Effects of light and temperature on isoprene emission at different leaf developmental stages of *Eschweilera coriacea* in central Amazon

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ABSTRACT

Isoprene emission from plants accounts for about one third of annual global volatile organic compound emissions. The largest source of isoprene for the global atmosphere is the Amazon Basin. This study aimed to identify and quantify the isoprene emission and photosynthesis at different levels of light intensity and leaf temperature, in three phenological phases (young mature leaf, old mature leaf and senescent leaf) of *Eschweilera coriacea* (Matamatá verdadeira), the species with the widest distribution in the central Amazon. *In situ* photosynthesis and isoprene emission measurements showed that young mature leaf had the highest rates at all light intensities and leaf temperatures. Additionally, it was observed that isoprene emission capacity (E_s) changed considerably over different leaf ages. This suggests that aging leads to a reduction of both leaf photosynthetic activity and isoprene production and emission. The algorithm of Guenther *et al.* (1999) provided good fits to the data when incident light was varied, however differences among E_S of all leaf ages influenced on quantic yield predicted by model. When leaf temperature was varied, algorithm prediction was not satisfactory for temperature higher than ~40 °C; this could be because our data did not show isoprene temperature optimum up to 45 °C. Our results are consistent with the hypothesis of the isoprene functional role in protecting plants from high temperatures and highlight the need to include leaf phenology effects in isoprene emission models.

KEYWORDS: light response curve, temperature response curve, leaf phenology, tropical species.

Efeitos da luz e da temperatura sobre a emissão de isopreno em diferentes estádios de desenvolvimento foliar de *Eschweilera coriacea* na Amazônia Central

RESUMO

O isopreno emitido pelas plantas corresponde em cerca de um terço das emissões globais de compostos orgânicos voláteis anualmente. A maior fonte de emissão de isopreno para a atmosfera global é a Bacia Amazônica. Este estudo objetivou identificar e quantificar a emissão de isopreno e fotossíntese em diferentes níveis de intensidade de luz e temperatura foliar, em três fases fenológicas (folha madura recente, folha madura tardia e folha senescente) de *Eschweilera coriacea* (Matamatá verdadeira) - a espécie com maior distribuição na Amazônia central. *In situ,* as medidas de fotossíntese e emissão de isopreno da folha madura recente apresentaram as maiores taxas em todos os níveis de luz e de temperatura. Adicionalmente, a capacidade de emissão de isopreno (E_S) mudou consideravelmente entre as diferentes idades foliares, sugerindo que o envelhecimento reduz a atividade fotossintética e a produção/emissão de isopreno. O algoritmo de Guenther *et al.* (1999) demonstrou bom ajuste para a emissão de isopreno em diferentes intensidades de luz, entretanto, diferenças na E_S entre as idades foliares influenciaram no rendimento quântico estimado pelo modelo. Em relação à temperatura foliar, a estimativa do algoritmo não foi satisfatória para as temperaturas acima de ~40 °C; isto provavelmente ocorreu pelo fato dos dados não apresentarem temperatura ótima até 45 °C. Nossos resultados são consistentes com a hipótese do isopreno ter um papel funcional para proteger as plantas de altas temperaturas e apontam a necessidade de incluir os efeitos da fenologia foliar em modelos de emissão de isopreno.

PALAVRAS-CHAVE: curva de luz, curva de temperatura, fenologia foliar, espécie tropical.

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INTRODUCTION

Terrestrial vegetation emits many non-methane Biogenic Volatile Organic Compounds (BVOCs), including hydrocarbons (isoprene, monoterpenes and sesquiterpenes) and oxygenated organic compounds (e.g., methanol and acetone) (Guenther 2002). These compounds have many effects on atmospheric chemistry and climate (Pacifico *et al.* 2009). Isoprene (2-methyl -1,3-butadiene, C_5H_8) is quantitatively the most important of the BVOCs, with an estimated global annual emission of about 400-600 TgC (see table 1 of Arneth *et al.* 2008). Terrestrial plant foliage is a source of > 90% of atmospheric isoprene and it is generally assumed that tropical forests provide most of the global isoprene budget (Guenther *et al.* 2006).

The ability to produce isoprene is widespread in the plant kingdom (Harley *et al.* 2004), and this production has been shown to depend on environmental factors, primarily light and temperature (Guenther *et al.* 1993; Sharkey and Singsaas 1995; Niinemets *et al.* 2010). Isoprene is not stored within the plant and although its function remains open to debate, experiments have demonstrated that high levels of isoprene within leaves confer protection against high temperatures (Singsaas *et al.* 1997; Peñuelas *et al.* 2005; Velikova and Loreto 2005), and oxidants (Loreto and Velikova, 2001). Studies with plants grown under different incident photosynthetic photon flux densities in constant temperature environments have also demonstrated positive correlations between light availability and isoprene emissions (Litvak *et al.* 1996; Hanson and Sharkey 2001).

In addition to effects of short-term environmental (light and temperature) fluctuations, leaf developmental variations also influence on isoprene production. Isoprene synthase activity, absent in very young leaves, increases gradually until full leaf maturation and decreases thereafter with the onset of leaf senescence (Schnitzler *et al.* 1997). The physiological activity of foliage varies in dependence on leaf ontogenetic stage, increasing rapidly in developing leaves and decreasing in senescing leaves undergoing programmed cell death (Niinemets *et al.* 2004). This decline in leaf physiological activity is also associated with strong reductions in foliage isoprene emission rates (Lehning *et al.* 2001; Mayrhofer *et al.* 2005; Sun *et al.* 2009).

The Amazon forest is the largest area of tropical rainforest and is representative of tropical forests in general, so that models predicting emissions of BVOCs from this critically important global biome provide necessary inputs to models of atmospheric chemistry and biogeochemical cycling at regional and global scales. Therefore, studies of isoprene emission in relation to environmental factors (light and temperature) and leaf developmental variations are important to improve emission models of isoprene and other important trace gas species. This is the first study that clearly shows isoprene emission at leaf level from *Eschweilera coriacea* (DC.) S. A. Mori, the most abundant tree species in the central Amazon. In addition, we characterized the effects of leaf temperature and light availability on photosynthesis and isoprene emission at different leaf developmental stages of this species.

MATERIALS AND METHODS

Site description

The species investigated occurs on a plateau of the Reserva Biológica do Cuieiras, a primary rainforest biological reserve located approximately 100 km north of Manaus, in the central Amazon Basin, in Amazonas, Brazil (2° 36'.67" S, 60° 12' 33.48" W, 130 m a.s.l.). This reserve has an area of about 230 km² and belongs to the National Institute for Amazon Research (INPA) (Andreae *et al.* 2002). Soils are well drained Oxisols and Ultisols on the plateau and slopes, respectively, and poorly drained Spodosols in the valleys (Ferraz *et al.* 1998; Luizão *et al.* 2004). The diversity of species is above 200 species ha^{-1} (Oliveira and Amaral 2005). The canopy is dense, resulting in greatly reduced light availability in the forest understory and on the forest floor (Oliveira *et al.* 2008). Annual rainfall is about 2400 mm and average air temperature ranges between 26 °C (in April) and 28 °C (in September). The wetter period extends from December to April, and the drier period from June to September, when rainfall is less than 100 mm per month (Araújo *et al.* 2002).

Isoprene data collection and analysis

Measurements of isoprene emission and photosynthesis of the canopy tree species *Eschweilera coriacea* were carried out in the months of October and November 2010, during the dry-to-wet season transition. Measurements were made on single leaves at three different developmental stages in the top layer of the canopy: young mature leaf (recently fully expanded), old mature leaf (longer period since full expansion) and senescent leaf. Although *E. coriacea* is the most abundant tree species in the central Amazon, photosynthesis and isoprene data collection were obtained in a single plant because of logistic difficult to get to canopy tree in the Amazon Forest. Nevertheless, these data may indicate the pattern of photosynthesis and isoprene emission in a tropical species, since data like these are very scarce.

Photosynthesis measurements were carried out between 8 and 12 h, using a commercial portable photosynthesis system (LI-6400, LI-COR, Inc, Lincoln, NE, USA). The flow rate of air into the leaf chamber was 400 μmol s^{-1}, and CO_2 and H_2O concentrations inside the chamber were 380±4 μmol mol^{-1} and 21±3 mmol mol^{-1}, respectively. The LI-6400 was set to photosynthetic photon flux density (PPFD) and leaf temperature variations.

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To measure isoprene emissions, air exiting the LI-6400 leaf chamber was routed to fill a 0.5 Tedlar sampling bag with single polypropylene septum fitting (SKC, Inc., Eighty Four, PA, USA); for each sampling one bag was filled to approximately 0.4 liter. In order to avoid chemical conversion of the primary emitted isoprene to oxygenated compounds inside the leaf enclosure and to remove isoprene from the incoming ambient air, activated charcoal filters (ORBO 32, Sigma-Aldrich, St. Louis, MO, USA) were installed at the air inlet of LI-6400 to remove ozone and hydrocarbons. Additionally, before each measurement a chamber blank sample was obtained from the empty leaf chamber. All tubing in contact with the sampling air was Teflon.

Isoprene concentrations (ppbv) in the bag samples were quantified within 3 hours after collection using a commercial high sensitivity proton transfer reaction mass spectrometry (PTR-MS, IONICON, Austria). The PTR-MS was operated in standard conditions and isoprene concentrations were determined as previously described (Jardine *et al.* 2011).

Once the volume mixing ratio of isoprene (ppbv) was obtained, leaf emission flux was determined using the following equation:

$$F = R_{ppbv} \times \frac{Q}{A} \qquad (1)$$

where F (nmol m^{-2} s^{-1}) is leaf flux of isoprene emission; R_{ppbv} (nmol mol^{-1}) is isoprene concentration of sample; Q is flow rate of air into the leaf chamber (400 x 10^{-6} mol s^{-1}); and A is the area of leaf within the chamber (0.0006 m²). Using this equation, units of isoprene emission were nmol m^{-2} s^{-1}. For conversion to units of mass, specific leaf mass was determined (g dry mass m^{-2}), where the dry weight was obtained using a microbalance (AY220, Shimadzu, Philippines) after drying leaves in a ventilated oven (65 °C) for 72 h. The mass units of isoprene emission were µg C g^{-1} h^{-1}.

Light and temperature response curves

Photosynthesis and isoprene emission rates were quantified at different levels of incident light (PPFD) and leaf temperature for three leaf developmental stages (young mature leaf, older mature leaf and senescent leaf). In the light response curves, measurements were made at nine increasing levels of irradiance (0, 50, 100, 250, 500, 750, 1000, 1500, 2000 µmol m^{-2} s^{-1}) under constant leaf temperature (30±1 °C). At each PPFD level, after the establishment of steady-state photosynthesis, a bag sample (approx. 0.4 l) of air exiting the chamber was collected in around one minute.

For temperature response curves, measurements were made under constant irradiance (1000 µmol m^{-2} s^{-1}) and leaf temperature was varied between approximately 25 °C and 45 °C. Because the thermoelectric temperature controllers could only regulate cuvette temperature within approximately 8 °C below and 10 °C above ambient temperature (e.g. Keller and Lerdau 1999), the actual range of each temperature response curve depended upon the ambient temperature at the time of measurement. At each leaf temperature, one sample was collected around one minute after leaf photosynthetic rate had reached steady state. For leaf temperatures above 30 °C, the collection was made 15 minutes after the establishment of apparent steady-state photosynthesis.

RESULTS

Isoprene emission capacity (E$_s$) for different developmental stages

Isoprene emission capacity (E_s), which is determined at standard conditions (30 °C and 1000 µmol m^{-2} s^{-1} of PPFD), obtained from the light response curve (Figure 1a) showed that E_s of the young mature leaf was 124% higher than E_s of the older mature leaf, and 92% higher than E_s of the senescent leaf (Table 1).
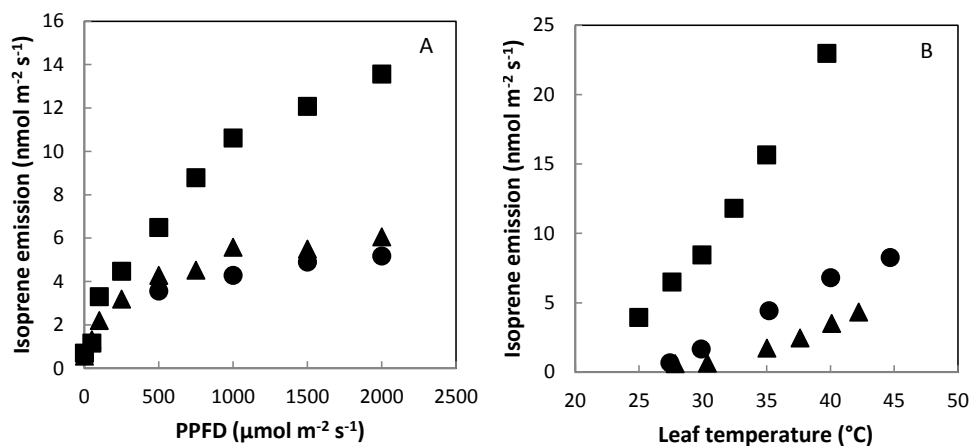

Figure 1 - Response of isoprene emission from different leaf developmental stages of *E. coriacea* to variations in PPFD (A) and leaf temperature (B). Senescent leaf (▲); old mature leaf (●); young mature leaf (■).

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Table 1 - Photosynthesis, isoprene emission capacity and percentage of carbon emitted in form of isoprene at standard conditions
(30 °C and 1000 μmol m^{-2} s^{-1} of PAR)

	Leaf Development Stage	Pn (μmol m^{-2} s^{-1})	Es (nmol m^{-2} s^{-1})	Specific Leaf Mass (g m^{-2})	Pn (μg C g^{-1} h^{-1})	Es (μg Cg^{-1} h^{-1})	% of fixed C emitted as isoprene
Temperature	YML	8.41	8.45	134.87	2700	13.54	0.5
Response	OML	3.33	1.67	123.55	1170	2.92	0.25
Curve	SL	2.79	0.65	137.7	877	1.03	0.12
Light	YML	9.96	9.6	142.41	3022	14.56	0.48
Response	OML	4.65	4.28	123.55	1628	7.49	0.46
Curve	SL	3.86	5	137.7	1211	10.07	0.83

Note: P$_N$ is photosynthetic rate; E$_s$ is isoprene emission capacity. OML = old mature leaf; SL = senescent leaf; YML = young mature leaf.

In addition, results showed lower values of photosynthesis and isoprene emission for older leaves in all light levels applied (Figure 2 a-c).

E_s obtained from the temperature response curves (Figure 1b) also showed that isoprene emission of young mature leaf was higher than E_s of older mature leaf, and higher than E_s of senescent leaf (Table 1). Young mature leaf showed the highest values of isoprene emission and photosynthesis in all leaf temperatures (Figure 2 d-f).

Light and temperature responses

Results showed that all leaf developmental stage responses of isoprene emission to varying light were similar to responses of photosynthesis, which approximates a rectangular hyperbola (Figure 2 a-c), highlighting the light dependence of isoprene emission.

Although the light response of isoprene and photosynthesis were similar, the light level at which the two processes saturated differed for all leaf ages. The light saturation of photosynthesis occurred at 1000 µmol m^{-2} s^{-1} of PPFD which was lower than light saturation of isoprene emission, so that the pattern of light-dependence for isoprene emission fails to show light saturation below 2000 µmol m^{-2} s^{-1}; emissions increased by about 5.5%, 9%, and 11% as light was raised from 1500 to 2000 µmol m^{-2} s^{-1} for older mature leaf, senescent leaf and young mature leaf, respectively (Figure 2 a-c).

The light response of isoprene for all leaf developmental stages was modeled using the light algorithm of Guenther *et al.* (1999) corrected by Monson *et al.* (2012),

$$emission\ rate = \varepsilon_0 \frac{\alpha \times C_{L_1} \times L}{\sqrt{1 + \frac{\alpha^2 \times L^2}{Cp_5^2}}} \qquad (2)$$

where ε_0 is the emission rate under standard conditions of 30 °C and PPFD equals to 1000 µmol m^{-2} s^{-1}; and α and C_{L1} are empirical coefficients; and C_{p5} is an additional coefficient for the mathematical correction proposed by Monson *et al.* (2012). For comparison, it was also shown predictions by the light function proposed by Keller and Lerdau (1999) for tropical tree species in Panama. The parameters obtained for the best fit to the data in Figure 3 correspond to values reported in Guenther *et al.* (1999) assuming LAI equals to 0.5 (leaves near the top of the canopy). The fit indicates that the attempts to model isoprene emission using the Guenther *et al.* (1999) light algorithm were successful (r^2= 0.94). However, the α obtained from the best fit to the data was different among all leaf ages. The predictions by Keller and Lerdau (1999) light function, in general, showed good agreement with the data (r^2= 0.94), however this light function overestimated isoprene emission up to 500 µmol m^{-2} s^{-1} of PPFD for all leaf ages and overestimated isoprene emission at the highest PPFD level (2000 µmol m^{-2} s^{-1}) for the senescent leaf (Figure 3).

Responses of isoprene emission to varying temperature for all leaf ages were different than photosynthesis responses (Figure 2 d-f). The temperature optimum of photosynthesis was about 37.5 °C for senescent leaf, ~32.5 °C for old mature leaf, and ~35°C for young mature leaf. In contrast, isoprene emission kept increasing exponentially with temperature, failing to exhibit an optimum even at temperatures well above photosynthesis temperature optimum.

The normalized temperature response of isoprene emission was modeled using the temperature algorithm of Guenther *et al.* (1999),

$$emission\ rate(T) = \frac{E_{opt} \times C_{T2}\ e^{C_{t1}x}}{C_{T2} - C_{T1}(1 - e^{C_{t1}x})} \qquad (3)$$

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where

$$X = \frac{\left(\frac{1}{T_{opt}}\right) - \left(\frac{1}{T_L}\right)}{R} \quad (4)$$

and T_L is leaf temperature (K), R is the gas constant (0.008314 kJ K^{-1} mol^{-1}), T_{opt} is the temperature optimum (K), E_{opt} is the emission rate (nmol m^{-2} s^{-1}) at T_{opt}, and C_{T1} (95 kJ mol^{-1}) and C_{T2} (230 kJ mol^{-1}) are empirical coefficients representing the energies of activation and deactivation, respectively. As data did not exhibit temperature optimum,

this parameter was fixed at 47 °C (320 K). Again, prediction of the modified algorithm suggested by Keller and Lerdau (1999) is shown for comparison (Figure 6).

Senescent leaf showed the highest normalized E_{opt}, hence this value was used to model the normalized isoprene emission for all leaf ages. Guenther *et al.* (1999) model showed a good fit to normalized data up ~40 ° C, after that modeled isoprene emission differed among all leaf ages. Keller and Lerdau (1999) model overestimated the isoprene emission at all leaf temperatures.
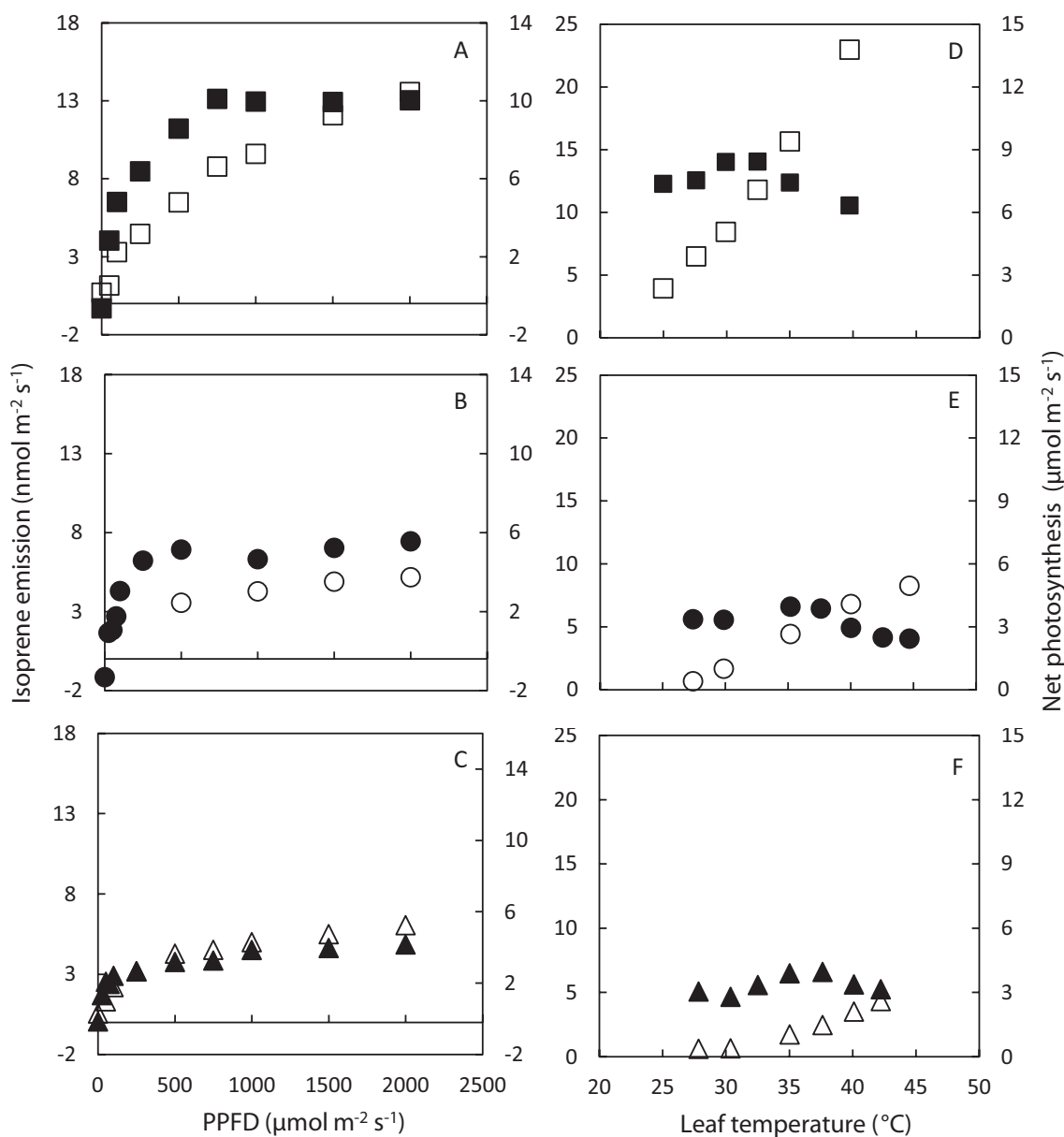

Figure 2 - Response of isoprene emission (open symbol) and net photosynthesis (dark symbol) from different leaf developmental stages of E. coriacea to variations in ligh availability and leaf temperature. Senescent leaf (▲); old mature leaf (●); young mature leaf (■).

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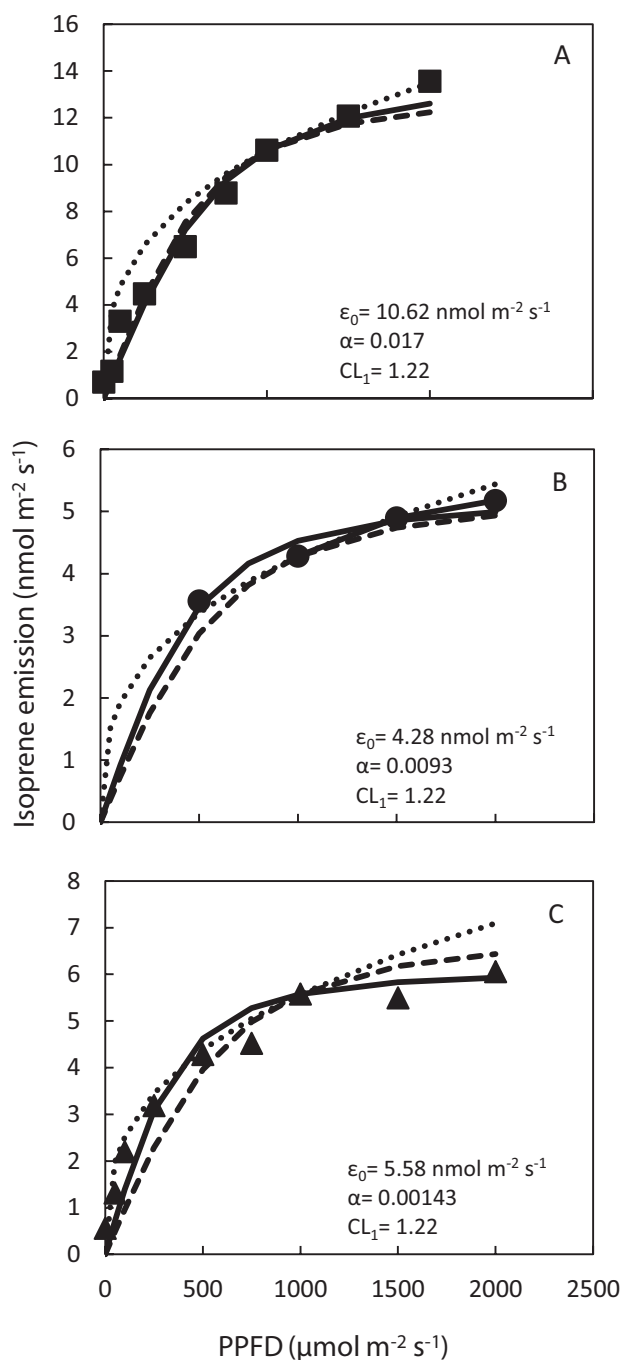

Figure 3 - Response of isoprene emission from different leaf developmental stages of E. coriacea to variations in PPFD. Parameters shown on each figure are based on non-linear least-square fits to the data (solid line) to Eq. (2). Model predictions are based on parameters (α = 0.00142 and CL1 = 1.22) suggested by Guenther et al. (1999) (dashed line); and based on the light function proposed by Keller and Lerdau (1999) (dotted line). Young mature leaf (A); old mature leaf (B); senescent leaf (C).

DISCUSSION

Isoprene emission capacity (E_s) for different developmental stages

Capacity of isoprene emission varied among different leaf ages, and the young mature leaf demonstrated the highest values of E_s. Our results are consistent with results for canopy tropical tree species *Hymenaea courbaril* in southwestern Amazon (Kuhn *et al.* 2004b), and indicate that inherent capacity of plant to synthesize isoprene changes during leaf development. This is because leaf physiological activity varies in dependence on leaf ontogenetic stage, increasing rapidly in developing leaves and decreasing in senescing leaves undergoing programmed cell death (e.g., Grassi et al. 2005; Niinemets et al. 2004).

It was observed that new leaves were beginning to flush even when older leaves were in the senescent stage, so that young mature leaves occurred mainly in the extreme top layer of crown and were exposed to very high levels of irradiance. The older non-senescent foliage gradually becomes shaded due to new foliage formation and expansion (Niinemets et al. 2006). In *Quercus ilex*, it has been demonstrated that, due to shading effects, 1-year-old leaves intercept 30% less light than the current-year leaves, and that leaves over 3-years-old of age intercept 80% less light than they intercepted during their formation (Niinemets et al. 2006). Thus, the observed reduction in isoprene emission with increasing leaf age is accompanied by a reduction in intercepted radiation, and it is difficult to separate direct effects of leaf age and effects of radiation changes. Because high light availability in the extreme top layer is accompanied by high temperatures, it is also possible to suggest a role for varying temperature.

Nevertheless, besides the changes in physiological activity due to enhanced shading, there is also evidence of lower photosynthetic activity of older foliage at any given light availability (Niinemets et al. 2006); and overall decline in leaf physiological activity is also associated with strong reductions in foliage isoprene emission rates (Lehning et al. 2001; Mayrhofer et al. 2005; Sun et al. 2009). Therefore, although the young mature leaves occur on the extreme top layer, which can shade older leaves, results showed lower values for photosynthesis and isoprene emission of older leaves at all light availability applied, suggesting that E_s resulted from reduced foliage physiological activity in dependence on leaf ontogenetic stage.

E_s of young mature leaf (8.45 nmol m^{-2} s^{-1} - from temperature response curve; 9.6 nmol m^{-2} s^{-1} - from light response curve) were on the low end of the range of E_s values reported for upper canopy tropical mature leaves from Panama, which demonstrated E_s ranging from 9 to 43 nmol m^{-2} s^{-1} for all species screened by Keller and Lerdau

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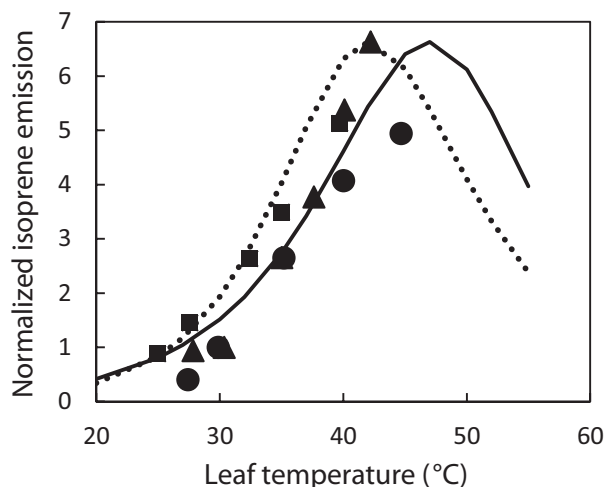developmental stages of *Eschweilera coriacea* in central Amazon

Figure 4 - Response of normalized isoprene emission (emission at 30 °C
set equal to 1.0) from different leaf developmental stages of E. coriacea to
variations in leaf temperature. Model predictions are based on parameters
suggested by Guenther et al. (1999) (solid line); and based on the algorithm
modified by Keller and Lerdau (1999) (dotted line). Senescent leaf (▲); old
mature leaf (●); young mature leaf (■).

(1999), and from 4 to 55 nmol m^{-2} s^{-1} for all species screened
by Lerdau and Throop (1999). Additionally, Harley *et al.*
(2004) showed results of E_s of mature leaf of *Mangifera indica*
equals to 66.5 µg C m^{-2} s^{-1}, and Kuhn *et al.* (2004b) showed
results of E_s of young mature leaves of *Hymenaea courbaril*
equals to 111.5 µg C m^{-2} s^{-1} in the southwestern Amazon,
which exhibited higher values of E_s than those presented for
young mature leaf in this study (14.56 µg C m^{-2} s^{-1} – from
light response curve; 13.54 µg C m^{-2} s^{-1} – from temperature
response curve).

Therefore, isoprene emission capacity does not vary only
due to leaf development, but also enzyme activity to produce
isoprene vary widely within and among species (Harley *et
al.*1999; Kesselmeier and Staudt 1999), and vary due to light
conditions and temperatures experienced by leaves in the days
preceding the measurements (Sharkey *et al.* 1999; Pétron *et
al.* 2001; Kuhn *et al.* 2004a).

Light and temperature responses

The response of isoprene emission and photosynthesis
to varying light in this study approximated a rectangular
hyperbola; this confirmed the light dependence of isoprene
production and emission, which was also verified for other
studies with tropical species (e.g. Keller and Lerdau 1999;
Lerdau and Throop 1999; Harley *et al.* 2004; Kuhn *et al.*
2004a).

Our results showed light saturation for photosynthesis (at
1000 µmol m^{-2} s^{-1} of PPFD), but did not show light saturation
for isoprene emission up to 2000 µmol m^{-2} s^{-1} (PPFD). This

observation is supported by data of Keller and Lerdau (1999),
and Lerdau and Throop (1999) who failed to observe light
saturation in some tropical species up to 2000 µmol m^{-2} s^{-1}
(PPFD). However, Harley *et al.* (2004) showed isoprene light
saturation for *M. indica* at 1500 µmol m^{-2} s^{-1}(PPFD), and
Kuhn *et al.* (2004b) revealed isoprene light saturation below
1000 µmol m^{-2} s^{-1} (PPFD) for *H. courbaril*.

Therefore, it appears that light saturation for isoprene
emission can vary between tropical species, even for species
adapted to the same light environment. However, the
reason(s) why photosynthesis saturates at lower light levels
than isoprene emission is currently not fully understood.
Grote and Niinemets (2008) suggest several possible reasons
why isoprene emission might saturate at higher irradiance
than photosynthesis, one of which is that at high light
photosynthesis may not be able to utilize all the ATP or
NADPH generated by electron transport (due to Rubisco
limitations) and that this excess remains available for isoprene
production (Niinemets *et al.*, 1999).

The light algorithm of Guenther *et al.* (1999) showed
a good fit to isoprene emission data, however the α values
were different among all leaf ages. Monson *et al.* (2012)
demonstrated that α depends on E_s and this dependence
become problematic when the isoprene emission rate vs. PPFD
dependence is used to derive α, especially in comparative
studies when leaves or species that show different E_s are
compared. In those cases, the derived value of α will not just
reflect inherent biochemical and photochemical constraints on
d(emission rate)/d(PPFD), but also will include an influential
component due to d(emission rate)/d(E_s). Therefore, as our
data showed differences among E_s of all leaf ages, changes
in the modeled α could be influenced by changes in the E_s.

Isoprene data, Guenther *et al.* (1999) algorithm and Keller
and Lerdau (1999) light function predictions agree closely at
1000 µmol m^{-2} s^{-1} (PPFD), however the Keller and Lerdau
(1999) predictions do not capture well the isoprene emission
up to 500 µmol m^{-2} s^{-1} (PPFD) and overestimated isoprene
emission at the highest level of PPFD especially for senescent
leaf. So, although Keller and Lerdau (1999) showed that this
light function for isoprene emission is adequate to upper
canopy tropical species, our results suggested that changes
in isoprene emission due to changes in leaf developmental
stages could not be captured by this light function. Thus,
more studies are needed to elucidate which changes should
be done in the models to capture leaf phenology effects on
isoprene emissions.

Besides the differences of E_s among all leaf ages, isoprene
emission vs. PPFD showed differences among all three leaf
developmental stages investigated. This indicates that the
light saturation for isoprene emission could be different
among leaf ages, demonstrating that biological changes due

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to aging can influence also on light response curve pattern of isoprene emission. These results disagree with previous results by Kuhn *et al.* (2004b) for *H. courbaril*, which state that only the inherent capacity of the plant to synthesize isoprene changes during leaf development, but not the instantaneous dependence on driving environmental factors.

It has been demonstrated that the shape of the isoprene temperature response, including the temperature optimum, depends on the rate of temperature increase (Singsaas and Sharkey 2000; Rasulov *et al.* 2010). However, the shape of the isoprene temperature response of this study agrees with data of Keller and Lerdau (1999) and Lerdau and Throop (1999), which showed that isoprene emission did not decrease until ~40 °C for different tropical species.

It was impossible to determine a distinct temperature optimum from our data, but on basis of data from old mature leaf it seems that the temperature optimum of isoprene emission of *E. coriacea* is above 45 °C, which is at least 10 °C higher than the temperature optimum for photosynthesis. These results differ from those presented by others studies with tropical species that suggested isoprene temperature optimum at about 40 °C (Lerdau and Keller 1997; Harley *et al.* 2004). This could mean that temperature optimum for isoprene emission of tropical species may vary considerably, so further field studies with different tropical canopy species are required to identify isoprene temperature optimum.

If the temperature optimum for isoprene emission is higher than 45 °C for *E. coriacea*, this may confer greater thermal protection, consistent with a role for isoprene in enhanced thermotolerance (Singsaas *et al.* 1997). Given that higher temperatures are expected in the coming decades under global warming, it is possible that species with high isoprene emissions and with high temperature optimum may be favored. However, more studies are required to support this discussion, since it was demonstrated that temperature optimum of isoprene emission is influenced by the rate of temperature rising and by the substrate availability (Rasulov *et al.* 2010).

The temperature algorithm of Guenther *et al.* (1999) did not show a good fit to normalized data at temperatures above ~40°C. Additionally, the algorithm modified by Keller and Lerdau (1999) overestimated the isoprene emission at all leaf temperatures. This is because our data did not show isoprene temperature optimum for any leaf developmental stages. Therefore, more studies will be necessary to identify the temperature optimum for different leaf developmental stages of this species and for others, since this could help to better understand the potential of Guenther *et al.* (1999) to model isoprene emission vs. temperature for tropical species.

CONCLUSIONS

Leaf ageing caused a reduction of both isoprene emission capacity and photosynthetic rates, which was attributed to reduction in metabolic activity. Although the data fit on the basis of the light algorithm (Guenther *et al.,* 1999) was satisfactory, the fit parameter α was different among all three leaf ages, likely due to differences in E_s. Moreover, because isoprene emissions from all leaves studied continued to increase with increasing leaf temperature, no optimum temperature was observable, but instead showed isoprene emissions continuing to increase up to 45 °C which is significantly higher than previously reported for tropical tree species. Thus, the temperature algorithm of Guenther *et al.* (1999) did not fit to normalized data well at temperatures above ~40°C for any leaf age. Therefore, in order to improve models that predict isoprene emission from tropical forest ecosystems, more experimental information is needed on how species-specific isoprene emission capacity changes during leaf developmental stages, and to identify the temperature optimum of isoprene emissions.

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