson *et al*., 1995; Niinemets & Reichstein, 2002, 2003). However, in light of accumulating evidence on light-dependent monoterpene emissions (e.g. Staudt & Seufert, 1995; Kesselmeier *et al*., 1997; Schuh *et al*., 1997; Kesselmeier & Staudt, 1999) some most recent inventories also included such a light-dependent category for monoterpene emissions (Otter *et al*., 2003). In a recent publication Kuhn *et al*. (2002b) have shown that also for the tropical tree species *Apeiba tibourbou* monoterpene emission was strongly light-dependent and strong diel pattern of vertical gradient measurements in and above the rainforest canopy gave evidence that this phenomenon is valid also on larger scales. A strong light dependence of monoterpene emissions may, if generalized for tropical tree species in common, have a strong impact on predicted magnitude and temporal distribution of these compounds.

In model-based global emission inventories VOC emissions of temperate regions respond to a strong annual cycle in leaf area index (LAI), photosynthetic active radiation (PAR) and temperature with considerably higher emissions in summer than in winter. In contrast, the tropics are assumed to stand out as a source year round, with minor variability mainly due to the annual LAI cycle of drought-deciduous trees (Guenther *et al*., 1995; Levis *et al*., 2003). Previous studies on seasonal variations of the emission capacity of temperate/Mediterranean vegetation highlighted the importance of implementing additional emission activity factors that account for leaf age/phenology and past environmental growth conditions (Janson, 1993; Monson *et al*., 1994; Bertin *et al*., 1997; Goldstein *et al*., 1998; Hakola *et al*., 1998; Penuelas & Llusia, 1999; Guenther *et al*., 1999b; Guenther *et al*., 2000; He *et al*., 2000; Staudt *et al*., 2000; Zhang *et al*., 2000; Petron *et al*., 2001; Sabillon & Cremades, 2001; Komenda & Koppmann, 2002; Ciccioli *et al*., 1997, 2003). But still, insufficient knowledge on the degree and factors controlling the seasonal variation of emission capacity is one of the currently most challenging reasons for high uncertainties associated with biogenic emission inventories. For tropical regions, most recent canopy scale measurements do considerably improve our understanding of factors controlling the instantaneous isoprenoid emission in tropical regions (Rinne *et al*., 2002; Greenberg *et al*., 2003; Harley *et al*., 2003). However, long-term seasonal variations of isoprenoid flux rates in tropical regions are hardly characterized (Serca *et al*., 2001), although such data are crucial for landscape emission estimates (Guenther *et al*., 1999b; Geron *et al*., 2000). For tropical genera, species-specific seasonal variations of the emission capacity are to our knowledge hitherto not reported at all in literature. In this paper we report about branch enclosure measurements performed in the dry season campaign of the Large Scale Biosphere Atmosphere Experiment in Amazônia (LBA-EUSTACH), which complement investigations carried out earlier during the wet season in Rondonia, Brazil

(Kuhn *et al*., 2002b). Diel courses of the trace gas exchange of three individuals of different tree species were investigated over the period of 2–3 consecutive days each. Species-specific seasonal differences in light-dependent monoterpene and isoprene emissions are shown and discussed in the context of the plant's primary productivity and the climatic conditions prevailing.

Materials and methods

Sampling sites and periods

Field measurements were made in a primary rainforest ecological reserve, Reserva Biológica Jarú (RBJ), ca. 100 km north of Ji-Paraná, in the south-west periphery of the Amazon Basin, in Rondônia, Brazil (10°08′43″S, 62°54′27″W, 107 m a.s.l.) (Kuhn *et al*., 2002b). The area is covered predominantly by primary tropical rain forest (*Floresta Ombrofila Aberta*), and dynamic branch enclosure measurements reported here were conducted close to a camp site in the middle of a 1 km^2 of secondary forest, surrounded by primary forest. The canopy height was about 8–10 m and enclosures were applied at the top of the canopy by means of a mobile 10 m scaffolding tower. This campaign was carried out at the end of the dry season in September/October 1999, still associated with high biomass burning activity. The site normally experiences a mean annual rainfall of 1600–2100 mm and has a pronounced dry season between June and August, while the wettest periods are between December and April (Gash *et al*., 1996; Andreae *et al*., 2002). The climate of the region during 1999 was strongly influenced by 'La Niña', which typically leads to high rainfall amounts in Amazônia. At our site, the yearly total rainfall amount of 1500 mm in 1999 was below normal, predominantly due to relatively dry conditions in the wet-to-dry season transition. Vegetation experienced a drought period between June and August, but rain started very early in the dry-to-wet season transition with double the average rainfall amount in September, preceding the measurements. For further details on the site and a meteorological overview of the LBA-EUSTACH 1999 campaigns see Andreae *et al*. (2002).

Three different tree species were investigated. Two species were drought-deciduous, *Hymenaea courbaril* L. var. (common name: Jatoba; family Caesalpinaceae), a widespread species found in tropical through subtropical forest life zones (Duke, 1978), and *A. tibourbou* (common name: Pente de Macaco; family Tiliaceae), frequent in forests throughout tropical America (Croat, 1978). For sunlit leaves of both species grown at the canopy top the same branches of the same individuals as in the wet season were investigated (Kuhn *et al*., 2002b). The trees had dropped their leaves in the dry season and bud break was about 4 weeks before the enclosure measurements reported here. The evergreen species *Sorocea guilleminiana* (common name: Jaca-branca, Jaca-brava; family Moraceae), which was exclusively investigated in the dry season, was growing in the understory and leaves investigated were adapted to shade conditions. This species is frequently found in the Amazon Region, east Venezuela and Guianas (Ribeiro & Berg, 1999). The cumulative LAI above the measured branch (ca. 5 m below canopy top) is estimated to be approximately 2–3. All of the enclosure measurements were carried out on mature, dark green and turgescent leaves. However, in the case of *A. tibourbou*, leaf size during the dry season experiment was still somewhat smaller than in the wet season.

According to a most recent compilation of tropical emission data by Harley *et al*. (2003), including a community VOC emission data base (ref: http://bvoc.acd.ucar.edu), about 38% of screened genera of the tropical family Moraceae, 33% of the family Caesalpinaceae, and 25% of the family Tiliaceae were found to emit significant amounts of isoprene.

Branch enclosure and ambient air measurements

An open, dynamic (flow-through) enclosure system flushed with ambient air (40 L min^{-1}) was used for the gas exchange measurements on the branch level (for details see Kuhn *et al*., 2002b). One individual branch of each of the three tree species was loosely mounted into one of two enclosures (volume ~75 L). Enclosed leaf areas were 0.10 m^2 for *H. courbaril*, 0.11 m^2 for *A. tibourbou* and 0.25 m^2 for *S. guilleminiana*. The following mean specific leaf weights (g leaf dry weight per m^2 leaf area) were determined during the dry season measurements: 70 g m^{-2} for *A. tibourbou*, 80 g m^{-2} for *H. courbaril*, and 92 g m^{-2} for *S. guilleminina*. An identical but empty reference enclosure was operated simultaneously. A Teflon propeller driven by a magnetically coupled motor attached outside (Kuhn *et al*., 2000) assured high turbulence inside the enclosure, hence a high leaf boundary conductance. The aerodynamic resistance inside the enclosures was calculated to be 30 s m^{-1}, and was small compared to the isoprenoid exchange. Automatic sampling units (made in-house) with pumps (KNF, Freiburg, Germany), mass flow controllers (MKS, München, Germany) and switching valves (Lee, Frankfurt, Germany) were used to collect air samples. Tests with ambient air samples were performed before and during the study and showed that there was no bias between the different sampling units. During the course of the experiment, three

samples were always taken simultaneously: one of the enclosures containing the branch, one of the empty reference enclosure, and a third one of the ambient air to check potential contamination problems by the enclosure system itself. Before each branch enclosure experiment, a series of air samples was collected from both empty enclosures. The three corresponding samples were indistinguishable within the analytical uncertainties for the compounds of interest (~10% RSD). Ozone scrubbers (multiple plies of MnO_2-coated copper mesh, Type ETO341FC003, Ansyco, Karlsruhe, Germany) were installed at the inlets of the enclosures to avoid chemical conversion of the primary emitted reactive VOC inside the enclosures. An additional ozone scrubber was used to remove ozone from the ambient air line to avoid possible ozone interference on the cartridges. Tests with ozone-free air performed in our laboratory revealed no bias between the samples collected with or without ozone scrubbers in dry air and humid air, indicating that the MnO_2-surface had no influence on the isoprenoid concentration. All inner surfaces of the pumps and equipment in contact with the sampling air were Teflon. Previous studies demonstrated that the applied Teflon film (FEP) shows no interference with trace gases tested and is fully light permeable in the spectral range of 300–900 nm. Air temperatures within and outside the enclosures were continuously recorded by teflonized micro-thermocouples (0.005″, Chromel–Constantan, Omega, Lancashire, UK), indicating a small temperature increase of 1–3 °C during daytime. Leaf temperatures were measured with the same type of thermocouples attached at the upper and the lower side of 2 individual leaves. Photosynthetically active radiation (PAR) was measured with a LICOR quantum sensor (Model SB 190, LICOR, Lincoln, NE, USA) positioned outside the chamber. Relative humidity was monitored with a combined temperature/relative humidity probe (Model Rotronics YA-100 F, Walz, Effeltrich, Germany). All continuously measured parameters were stored as 5 min averages on a data logger (Model 21X, Campbell Scientific Inc., Longhborough, UK).

Quantification of CO_2 (assimilation/respiration) and H_2O (transpiration) exchange was achieved using a standard infrared dual-channel gas analyzer (Model 6262, LICOR, Lincoln, NE, USA) operated in the differential mode. The analyzer was maintained in an insulated, temperature controlled box at 40 °C to prevent signal fluctuations due to temperature effects as well as water condensation inside the instrument due to the high relative humidity. Furthermore, all tubing downstream of the enclosures was insulated and heated above ambient temperature to about 40 °C. Each branch was monitored for a period of 2–3 days, allowing for an adaptation time (after enclosing a branch) of at least one day. For determination of the trace gas flux, air samples were taken continuously from inside of both enclosures for isoprenoid analysis (0.1 L min^{-1}) and for the CO_2/H_2O-analyzer (1 L min^{-1}).

Trace gas collection and analysis

Emission rates were quantified every 1–2 h during daytime, with a lower time resolution during night. The sampling flow rate (40 min at 100 mL min^{-1}) was continuously monitored using electronic mass flow controllers. The heated Teflon (PFA) inlet lines were continuously flushed with sample air. Field blanks were regularly collected during the study, with about 1 blank cartridge for every 10 real samples. Isoprenoid analysis was routinely carried out using a thermal desorption GC/FID system. On an event basis a GC/MS system was used to qualitatively confirm the measurement results. For the GC/FID system (Autosystem XL with ATD400 Thermal Desorber, Perkin-Elmer, Rodgau, Germany), samples were collected by drawing air through fused silica-lined stainless-steel cartridges (89 mm length, 5.33 mm I.D., Silicosteel, Restek, Bellefonte, USA) packed with sequential adsorbent beds of 130 mg Carbograph 1 (90 $m^2 g^{-1}$, Lara s.r.l., Rome, Italy) followed by 130 mg Carbograph 5 (560 $m^2 g^{-1}$). For details of subsequent analysis, see Kuhn *et al*. (2002b). The detection limit of this method was estimated as the greater of the variability in the blank levels (at the 95% confidence level) and a chromatographic peak ~3 times the noise for each compound, and was typically ~30 ppt for isoprene and <10 ppt for monoterpenes (with some variation among individual compounds). The error of the individual concentrations measurements was calculated by an error propagation of the analytical error and the error of the mass flow, respectively. Typical uncertainties were ~10% for isoprene at 1 ppb, and ranged from ~5% to 30% at 100 ppt for monoterpenes, depending on the individual monoterpene peak resolution and blank variability. For the GC/MS system (HP 5890 with HP 5970B mass selective detector, Hewlett-Packard, Palo Alto, USA), isoprenoids were collected using glass cartridges (160 mm length, 3 mm I.D.) packed with 118 mg Carbopack C (12 $m^2 g^{-1}$, Supelco, Bellefonte, USA), 60 mg Carbograph 1 (90 $m^2 g^{-1}$, Lara s.r.l., Rome, Italy), and 115 mg Carbograph 5 (560 $m^2 g^{-1}$) in sequential beds. Sample flow rate was 250 ml min^{-1} and the sample volume was 5.0 L. For details on the subsequent analysis technique, see Ciccioli *et al*. (1992).

The gas exchange rates (F) were calculated according to Eqn (1) from the measured concentration difference between the enclosures ($\Delta c = c_{sample} - c_{ref}$), the chamber

flush rate (Q) and the enclosed leaf area and/or dry weight (A), respectively.

$$F = \Delta c \cdot \frac{Q}{A} \quad (1)$$

For the error calculation of the exchange rates, an error propagation method was used integrating the calculated error of each individual concentration measurement, the error of the mass flow, and the leaf area, respectively (Kesselmeier *et al*., 1997; Kuhn *et al*., 1999). Projected leaf area and leaf dry weight were determined at the end of the experiments. Leaf area was measured by a calibrated scanner system (ScanJET IICX with DeskSCAN II, both Hewlett-Packard, USA, and SIZE 1.10, Müller, Mainz, Germany), and dry weight was determined using a micro-balance (PM 400, Mettler-Toledo, Giessen, Germany), after drying the leaves in a ventilated oven (90 °C) for two days.

Application of light and temperature algorithm

In emission inventories, the instantaneous emission rates for monoterpenes are calculated by multiplying a standard emission factor (E_s; also called emission capacity, emission potential or basal emission rate) with an exponential function depending only on temperature (Tingey *et al*., 1981; Guenther *et al*., 1993), whereas the emission of isoprene was calculated by multiplying a standard emission factor with functions of both a temperature dependence (C_T) and a light dependence (C_L) (Guenther *et al*., 1993):

$$E = E_S C_T C_L, \quad (2)$$

where E is the isoprene emission rate at the current leaf temperature and PAR intensity, and E_S is the emission rate at standard temperature (303 K) and standard PAR intensity (1000 $\mu mol\, m^{-2}\, s^{-1}$). This algorithm will be referred to as G93 in the text. In light of accumulating evidence on light-dependent monoterpene emissions, some most recent inventories also included such a category for monoterpene emissions (e.g. Otter *et al*., 2003). Additional requirements to describe long-term variations in emission capacity are evident, and improved physiological and biochemical understanding is urgently needed (Schnitzler *et al*., 1997; Guenther *et al*., 2000). Comparison of E_S derived from measurements on the branch level with leaf level measurements normally is problematic because partly shaded leaves on the lower portion of a branch have a considerably lower emission rate than leaves that are in direct sunlight. As the leaf surface area of sunlit leaves enclosed in our branch enclosures was always less than 0.11 m^2 and the leaves were widely spread, shading is assumed to be of minor importance, but let us consider our branch level results as a lower bound estimate if compared to leaf level experiments. For the sunlit species, shading from other branches can be excluded, as the branches were growing at the top of canopy.

Results

Diel emission pattern

Net assimilation and transpiration reflected typical diel pattern of the plant physiological processes as a function of light intensity, temperature and relative humidity. The maximum daytime carbon assimilation rates of sunlit leaves of *A. tibourbou* and *H. courbaril* at the canopy top were 7.8 $\mu mol\, m^{-2}\, s^{-1}$ (4.7 $mgC\, g^{-1}\, h^{-1}$) and 10.4 $\mu mol\ m^{-2}\, s^{-1}$ (5.6 $mgC\, g^{-1}\, h^{-1}$), respectively. The mean total daily net primary production (NPP) on the branch level was about 23.9 $mgC\, g^{-1}$ (1.7 $gC\, m^{-2}$) for *A. tibourbou* and about 37.0 $mgC\, g^{-1}$ (2.9 $gC\, m^{-2}$) for *H. courbaril* (Table 1). For the shade-adapted understory species *S. guilleminiana* a much lower maximal assimilation rate of 0.8 $mgC\, g^{-1}\, h^{-1}$ (1.7 $\mu mol\, m^{-2}\, s^{-1}$) was observed, with a mean total daily net primary production of only 3.4 $mgC\, g^{-1}$ (0.3 $gC\, m^{-2}$). Low NPP numbers were associated with significantly lower stomatal conductance, both characteristic for the low-light regime. On both days investigated, the stomatal conductance considerably decreased after 1000 LT in order to partially balance increased transpiration rates, and the higher stomatal resistance resulted in a drastic reduction in CO_2 uptake (Figs 3 and 5). Low photosynthetic activity and relatively high transpiration rates yielded considerably lower water use efficiency (carbon gain per water loss) for the shade-adapted species.

A large inter-specific variation in VOC emission composition and magnitude was observed. *A. tibourbou* was found to emit exclusively monoterpenes (Fig. 1). Mature leaves of *H. courbaril* and *S. guilleminiana*, in contrast, emitted only isoprene and no monoterpenes (Figs 2 and 3). Daily integrated isoprene emission from sunlit leaves of *H. courbaril* and from shade-adapted leaves of *S. guilleminiana* differed about one order of magnitude, as did CO_2-assimilation (Table 1). Maximum isoprene emission rates were 260.6 $\mu gC\, g^{-1}\, h^{-1}$ (96.4 $nmol\, m^{-2}\, s^{-1}$) and 40.4 $\mu gC\, g^{-1}\, h^{-1}$ (17.2 $nmol\ m^{-2}\, s^{-1}$). Maximum emission rate for the sum of monoterpenes emitted from *A. tibourbou* was 4.7 $\mu gC\, g^{-1}\, h^{-1}$ (0.8 $nmol\, m^{-2}\, s^{-1}$). About 80% of the total monoterpenes emitted was comprised of only 5 compounds. While the total sum of monoterpenes showed a distinct diel cycle, the relative proportion of composition (on average: $33 \pm 14\%$ sabinene, $28 \pm 7\%$ α-pinene, $15 \pm 3\%$ β-pinene, $14 \pm 12\%$ camphene, and $9 \pm 4\%$ limonene) did not change considerably during

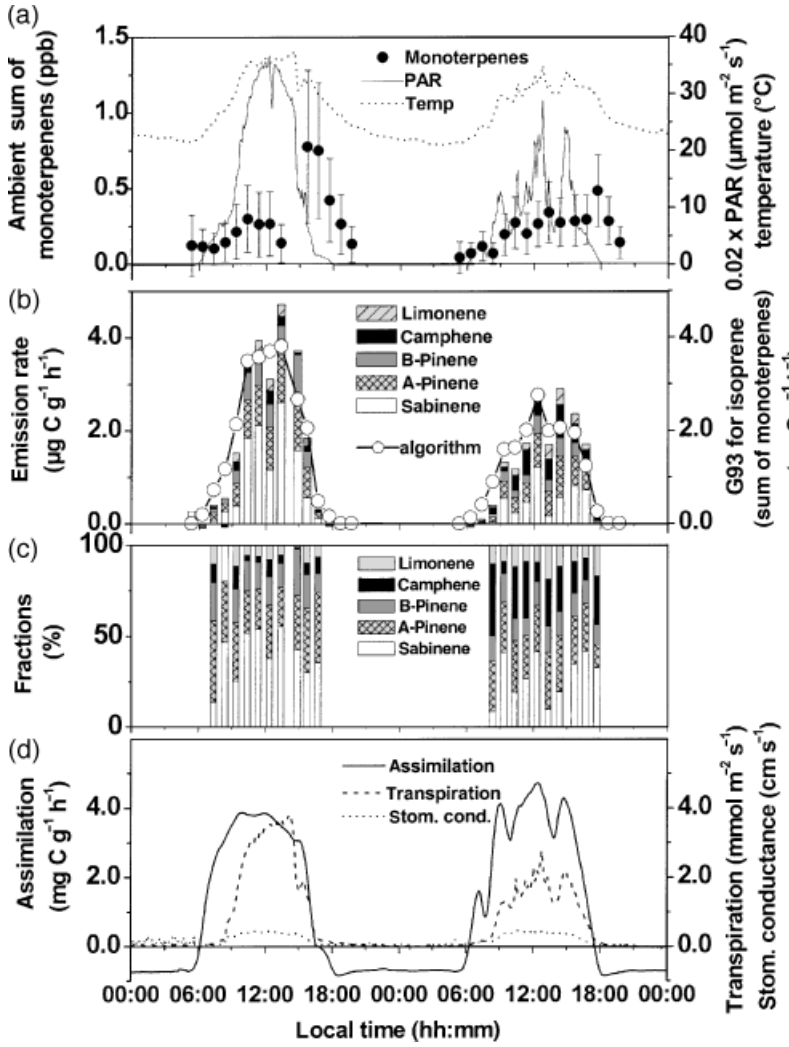

Fig. 1 Diel pattern of the dominant five monoterpene compounds emitted from *A. tibourbou* on 18–19 Oct 1999 are presented with data of photosynthetic active radiation (PAR), leaf temperature (temp) and the ambient air mixing ratios of the sum of monoterpenes, in conjunction with physiological data of assimilation, transpiration and calculated stomatal conductance (stom. cond.). Measured emission rates are compared with the diel course predicted for monoterpenes using the G93 algorithm for isoprene (panel b). In panel c the fractions (% of total) of individual monoterpenes are shown. The shift in monoterpene ambient mixing ratios on 18 Oct 1999 (panel a) may be explained by a shift in wind direction from westerly ($270 \pm 45^{\circ}$) to easterly ($90 \pm 45^{\circ}$) winds after 1430 LT, which was not the case on 19 Oct. 1999. There is a sufficient fetch over primary rain forest for several tens of km in the main wind direction (i.e. from NW over N to SSE) at this site, whereas the fetch over forest is limited in the remaining section. The Rio Machado, which partly forms the boundary of the RBJ, is westerly of the campsite. Error bars are calculated by an error propagation method for the absolute (ambient) measurements.

the day, i.e. was independent of temperature and light intensity. The remaining fraction investigated, but not accounted for in our calculations, was composed of myrcene, α-phellandrene, α-terpinene, *p*-cymene, and γ-terpinene. The calculated standard emission factor E_S (in $\mu gC\,g^{-1}\,h^{-1}$), i.e. the emission rate normalized to standard light ($1000\,\mu mol\,m^{-2}\,s^{-1}$) and temperature (30 °C), were 2.1 for the sum of monoterpene emission from *A. tibourbou* and 111.5 and 24.0 for isoprene emission from *H. courbaril* and *S. guilleminiana*, respectively. Despite these differences in emission composition and magnitude, strong similarities were observed in the diel pattern of isoprenoid emission of all tree species. Emission of all isoprenoids was negligible during nighttime, increased through the morning with increasing air temperature and light, and declined in the afternoon. Consequently the same algorithm (G93, Eqn (2)) was successfully applied to model both monoterpene and isoprene emissions (Figs 1–3). The correlations of measured and modelled values are

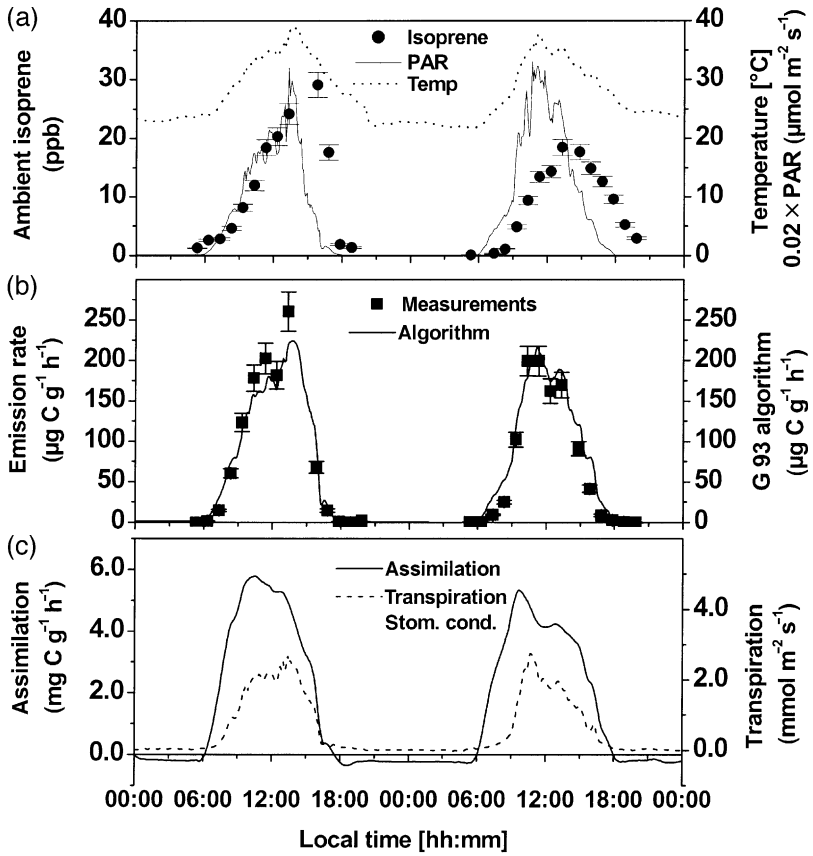

Fig. 2 Diel pattern of observed isoprene emission rates from *H. courbaril* on 22–23 Oct 1999 are presented with data of photosynthetic active radiation (PAR), leaf temperature (temp) and the ambient air isoprene mixing ratios, in conjunction with physiological data of assimilation, transpiration and calculated stomatal conductance (stom. cond.). Measured emission rates are compared with the diel course predicted by the G93 algorithm for isoprene in panel b. Ambient concentrations were in close vicinity and downwind of the strong isoprene emitter and may not reflect average ambient air mixing ratios. Error bars are calculated by an error propagation method for the absolute (ambient) and the difference (enclosure) measurements, respectively.

plotted in Fig. 4 and show good agreement for both seasons investigated.

Light and temperature dependence of isoprenoid emission

Monoterpene emissions from *A. tibourbou* and isoprene emissions from *H. courbaril* and *S. guilleminiana* plotted vs. the corresponding photosynthetic active radiation are shown in Fig. 5. The emission rates were normalized to standard temperature (30 °C) by dividing by the temperature correction factor C_T (Eqn (2)) to extract the dependence on light intensity. Additional division by the standard emission factor allowed better comparison between the response of isoprenoid emission from different trees and different seasons. Applying this method (wet season data from Kuhn *et al.*, 2002b), revealed that only the emission capacity, but not the underlying controlling functions were subject to seasonal changes. Both the monoterpene and the isoprene emission of sunlit leaves exhibited the same strong response vs. light, i.e. no emission in the dark, an increase at low intensities approaching saturation at high PAR. In both seasons and for all trees investigated, the light dependence of isoprene and monoterpene appeared to reflect that of photosynthetic activity. For sunlit leaves of *H. courbaril* and *A. tibourbou* exposed to higher light intensities, both isoprenoid emission and CO_2 assimilation seem to level off at 500–1000 $\mu mol\, m^{-2} s^{-1}$. For the shade-adapted *S. guilliminiana* (Fig. 5, panel C) light intensity did not exceed 500 $\mu mol\, m^{-2} s^{-1}$ during measurements and thus light saturation could not be observed. However, it is interesting to note that on both days the CO_2 assimilation of the shade-adapted tree increased linearly with increasing light in the early morning, but then was sharply reduced in the late morning hours due to increased stomatal resistance in order to prevent excessive water loss. In the afternoon photosynthetic

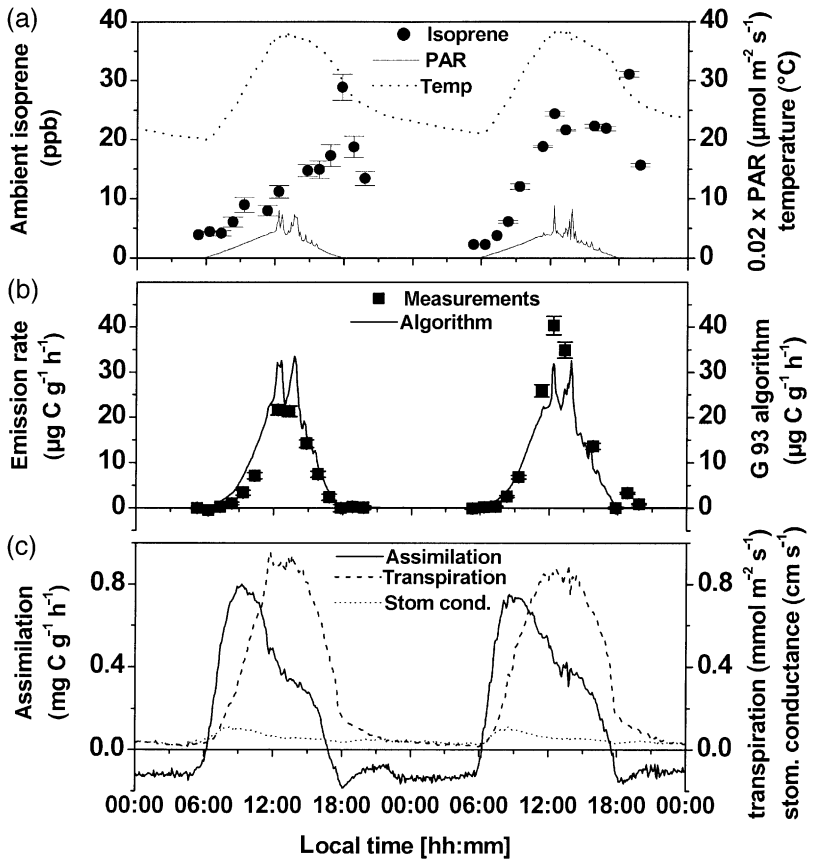

Fig. 3 Diel pattern of observed isoprene emission rates from *S. guilleminiana* on 10–11 Oct 1999 are presented with data of photosynthetic active radiation (PAR, on the height of the enclosure; note that the radiation intensity on canopy top was comparable to the values on 18–19 Oct), leaf temperature (temp) and the ambient air isoprene mixing ratios, in conjunction with physiological data of assimilation, transpiration and calculated stomatal conductance (stom. cond.). Ambient concentrations were in close vicinity and downwind of the strong isoprene emitter and may not reflect average ambient air mixing ratios. Error bars are calculated by an error propagation method for the absolute (ambient) and the difference (enclosure) measurements, respectively. Note: the scales of the isoprene emission rates and the physiological data are different than in Fig. 2.

activity then gradually declined to a lower level, revealing a strong hysteresis of CO_2 exchange. In contrast, the isoprene emission rate was not influenced by stomatal aperture.

Carbon balance

Biosynthesis of isoprenoids is based on photosynthetically fixed carbon. The ratio between carbon gain by CO_2 assimilation and the carbon loss by isoprenoid emission, i.e. the amount of instantaneous relative carbon loss, varied considerably during the day and exhibited a distinct diel course (Fig. 6). Peak values occurred during midday and were 0.13% for monoterpene emissions of *A. tibourbou*, and 5.2% and 8.9% for isoprene emissions of *H. courbaril* and *S. guilleminiana*, respectively. The mean values of daily integrated (24 h) carbon loss by isoprenoid emission in relation to the photosynthetically fixed carbon (carbon balance) on the branch level were found to be 0.1% for monoterpene emissions by *A. tibourbou* and 3.5% and 4.3% for isoprene emissions by *H. courbaril* and *S. guilleminiana*, respectively (Table 1).

Discussion

Species-specific differences observed in the dry season

The daytime assimilation rates and other primary physiological parameters like transpiration and stomatal conductance of sunlit leaves were comparable to those reported for tropical vegetation (Koyama, 1978; Pearcy, 1987; Koch *et al.*, 1994; McWilliams *et al.*, 1996). Low values for photosynthesis, transpiration and stomatal conductance found for shade-adapted species *S. guilleminiana* are consistent with strong vertical in-canopy gradients of primary productivity found in forest canopies. For a detailed discussion of the primary

Table 1 Inter-specific comparison of daily integrated values (24 h) of carbon gain by net primary productivity (NPP), carbon loss by isoprenoid emission and carbon recycled by uptake of volatile organic acids (VOC) (*Kuhn *et al.*, 2002a) and aldehydes (†extracted from Rottenberger *et al.*, 2003) measured in the dry season

Tree species (emission type, light condition) date	Net primary productivity ($24\,h^{-1}$) (NPP) ($mg\,C\,g^{-1}\,dw$) ($g\,C\,m^{-2}$)	Isoprenoid emission ($24\,h^{-1}$) ($mg\,C\,g^{-1}\,dw$) ($mg\,C\,m^{-2}$)	Fraction of C emitted as isoprenoids (%C_{VOC}/C_{CO_2})	Fraction of C re-cycled by uptake of volatile organic acids* (%C_{acid}/C_{CO_2})	Fraction of C re-cycled by uptake of volatile aldehydes† (%C_{ald}/C_{CO_2})	Carbon balance total VOC (%$C_{totalVOC}/C_{CO_2}$)
Apeiba tibourbou (monoterpenes, sunlit)						
18 Oct 1999	22.1 (156)	0.03 (1.9)	0.13	0.022	0.014	0.08
19 Oct 1999	25.6 (1.80)	0.02 (1.3)	0.08	0.020	0	0.05
Hymenaea courbaril (isoprene, sunlit)						
22 Oct 1999	38.3 (3.06)	1.33 (106.3)	3.48	0.009	0.007	3.46
23 Oct 1999	35.6 (2.85)	1.26 (100.8)	3.54	0.010	0.004	3.53
Sorocea guilleminiana (isoprene, shaded)						
10 Oct 1999	3.2 (0.30)	0.15 (13.5)	4.52	0.165	0.052	4.30
11 Oct 1999	3.5 (0.32)	0.14 (13.3)	4.15	0.134	0.072	3.94

Values during sampling breaks for VOC measurements were interpolated. Units are given in mass of carbon, and based on dry weight or leaf area, respectively.

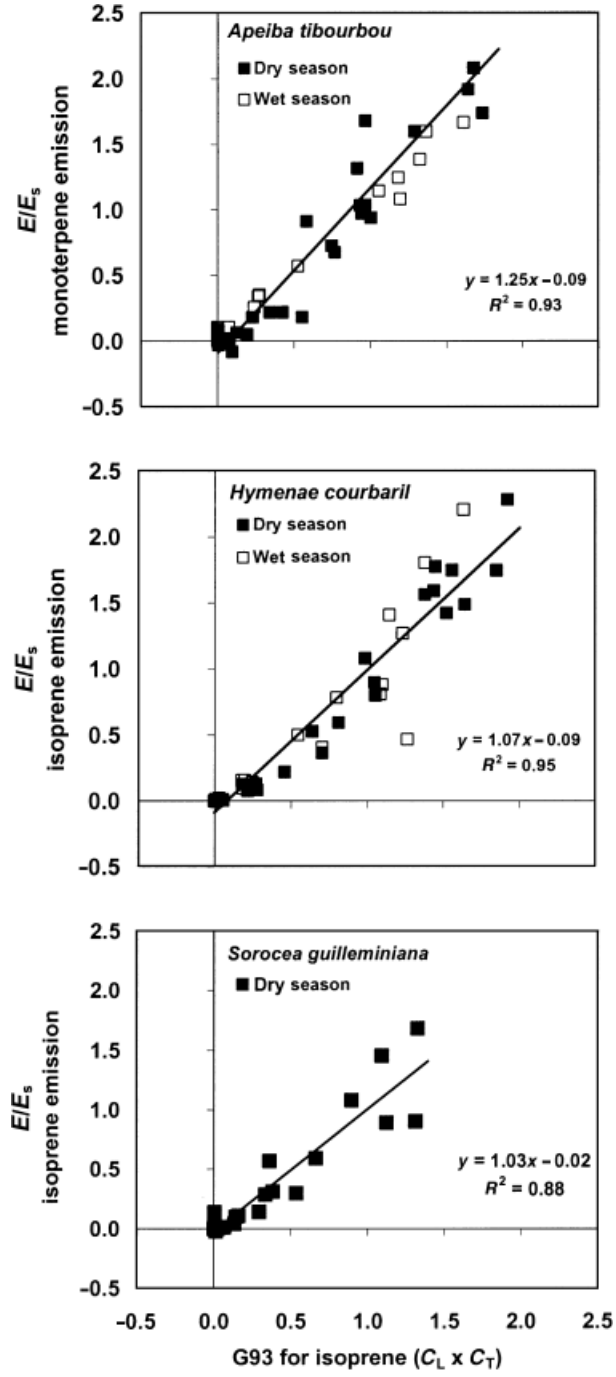

Fig. 4 Comparison of observed normalized isoprenoid emission rates (E/E_s, see Eqn (2)) and the emission predicted by the G93 algorithm for isoprene. Wet season data (open symbols) are from Kuhn *et al*. (2002b).

physiological parameters and differences in climatic data, see Rottenberger *et al*. (2003).

The three selected tree species strongly differed in isoprenoid emission type (monoterpene vs. isoprene emitter; sunlit vs. shade-adapted isoprene emitter) and

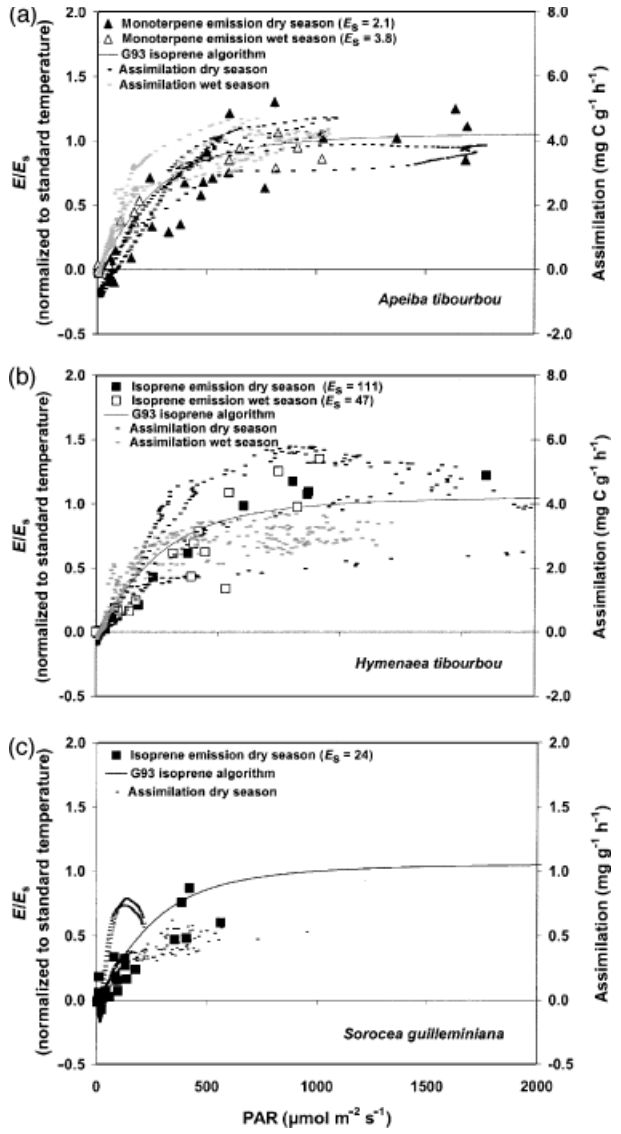

Fig. 5 Response of monoterpene emission of *A. tibourbou* (panel a), and isoprene emissions of *H. courbaril* (panel b) and *S. guilleminiana* (panel c) to the corresponding light intensities (PAR) in the wet season (open symbols, see Kuhn *et al*., 2002b) and the dry season (solid symbols, this work). The solid lines indicate the dependence on light predicted by the G93 algorithm for isoprene. The measured VOC emission rates were normalized to standard temperature (30 °C) by dividing by the temperature correction factor C_T (Eqn (2)). Additional division by the standard emission factor allowed better comparison between the response of isoprenoid emission from different trees and different seasons. For comparison the photosynthetic rates (not corrected for temperature) are also plotted for the wet (small grey dots) and the dry season (small black dots).

span a wide range of instantaneous VOC emission rates (Table 1) and standard emission capacity (E_S of 2.1, 24.0, and 111.5 µgC $g^{-1} h^{-1}$ for *A. tibourbou*, *S. guilleminiana*, and *H. courbaril*, respectively; Table 2). Comparing emission rates reveals about 10–50 times higher values for isoprene compared to monoterpenes. This species-specific difference in emission rates directly corresponded to the observed differences in VOC ambient mixing ratios close to the canopy top at the RBJ site (panels A in Figs 1 and 2; see also Kesselmeier *et al*., 2002b). Isoprene mixing ratios were more than one order of magnitude higher than for monoterpenes during both, the wet and the dry season, which points to a good representation of the emission types present at this site.

Low isoprene emission of the shade-adapted species *S. guilleminiana* is consistent with literature data for temperate (Harley *et al*., 1996; Sharkey *et al*., 1996; Bertin *et al*., 1997; Harley *et al*., 1997) as well as for tropical sites (Lerdau & Keller, 1997; Lerdau & Throop, 2000; Geron *et al*., 2002). Moreover, observations made in a tropical forest in Panama by Lerdau & Throop (1999) showed that some trees growing in the shade did not emit isoprene even though the same species were high isoprene emitters when grown in full sun. The leaf light environment has strong effects on foliar characteristics and photosynthesis. In general foliar biomass per area (specific leaf mass, SLM) increases with canopy height, and consequently the changes in morphology alter the variation of trace gas exchange if expressed on an area basis (Bertin *et al*., 1997; Reich *et al*., 1997, 1998). In contrast to photosynthesis, the isoprene emission capacity was found to change both on area and mass basis, although the difference gets smaller when expressed on mass basis (Harley *et al*., 1996; Lerdau & Throop, 2000). Here, the instantaneous photosynthesis and isoprene emission of the tree species investigated at the canopy top (*H. courbaril*) and the shade-adapted species (*S. guilleminiana*) changed in similar manner, both on area- and mass-basis (Table 1). As isoprene and monoterpene emissions are discussed to confer protection of the photosynthetic apparatus against high temperatures (Sharkey & Singsaas, 1995; Loreto *et al*., 1998; Delfine *et al*., 2000) and ozone (Loreto *et al*., 2001), and given the fact that both leaf temperatures and ozone concentrations are higher at the top of the canopy, a requirement for greater thermo and ozone tolerance may provide a functional explanation for increased isoprene emission capacity in the upper canopy.

Strong light dependence of monoterpene and isoprene emissions

Despite their different isoprenoid emission composition, the diel emission pattern of all three tree species reacted similarly towards the environmental conditions

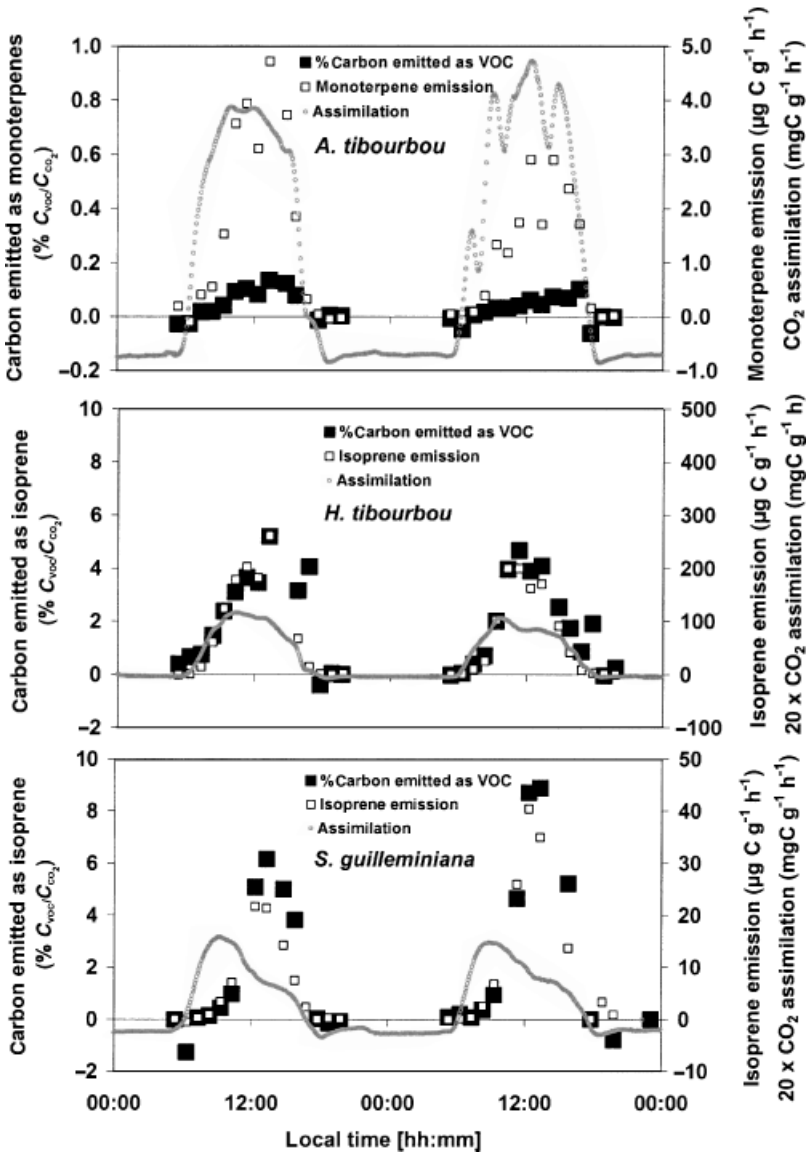

Fig. 6 Diel course of carbon lost as monoterpene emissions from *A. tibourbou* (18–19 Oct 1999) and isoprene emission from *H. courbaril* (22–23 Oct 1999) and *S. guilleminiana* (10–11 Oct 1999) per carbon assimilated by photosynthesis. For comparison the respective isoprene and monoterpene emission rates and the photosynthetic activity are also plotted.

of fluctuating light and temperature, demonstrating that closely related biosynthesis/emission pathways are involved. Even though temperatures were relatively high during night, we did not observe nighttime monoterpene release from *A. tibourbou*. This species obviously lack specific storage pools and emitted monoterpenes result from direct production prior to emission. Consequently, as indicated by the linear regression analysis of the normalized observed flux rates vs. the G93 algorithm (Fig. 4), modelling monoterpene emission analogously to isoprene (Eqn (2)) provided a good fit to the observed data in both seasons. Standardization of the emission rates of both seasons showed that long-term seasonal adaptations involve only a change in the standard emission factor without modifying the function of light response (Fig. 4). Isoprene and monoterpene emissions exhibited a light saturation similar to net CO_2 uptake, indicating closely related primary and secondary metabolic pathways (Lichtenthaler, 1999). The *de novo* synthesis of both, isoprene and monoterpenes, ultimately relies on photosynthetic electron transport, which provides energy and reductive equivalents. The observed light dependence of the instantaneous VOC emission may give further evidence that correlations between photosynthetic characteristics and isoprenoid emission rates

Table 2 Seasonal differences in standard emission factors and daily integrated (24 h) mean values of net primary productivity (NPP), carbon loss by isoprenoid emission and carbon recycled by uptake of volatile organic acids (VOC) (*Kuhn *et al.*, 2002a) and aldehydes (**extracted from Rottenberger *et al.*, 2003)

Tree species (emission type, canopy position) season	Isoprenoid standard emission factor E_S ($\mu g C\, g^{-1}\, dw\, h^{-1}$) ($nmol\, m^{-2}\, s^{-1}$)	Net primary productivity ($24\, h^{-1}$) (NPP) ($mg\, C\, g^{-1}\, dw$) ($g\, C\, m^{-2}$)	Isoprenoid emission ($24\, h^{-1}$) ($mg\, C\, g^{-1}\, dw$) ($mg\, C\, m^{-2}$)	Fraction of C emitted as isoprenoids ($\%\, C_{VOC}/C_{CO_2}$)	Fraction of C re-cycled by uptake of organic acids* ($\%\, C_{acid}/C_{CO_2}$)	Fraction of C re-cycled by uptake of aldehydes** ($\%\, C_{ald}/C_{CO_2}$)	Carbon balance total VOC ($\%\, C_{tot\,VOC}/C_{CO_2}$)
Apeiba tibourbou (monoterpenes, sunlit canopy top)							
Dry season	2.1 (0.3)	22.1–25.6 (1.6–1.8)	0.02–0.03 (1.3–1.9)	0.07–0.12	0.020–0.022	0.00–0.014	0.06–0.12
Wet season	3.6 (0.8)	26.9–30.9 (2.4–2.6)	0.02–0.03 (2.1–2.6)	0.09–0.10	0.002–0.003	0.001–0.002	0.09–0.10
Hymenaea courbaril (isoprene, sunlit canopy top)							
Dry season	111.5 (41.2)	35.6–38.3 (2.9–3.1)	1.26–1.33 (100.8–106.3)	3.4–3.5	0.009–0.010	0.0040–0.0070	3.4–3.5
Wet season	45.4 (24.9)	22.3–24.9 (2.6–2.9)	0.41–0.46 (48.2–54.0)	1.6–2.1	0.004–0.010	0.0002–0.0003	1.6–2.1
Sorocea guilleminiana (isoprene, low-light understory)							
Dry season	24.0 (10.2)	3.2–3.5 (0.30–0.32)	0.14–0.15 (13.3–13.5)	4.2–4.5	0.13–0.17	0.05–0.07	4.0–4.5

Wet season isoprenoid data from Kuhn *et al.* (2002b).

may be applied to model the instantaneous VOC emissions from species that lack storage compartments within the leaves, especially under non-stress environmental conditions (Lerdau & Throop, 1999; Niinemets *et al.*, 2002). However the lack of a correlation between seasonal development of isoprenoid emissions and seasonal variation of net photosynthesis rates as found by Sharkey *et al.* (1999) for a temperate forest and by Serca *et al.* (2001) for a tropical forest site in the Congo demonstrates potential shortcomings of this approach.

Further support for light-dependent monoterpene emissions can also be found in the strong diel course of ambient air mixing ratios. As shown in Fig. 1 (panel A) the sum of ambient monoterpenes close to the canopy top during nighttime was not distinguishable from zero in the range of uncertainty and followed the pattern of light intensity during daytime. As was observed in the wet season (Kuhn *et al.*, 2002b), the diel course of monoterpenes followed closely that of isoprene, and indicated a light-dependent source even on a larger scale, especially if taking into account the small amplitude of diel temperature variation. Nighttime emissions of monoterpenes into the shallow nocturnal boundary layer would result in higher surface mixing ratios than during daytime, when mixing through a deeper boundary layer and chemical losses would decrease ambient concentrations (Kuhn *et al.*, 2002b). Recent canopy flux measurements in the Amazon by Rinne *et al.* (2002) and eddy flux and leaf level measurements in a semiarid woodland site in Botswana by Greenberg *et al.* (2003) both confirmed that a strong light dependence of monoterpene emissions may be widespread among tropical tree species, with strong influence on predicted temporal distribution and magnitude of emitted monoterpenes.

Intra-specific seasonal variation in VOC emission

The instantaneous environmental conditions, including light, temperature and rainfall amount, were not much different between the dry-to-wet season transition and the wet-to-dry season transition in 1999 (Andreae *et al.*, 2002). Consequently, the seasonal variation in the net primary production (NPP) and transpiration rates on the branch level of the individual species (Table 2) as well as on canopy scale (Andreae *et al.*, 2002) was not profound. This is in accordance with McWilliams *et al.* (1996) and Roberts *et al.* (1990), who found that the CO_2 gas exchange in the Amazon region is not affected greatly by seasonal changes in rainfall amount or soil moisture. For a detailed discussion on different strategies of primary physiology to respond to the changes in environmental conditions at our site see Rottenberger *et al.* (2003).

In contrast to the relatively small changes in primary physiology, the isoprene and the monoterpene emissions reacted differently towards long-term, seasonal variations in growth conditions. Whereas the isoprene emission rates of young mature leaves of *H. courbaril* investigated in the dry season were more than twofold the emission rate of older mature leaves in the wet season, the monoterpene emission rates from *A. tibourbou* decreased (Table 2). The seasonal differences in isoprenoid emission rates can not be reconciled solely with instantaneous meteorological data of light and temperature. The leaves rather undergo physiological and developmental changes over the course of the growing season and the isoprenoid basal emission rates vary accordingly. For extra-tropical regions there is evidence of seasonal variation in isoprenoid emission capacity due to long-term responses to plant phenological processes (Hakola *et al*., 1998) and modification in past and current growth conditions like temperature, light intensity, nutrition status and water availability (Sharkey & Loreto, 1993; Bertin & Staudt, 1996; Fang *et al*., 1996; Litvak *et al*., 1996; Bertin *et al*., 1997; Goldstein *et al*., 1998; Penuelas & Llusia, 1999; He *et al*., 2000; Staudt *et al*., 2000; Zhang *et al*., 2000; Sabillon & Cremades, 2001; Serca *et al*., 2001; Komenda & Koppmann, 2002; Ciccioli *et al*., 2003). However, the seasonal variation of isoprenoid emission appears to be species-specific, and hitherto there are hardly any data reported on species-specific seasonal variations of isoprenoid emissions of tropical vegetation.

Our results of a doubling of isoprene emission capacity of *H. courbaril* at the end of the dry season are in close agreement with canopy-scale flux measurements during the EXPRESSO program in a tropical forest site in Congo, which are hitherto the only studies on seasonal variations of emission flux rates in tropical areas (Klinger *et al*., 1998; Serca *et al*., 2001). For both sites the seasonal changes of isoprene ambient air mixing ratios and emission rates were accompanied by only minor differences in the overall environmental conditions, i.e. mean temperature, relative humidity and water availability. In earlier studies, variation in isoprenoid emission was often explained by water stress effects, and might suitably confer a reasonable explanation for seasonal differences of drought-deciduous tropical vegetation. Both assimilation rates and isoprenoid emission rates were found to be negatively affected by drought, whereas the sensitivity of the isoprenoid emission rate seems to be smaller than for stomatal conductance and assimilation rates (Tingey *et al*., 1981; Sharkey & Loreto, 1993; Bertin & Staudt, 1996; Fang *et al*., 1996; Lerdau & Keller, 1997; Guenther *et al*., 1999a; Llusia & Penuelas, 2000; Staudt *et al*., 2002). However, this behaviour would rather support lower isoprene emission rates in the end of the dry season, contradictory to our findings. Decreased leaf water potential causing reduced stomatal conductance and lower transpiration rates, leading to lower evaporative cooling and hence higher leaf temperatures, as suggested by Guenther *et al*. (1999b), cannot account for increased isoprene emissions in the end of the dry season in the case of *H. courbaril*. The rainfall amount at the site in the weeks preceding our enclosure measurements was comparable to that during the wet season investigations and water stress may be excluded as potential reason for increased isoprene emissions. Indeed an increase in water vapour deficit in the dry season resulted in a reduction of stomatal conductance of *H. courbaril*, but was associated with transpiration rates comparable to those observed in the wet season.

When interpreting seasonal variations in emission capacities, one has to keep in mind that differences in specific leaf weight (leaf dry mass per unit area) are not only subject to differences in long-term light availability in its canopy position (sun/shade adaptation, see above), but also occur during leaf phenology, i.e. the seasonal development of the leaf. Whereas the specific leaf mass was $80\,g\,m^{-2}$ for *H. courbaril* in the dry season (about 1 month after bud break), changes in leaf morphology had led to $118.5\,g\,m^{-2}$ in the wet season (7 months after bud break), respectively. This way, net primary productivity of *H. courbaril* is significantly decreased from the dry to the wet season if calculated on a dry weight basis, but not if based on a leaf area basis. Accordingly, the decrease of isoprene emission from the dry to the wet season is about threefold if based on dry weight, but only twofold if based on the leaf surface area (Table 2).

Long-term changes in isoprene emission capacity are obviously triggered by additional environmental parameters other than those influencing primary metabolism. In contrast to short-term controls over the isoprene emission capacity, long-term and seasonal variations in isoprene emissions seem to correlate with the inherent capacity of the leaves to synthesize isoprene (Monson *et al*., 1994; Schnitzler *et al*., 1997). The changes in basal emission capacity depend on the developmental stage of the leaves and have been correlated with extractable isoprene synthase activity (Kuzma & Fall, 1993; Lehning *et al*., 1999) or with the availability of the substrate dimethylallyl diphosphate (DMAPP; Geron *et al*., 2000; Brüggemann & Schnitzler, 2002). These changes can be triggered by nutrient availability (Litvak *et al*., 1996) and the light environment over several days (Sharkey *et al*., 1999), but most research focussed on the greater impact of the past and current growth temperature regime (Monson *et al*., 1994; Sharkey *et al*., 1999; Geron *et al*., 2000; Lehning

et al., 2001). Petron *et al*. (2001) demonstrated that the isoprene emission capacity of *Quercus macrocarpa* doubled when growth temperature 3–6 weeks preceding the emission measurements was increased by only 5 °C. Even though the environmental conditions during the measurements in the present study were similar (as indicated by the dotted line in Fig. 7), the ambient temperatures in the days and weeks preceding our exchange measurements were somewhat higher in the dry season than in the wet season (2–5 °C, depending on the time period taken into account). Moreover, increased growth temperature were found to result in higher temperature optima of isoprene emissions (Monson *et al*., 1992; Petron *et al*., 2001), and thus an increase of 5 °C from 25 °C to 30 °C reported by Petron *et al*. (2001) may easily be transferred to the observed increase from 30 °C to 35 °C in the tropical case, where species are adjusted to higher average temperatures all over the year. This way we assume that the moderate increase in temperature preceding our enclosure measurements in the dry season may help to explain the doubling of isoprene emission by *H. courbaril*.

Another rationale for increased isoprene emission may be found in higher ozone concentrations observed during the dry season, which were triggered by high biomass burning activity (average daytime >40 ppb, as compared to <10 ppb in the wet season; Kesselmeier *et al*., 2002b). Even though we scrubbed ozone in the air entering the enclosures and the branch under investigation was not subject to high ozone concentrations, the observed high isoprene emissions may reflect a higher overall oxidant protection status of the whole tree. Recent work provides evidence that VOC may be involved in protection against oxidizing agents like ozone (Zeidler *et al*., 1997; Loreto *et al*., 2001; Penuelas & Llusia, 2001). The influence of elevated atmospheric ozone on the emission capacity of plants, as an abiotic stress factor, is currently under debate (Kimmerer & Kozlowski, 1982; Heiden *et al*., 1999, 2003; Llusia *et al*., 2002; Wildt *et al*., 2003), and it seems that ozone

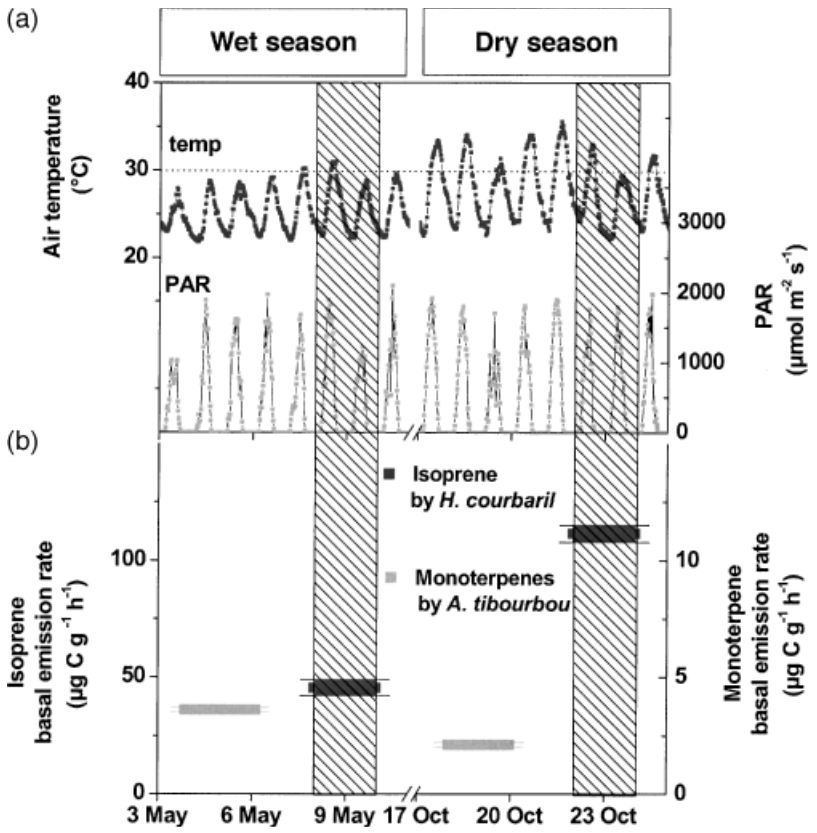

Fig. 7 Seasonal differences of standard emission factors (normalized to 1000 µmol m^{-2} s^{-1} of PAR and 30 °C leaf temperature) of monoterpene emission from *A. tibourbou* and isoprene emission from *H. courbaril* (panel b), in conjunction with data of ambient temperature and photosynthetic active radiation (PAR) in the days proceeding the measurements (panel a). During the period of the enclosure measurements of *H. courbaril* (patterned boxes) the average temperatures were essentially identical (as indicated by the dotted line in panel a), but were increased in the days proceeding the measurement periods. The meteorological parameters are from a tropical rainforest tower site 10 km north of the RBJ camp site (Andreae *et al*., 2002).

fumigation results in species-specific responses. It is striking that, on a regional scale, the maximum in atmospheric ozone formation potential occurs at the same times when isoprene emission potentials are greatest (Penuelas *et al*., 1999). Thus, the seasonal variation in atmospheric ozone production efficiency was often attributed to changes of isoprene emissions and ambient concentrations (Hirsch *et al*., 1996). While VOC emissions may have led to large-scale atmospheric depletion of tropospheric ozone by way of VOC ozonolysis in non-polluted, pre-industrial times (low NO_x regime), this strategy of increased VOC emission as defence towards high ozone events has the opposite effect in anthropogenically polluted, high NO_x environments, as the ozone production potential of VOC is very sensitive towards the NO_x mixing ratios (Atkinson, 2000). An increase in VOC emission as defensive response to high ozone concentrations would result in a positive feedback mechanism for tropospheric ozone formation.

In contrast to the increase of isoprene emission of *H. courbaril*, monoterpene emission capacity for *A. tibourbou* was decreased in the dry season compared to the wet season (Table 2). The emission composition was not much different for *A. tibourbou*, except that the role of myrcene among the dominant five monoterpenes emitted was taken over by camphene. Like for *H. courbaril*, the stomatal conductance was also reduced in the dry season for *A. tibourbou*, but could not compensate for the increased water vapour deficit, which led to an increase in transpiration rates. Associated with a slightly reduced primary productivity (NPP) this led to reduced water use efficiency. But the reduced stomatal conductance cannot account for the decline in monoterpene emission of *A. tibourbou*. Although there is conclusive evidence indicating that light-dependent foliar terpenes penetrate the intercellular space of the leaf and exit the plant predominantly via the stomata (Loreto *et al*., 1996), high intercellular isoprenoid biosynthesis/concentrations are assumed to counterbalance stomatal closure with an increase in the leaf-to-air isoprenoid vapour pressure gradient (Fall & Monson, 1992). This theory is also strictly confirmed by the diel course of stomatal conductance of *S. guilleminiana*, which had a major effect on the CO_2 uptake rate but not on the isoprene emission rate. Although the decreasing stomatal conductance during the midday hours resulted in strong hysteresis of CO_2 uptake, it did not affect the isoprene emissions at all (Figs 3 and 5).

Like for isoprene, increased thermo and ozone tolerance was also discussed for monoterpenes (Loreto *et al*., 1998; Singsaas & Sharkey, 2000; Loreto *et al*., 2001) and an increase of monoterpene emission rates after ozone fumigation was observed by some researchers (Heiden *et al*., 1999; Penuelas *et al*., 1999; Llusia *et al*., 2002). We did not find a positive response to the seasonal changes of atmospheric oxidative capacity, either in direct emission strength of *A. tibourbou* or in ambient monoterpene concentrations (Kesselmeier *et al*., 2002b), lending no support to this hypothesis. However, a dramatic increase in concentration and distribution of oxidative compounds (OH, O_3, H_2O_2, HO_2) due to extensive biomass burning activity may have shorten the chemical lifetime of highly reactive monoterpene compounds and counterbalance accumulation in the ambient air. In general, these compounds greatly interact and detailed chemical modelling is necessary to get further insight (Jacob & Wofsy, 1988; Guenther *et al*., 1999b).

One limitation of the interpretation of seasonal differences as reported in this field study is that the drivers affecting the emission potential are correlative. For both tree species investigated in the two seasons we can not differentiate between climatological effects in the days preceding our measurements, or phenological effects as budbreak was only about 4 weeks before the enclosure measurements reported here. Especially for *A. tibourbou*, even though leaves were fully green and turgescent, leaf size during the enclosure measurements was somewhat smaller in the dry season, and we cannot exclude short-term phenological effects being responsible for the lower NPP, and the rather decreased monoterpene emission compared to the wet season. Furthermore, with only one individual measured per species our conclusions can only be preliminary and long-term replicates are needed to confirm our findings.

VOC emission in relation to plant carbon fixation

There is growing interest on the role of VOC fluxes in the context of carbon transfer from the terrestrial biosphere to the atmospheric CO_2 reservoir. Whereas biogenic VOC fluxes are small in relation to the total numbers of carbon emission and deposition, the amount of carbon lost as VOC can be highly significant relative to the net ecosystem productivity (NEP), and are in the same order of magnitude as net biome productivity (NBP). Therefore investigations on the carbon balance from the leaf level up to the canopy scale are crucially needed, and should be considered in carbon flux models (Guenther 2002; Kesselmeier *et al*., 2002a).

Even though totally different in the total numbers of emission rates, the carbon balance of both isoprene emitting species was comparable (3.5% and 4.3%) and were about an order of magnitude larger as for the monoterpene emitter (0.1%). Seasonal differences in the percentage of carbon emitted in form of VOC was only

found for the strong isoprene emitter *H. courbaril* (3.5% in the dry season vs. 2.1% in the wet season, respectively), whereas the monoterpene emitter *A. tibourbou* revealed a similar carbon balance in both seasons (0.1%).

The substantial loss of photosynthetically fixed carbon in form of isoprene agrees very well with values for the tropical species *Mangifera indica* (mango tree) of 3.3–4.4% reported by Harley *et al*. (2003) and is in the upper range of carbon loss reported for temperate isoprenoid emitters (for a review, see Kesselmeier *et al*., 2002a). For all species the fraction of carbon re-emitted by isoprenoid emission was not constant during the day, but exhibited a strong diel course. Photosynthetic activity started earlier in the morning and declined almost simultaneously to the decrease in isoprenoid emission in the late afternoon (Fig. 6). This can be attributed to the low light compensation point of CO_2 exchange (Fig. 5), and the fact that isoprenoid emission tends to have a higher temperature maximum. Thus the amount of isoprene emitted relative to CO_2 uptake is enhanced especially for temperatures above the temperature optimum of photosynthesis (Guenther, 2002).

In the context of the global atmospheric carbon cycle it is important to note that there is evidence that part of the carbon transfer back from the atmosphere into the biosphere can be short-cut by plant uptake/deposition of partially oxygenated compounds after intermediate atmospheric chemical conversion (Kesselmeier *et al*., 2002a). As indicated in Table 1 and 2, a direct comparison with published data of the stomatal uptake of short-chain organic acids (Kuhn *et al*., 2002a) and aldehydes (Rottenberger *et al*., 2003) on the same trees demonstrates that the uptake of carbon other than CO_2 may be small on the branch level. The fraction of carbon re-cycled by uptake of short-chain oxygenated volatiles hardly exceeded 0.03% of NPP for the sunlit leaves investigated here. Only for the shade-adapted tree species *S. guilleminiana* the re-cycling of organic carbon became significant with values up to 0.2% mainly due to its relatively low photosynthetic performance. As the relative proportion of shaded leaves is dominating in forest canopies with high LAI like the tropical rainforest, the impact on the ecosystem scale, also including soil surface with dead leaf litter cover, needs to be further investigated.

Conclusion

Whereas tropical ecosystems generally show less seasonal behaviour in environmental conditions like temperature and light and also in the primary physiology than temperate ecosystems, we observed strong differences of branch-level VOC emissions. Although it is difficult to generalize due to the vast biodiversity in tropical rain forests and only one individual measured per tree species, our findings are in good agreement with observations of a concurrent doubling of atmospheric mixing ratios at this site, and a corresponding threefold seasonal increase in isoprene fluxes and ambient mixing ratios observed in another tropical forest site in the Congo. Our conclusions can only be preliminary and true replication is needed to confirm significant differences as reported here. But the inadequacy of using one single standard emission rate to represent an entire seasonal cycle seems apparent. Seasonal variation in VOC emission capacity is assumed not only to depend on instantaneous light and temperature, but is influenced by a number of physiological and environmental drivers including the seasonal dependence of leaf maturation, changes in leaf growth environment of the plants, and water and nutrient availability, independently of foliar area development. Our observations may confirm earlier laboratory investigations showing that the growth temperature regime during the proceeding days and weeks may be applied to predict the enzymatic status and isoprene emission capacity. As isoprene emissions are discussed to confer protection against high temperatures and high ozone, and given the fact that both leaf temperatures and ozone concentrations were higher in the dry season than in wet season, a requirement for greater thermo and ozone tolerance may provide a functional explanation for increased isoprene emissions in the dry season. But the various drivers potentially controlling VOC emission capacities have not yet been well characterized, and they all change seasonally in concert. Consequently their individual impacts can hardly be incorporated in landscape emission models (Guenther *et al*., 1999b; Otter *et al*., 2002).

Simultaneous canopy scale measurements of isoprenoid emission and CO_2 exchange are needed to get a better insight in the role of VOC in the global carbon budget. As both respond differently towards biophysical drivers, the percentage of carbon released as isoprene is assumed to increase with temperature (Goldstein *et al*., 1998; Guenther, 2002). This way the carbon balance exhibits a positive feedback of global warming and future scenarios predict an even higher fraction of organic carbon re-emitted on a global scale. On-going land-use change especially in the tropics will result in further perturbations of the carbon and other biogeochemical cycles. More long-term measurements are needed to better characterize seasonal and interannual variability to estimate present and future impact of biogenic VOC fluxes especially in respect to the global atmospheric carbon budget.

Acknowledgements

We thank the staff at INCRA (Instituto Nacional de Colonizacao e Reforma Agraria) and IBAMA (Instituto Brasileiro de Meio Ambiente e Recursos Naturais Renovaveis) in Ji-Paraná for their help in installing and maintaining the infrastructure and Ricardo Dallarosa and Ieda Amaral (Instituto National de Pesquisas da Amazônia, INPA) for their help in species determination. We are indebted to Beatrice E. Gomes (Universidade Federal de Rondônia, Ji-Paraná) for her kind support and are grateful to Ulrich Renk, Monika Scheibe, S. Nei Leite, and Sergio T. Oliva for their assistance in the field, and Carol Strametz for language assistance. This research is supported by the Max Planck Society and the 'Environmental and Climate Program' (EUSTACH-LBA; ENV4-CT97-0566) of the European Union.

References

Andreae MO, Artaxo P, Brandao C *et al*. (2002) Biogeochemical cycling of carbon, water, energy, trace gases, and aerosols in Amazonia: the LBA-EUSTACH experiments. *Journal of Geophysical Research-Atmospheres*, **107 (D20)**, doi: 10.1029/2001JD000524.

Atkinson R (2000) Atmospheric chemistry of VOCs and NO_x. *Atmospheric Environment*, **34**, 2063–2101.

Bertin N, Staudt M (1996) Effect of water stress on monoterpene emissions from young potted holm oak (*Quercus ilex* L.) trees. *Oecologia*, **107**, 456–462.

Bertin N, Staudt M, Hansen U *et al*. (1997) Diurnal and seasonal course of monoterpene emissions from *Quercus ilex* (L.) under natural conditions – Applications of light and temperature algorithms. *Atmospheric Environment*, **31**, 135–144.

Brüggemann N, Schnitzler JP (2002) Diurnal variation of dimethylallyl diphosphate concentrations in oak (*Quercus robur*) leaves. *Physicologia Plantarum*, **115**, 190–196.

Ciccioli P, Brancaleoni E, Frattoni M *et al*. (2003) Relaxed eddy accumulation, a new technique for measuring emission and deposition fluxes of volatile organic compounds by capillary gas chromatography and mass spectrometry. *Journal of Chromatography A*, **985**, 283–296.

Ciccioli P, Cecinato A, Brancaleoni E *et al*. (1992) Use of carbon adsorption traps combined with high-resolution gas-chromatography–mass-spectrometry for the analysis of polar and non-polar C-4-C-14 hydrocarbons involved in photochemical smog formation. *Journal of High Resolution Chromatography*, **15**, 75–84.

Ciccioli P, Fabozzi C, Brancaleoni E *et al*. (1997) Use of the isoprene algorithm for predicting the monoterpene emission from the Mediterranean holm oak *Quercus ilex* L: Performance and limits of this approach. *Journal of Geophysical Research-Atmospheres*, **102**, 23319–23328.

Collins WD, Rasch PJ, Eaton BE *et al*. (2002) Simulation of aerosol distributions and radiative forcing for INDOEX: Regional climate impacts. *Journal of Geophysical Research-Atmospheres*, **107 (D19)**, doi: 10.1029/2000JD000032.

Croat TB (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford.

Delfine S, Csiky O, Seufert G (2000) Fumigation with exogenous monoterpenes of a non-isoprenoid-emitting oak (Quercus suber): monoterpene acquisition, translocation, and effect on the photosynthetic properties at high temperatures. *New Phytologist*, **146**, 27–36.

Duke JA (1978) The quest for tolerant germplasm. In: *ASA Special Symposium 32, Crop Tolerance to Suboptimal Land Conditions* (ed. Jung GA), pp. 1–61. American Society of Acronomy, WI, USA.

Fall R, Monson RK (1992) Isoprene emission rate and intercellular isoprene concentration as influenced by stomatal distribution and conductance. *Plant Physiology*, **100**, 987–992.

Fang CW, Monson RK, Cowling EB (1996) Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles. *Tree Physiology*, **16**, 441–446.

Gash JHC, Nobre CA, Roberts JM *et al*. (1996) An overview of ABRACOS. In: *Amazonian Deforestation and Climate* (eds Gash JHC), pp. 1–14. John Wiley, New York.

Geron C, Guenther A, Greenberg J *et al*. (2002) Biogenic volatile organic compound emissions from a lowland tropical wet forest in Costa Rica. *Atmospheric Environment*, **36**, 3793–3802.

Geron C, Guenther A, Sharkey T *et al*. (2000) Temporal variability in basal isoprene emission factor. *Tree Physiology*, **20**, 799–805.

Goldstein AH, Goulden ML, Munger JW *et al*. (1998) Seasonal course of isoprene emissions from a midlatitude deciduous forest. *Journal of Geophysical Research-Atmospheres*, **103**, 31045–31056.

Greenberg JP, Guenther A, Wiedinmyer C *et al*. (2003) Biognic VOC emissions from disturbed and undisturbed Amazonian landscapes. *Global Change Biology* (this issue).

Guenther A (2002) The contribution of reactive carbon emissions from vegetation to the carbon balance of terrestrial ecosystems. *Chemosphere*, **49**, 837–844.

Guenther A, Archer S, Greenberg J *et al*. (1999a) Biogenic hydrocarbon emissions and landcover/climate change in a subtropical savanna. *Physics and Chemistry of the Earth Part B – Hydrology Oceans and Atmosphere*, **24**, 659–667.

Guenther A, Baugh B, Brasseur G *et al*. (1999b) Isoprene emission estimates and uncertainties for the Central African EXPRESSO study domain. *Journal of Geophysical Research-Atmospheres*, **104**, 30625–30639.

Guenther A, Geron C, Pierce T *et al*. (2000) Natural emissions of non-methane volatile organic compounds; carbon monoxide, and oxides of nitrogen from North America. *Atmospheric Environment*, **34**, 2205–2230.

Guenther A, Hewitt CN, Erickson D *et al*. (1995) A global model of natural volatile organic compound emissions. *Journal of Geophysical Research-Atmospheres*, **100**, 8873–8892.

Guenther AB, Monson RK, Fall R (1991) Isoprene and monoterpene emission rate variability – observations with Eucalyptus and emission rate algorithm development. *Journal of Geophysical Research-Atmospheres*, **96**, 10799–10808.

Guenther AB, Zimmerman PR, Harley PC *et al*. (1993) Isoprene and monoterpene emission rate variability – model evaluations and sensitivity analyses. *Journal of Geophysical Research – Atmospheres*, **98**, 12609–12617.

Hakola H, Rinne J, Laurila T (1998) The hydrocarbon emission rates of tea-leafed willow (*Salix phylicifolia*), silver birch (*Betula pendula*) and European aspen (*Populus tremula*). *Atmospheric Environment*, **32**, 1825–1833.

Harley P, Guenther A, Zimmerman P (1996) Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology*, **16**, 25–32.

Harley P, Guenther A, Zimmerman P (1997) Environmental controls over isoprene emission in deciduous oak canopies. *Tree Physiology*, **17**, 705–714.

Harley P, Vasconcellos P, Vierling L *et al*. (2003) Variation in potential for isoprene emissions among Neotropical forest sites. *Global Change Biology* (this issue).

He CR, Murray F, Lyons T (2000) Monoterpene and isoprene emissions from 15 Eucalyptus species in Australia. *Atmospheric Environment*, **34**, 645–655.

Heiden AC, Kobel K, Langebartels C *et al*. (2003) Emissions of oxygenated volatile organic compounds from plants, Part I: emissions from lipoxygenase activity. *Journal of Atmospheric Chemistry*, **45**, 143–172.

Heiden AC, Hoffmann T, Kahl J *et al*. (1999) Emission of volatile organic compounds from ozone-exposed plants. *Ecological Applications*, **9**, 1160–1167.

Hirsch AI, Munger JW, Jacob DJ *et al*. (1996) Seasonal variation of the ozone production efficiency per unit NO_x at Harvard Forest, Massachusetts. *Journal of Geophysical Research-Atmospheres*, **101**, 12659–12666.

Jacob DJ, Wofsy SC (1988) Photochemistry of biogenic emissions over the Amazon forest. *Journal of Geophysical Research-Atmospheres*, **93**, 1477–1486.

Janson RW (1993) Monoterpene emissions from Scots Pine and Norwegian Spruce. *Journal of Geophysical Research – Atmospheres*, **98**, 2839–2850.

Keene WC, Galloway JN, Holden JD (1983) Measurement of weak organic acidity in precipitation from remote areas of the world. *Journal of Geophysical Research-Oceans and Atmospheres*, **88**, 5122–5130.

Kesselmeier J, Bode K, Hofmann U *et al*. (1997) Emission of short chained organic acids, aldehydes and monoterpenes from *Quercus ilex* L. and *Pinus pinea* L. in relation to physiological activities, carbon budget and emission algorithms. *Atmospheric Environment*, **31**, 119–133.

Kesselmeier J, Ciccioli P, Kuhn U *et al*. (2002a) Volatile organic compound emissions in relation to plant carbon fixation and the terrestrial carbon budget. *Global Biogeochemical Cycles*, **16**, doi: 10.1029/2001GB001813.

Kesselmeier J, Kuhn U, Rottenberger S *et al*. (2002b) Concentrations and species composition of atmospheric volatile organic compounds (VOCs) as observed during the wet and dry season in Rondônia (Amazonia). *Journal of Geophysical Research-Atmospheres*, **107 (D20)**, doi: 10.1029/2000JD000267.

Kesselmeier J, Staudt M (1999) Biogenic volatile organic compounds (VOC) between plants and the atmosphere: an overview on emission, physiology and ecology. *Journal of Atmospheric Chemistry*, **33**, 23–88.

Kimmerer TW, Kozlowski TT (1982) Ethylene, ethane, acetaldehyde, and ethanol-production by plants under stress. *Plant Physiology*, **69**, 840–847.

Klinger LF, Greenberg J, Guenther A *et al*. (1998) Patterns in volatile organic compound emissions along a savanna-rainforest gradient in central Africa. *Journal of Geophysical Research-Atmospheres*, **103**, 1443–1454.

Koch GW, Amthor JS, Goulden ML (1994) Diurnal patterns of leaf photosynthesis, conductance and water potential at the top of a lowland rain-forest canopy in Cameroon–measurements from the Radeau-Des-Cimes. *Tree Physiology*, **14**, 347–360.

Komenda M, Koppmann R (2002) Monoterpene emissions from Scots pine (*Pinus sylvestris*): Field studies of emission rate variabilities. *Journal of Geophysical Research-Atmospheres*, **107 (D13)**, doi: 10.1029/2001JD000691.

Koyama H (1978) Photosynthsis studies in Pasoh forest. *Malayan Nature Journal*, **30**, 253–258.

Kuhn U, Ammann C, Wolf A (1999) Carbonyl sulfide exchange on an ecosystem scale: soil represents a dominant sink for atmospheric COS. *Atmospheric Environment*, **33**, 995–1008.

Kuhn U, Rottenberger S, Biesenthal T *et al*. (2002a) Exchange of short-chain monocarboxylic acids by vegetation at a remote tropical forest site in Amazonia. *Journal of Geophysical Research-Atmospheres*, **107 (D20)**, doi: 10.1029/2000JD000303.

Kuhn U., Rottenberger S., Biesenthal T. (2002b) Isoprene and monoterpene emissions of Amazonian tree species during the wet season: Direct and indirect investigations on controlling environmental functions. *Journal of Geophysical Research-Atmospheres*, **107 (D20)**, doi: 10.1029/2001JD000303.

Kuhn U, Wolf A, Gries C *et al*. (2000) Field measurements on the exchange of carbonyl sulfide between lichens and the atmosphere. *Atmospheric Environment*, **34**, 4867–4878.

Kuzma J, Fall R (1993) Leaf isoprene emission rate is dependent on leaf development and the level of isoprene synthase. *Plant Physiology*, **101**, 435–440.

Lehning A, Zimmer I, Steinbrecher R *et al*. (1999) Isoprene synthase activity and its relation to isoprene emission in *Quercus robur* L-leaves. *Plant Cell and Environment*, **22**, 495–504.

Lehning A, Zimmer W, Zimmer I *et al*. (2001) Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates. *Journal of Geophysical Research-Atmospheres*, **106**, 3157–3166.

Lerdau M, Keller M (1997) Controls on isoprene emission from trees in a subtropical dry forest. *Plant Cell and Environment*, **20**, 569–578.

Lerdau M, Throop HL (2000) Sources of variability in isoprene emission and photosynthesis in two species of tropical wet forest trees. *Biotropica*, **32**, 670–676.

Lerdau M, Throop HL (1999) Isoprene emission and photosynthesis in a tropical forest canopy: implications for model development. *Ecological Applications*, **9**, 1109–1117.

Levis S, Wiedinmyer C, Bonan GB *et al*. (2003) Simulating biogenic volatile organic compound emission in the Community Climate System Model. *Journal of Geophysical Research-Atmospheres*, **108 (D21)**, doi: 10.1029/2002DJ003203.

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 47–65.

Litvak ME, Loreto F, Harley PC *et al*. (1996) The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. *Plant Cell and Environment*, **19**, 549–559.

Llusia J, Penuelas J (2000) Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions. *American Journal of Botany*, **87**, 133–140.

Llusia J, Penuelas J, Gimeno BS (2002) Seasonal and species-specific response of VOC emissions by Mediterranean woody plant to elevated ozone concentrations. *Atmospheric Environment*, **36**, 3931–3938.

Loreto F, Ciccioli P, Cecinato A *et al*. (1996) Influence of environmental factors and air composition on the emission of alpha-pinene from *Quercus ilex* leaves. *Plant Physiology*, **110**, 267–275.

Loreto F, Forster A, Durr M *et al*. (1998) On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant Cell and Environment*, **21**, 101–107.

Loreto F, Mannozzi M, Maris C *et al*. (2001) Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology*, **126**, 993–1000.

McWilliams ALC, Cabral OMR, Gomes BM *et al*. (1996) Forest and pasture leaf-gas exchange in South-West Amazonia. In: *Amazonian Deforestation and Climate* (ed. Gash JHC *et al*.), pp 265–285. John Wiley, New York, USA.

Monson RK, Harley PC, Litvak ME *et al*. (1994) Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia*, **99**, 260–270.

Monson RK, Holland EA (2001) Biogenic trace gas fluxes and their control over tropospheric chemistry. *Annual Review of Ecology and Systematics*, **32**, 547–576.

Monson RK, Jaeger CH, Adams WW *et al*. (1992) Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiology*, **98**, 1175–1180.

Monson RK, Lerdau MT, Sharkey TD *et al*. (1995) Biological aspects of constructing volatile organic compound emission inventories. *Atmospheric Environment*, **29**, 2989–3002.

Niinemets Ü, Hauff K, Bertin N *et al*. (2002) Monoterpene emissions in relation to foliar photosynthetic and structural variables in Mediterranean evergreen Quercus species. *New Phytologist*, **153**, 243–256.

Niinemets Ü, Reichstein M (2002) A model analysis of the effects of nonspecific monoterpenoid storage in leaf tissues on emission kinetics and composition in Mediterranean sclerophyllous Quercus species. *Global Biogeochemical Cycles*, **16**, doi: 10.1029/2002GB001927.

Niinemets Ü, Reichstein M (2003) Controls on the emission of plant volatiles through stomata: differential sensitivity of emission rates to stomatal closure explained. *Journal of Geophysical Research-Atmospheres*, **108**, **(D7)**, doi: 10.1029/2002JD002620.

Otter LB, Guenther A, Greenberg J (2002) Seasonal and spatial variations in biogenic hydrocarbon emissions from southern African savannas and woodlands. *Atmospheric Environment*, **36**, 4265–4275.

Otter LB, Guenther A, Wiedinmyer C *et al*. (2003) Spatial and temporal variation in biogenic volatile organic compound emissions for Africa south of the equator. *Journal of Geophysical Research-Atmospheres*, **108**, **(D13)**, doi: 10.1029/2002JD002609.

Pearcy RW (1987) Photosynthetic gas exchange responses of Australian tropical forest trees in canopy, gap and understory micro-environments. *Functional Ecology*, **1**.

Penuelas J, Llusia J (1999) Seasonal emission of monoterpenes by the Mediterranean tree Quercus ilex in field conditions: relations with photosynthetic rates, temperature and volatility. *Physiologia Plantarum*, **105**, 641–647.

Penuelas J, Llusia J (2001) The complexity of factors driving volatile organic compound emissions by plants. *Biologia Plantarum*, **44**, 481–487.

Penuelas J, Llusia J, Gimeno BS (1999) Effects of ozone concentrations on biogenic volatile organic compounds emission in the Mediterranean region. *Environmental Pollution*, **105**, 17–23.

Petron G, Harley P, Greenberg J *et al*. (2001) Seasonal temperature variations influence isoprene emission. *Geophysical Research Letters*, **28**, 1707–1710.

Poisson N, Kanakidou M, Crutzen PJ (2000) Impact of non-methane hydrocarbons on tropospheric chemistry and the oxidizing power of the global troposphere: 3-dimensional modelling results. *Journal of Atmospheric Chemistry*, **36**, 157–230.

Reich PB, Ellsworth DS, Walters MB (1998) Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology*, **12**, 948–958.

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 13730–13734.

Ribeiro JELS, Berg CC (1999) *Guia de identificacao das palntas vasculares de uma floresta de terra-firme na Amazonia Central*. Manaus, Brazil.

Rinne HJI, Guenther AB, Greenberg JP, Harley PC (2002) Isoprene and monoterpene fluxes measured above Amazonian rainforest and their dependence on light and temperature. *Atmospheric Environment*, **36**, 2421–2426.

Roberts J, Cabral OMR, Aguiar LF (1990) Stomatal and boundary-layer conductance in an Amazonian terra firme rain forest. *Journal of Applied Ecology*, **27**, 336–353.

Rottenberger S, Kuhn U, Wolf A *et al*. (2003) Exchange of short-chain aldehydes between Amazonian vegetation and the atmosphere at a remote forest site in Brazil. *Ecological Applications*, in press.

Sabillon D, Cremades LV (2001) Diurnal and seasonal variation of monoterpene emission rates for two typical Mediterranean species (*Pinus pinea* and *Quercus ilex*) from field measurements-relationship with temperature and PAR. *Atmospheric Environment*, **35**, 4419–4431.

Schnitzler JP, Lehning A, Steinbrecher R (1997) Seasonal pattern of isoprene synthase activity in *Quercus robur* leaves and its significance for modeling isoprene emission rates. *Botanica Acta*, **110**, 240–243.

Schuh G, Heiden AC, Hoffmann T *et al*. (1997) Emissions of volatile organic compounds from sunflower and beech: dependence on temperature and light intensity. *Journal of Atmospheric Chemistry*, **27**, 291–318.

Serca D, Guenther A, Klinger L *et al*. (2001) EXPRESSO flux measurements at upland and lowland Congo tropical forest site. *Tellus Series B – Chemical and Physical Meteorology*, **53**, 220–234.

Sharkey TD, Loreto F (1993) Water-stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of Kudzu leaves. *Oecologia*, **95**, 328–333.

Sharkey TD, Singsaas EL (1995) Why plants emit isoprene. *Nature*, **374**, 769–769.

Sharkey TD, Singsaas EL, Lerdau MT *et al*. (1999) Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecological Applications*, **9**, 1132–1137.

Sharkey TD, Singsaas EL, Vanderveer PJ *et al*. (1996) Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiology*, **16**, 649–654.

Singsaas EL, Sharkey TD (2000) The effects of high temperature on isoprene synthesis in oak leaves. *Plant Cell and Environment*, **23**, 751–757.

Staudt M, Bertin N, Frenzel B *et al*. (2000) Seasonal variation in amount and composition of monoterpenes emitted by young *Pinus pinea* trees – implications for emission modeling. *Journal of Atmospheric Chemistry*, **35**, 77–99.

Staudt M, Rambal S, Joffre R *et al*. (2002) Impact of drought on seasonal monoterpene emissions from *Quercus ilex* in southern France. *Journal of Geophysical Research – Atmospheres*, **107 (D21)**, doi: 10.1029/2001JD002043.

Staudt M, Seufert G (1995) Light-dependent emission of monoterpenes by holm oak (*Quercus-ilex* L.). *Naturwissenschaften*, **82**, 89–92.

Tingey DT, Evans R, Gumpertz M (1981) Effects of environmental conditions on isoprene emission from Live Oak. *Planta*, **152**, 565–570.

Wildt J, Kobel K, Schuh-Thomas G *et al*. (2003) Emissions of oxygenated volatile organic compounds from plants, Part II: Emissions from saturated aldehydes. *Journal of Atmospheric Chemistry*, **45**, 173–196.

Zeidler JG, Lichtenthaler HK, May HU *et al*. (1997) Is isoprene emitted by plants synthesized via the novel isopentenyl pyrophosphate pathway. *Zeitschrift Fur Naturforschung C – A Journal of Biosciences*, **52**, 15–23.

Zhang XS, Mu YJ, Song WZ, Zhuang YH (2000) Seasonal variations of isoprene emissions from deciduous trees. *Atmospheric Environment*, **34**, 3027–3032.