Title: Methanol and Isoprene Emissions from the Fast Growing Tropical Pioneer Species *Vismia guianensis* (Aubl.) Pers. (Hypericaceae) in the central Amazon Forest

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Key Points

- High light-dependent isoprene emissions were observed from mature *V. guianensis* leaves in the central Amazon
- As predicted by energetic models, isoprene emission increased non-linearly with net photosynthesis
- High leaf temperatures resulted in the classic uncoupling of net photosynthesis from isoprene emissions
- Leaf phenology differentially controls methanol and isoprene emissions

Abstract

Isoprene (Is) emissions by plants represent a loss of carbon and energy resources leading to the initial hypothesis that fast growing pioneer species in secondary tropical forests allocate carbon primarily to growth at the expense of isoprenoid defenses. In this study, we quantified leaf isoprene and methanol emissions from the abundant pantropical pioneer tree species *Vismia guianensis* and ambient isoprene concentrations above a diverse secondary forest in the central Amazon. As photosynthetically active radiation (PAR) was varied (0 to 3,000 µmol m^{-2} s^{-1}) under standard leaf temperature (30 °C), isoprene emissions from *V. guianensis* increased without saturation up to 80 nmol m^{-2} s^{-1}. A non-linear increase in isoprene emissions with respect to net photosynthesis (Pn) resulted with the fraction of Pn dedicated to isoprene emissions increasing with light intensity (up to 2% of Pn). Emission responses to temperature under standard light conditions (PAR of 1,000 µmol m^{-2} s^{-1}) resulted in the classic uncoupling of isoprene emissions ($T_{opt,iso} > 40$ ºC) from net photosynthesis ($T_{opt, Pn} = 30.0$-32.5 °C) with up to 7% of Pn emitted as isoprene at 40 °C. Under standard environmental conditions of PAR and leaf temperature, young *V. guianensis* leaves showed high methanol emissions, low Pn, and low isoprene emissions. In contrast, mature leaves showed high Pn, high isoprene emissions, and low methanol emissions, highlighting the differential control of leaf phenology over methanol and isoprene emissions. High daytime ambient isoprene concentrations (11 ppbv) were observed above a secondary Amazon rainforest suggesting that isoprene emissions are common among neotropical pioneer species. The results are not consistent with the initial hypothesis and support a functional role of methanol during leaf expansion and the establishment of photosynthetic machinery, and a protective role of isoprene for photosynthesis during high temperature extremes regularly experienced in secondary rainforest ecosystems.

Keywords: Pioneer species, tropical forest, growth and defense, volatile isoprenoids

1. Introduction

Due to its vast territorial expansion, high species diversity, and long growing season, the Amazon forest in South America is responsible for an estimated 15% of global terrestrial photosynthesis (Malhi et al., 2008). However, increased deforestation, degradation and natural disturbances have changed this scenario, exerting strong control on the evolution of atmospheric CO_2 (Pan et al., 2011;Malhi et al., 2008). A recent analysis of biomass dynamics revealed a long-term trend of increased mortality-driven shortening of carbon residence times in the Amazon forest (Brienen et al., 2015). This effect has been attributed to increased climate variability, as recurrent drought episodes occurred in the region (Phillips et al., 2009;Lewis et al., 2011). Amazon carbon sink suppression during the intense drought period in 2005 was associated with a decrease in biomass gain and increased vegetation mortality (Phillips et al., 2009). Changes in forest turnover rate can directly affect forest composition and structure; the creation of forest gaps leads to the release of suppressed trees and increased pioneer species recruitment rates (Bugmann, 2001). Tropical forest regrowth has been identified as a strong terrestrial carbon sink that can partly counterbalance carbon losses by deforestation and forest degradation (Pan et al., 2011). If tropical forests are becoming more dynamic, gap-phase processes can therefore play a more central role in determining carbon residence times, which have been described as the largest uncertainty in terrestrial vegetation responses to climate and elevated CO_2 (Friend et al., 2014).

The classic Neotropical pioneer genera *Vismia* and *Cecropia* dominate large rainforest disturbance gaps in the Amazon Basin (Chambers et al., 2009) where they help accelerate the regeneration of secondary forests by influencing forest successional pathways (Uhl et al., 1988;Vieira et al., 2003;Zalamea et al., 2008). Their success in secondary forests is related to their ability to maintain high rates of net photosynthesis (Pn) and growth under conditions of full sunlight, high leaf temperatures, and low nutrient availability, often characteristic of tropical landscapes impacted by natural (Chambers et al., 2009) and human (Mesquita et al., 2001) disturbances. Under optimal environmental conditions for photosynthesis, emissions of volatile isoprenoids by leaves of many plant species can account for a few percent of Pn (Kesselmeier et al., 2002). However, under stress conditions that diminish Pn but increase isoprene (Is) emissions such as high leaf temperatures, emissions of Is can account for over 50% of Pn (Jardine et al., 2014). While investments into Is production remains poorly understood among tropical plants (Harley et al., 2004), the pattern of the photosynthetic carbon allocation has been discussed through carbon-nutrient balance and growth-differentiation balance hypotheses (Stamp, 2004;Glynn et al., 2007;Massad et al., 2012). These hypotheses predict the existence of trade-off between investment in growth *versus* plant defense.

Thus, a hypothesis can be considered that fast growing pioneer tree species in secondary forests do not produce volatile isoprenoids as secondary metabolites and instead dedicate these carbon and energy resources to primary metabolites for enhanced biomass production and growth or increased catabolism for energy generation during growth and maintenance respiration. However, this hypothesis is not well supported in the literature as early successional pioneer species have been observed with high volatile isoprenoid emission rates (Klinger et al., 1998;Jardine et al., 2015).

An alternative hypothesis that is well supported in the literature is that investment of carbon and energy resources into Is and monoterpene production and emissions by secondary forest species protects photosynthesis during abiotic stress including high temperature stress, possibly through antioxidant and energy/reducing equivalent consumption mechanisms (Vickers et al., 2009a;Vickers et al., 2009b;Jardine et al., 2012b;Penuelas and Llusia, 2002;Grote et al., 2014;Loreto and Velikova, 2001). Additional studies revealed possible connections between volatile isoprenoid emissions and increased photorespiration during high leaf temperatures (Jardine et al., 2014) and drought stress (Dani et al., 2014). Consistent with these potentially important functional roles for pioneer species, a leaf and branch survey at four neo-tropical sites (Harley et al., 2004) suggested that *Vismia guianensis* is an Is producer with a single leaf emission value of 48 µg C g^{-1} hr^{-1} reported (or 11 nmol m^{-2} s^{-1} assuming a specific leaf area of 20 m^2 kg^{-1}) (Dias-Filho, 1995). A survey of Is emissions from tropical central Africa suggested that Is emissions are higher in early successional forest communities relative to primary forests (Klinger et al., 1998). In addition to Is, more recent field studies of *Cecropia sciadophylla* in the Amazon suggest that not only can a fast growing tropical pioneer tree species emit volatile isoprenoids, but that leaf emission rates of highly reactive monoterpenes such as cis and trans-β-ocimene were among the highest yet observed from trees globally (Jardine et al., 2015).

Although volatile isoprenoid emissions are highly species specific, methanol emissions appear to be a universal feature in plants attributed to the hydrolysis of cell wall methyl esters during changes in cell wall chemical and physical properties (Fall, 2003;Fall and Benson, 1996). Leaf methanol emissions have been shown to closely correlate with plant growth rates, especially at the early stages of leaf development with young leaves consistently observed to be higher emitters than mature leaves (Hüve et al., 2007;Nemecek-Marshall et al., 1995). In contrast, Is emission capacity has been reported to increase considerably throughout leaf development in close connection with photosynthetic capacity (Alves et al., 2014). However, phenological controls on both methanol and Is emissions have not been studied together in tropical plants.

As a part of Observations and Modeling of the Green Ocean Amazon (GoAmazon 2014/5) (Martin et al., 2015), we hypothesized that despite the high carbon and energy costs that could otherwise be used for growth and maintenance, the highly abundant pantropical pioneer tree species *V.*

guianensis dedicates a significant fraction of recent photoassimilated carbon to volatile isoprenoid emissions, due to their protective properties under abiotic stress. We further hypothesized that this fraction changes as a function of light intensity due to the well-documented light suppression of mitochondrial respiration at low light (e.g. the Kok effect) which results in a large increase in Pn but only a relatively small increase in gross photosynthesis (Sharp et al., 1984). In addition, recent mechanistic Is emission models suggest that during high-light conditions where Pn is light-saturated, emissions of Is (and other volatile isoprenoids) may continue to increase with increasing light due to increased excess available energy and reducing power for the methylerythritol 4-phosphate (MEP) pathway (Grote et al., 2014). Consistent with this model, a survey of tropical vegetation revealed strong light-dependent Is emission rates 2-3 times higher than those of temperate species (Lerdau and Keller, 1997). Moreover, tropical leaves did not demonstrate a light-saturation in Is emissions which continued to increase with light up to 2,500 mol m^{-2} s^{-1}, the highest PAR fluxes studied (Lerdau and Keller, 1997). Thus, although biochemical and modeling studies have identified mechanisms expected to cause significant deviations between a constant linear ratio of volatile isoprenoid emissions and Pn as a function of light, experimental observations in the tropics are extremely limited to investigate these processes. Moreover, as laboratory studies have shown the classic uncoupling between net photosynthesis and Is emissions occurs in tropical plants at high leaf temperatures, little *in situ* information exists on this phenomenon in the tropics.

Here we first present new *in situ* observations during 2014 of leaf Is emissions and Pn as a function of light intensity from *V. guianensis* in the central Amazon together with a reanalysis of light-dependent monoterpene emissions from *C. sciadophylla* leaves in relation to Pn (Jardine et al., 2015). Second, we present the results leaf Is emissions and Pn responses in *V. guianensis* as a function of leaf temperature during 2015. Third, by taking advantage of the rapidly developing leaves of *V. guianensis*, we also test the hypothesis that leaf phenology differentially impacts methanol versus isoprenoid emissions. Finally, in order to further evaluate the potential for secondary tropical forests to be important atmospheric sources of isoprene, we present limited measurements of ambient daytime Is concentrations above a secondary rainforest ecosystem in the central Amazon. We end by discussing the potential physiological roles of volatile isoprenoids and methanol in secondary tropical rainforest ecosystems.

2. Material and methods

In this study, seven individuals of *Vismia guianensis* (Aubl.) Pers., a pioneer tree species from the Hypericaceae family, were studied in the Reserva Biológica do Cuieiras (ZF2), a primary rainforest biological reserve located approximately 60 km northwest of Manaus, in the central Amazon Basin, Brazil. This reserve has an area of primary rainforest of roughly 230 km² and is managed by the National Institute for Amazon Research (INPA). A nearby secondary rainforest ecosystem (ZF3 reserve), located approximately 105 km northwest of Manaus, was also studied for ambient concentrations of Is at the top of the canopy (~25 m) as a part of the biological dynamics of forest fragments project (Gascon et al., 2001) (See **Fig. 1**).

2.1 Ambient Concentrations of Is above the Secondary Forest Canopy

Six ambient air thermal desorption tube samples (150 ml/min for 15 min) were sequentially collected at the canopy height of 25 m on a walk up tower at the ZF3 site (coordinates: 02º 23’26.5” S and 59º53’0.7” W) on 23 April 2015 between 11:38-13:18. No samples were collected on two additional thermal desorption tubes for background analysis. The thermal desorption tubes were purchased commercially and filled with Quartz wool, Tenax TA, and Carbograph 5TD adsorbents (Markes International, UK) and analyzed for Is concentrations using a thermal desorption system interfaced with a gas chromatograph-mass spectrometer system (GC-MS) at INPA in Manaus, Brazil as previously described (Jardine et al., 2014).

2.2 Emission Responses to Light

Emission responses to light for *V. guianensis* leaves under constant leaf temperature (30 °C) and reference [CO_2] (400 ppm) were collected both in the field on intact branches and in the lab on detached branches during July of 2014. Field observations of Is emissions and Pn for *V. guianensis* leaves as a function of PAR intensity (0-2000 μmol m^{-2} s^{-1}) under constant leaf temperature (30 ºC) were based on the coupling of the LI-6400XT with a portable thermal desorption tube sample collector as previously described (Jardine et al., 2015). Briefly, PAR values of 0, 100, 250, 500, 1000, 2000 μmol m^{-2} s^{-1} were established for 10 min each with a thermal desorption tube sample collected for each light level (75 ml min^{-1} x 10 min). Blank tubes were also collected without a leaf in the enclosure at the beginning of the experiment when the light level was 0 μmol m^{-2} s^{-1}. Is emissions were quantified using a thermal desorption GC-MS as previously described (Jardine et al., 2014). Emission responses to light were collected for two young and two young-mature leaves from intact branches in the field (one individual). In addition, light induced emission responses of detached branches (two individuals) were analyzed in the laboratory using PTR-MS. For these laboratory experiments, an additional light level of 3000 µmol m^{-2} s^{-1} PAR was included to evaluate the saturation of Pn and Is emissions at high light intensities. Both the GC-MS and PTR-MS systems were calibrated to Is using dynamic dilution of a commercial compressed gas standard (2.0 ppm Is, Apel-Riemer Environmental). Monthly GC-MS calibrations through a period that encompassed the July 2014 and 2015 field experiments (Nov 2013 - July 2015) demonstrated a high precision of Is quantitation by GC-MS; Is m/z 67 calibration slopes showed a relative standard deviation of 19.2%.

2.3 Emission Responses to Temperature

Emission responses to temperature for *V. guianensis* leaves under constant PAR (1000 µmol m^{-2} s^{-1}) and reference [CO_2] (400 ppm) were collected on intact branches and in the field during July 2015. Field observations of Is emissions and Pn for *V. guianensis* leaves as a function of leaf temperature were conducted using the combined LI-6400XT/GC-MS system as described in section 2.2. Leaf temperatures (25, 27.5, 30.0, 32.5, 35, 37.5, and 40 ºC) were established for 10 min each with a thermal desorption tube sample collected for each temperature (50-75 ml min^{-1} x 10 min). Blank tubes were also collected without a leaf in the enclosure at the initial temperature of 25 ºC at the beginning of the experiment. Is emissions and Pn were quantified from 5 young-mature leaves from intact branches in the field (one leaf per individual).

2.4 Pn combined with Is and methanol emissions as a function of leaf age in *V. guianensis*

In addition to being highly abundant in disturbed Amazon secondary forests (Mesquita, 2000), *V. guianensis* was selected because of its high leaf development rates, which produce two new apposing leaves roughly every month (G. Martins, personal communication). Leaves used were classified in three stages: young, young-mature, and mature. For all plants, young leaves occurred at the top of the branch or the first leaf stage, young-mature leaves occurring in the second leaf stage, and mature leaves occurring in the third leaf stage. For each leaf age experiment conducted during July 2014 in the field laboratory (six total individuals, one experiment per day), large branches roughly 1 m tall were detached from the tree around noon and immediately placed and recut in tap water before being transported to the field laboratory and analyzed for gas exchange within 15 minutes of being cut. Upon arriving in the laboratory, branches were placed under an LED plant growth light with between 300-600 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at branch height. As air temperature of the laboratory was roughly 25 ºC, the PAR and air temperature environment in the laboratory was likely lower than the natural conditions under which the branch was removed. Leaf gas exchange measurements were initiated by placing a young leaf (first leaf stage) in the enclosure of a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA) interfaced with a proton transfer reaction – mass spectrometer (PTR-MS, Ionicon Analytik, Austria) as previously described (Jardine et al., 2014). Is and methanol emissions were quantified using the mass to charge ratios m/z 69 and 33 respectively using PTR-MS, while Pn, stomatal conductance, and transpiration rates were quantified using the LI-6400XT. For each leaf age experiment, a single young leaf (first leaf stage), young-mature leaf (second leaf stage), and mature leaf (third leaf stage) were sequentially placed inside the chamber for 15 min each. Before and after each leaf measurement, background measurements were collected for several minutes with an empty leaf chamber. Thus, the time required for each leaf age experiment was roughly one hour beginning around noon, during July of 2014. These leaf measurements were carried out under constant PAR flux (1000 µmol m^{-2} s^{-1}), leaf temperature (30 °C), and reference [CO_2] (400 ppm).

3 Results and Discussion

3.1 Ambient Concentrations of Is above the Secondary Forest Canopy

To evaluate for the first time the potential role of secondary forests as source of Is to the lower tropical troposphere, we measured daytime ambient concentrations of Is at the top of a ~25 m canopy in the ZF3 rainforest fragment site (Gascon et al., 2001). Daytime ambient Is concentrations above secondary forest canopy at ZF3 where high (>10 ppb) and increased from 10.0 ppb at 11:38 to 10.9 ppb by 12:30. This was followed by a decrease to 10.5 ppb by 13:03, possibly due to the reduction of light and temperature from afternoon cloud formation. As Is concentrations from primary forests in the Amazon have been reported between 6-10 ppb (Jardine et al., 2012b;Karl et al., 2009), these observations are consistent with the idea that tropical secondary forests represent an important source of Is in the lower troposphere.

3.2 Emission Responses to Light

In order to investigate the possibility that the highly abundant pantropical pioneer species *V. guianensis* dedicates a significant fraction of Pn to volatile isoprenoid emissions to the atmosphere, we first conducted controlled light experiments on intact branches in the field using a new portable photosynthesis and volatile organic compound emission system based on thermal desorption GC-MS (Jardine et al., 2015). The results show that during the 2014 rainy season in the central Amazon (13-May-2014), light-stimulation of Pn up to 15 μmol m^{-2} s^{-1} in young-mature leaves were associated with Is emissions which continued to increase with light up to the maximum PAR intensity (2000 μmol m^{-2} s^{-1}) where emission rates were 30 nmol m^{-2} s^{-1}. These emission rates are higher than those reported by Harley *et al.*, 2004 of roughly 11 nmol m^{-2} s^{-1} from a *V. guianensis* leaf in the National Forest Tapajós, near Santarém, Brazil (Harley et al., 2004). This emission rate is similar however, with Is emissions observed by PTR-MS in this study during the 2014 dry season which ranged from 20-45 nmol m^{-2} s^{-1} under standard conditions of PAR and leaf temperature, and up to 80 nmol m^{-2} s^{-1} at maximum PAR fluxes of 3000 μmol m^{-2} s^{-1} (see **Fig. 2**).

Although GC-MS results confirm that *V. guianensis* is a strong Is emitting species, Is collections on thermal desorption tubes at each environmental light level represent the average emission rate during each 10 min sample collection. Therefore, to analyze the relationship between Pn and Is emissions as a function of PAR in greater temporal detail, real-time PTR-MS measurements of Is emissions were collected simultaneously with real-time Pn measurements (**Fig. 2a**). The PTR-MS system was installed in the field laboratory and detached branches of *V. guianensis* growing just outside of the laboratory were utilized. Similar to the GC-MS measurements, *V. guianensis* showed negligible Is emissions in the dark where Pn was negative (likely due to mitochondrial respiration and the absence of photosynthesis). Moreover, upon first switching on the light, Pn sharply increased from slightly negative in the dark to around 5.0 μmol m^{-2} s^{-1} at a PAR flux of 100 μmol m^{-2} s^{-1}. With every increase in PAR up to the maximum of 3000 μmol m^{-2} s^{-1}, Is emissions continued to increase without any sign of saturation. In contrast, although Pn also increased with PAR, it essentially saturated at PAR fluxes above 2000 μmol m^{-2} s^{-1}. Thus, when carbon flux emitted as Is was plotted against Pn, a strong non-linear relationship was observed (**Fig. 2b**). As Pn increased with PAR, the fraction of Pn dedicated to Is emissions increased up to 1.9%. This non-linear effect could not be explained by an increase in leaf temperature as PAR increased; throughout the range of PAR values, leaf temperatures remained between 30 +/- 1 ºC.

In light of the non-linear relationship between the fraction of Pn dedicated to Is emissions and Pn for the pioneer species *V. guianensis*, we analyzed previously reported light-dependent monoterpene data from the abundant pantropical pioneer species *C. sciadophylla* for a similar non-linear relationship (Jardine et al., 2015). The results of the *C. sciadophylla* reanalysis also revealed a strong non-linear relationship between the fraction of Pn emitted as monoterpenes and Pn during controlled light experiments (graph not shown). The fraction of Pn dedicated to monoterpene emissions continued to increase with PAR up to 1.9% at maximum PAR. Thus, both *V. guianensis* and *C. sciadophylla* dedicate roughly 2% of Pn to volatile isoprenoid emissions at 30 °C leaf temperature and show a strong increase in the fraction of Pn dedicated to volatile isoprenoid emissions as PAR increases. These observations are consistent with a growing body of evidence that the fraction of assimilated carbon transformed to volatile isoprenoids increases with leaf energetic status (including high light and low atmospheric CO_2 concentrations) (Morfopoulos et al., 2014). While not captured by common Is emission algorithms, the increased allocation of Pn to volatile isoprenoid emissions is captured by energetic models of Is emissions (Morfopoulos et al., 2014). These observations imply that the functional roles of volatile isoprenoids are particularly important under high light conditions and could potentially be explained by a competition between photosynthesis and the MEP pathway for adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) generated by the light reactions of photosynthesis (Grote et al., 2014).

Under low light conditions, the Benson-Calvin cycle dominates the consumption of ATP and NADPH resulting in relatively large increases in Pn with a correspondingly small increase in volatile isoprenoid production. In contrast, under light saturated conditions for Pn, excess ATP and NADPH are consumed by the MEP pathway resulting in a relatively small increase in Pn with a correspondingly large increase in volatile isoprenoid production. Finally, the Kok effect may further contribute to this non-linear relationship at low light levels (Sharp et al., 1984). Low PAR fluxes around the light compensation point for Pn have been shown to partially suppress mitochondrial respiration which results in a relatively small increase in gross photosynthesis and a correspondingly large increase in Pn. Thus, at low light levels, this would further contribute to a relatively large increase in Pn with a correspondingly small increase in Is emissions.

3.3 Emission Responses to Temperature

A strong uncoupling of Is emissions and Pn was observed as a function of leaf temperature in each of the *V. guianensis* leaves studied from 5 individuals (**Fig. 3**). For 3 of the 5 leaves, Pn increased together with temperature and showed a clear optimum temperature of 30-32.5 °C and decreased at higher temperatures. The other two leaves showed decreases in Pn as temperatures increased above 25 °C. Thus, a relatively high standard deviation occurred at the lowest leaf temperature (25 °C) and a clear optimum in Pn between 30-32.5 °C was generally not observable from the average. Nonetheless, above 30-32.5°C, all 5 leaves showed a strong decrease in Pn.

In contrast, Is emissions from all 5 leaves increased with leaf temperatures above 25 ºC; Is emissions continued to increase even while Pn was strongly suppressed up to the highest leaf temperatures studied (40 ºC). Therefore, distinct temperature optima for Pn (30.0-32.5 ºC) and Is (>40 ºC) exists for *V. guianensis* leaves. This classic uncoupling has been shown to be influenced by the use of ‘alternate’ Is carbon sources including potential extrachloroplastic substrates (Rosenstiel et al., 2004;Loreto et al., 2004;Karl et al., 2002) as well as the integration of photorespiratory substrates into the Calvin Cycle and the re-assimilation of internally produced CO_2 (e.g. respiration, photorespiration) (Jardine et al., 2014;Jardine et al., 2010). At the highest leaf temperature studied (40 ºC), 7% of the Pn on average was emitted from *V. guianensis* leaves in the form of Is.

Interestingly, *V. guianensis* also produces large quantities of red latex as an herbivore deterrent (Almeida-Cortez and Melo-de-Pinna, 2006). Although latex, or cis-polyisoprene, is produced within the cytosol by the mevalonate pathway whereas Is is produced within the chloroplasts by the MEP pathway, exchange of common intermediates such as isopentenyl diphosphate (IDP) may occur (Chow et al., 2007). These data build on previous studies in Asia, which demonstrated that latex producing trees can be strong emitters of volatile isoprenoids (Wang et al., 2007;Baker et al., 2005).

3.4 Pn combined with Is and methanol emissions as a function of leaf age in *V. guianensis*

A well-defined pattern of photosynthesis and Is /methanol emissions was observed according the leaf age of *V. guianensis* as shown in real-time during two example leaf age experiments (**Fig. 4**) and as an average of six leaf age experiments (**Fig. 5**). The results show that young leaves had low Pn rates (2.7 +/- 2.2 μmol m^{-2} s^{-1}) high methanol emissions (24 +/- 13 nmol m^{-2} s^{-1}) but low to undetectable Is emissions (2 .1 +/- 0.2 nmol m^{-2} s^{-1}). In contrast, young-mature leaves had high Pn rates (12.9 +/- 5.2 μmol m^{-2} s^{-1}) low methanol emissions (3.3 +/- 1.5 nmol m^{-2} s^{-1}) but high Is emissions (35.7 +/- 9.1 nmol m^{-2} s^{-1}). In addition, mature leaves also showed the same pattern as young-mature leaves with high Pn rates (10.6 +/- 5.2 μmol m^{-2} s^{-1}) low methanol emissions (3.0 +/- 1.1 nmol m^{-2} s^{-1}) but high Is emissions (39.8 +/- 6.0 nmol m^{-2} s^{-1}).

There is a wide range of morphological characteristic, chemical composition and physiological activities of leaves depending on the developmental stage of the plant or tissue (Forrest and Miller-Rushing, 2010;Richardson et al., 2013). In the case of photosynthesis, increased light harvesting components, electron transport rates, and carboxylation efficiency occur in parallel with the growth and development of leaves (Reich et al., 2009;Kikuzawa, 1995;Merilo et al., 2009). The lower Pn rates observed in young *V. guianensis* leaves compared to mature leaves (**Fig. 5**) is a pattern that is routinely observed in numerous studies (Alves et al., 2014;Reich et al., 1991). The relationship between photosynthetic activity and leaf development can be explained, in part, by the development of chloroplasts (Massad et al., 2012;Hikosaka, 2003). Previous studies have confirmed increased levels of amino acids, proteins, nucleic acids and pigments during leaf development, together with greater rates of carboxylation (Lohman et al., 1994;Buchanan-Wollaston and Ainsworth, 1997;Egli and Schmid, 1999). Is from *V. guianensis* leaves observed in this study also followed a similar developmental pattern with that of Pn rates. This result is consistent with previous observations that in the early stages of leaf growth, photoassimilates tend to be partitioned towards growth compounds at the expense of defense compounds (Massad et al., 2012). This behavior can partially be explained by a limitation in substrate for Is in young leaves because photosynthesis is one of the main processes responsible for providing the required carbon intermediates, reducing equivalents, and ATP needed to produce dimethylallyl pyrophosphate (DMAPP) required for Is biosynthesis (Loivamaki et al., 2007;Sun et al., 2013). On the other hand, the demand for DMAPP is very high during leaf expansion because this compound is essential for the synthesis of all plant isoprenoids including photosynthetic pigments (Hannoufa and Hossain, 2012;Domonkos et al., 2013;Eisenreich et al., 2004;Opitz et al., 2014). Thus, due to the limited availability of DMAPP in young rapidly expanding leaves, a competition occurs for this substrate such that a larger fraction is allocated towards photosynthetic pigments (Rasulov et al., 2014). However, once the photosynthetic machinery is in place, a larger fraction of DMAPP may be dedicated to Is production and emissions (Rasulov et al., 2014). Once the photosynthetic machinery is established, high Is production rates may help protect against photoinhibition and photooxidation by consuming excess energy and reducing equivalents during conditions of light saturation for photosynthesis. Moreover, the antioxidant properties of Is have been well demonstrated (Jardine et al., 2012a;Vickers et al., 2009a;Loreto et al., 2001;Affek and Yakir, 2002). Is production lowers lipid peroxidation, quenches reactive oxygen species, and protects photosynthesis under oxidative stress (Loreto and Velikova, 2001). Previous studies have demonstrated the protective role of Is for photosynthesis during high leaf temperature stress (Sharkey et al., 2001) and a potential antioxidant mechanism was supported by the detection of Is oxidation products for high-temperature stress (Jardine et al., 2012a;Jardine et al., 2013).

In contrast to Pn and Is, emissions of methanol from *V. guianensis* leaves were eight times higher in young leaves than in mature leaves (**Fig. 5**), similar to patterns found in the literature from leaves of mid-latitude trees (Harley et al., 2007;Nemecek-Marshall et al., 1995;Hüve et al., 2007). Emissions of methanol have been closely associated with leaf growth rates (Hüve et al., 2007) caused primarily by the expansion of cell walls (Fall, 2003). As a consequence, young rapidly expanding leaves have consistently been observed to have higher methanol emissions than mature leaves (Hüve et al., 2007;Nemecek-Marshall et al., 1995). Methanol production is thought to be initiated during pectin demethylation reactions catalyzed by the enzyme pectin methylesterase (Bai et al., 2014). In this reaction, the hydrolysis of galacturonic acid methyl esters strengthens the cell wall while liberating methanol as a by-product (Hüve et al., 2007;Harley et al., 2007;Bai et al., 2014;Hanson and Roje, 2001). High rates of methanol emission may be associated with higher catalytic activity of this enzyme (Galbally and Kirstine, 2002;Hüve et al., 2007). In addition to growth processes, high methanol emissions have also been observed during stress and senescence processes (Cosgrove, 2005, 1999), possibly also mediated by pectin demethylation reactions during physicochemical changes to cell walls. Thus, the high methanol emissions from young leaves of *V. guianensis* may be due to both growth and stress processes.

3.5 Potential Roles of Volatile Isoprenoids and Methanol in Secondary Tropical Ecosystems

High emission rates of volatile isoprenoids have been observed from dominant central Amazon pioneer species including *V. guianensis* (this study) and *C. sciadophylla* (Jardine et al., 2015) as well early successional species in central Africa (Klinger et al., 1998). Although a systematic survey of pioneer tree species in the tropics is needed, the potential for widespread occurrence of Is emissions from secondary forest tree species is supported by the single mid-day set of observations of high ambient Is concentrations (11.0 ppbv) above a diverse secondary rainforest canopy in the central Amazon. The observations of increased Pn allocation to volatile isoprenoid emissions as a function of light intensity provides additional support for a functional role of volatile isoprenoid biosynthesis in minimizing photoinhibition by consuming excess photosynthetic energy and reducing equivalents (Morfopoulos et al., 2014) as well as other potential direct and indirect antioxidant activities (Jardine et al., 2012a;Vickers et al., 2009a;Velikova and Loreto, 2005).

The non-linear relationship between Is emissions and Pn in *V. guianensis* leaves is consistent with a suppression of mitochondrial respiration at low light (Sharp et al., 1984) and an increased dedication of photoassimilated carbon to Is biosynthesis via the methylerythritol 4-phosphate (MEP) pathway under light saturating conditions of Pn, possibly due to the utilization of excess available energy and reducing equivalents (Morfopoulos et al., 2014). These observations are consistent with volatile isoprenoids offering substantial protection to the photosynthetic machinery against photoinhibition and oxidative damage under stress conditions such as high-light and leaf temperature environments that are regularly experienced by secondary forests. In the case of *V. guianensis*, we observed that Is emissions and photosynthesis rates increase together throughout leaf development while methanol emissions decreased. Although not easily distinguished in the present data set due to the low temporal resolution of leaf development observations (roughly monthly resolved ages categorized into young, young-mature, and mature), Pn has been shown to proceed Is emissions in young developing leaves by several days to several weeks (Monson et al., 1994;Kuzma and Fall, 1993;Grinspoon et al., 1991). Methanol emission patterns are also consistent to previous observations which have shown strong positive relationships between leaf expansion rates and methanol emissions (Grinspoon et al., 1991). Similar observations have been made at the ecosystem scale in a mixed hardwood forest in northern Michigan, US during the spring growing season where a strong enhancement in ecosystem emissions of methanol were observed together with an increase in leaf area index (Karl et al., 2003).

4. Conclusion

The findings of this study show that abundant secondary rainforest tree species (e.g. *V. guianensis* and *C. sciadophylla*) contribute high emissions of volatile isoprenoids to the atmosphere that represent up to 2% of Pn under the standard leaf temperature of 30 ºC and up to 7% under 40 ºC, the carbon and energy costs notwithstanding. Thus, the hypothesis that fast growing pioneer tree species in secondary tropical forests do not produce volatile isoprenoids, and instead dedicate these carbon and energy resources to enhanced growth and respiration demands, is not supported.

High emission rates of volatile isoprenoids have been observed from dominant central Amazon pioneer species including *V. guianensis* (this study) and *C. sciadophylla* (Jardine et al., 2015) as well early successional species in central Africa (Klinger et al., 1998). Although a systematic survey of pioneer tree species in the Amazon is needed, the potential for widespread occurrence of Is emissions from secondary forest tree species is supported by the single mid-day set of observations of high ambient Is concentrations (11.0 ppbv) above a diverse secondary rainforest canopy in the central Amazon. The observations of increased Pn allocation to volatile isoprenoid emissions as a function of light intensity provides additional support for a functional role of volatile isoprenoid biosynthesis in minimizing photoinhibition by consuming excess photosynthetic energy and reducing equivalents (Morfopoulos et al., 2014) as well as other potential direct and indirect antioxidant activities (Jardine et al., 2012a;Vickers et al., 2009a;Velikova and Loreto, 2005). Together with previous studies, our observations support a functional role for methanol production during cell wall expansion during growth (Fall, 2003) and the establishment of photosynthetic machinery and a defense role for volatile isoprenoid production to help protect this photosynthetic machinery against the abiotic stresses (Vickers et al., 2009a) that are commonly experienced in secondary rainforest ecosystems.

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The data used in this manuscript is available for download for research and educational purposes at the following web link maintained by Lawrence Berkeley National Laboratory (https://www.dropbox.com/sh/hr5ucem69m6dbhq/AABIK5QrnfRMg4UhNLWnAJYya?dl=0). We acknowledge the support from the Central Office of the Large Scale Biosphere Atmosphere Experiment in Amazonia (LBA), the Instituto Nacional de Pesquisas da Amazonia (INPA), and the Universidade do Estado do Amazonia (UEA). We would like to especially thank INPA researchers Giordane Martins and Ana Paula Florentino for introducing our team to the fascinating science involving the fast growing pioneer species *Vismia guianensis*. This material is based upon work supported as part of the GoAmazon 2014/5 and the Next Generation Ecosystem Experiments-Tropics (NGEE-Tropics) funded by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research through contract No. DE-AC02-05CH11231 to LBNL, as part of DOE's Terrestrial Ecosystem Science Program.

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Figures

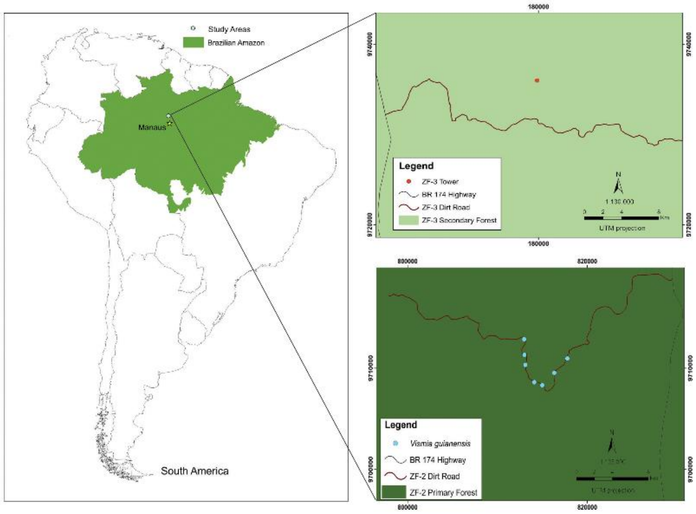

Figure 1: Location of the primary forest in the Reserva Biológica do Cuieras (ZF2) and the secondary forest (ZF3) in the biological dynamics of forest fragments project near Manaus, Brazil.

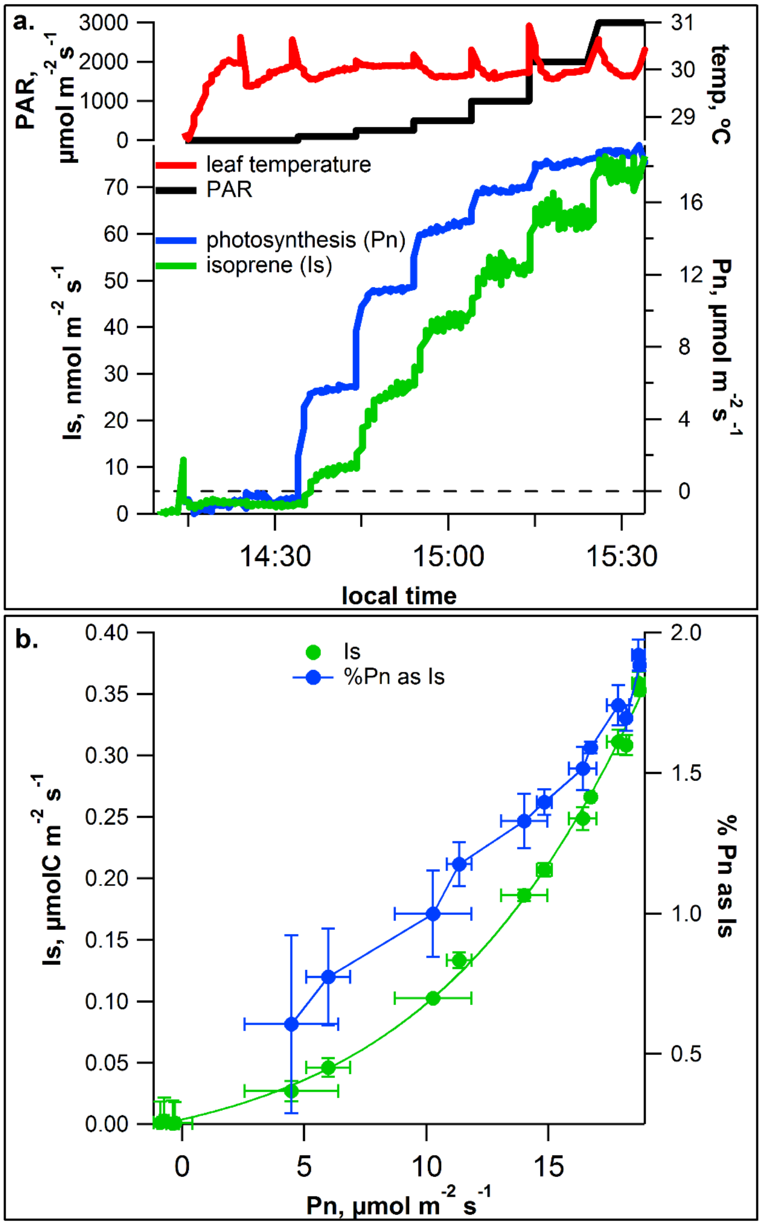

Figure 2: Real-time leaf net photosynthesis and isoprene emissions as a function of light intensity in the fast growing pantropical pioneer species *V. guianensis*. (**a**) Example time series plot of net photosynthesis (Pn), isoprene emission (Is) together with leaf temperature (temp) and photosynthetically active radiation (PAR) during a controlled light experiment under constant leaf temperature (30 ºC +/- 1 ºC). (**b**) Is expressed in µmolC m^{-2} s^{-1} plotted against Pn (µmol m^{-2} s^{-1}). Also shown is the increase in %Pn dedicated to Is as a function of Pn. Pn was determined by a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA) and Is by a proton transfer reaction – mass spectrometer (PTR-MS, Ionicon Analytik, Austria).

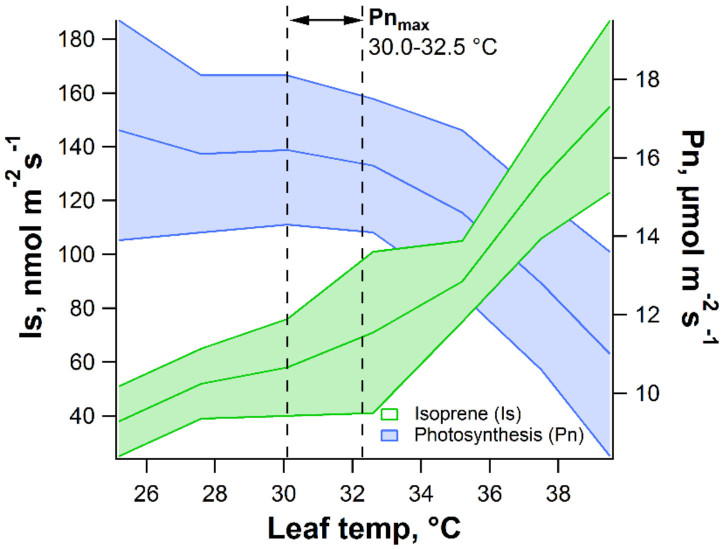

Figure 3: Average net photosynthesis (Pn) and isoprene emissions (Is) from *V. guianensis* leaves as a function of leaf temperature (average +/- 1 standard deviation, n = 5 leaves) under constant PAR of 1000 µmol m^{-2} s^{-1}and 400 ppm reference [CO$_2$]. Note the decline in Pn and the increase in Is with leaf temperature above 30.0-32.5 °C, where the majority of leaves showed an optimum in Pn. Pn was determined with a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA) and Is was determined using thermal desorption GC-MS.

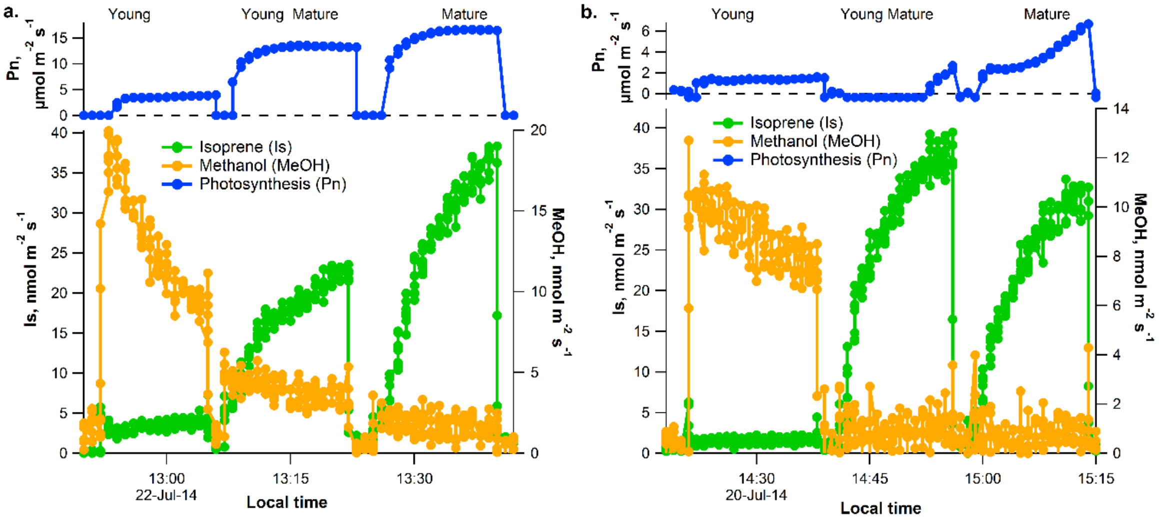

Figure 4: Example leaf age experiments of net photosynthesis (Pn, blue circles), methanol emissions (MeOH, orange circles) and isoprene emissions (Is, green circles) from two *V. guianensis* individuals. For each plant shown in **a.** and **b.** a young, young mature, and mature leaf were sequentially placed in the leaf enclosure for 15 min each after exposed to laboratory conditions following transport from the field. Throughout the leaf age experiments, PAR, leaf temperature and reference [CO$_2$] were held constant at 1000 µmol m^{-2} s^{-1}, 30 °C and 400 ppm, respectively. Pn was determined by a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA). Is and MeOH by a proton transfer reaction – mass spectrometer (PTR-MS, Ionicon Analytik, Austria).

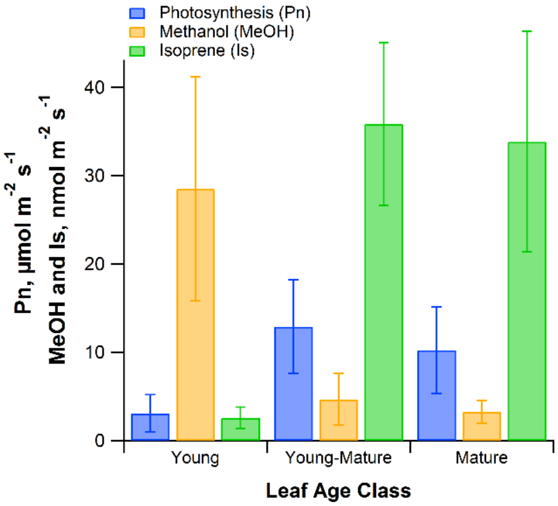

Figure 5: Average net photosynthesis rates (Pn, blue bars) together with leaf emissions of Isoprene (Is, green bars) and methanol (MeOH, orange bars) for six *V. guianensis* individuals. For each individual, volatile emissions and net photosynthesis rates were determined from young, young-mature, and mature leaves. Error bars represent +/- one standard deviation (six individuals, one branch per individual, one young, one young-mature, and one mature leaf per branch). Pn was determined by a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA). Is and MeOH by a proton transfer reaction – mass spectrometer (PTR-MS, Ionicon Analytik, Austria).