Leaf level VOC emissions of single plants from Amazonian and Mediterranean ecosystems; Ontogeny and flooding as stress factor for VOC emissions

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Die Vegetation ist die wichtigste Quelle von organischen flüchtigen Verbindungen (auf Englisch volatile organic compounds,VOCs), die einen bemerkenswerten Einfluss auf der Chemie und Physik der Atmosphäre haben. VOCs beeinflussen die oxidative Kapazität der Atmosphäre und tragen zu der Bildung und zum Wachstum von sekundären organischen Aerosolen bei, welche einerseits eine Streuung und Reflektierung der Energie verursachen und andererseits sich an der Bildung und Entwicklung von Wolken beteiligen. Ziel dieser Arbeit war die Beschreibung und der Vergleich von VOC Emissionen aus Pflanzen aus zwei verschiedenen Ökosystemen: Mediterranes Ökosystem und Tropisches Ökosystem. Für diese Aufgabe wurden gewöhnliche Pflanzen von beiden Ökosystemen untersucht. Siebzehn Pflanzenspezies aus der Mittelmeergebiet, welches bekannt ist für seine Vielfalt an VOC emittierenden Pflanzen, wurden in die Untersuchungen einbezogen. Im Gegensatz zum mediterranen Ökosystem sind nur wenig Information verfügbar über VOC Emissionen aus Blättern tropischer Baumspezies. Vor diesem Hintergrund wurden sechsundzwanzig Baumspezies aus verschiedenen Ökotypen des Amazonasbeckens (Terra firme, Várzea und Igapó) wurden auf VOC Emissionen auf Blattebene mit einem Küvetten-System untersucht. Analysen von flüchtigen organischen Verbindungen wurden online mit PTR-MS und offline mittels Sammlung auf entsprechenden Adsorbern (Kartuschen) und nachfolgender GC-FID Analyse untersucht. Die höchsten Emissionen wurden für Isoprene beobachtete, gefolgt durch Monoterpene, Methanol und Aceton. Die meisten Mittelmeer Spezies emittierten eine hohe Vielfalt an Monoterpenspezies, hingegen zeigten nur fünf tropische Pflanzenspezies eine Monoterpene mit einen sehr konservativen Emissionsprofil (α-Pinen>Limonen>Sabinen >ß-Pinen). Mittelmeerpflanzen zeigten zusätzlich Emissionen von Sesquiterpenen, während bei der Pflanzen des Amazonas Beckens keine Sesquiterpenemissionen gefunden wurden. Dieser letzte Befund könnte aber auch durch eine niedrigere Sensitivität des Messsystems während der Arbeiten im Amazonasgebiet erklärt werden. Zusätzlich zu den Isoprenoidemissionen waren Methanolemissionen als Indikator für Wachtumsvorgänge sehr verbreitet in den meisten Pflanzenspezies aus tropischen und mediterranen Gebieten. Einige Pflanzenspezies beider Ökosystemen zeigten Acetonemissionen.

VOC Emissionen werde durch eine große Vielfalt an biotischen und abiotischen Faktoren wie Lichtintensität, Temperatur, CO_2 und Trockenheit beeinflusst. Ein anderer, öfter übersehener Faktor, der aber sehr wichtig ist für das Amazonas Becken, ist die regelmäßige Überflutung. In dieser Untersuchung wir fanden heraus, dass am Anfang einer Wurzelanoxie, die durch die Überflutung verursacht wurde, Ethanol und Acetaldehyd emittiert werden können, vor allem in Pflanzenspezies, die schlechter an eine unzureichende Sauerstoffversorgung bei Flutung adaptiert sind, wie z.B. *Vatairea guianensis*. Die Spezies *Hevea spruceana*, welche besser an Überflutung adaptiert ist, könnte möglicherweise der gebildete Ethanol sofort remetabolisieren ohne es zu emittieren. Nach einer langen Periode einer Überflutung konnte allerdings keine Emission mehr beobachtet werden, was auf eine vollständige Adaptation mit zunehmender Dauer schließen lässt. Als Reaktion auf den ausgelösten Stress können Isoprenoidemissionen ebenfalls kurzfristig nach einigen Tage an Überflutung zunehmen, fallen dann aber dann nach einer langen Periode zusammen mit der Photosynthese, Transpiration und stomatäre Leitfähigkeit deutlich ab.

Pflanzen Ontogenese ist anscheinend von Bedeutung für die Qualität und Quantität von VOC Emissionen. Aus diesem Grund wurden junge und erwachsene Blätter einiger gut charakterisierten Pflanzen Spezies aus dem Mittelmeerraum auf VOC Emissionen untersucht. Standard Emissionsfaktoren von Isopren waren niedriger in jungen Blättern als in erwachsene

Blätter. Hingegen wurden höhere Monoterpen- und Sesquiterpenemissionen in jungen Blätter einiger Pflanzenspezies gefunden. Dieser Befund deutet auf eine potentielle Rolle dieser VOCs als Abwehrkomponenten gegen Pflanzenfresser oder Pathogene bei jungen Blätter hin. In einigen Fällen variierte auch die Zusammensetzung der Monoterpen- und Sesquiterpenspezies bei jungen und erwachsenen Blättern. Methanolemissionen waren, wie erwartet, höher in jungen Blättern als in ausgewachsenen Blättern, was mit der Demethylierung von Pectin bei der Zellwandreifung erklärt werden kann. Diese Befunde zu Änderungen der Emissionskapazität der Vegetation können für zukünftige Modellierungen herangezogen werden.

ABSTRACT

Terrestrial vegetation is the most important source of atmospheric volatile organic compounds (VOC) with significant influence on the chemistry and physics of the atmosphere. VOC influence the oxidative capacity of the atmosphere and contribute to the formation and growth of secondary organic aerosols affecting scatter and reflectance of energy as well as cloud development and precipitation. The aim of this work was to achieve a description and comparison of VOC emission from tropical and Mediterranean vegetation. For this task common plant species of both ecosystems were investigated. Sixteen plant species from the Mediterranean area, which is known for its special diversity in VOC emitting plant species, were chosen. In contrast, little information is currently available regarding emissions of VOCs from tropical tree species at leaf level; twenty-six tree species from different environments of the Amazon basin, i.e.Terra firme, Várzea and Igapó, were screened for VOCs emission at leaf level with a branch enclosure system. Analysis of volatile organics was performed online by PTR-MS and offline by collection on adsorbent tubes and subsequent GC-FID analysis. Isoprene was quantitatively the most dominant emitted compound followed by monoterpenes, methanol and acetone. Most of the Mediterranean species emitted a variety of monoterpenes whereas only five tropical species were monoterpene emitters conserving a quite conservative emission pattern (α-pinene>limonene>sabinene >ß-pinene). Mediterranean plants showed additionally emissions of sesquiterpenes, whereas in the Amazon region no sesquiterpenes were detected probably due to lack of sensitivity of the measuring systems. On the other hand, methanol emissions were common in most of the tropical and Mediterranean species as an indicator of growth, and a few species from both ecosystems showed acetone emissions.

A variety of biotic and abiotic factors affect VOC emissions, such as light intensity, temperature, CO_2 and drought. Another, usually overlooked stress factor, and very important for the Amazon region, is flooding. In this study we found out that at the beginning of root anoxia due to inundation, emissions of ethanol and acetaldehyde can be detected, especially in less anoxia adapted species such as *Vatairea guianensis*. The better adapted species *Hevea spruceana* could probably directly remetabolize the formed ethanol, without emitting it. After long periods of inundation a total adaptation to flooding could be assumed in those cases where no ethanol or/and acetaldehyde emissions were detected any more. On the other hand, isoprenoid emissions can shortly increase after some days of flooding but then after a long period of waterlogging emissions tend to decrease considerably, as well as photosynthesis, transpiration and stomatal conductance.

Plant ontogeny also seems to be of importance for the VOC emission in terms of quality and quantity. Therefore, VOC emissions of young and mature leaves of principally already well characterized plant species from the Mediterranean area were studied in detail for VOC emissions. Standard emission factors of isoprene were lower in young leaves than in mature leaves. Contrasting, higher monoterpene and sesquiterpene emissions were found in case of several plant species, indicating a potential role for these VOC species as defense compounds against herbivores or pathogens during the youth period. Monoterpene and sesquiterpene species composition varied also in some cases with maturity. On the other hand, methanol emissions were, as expected, higher in young leaves than in mature leaves, corroborating the theory that methanol is the product of demethylation of pectine by cell wall maturation. These changes in emission capacity of vegetation can be accounted for in future model estimates

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CHAPTER 1: INTRODUCTION

The Earth's atmosphere primarily consists of the gases N_2 (78%), O_2 (21%), Ar (1%), whose abundance are controlled over geologic time scales by the biosphere. The biosphere is that part of the earth in which living organisms exist or that is capable of supporting life. The biosphere is in close interaction with the atmosphere and contributes in many ways into the chemical and physical events of the troposphere; which is the lowest layer of the atmosphere (figure 1.1). The troposphere is bounded above by the tropopause, which is a boundary marked by stable temperatures. Above the troposphere we find the stratosphere. In the troposphere, temperature usually declines with increasing altitude. This layer is denser than the layers of the atmosphere above, and contains up to 75% of the mass of the atmosphere. Water vapour is the next most abundant constituent, which is found mainly in the lower atmosphere and whose concentration is highly variable. The remaining gaseous constituents, which are the trace gases, comprise less than 1% of the atmosphere. These trace gases play a crucial role in the Earth's radiative balance and the chemical properties of the atmosphere.

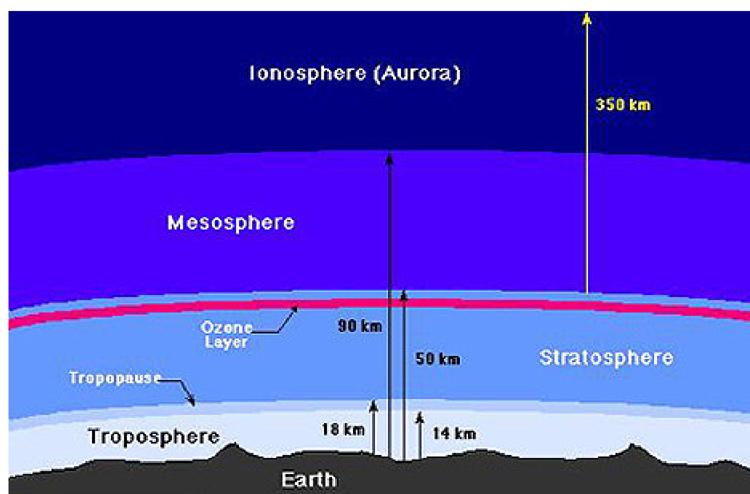


Figure 1.1. Representation of atmosphere layers. (Image from http://www.globalwarmingclassroom.info/images/atmosphere%5B1%5D.jpg)

Vegetation colonizes all kind of habitats in the biosphere. Important contributions of the terrestrial vegetation are e.g. the fixation of carbon dioxide, which is supposed to be an important sink for carbon. But the vegetation plays also an important role in the field of trace gases, such as volatile organic compounds (VOCs), which have atmospheric concentrations 3-6 orders of magnitude lower than CO_2.

Generally, the term of VOCs is used to denote the entire set of vapour-phase atmospheric organics excluding CO and CO_2 (Seinfeld and Pandis, 1998). However, there is a variety of denominations for the term VOCs. For example, the EPA (Environmental Protection Agency) from the USA simply defines VOCs as "any organic compound which has a vapour pressure

above 1.3 hPa in ambient conditions". Methane and other compounds may be excluded from this terminology by this particular definition, but some authors distinguish also between VOCs and "Non Methane VOC" (NMVOC, e.g. Monson (2002)).

VOCs can have an anthropogenic or a biogenic origin, being in both cases central to the atmospheric chemistry. In order to distinguish VOCs that are naturally emitted from the biosphere, the term "Biogenic VOC" (BVOC) has been defined. Measurements in forest and agricultural areas coupled with emission studies from selected individual trees and agricultural crops have demonstrated the ubiquitous nature of biogenic emissions and the variety of organic compounds that can be emitted (e. g. Steinbrecher *et al.* (2009), Kesselmeier and Staudt (1999)). The largest source of VOCs, by far, is the natural emission from vegetation (Guenther *et al.*, 2006). On the other hand, anthropogenic sources of VOC include fuel production, distribution, and combustion, with the largest source being emissions (i) from motor vehicles due to either evaporation or incomplete combustion of fuel, and (ii) from biomass burning. Thousands of different compounds with varying lifetimes (from minutes to days) and chemical behaviour have been observed in the atmosphere, so most models of tropospheric chemistry include some chemical speciation of VOC. According to the IPCC Report 2001, natural emissions occur predominantly in the tropics (23°S to 23°N) with smaller amounts emitted in the northern mid-latitudes and boreal regions mainly in the warmer seasons. Anthropogenic emissions occur in heavily populated, industrialised regions (95% in the Northern Hemisphere peaking at 40°N to 50°N), where natural emissions are relatively low, therefore they have significant impacts on regional chemistry despite small global emissions.

A compilation of organic compounds in the atmosphere documented a total of 367 different compounds that are released to the atmosphere from vegetation sources (Manahan, 2000). Other natural sources include microorganisms, forest fires, animal waste and volcanoes. Global mean abundance of the most important VOC emitted by vegetation is represented by table 1.1. The estimates of global flux of biogenic VOCs from the biosphere are very uncertain, but may be 700-1000 x 10^{12} g (C) per year, being isoprene the most abundant compound followed by monoterpenes and other VOCs (Laothawornkitkul *et al.*, 2009).

Table 1.1 The major classes of biogenic volatile organic compounds (BVOCs), the major groups of BVOC-emitting plants and estimates of current and future BVOC fluxes into the atmosphere (Laothawornkitkul *et al.*, 2009)

BOVC species	Present estimated annual global emission (10^{12} g C)	Future estimated annual global emission (10^{12} g C)	Atmospheric lifetime (d)	Example	Major emitting plants
Total	700–1000	1251–1288			
Isoprene	412–601	638–689	0.2		*Populus*, *Salix*, *Platanus*, *Cocos*, *Elaeis*, *Casuarina*, *Picea* and *Eucalyptus*
Monoterpene	33–480	265–316	0.1–0.2	β-Pinene, α-pinene, limonene	*Lycopersicon*, *Quercus*, *Cistus*, *Malus*, *Pinus* and *Trichostema*
Other reactive BVOCs	~260	~56–159 (only for acetaldehyde and formaldehyde)	< 1	Acetaldehyde, 2-methyl-3-buten-2-ol and hexenal family	Grassland (mix of C_3 plants), *Vitis*, *Brassica*, *Secale* and *Betula*
Other less reactive BVOCs	~260	~292–514 (only for methanol, acetone, formic acid and acetic acid)	> 1	Methanol, ethanol, formic acid, acetic acid and acetone	Grassland (mix of C_3 plants), *Vitis*, *Brassica*, *Secale* and *Betula*
Ethylene	8–25		1.9		

Source: (Guenther *et al.*, 1995; Kirstine *et al.*, 1998; Fall, 1999; Fukui & Doskey, 2000; Lathiere *et al.*, 2005; Arneth *et al.*, 2008a; Davison *et al.*, 2008), http://bai.acd.ucar.edu/Data/BVOC/index.shtml and http://www.es.lancs.ac.uk/cnhgroup/download.html.

Model estimates showed past, present and future scenarios for biogenic VOC (see table 1.2) (Lathiere *et al.*, 2005). In the future, tropical regions, which produce currently most of the biogenic VOC, would decrease its contribution considerably due to the reduction of tropical tree coverage. Nevertheless, they remain a high emission source, whereas European areas would increase its contribution due to expansion of temperate and boreal forest (see figure 1.2).

Table 1.2 Biogenic VOC Emissions (TgC/yr) Calculated by ORCHIDEE for the Preindustrial and Present-Day With Static Vegetation Distribution, and for the LGM, Present-Day and Future With Dynamic Vegetation[a] (Lathiere *et al.*, 2005)

	Static mode		Dynamic mode		
	Preindustrial	Present	LGM	Present	Future
Isoprene	409 (+2)	402	226 (−55)	502	638 (+27)
Monoterpenes	127 (−3)	131	49 (−72)	175	265 (+51)
Methanol	91 (−22)	116	27 (−79)	132	192 (+45)
Acetone	46 (−2)	47	18 (−71)	63	96 (+52)
Acetaldehyde	16	16	6 (−73)	22	33 (+50)
Formaldehyde	11	11	4 (−73)	15	23 (+53)
Formic acid	1.6	1.6	0.6 (−73)	2.2	3.3 (+50)
Acetic acid	0.3	0.3	0.1 (−75)	0.4	0.7 (+75)
TOTAL	702 (−3)	725	331 (−64)	912	1251 (+37)

[a]Percentage change in emissions relative to present-day is shown in parentheses.

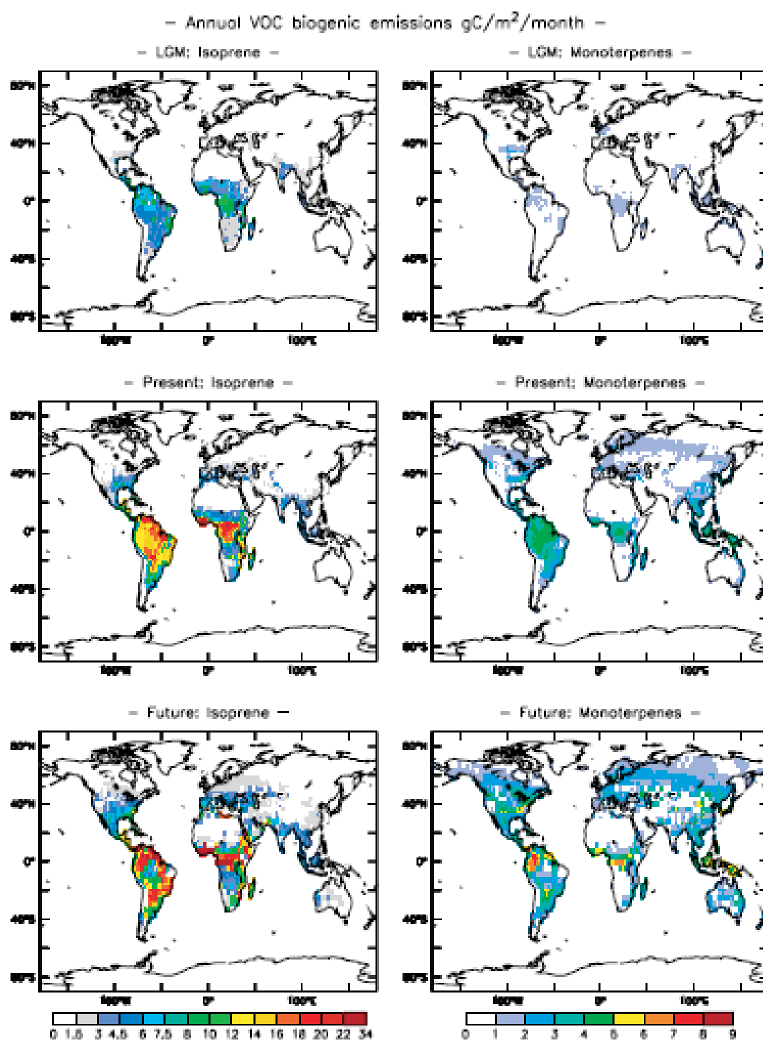


Figure 1.2 Distribution of annual biogenic emissions (gC/m^2/month) for the LGM (Last Glacial Maximum) (first line), present-day (second line) and future (third line) for (left) isoprene and (right) monoterpenes (Lathiere *et al.*, 2005).

1.1 VOC AND THEIR PARTICIPATION ON THE BIOGEOCHEMICAL CYCLE OF CARBON

Life on Earth is carbon based. This compound has a central role in the complicated biogeochemical processes of ecosystems, contributing for about half of the dry mass of life on Earth and the organic matter that accumulates in soils and sediments when organisms die (Chapin III *et al.*, 2006). The carbon cycle includes four main reservoirs of stored carbon: (1) CO_2 and other hydrocarbons in the atmosphere (~780 Pg); (2) organic compounds in living or recently died organisms (vegetation ~550 Pg, soils and litter 1599-2300 Pg and lithosphere carbonates > 60000000 Pg); (3) dissolved carbon dioxide in the oceans and other bodies of water (~39700 Pg); and (4) calcium carbonate in limestone and in buried organic matter (e. g. natural gas, petroleum, coal) (~5000-10000 Pg) (see figure 1.3).

Plants absorb carbon from the atmosphere by photosynthesis and fix it in form of carbohydrates, which are exploited for further use, i. e. energy production and growth. By this process plants absorbs around 120 Pg a^{-1} from the atmosphere per year. Heterotrophic and autotrophic respiration are releasing this carbon again with a share of approximately 60 Pg a^{-1} for each process. Additionally, plants may also release substantial amounts of carbon into the atmosphere in form of VOC (Kesselmeier *et al.*, 2002a). When plants and animals die, carbon is released in the atmosphere as CO_2 and other hydrocarbons. Hydrocarbons can also partly redeposit as sediment, where they are trapped and can form deposits of coal and petroleum. CO_2 from the atmosphere also dissolves in oceans and other bodies of water. Aquatic plants use the CO_2 for their photosynthesis and many aquatic animals use it to make shells of calcium carbonate. The shells of dead organisms (e.g. phytoplankton or coral reefs) accumulate on the sea floor and can form limestone that is part of the sedimentary cycle.

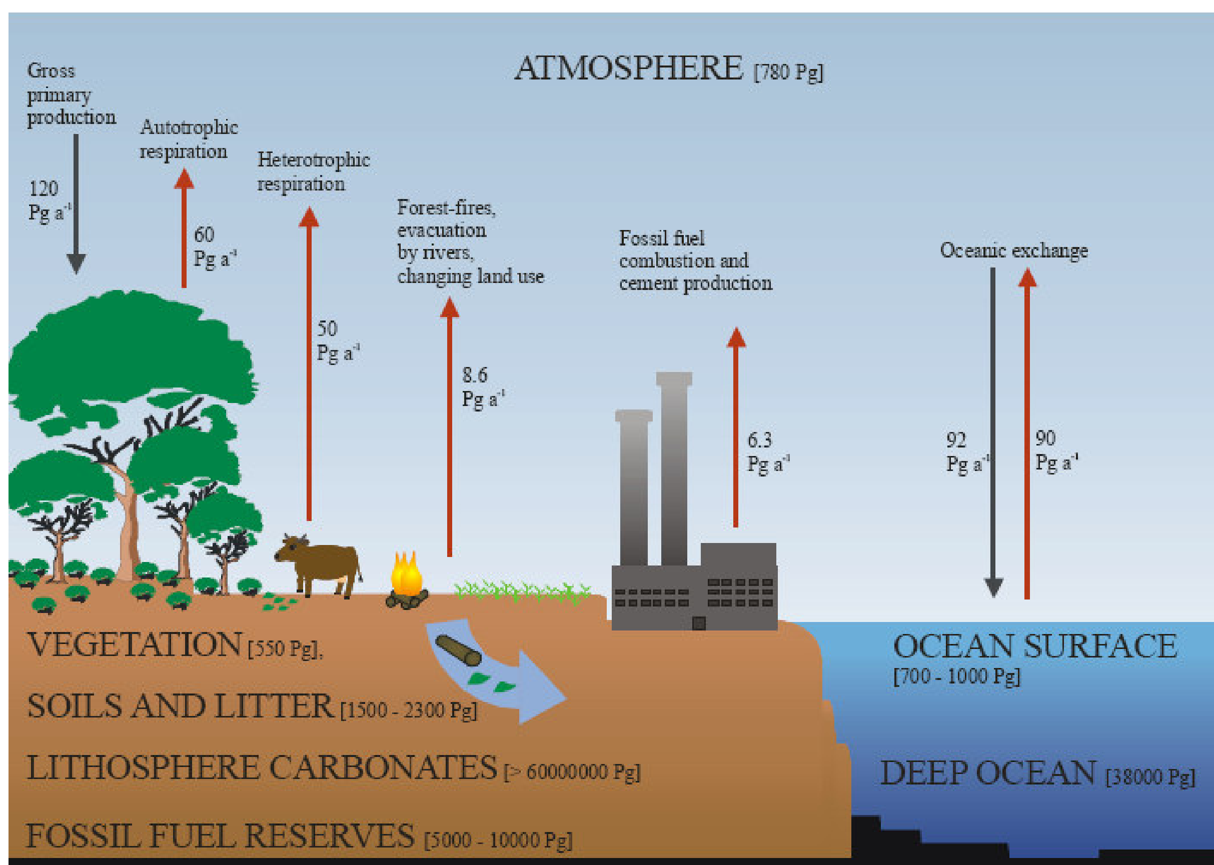


Figure 1.3 Biogechemical Carbon Cycle (Dindorf, 2006). Carbon reservoirs are shown in parentheses in units of Pg (= 1015g). Emission is indicated by red arrows, deposition by black arrows, both in units of Pg a^{-1}.

In the last decades, the interest for the carbon cycle has been increased by the society because of the observed increase in levels of atmospheric CO_2 (from ~280 ppm in 1800 to ~315 ppm in 1957 to ~356 ppm in 1993 to ~366 ppm in 1998 to ~379 ppm in 2005) due to

anthropogenic influences (Wigley and Schimel, 2000). CO_2 is influenced by the biological and physical controls over C absorption, sequestration and release by ecosystems. Furthermore, CO_2 contributes together with a variety of trace gases such as methane, N_2O and water vapor to the natural greenhouse effect that keeps the planet warm enough to evolve and support life (Schlesinger, 2003). The disturbance of the equilibrium produced by the anthropogenic influences enhances the heat trapping capacity of CO_2 and thus affects the dynamics of global climate systems (Cox *et al.*, 2000; Fung *et al.*, 2005).

The largest carbon pools in the world are presented by sedimentary carbonates (>60000000 Pg), world's ocean (38000 Pg) and fossil fuel reserves (5000-10000 Pg). The formations of these components occur over millions of years. But humans use e. g. fossil fuels to produce electricity and heat and consume them in a very short time of period, releasing huge amounts of the much more active form of carbon, CO_2 to the atmosphere. The problem now is that the carbon cycle is lopsided. It took hundreds of millions of years to sequester carbon deep into the earth and under the ocean floor, and humans have released much of that carbon into the atmosphere during the last century. A consequence of the enhancement of CO_2 in the atmosphere is the global warming and thus the climate change. Another very important consequence is the increase of the CO_2 partial pressure in the seawater (also known as hypercapnia). This can have an impact on marine organisms both via decreased calcium carbonate ($CaCO_3$) saturation, which affects calcification rates of many organisms, and via disturbance to acid-base (metabolic) physiology, affecting coral reefs and planktonic coccolithophores (Fabry *et al.*, 2008; Hoegh-Guldberg *et al.*, 2007).

1.2 VOC AND CLIMATE CHANGE

In the last decades the climate change was a very hot topic. At least since the last IPCC report (2007) almost the whole scientific community agrees on the anthropogenic contribution to this change. One of the important contributions to the climate change is the denominated global warming. This is caused by the influence of the greenhouse gases, like CO_2, CH_4 and water vapor. The greenhouse effect is needed to maintain a temperature-range in the atmosphere that allows life. In a very complicated cycle the atmospheric concentration of these gases was naturally maintained almost constant in the last centuries. Since the industrial revolution at the beginning of the 20th century the anthropogenic influence on this cycle has increased continuously. The worry about the economical, social and biological consequences of this influence on the climate has increased the interest on this topic in several sectors of the

society. The atmospheric chemistry involved in this process is very complicated and there are many different compounds that influence directly or indirectly the green house effect. Volatile organic compounds (VOCs) are one of the groups of gases that can exert influence on the greenhouse gases. Although NMVOCs are not included in global warming potential-weighted greenhouse gas emission totals, they are reported on greenhouse gas inventories (IPCC 2006). In the presence of sunlight these gases can contribute to the formation of the greenhouse gas Ozone (O_3). Therefore they are often called "Ozone precursors". VOC can also modify the atmospheric chemistry so that greenhouse gases residence times can be affected.

Not only anthropogenic sources are involved in the cycle, also biogenic sources represent important contributors for VOC. Due to the biodiversity of the VOC sources, the quantification of the VOC contribution from biogenic sources is a very arduous task. Nevertheless, it is necessary if we want to make reliable predictions.

Biogenic VOCs also play a very important role in the atmospheric chemistry and influence climate being strong precursors of secondary gaseous pollutants and being implicated in the formation and growth of secondary organic aerosols affecting cloud development and precipitation (Andreae and Crutzen, 1997).

1.3 ATMOSPHERIC CHEMISTRY OF VOC

Volatile organic compounds (VOCs) influence the chemical and physical properties of the atmosphere. More than 1000 different VOCs are released from natural or man-made (anthropogenic) sources into the atmosphere. A large proportion of these compounds stems from plants. Hence, the composition of the atmosphere is largely affected by the biogenic activity.

Since the atmospheric chemistry of VOC regulates the oxidative capacity of the atmosphere (Poisson *et al.*, 2000), the study of the emission of these compound by plants is of great importance (Fehsenfeld *et al.*, 1992). The most abundant VOC emitted by plants are the isoprenoids isoprene and monoterpenes followed by other VOC like oxygenated VOCs (methanol, ethanol, formaldehyde, acetaldehyde, acetone, formic acid, and acetic acid) and sesquiterpenes (Guenther *et al.*, 1995; Steinbrecher *et al.*, 2009).

1.3.1 ISOPRENOIDS: ISOPRENE, MONOTERPENES AND SESQUITERPENES

Although isoprene and monoterpenes are not classified as greenhouse gases, they can modify the atmospheric chemistry, affecting the residence time of greenhouse gases. Isoprene and monoterpenes are unsaturated organic compounds that can be quickly removed by reactions

with hydroxyl radicals, nitrate radicals and ozone from the atmosphere. Their short lifetimes of a few hours in the atmosphere (Atkinson and Arey, 2003) explain their significance for the chemistry of the atmosphere. Particularly their reaction with OH radicals, nitrate radicals and ozone was investigated very intensive in the last two decades.

There are two main reaction mechanisms (Atkinson, 1997):
1) Addition of OH radicals, nitrate radicals and ozone to C = C bonds.
2) H-atom removal of C-H bonds, removed by hydroxyl radicals and nitrogen oxides.

The first group of mechanisms affects primarily isoprene, monoterpenes and sesquiterpenes. The reactions of ozone with alkenes, such as isoprene, monoterpenes and 2-methyl-3-butene-2 -ol, which have C = C bonds, may be an additional source of OH radicals in the troposphere present even during the night (Paulson, 1996). Furthermore Hydroxyl radicals can be formed in the atmosphere from the photolytic reactions of ozone.

In the presence of nitrogen oxides, which are often of anthropogenic origin, the oxidation of volatile organic compounds such as isoprene and monoterpenes, in the atmosphere by photolytic reactions can lead to the production of ozone and other polluting compounds (Daum, 2000). Some of the degradation products of isoprene reactions with hydroxyl radicals, nitrate radicals and ozone in the atmosphere are organic acids, CO, methacrolein and methyl vinyl ketone. When the concentration of nitrogen oxides is high, the degradation of isoprene can result in the formation of peroxyacetyl nitrate (PAN) and Methylperoxyacetylnitrat (MPAN). These compounds are phytotoxic at concentrations of a few ppb and represent the main components of the urban smog (Sun, 1995). By absence of nitrogen oxides, peroxy radicals, formed by the oxidation of isoprene with OH radicals, can recombine into peroxides. The peroxy radicals from this pathway are removed by deposition processes, so that radicals are lost, whereas the recycling through the pathway mentioned above in the presence of nitrogen oxides, comes with ozone formation. A third alternative pathway has been recently proposed to be relevant in unpolluted low NO environments. Here, isoprene chemistry can directly recycle radicals, and the degradation products yield OH more efficiently than was assumed before in atmospheric models (Lelieveld *et al.*, 2008).

By ozonolysis of monoterpenes, hydroxyl radicals and nitrate radicals are formed in a multitude of different reaction products (Atkinson and Arey, 2003) and are responsible for a large number of atmospheric oxidation processes. An example of these reactions is given by

the formation of a peroxo compound through the addition of ozone to α-pinene. The resulting Criegee radicals continue reacting to pinone acid and pinonealdehyde or adipic acid and norpin acid opening its ring structure. The reaction of hydroxyl radicals with α-pinene forms again pinonealdehyde in high yield through different reaction paths. In addition to these long-chain compounds short-chain components occur as oxidative end products of monoterpenes, such as acetone, formaldehyde and formic acid. Depending on the volatility of all these compounds, they can change from the gas phase into the particulate phase, thus contributing to the new formation or growth of aerosol particles (Andreae and Crutzen, 1997; Noziere and Barnes, 1998; Seinfeld and Pankow, 2003). This phenomenon of particle formation was already discussed by Went (1960). Frits Went associated for the first time emissions of isoprenoids from plants with the "blue haze" as sometimes observed over forested areas. Nowadays we know that even isoprene can be involved in the particle formation (Claeys *et al.*, 2004a).

Atmospheric oxidation of sesquiterpenes occurs also by ozonolysis and by reaction with OH and NO_3 radicals. Sesquiterpene atmospheric lifetimes from reaction with ambient oxidants are expected to be short. For instance, sesquiterpene lifetimes of ~1-2 minutes were reported for trans-caryophyllene and α-humulene (Atkinson and Arey, 2003; Shu and Atkinson, 1995). Sesquiterpene are suspected to play an important role in biogenic aerosol formation as e.g. trans-caryophyllene that was found to reside almost completely in the aerosol phase (Bonn and Moortgat, 2003; Hoffmann *et al.*, 1997). Oxidation products from gas phase trans-caryophyllene have been identified as 3,3-dimethyl-*γ*-methylene-2-(3-oxobutyl) cyclobutanebutanal, 3,3-dimethyl- *γ* -oxo-2-(3-oxobutyl) cyclobutanebutanal and formaldehyde (Calogirou *et al.*, 1997; Nguyen *et al.*, 2009). Furthermore, sesquiterpenoid compounds (nerolidol, farnesol) typically released from epicuticular waxes, for example from Mediterranean plants (*Quercus ilex, Citrus sinensis, Quercus suber, Quercus freinette, Pinus pinea*) react with ozone yielding significant emissions of geranylacetone, 6-methyl-5-hepten-2-one, 4-oxopentanal and acetone (Fruekilde *et al.*, 1998). On the other hand, tobacco plants (Nicotiana tabacum) show strong sesquiterpene emissions after being exposed to elevated ozone levels (120-170 ppbv) (Heiden *et al.*, 1999a).

1.3.2 OXYGENATED VOCs: METHANOL AND ACETONE

Oxygenated VOCs such as methanol and acetone have a long lifetime of >1 day in comparison with isoprenoids (Guenther *et al.*, 1995; Kesselmeier and Staudt, 1999). Methanol

can undergo photochemical reactions, beginning with abstraction of hydrogen by the hydroxyl radical. Mechanisms for scavenging alcohols from the atmosphere are relatively efficient because alcohols are quite water soluble. In addition to reacting with HO radicals, alkenyl radicals react strongly with atmospheric ozone, which adds across the double bond. Acetone, that is the most abundant atmospheric ketone, is usually generated as a product of the atmospheric oxidation of propane, isobutene, and other hydrocarbons, but can also be directly emitted from plants (21 %), by biomass burning (26 %) and there is a contribution of 3% from direct anthropogenic emissions (Singh *et al.*, 1994). Acetone photolyzes in the atmosphere, producing the PAN precursor acetyl radical. It is believed that the mechanism for the removal of the higher ketones from the atmosphere involves an initial reaction with OH radical.

1.4 BIOGENIC VOC

1.4.1 METABOLIC FUNCTION OF VOC IN THE PLANTS

The variety of pathways for the biosynthesis of VOCs is discussed in the literature and was recently reviewed by Laothawornkitkul et al. (2009). Figure 1.4, taken from this review, gives an overview about the complex mixture of VOC species and their biosynthesis. Biogenic VOCs are released from above- and below-ground plant organs. In general, flowers and fruits release the widest variety of VOCs, with emission rates peaking on maturation (Dixon and Hewett, 2000; Knudsen *et al.*, 2006; Knudsen and Gershenzon, 2006; Soares *et al.*, 2007). On the other hand, leaves have the greatest mass emission rates, releasing a mixture of terpenoids, including isoprene, monoterpenes, sesquiterpenes and some diterpenes, but also oxygenated compounds like formaldehyde, acetaldehyde, acetone, methanol, ethanol, methylbutenol, etc (Duhl *et al.*, 2008; Kesselmeier and Staudt, 1999; Seco *et al.*, 2007).

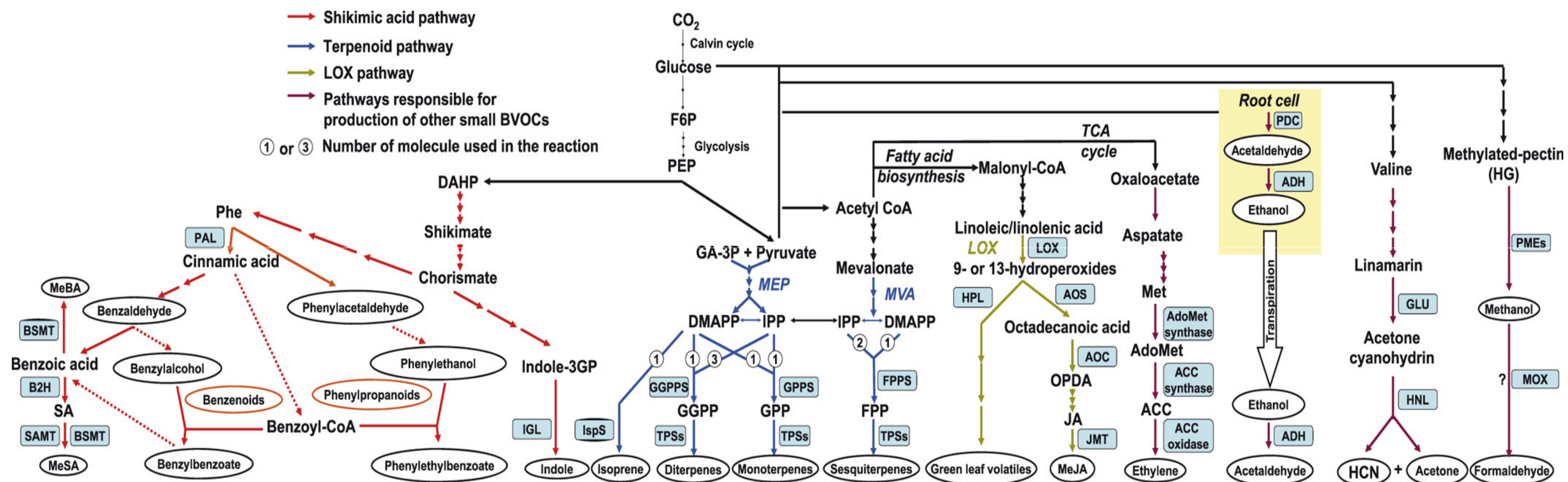


Figure 1.4 Simplified description of the metabolic pathways of biogenic volatile organic compound (BVOC) biosynthesis in leaves and/or flowers and roots. Volatile compounds are shown inside oval-shaped areas and the enzymes responsible for BVOC synthesis are shown in boxes (adapted from Dudareva *et al.*, 2006). ACC, 1-aminocyclopropane-1-carboxylic acid; ADH, alcohol dehydrogenase; AdoMet, *S*-adenosyl-L-methionine; ALDH, aldehyde dehydrogenase; AOC, allene oxide cyclase; AOS, allene oxide synthase; B2H, benzoic acid-2-hydroxylase; BSMT, *S*-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase; CoA, coenzyme A; DAHP, 3-deoxy-D-arabino-heptulosonate; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; FPPS, FPP synthase; F6P, fructose-6-phosphate; GA-3P, glyceraldehyde-3-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; GLU, β-glucosidase; GPP, geranyl diphosphate; GPPS, GPP synthase; HCN, hydrogen cyanide; HG, homogalacturonic acid; HNL, hydroxynitrile lyase; HPL, fatty acid hydroperoxide lyase; IGL, indole-3GP lyase; indole-3GP, indole 3-glycerol phosphate; IPP, isopentenyl diphosphate; IspS, isoprene synthase; JA, jasmonic acid; JMT, jasmonic acid carboxyl methyltransferase; LOX, lipoxygenase; MeBA, methyl benzoate; MeJA, methyl jasmonate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MeSA, methyl salicylate; Met, methionine; MOX, methanol oxidase; MVA, mevalonate; OPDA, 12-oxo-phytodienoic acid; PAL, phenylalanine ammonia lyase; PDC, pyruvate decarboxylase; PEP, phosphoenolpyruvate; Phe, phenylalanine; PMEs, wall-localized pectin methylesterases; SA, salicylic acid; SAMT, *S*-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase; TCA, tricarboxylic acid or citric acid; TPSs, terpene synthases. Figure and Legend were taken from Laothawornkitkul *et al.* (2009).

Isoprenoids (or terpenoids) represent a large group of natural substances, which derive from the biosynthesized isopentenyl pyrophosphate (IPP). Plants have two possible routes to form IPP:

1) The mevalonate pathway, which takes place in the cytoplasm, where terpenes are biosynthesized from the primary metabolite acetyl CoA with mevalonic acid as an intermediate.

2) The non-mevalonate-pathway, which takes place in the plastids, where terpernes are biosynthesized from the primary metabolite pyruvate and D-3-Phosphoglycerinaldehyd with 1-deoxy-D-xylulose-5-phosphate as an intermediate.

The mevalonate pathway supplies the biosynthesis of sesquiterpenes, triterpenes and polyterpenes. Through the non-mevalonate pathway, by contrast, isoprene, monoterpenes, diterpenes, tetraterpenes and some other compounds are synthesized (see figure 1.4). The smallest representative of the produced isoprenoids is the C5 compound isoprene. This is formed from dimethylallyl pyrophosphate (DMAPP) by the catalytic reaction with isoprene synthase in the chloroplast. Isopentenyl pyrophosphate (IPP) is in equilibrium with the isomeric form DMAPP via the IPP isomerase. The precursor of the monoterpenes geranyldiphosphate (GPP) is formed by the addition of a cation formed enzymaticly from DMAPP and IPP (head to tail reaction). Similarly, farnesyldiphosphate (FPP) and geranylgeranyl diphosphate (GGPP), the precursor of sesquiterpenes and diterpenes, are synthesized by the addition of IPP and GPP or FPP (see figure 1.4). The enzymes catalyzing these reactions are denominated prenyltransferases. The linear molecules GPP (C10), FPP (C15) and GGPP (C20) are the precursor materials for the various transformations of the molecules of mono-, sesqui-and diterpenes. Triterpenes are formed by tail to tail dimerization of two molecules of FPP. Similar to the triterpenes, tetraterpenes are also formed by tail to tail dimerization of two GGPP molecules. Oligoterpenes, as side chains of i. a. plastoquinone, phylloquinone and ubiquinone, are formed in the non-mevalonate pathway and consist of 5 to 15 C_5 units. Various polyterpenes, derived from the mevalonate pathway, like rubber and gutta-percha are formed by successive condensation of a large number of C5 units, for rubber up to 5000 units.

A number of low-molecular weight ($C<5$) BVOCs are also emitted by plants, for example methanol, ethylene, formaldehyde, ethanol, acetone and acetaldehyde (see figure 1.4). The production of the C1 compound methanol seems to be related to plant growth and

development. Methanol has been described to be formed by the demethoxylation of pectine on the cell wall by cell wall expansion, (Fall and Benson, 1996; Hüve *et al.*, 2007). This reaction might be catalyzed by a wall located pectin methyl esterases (PME) which, among other functions, catalyzes the demethoxylation of pectin to form carboxylated pectin, thereby releasing methanol (Ricard and Noat, 1986). In addition, methanol in plants is also derived from the action of protein methyl transferases and protein repair reactions (Mudgett and Clarke, 1993), as well as from the tetrahydrofolate metabolism (Cossins, 1987) and the degradation of lignin in plant secondary cell walls by fungi (Ander and Eriksson, 1985). However, methanol in plants may also contribute to the production of formaldehyde (see figure 1.4). Furthermore, ethanol seems to be emitted as a response of root anoxia. In case of plants in floodplain areas under root anoxia plants undergo alcoholic fermentation in the cytoplasm by suppression of oxidative respiration due to the lack of oxygen. This switch activates a combined action of pyruvate decarboxylase and alcohol dehydrogenase. Ethanol is then transported by the transpiration stream and emitted through the leaves. The major portion of ethanol in the leaves is oxidized by alcohol dehydroganese (Halliwell and Gutteridge, 1989). As an intermediate of ethanol oxidation, acetaldehyde is produced and further oxidized by the action of aldehyde dehydrogenase. This leads to the synthesis of acetate which may be converted to acetyl-CoA by acetyl-CoA synthetase, and then enters the general metabolism pathways such as the TCA cycle or lipid synthesis (Macdonald and Kimmerer, 1993). Contrasting, the metabolic pathways for acetone formation in plants are not yet clearly understood. One of the possible metabolic pathways described for acetone is the cyanogenic pathway, activated to deter herbivores, which induces the production of hydrogen cyanide (HCN) and, as a by-product, acetone (Fall, 2003). Several thousand plant species synthesize cyanogenic glycosides (Vetter, 2000). However other possible acetone production pathways cannot be excluded, such as the production of acetone from acetotacetate that has been supposed for conifer buds by Macdonald and Fall (1993b) and is also reported for soil bacteria (Fall, 2003).

1.4.2 ECOPHYSIOLOGICAL ROLE OF VOC IN THE PLANTS

The biochemical regulation and function of most BVOCs are not well known. A variety of theories have been proposed. For many years, these compounds were thought to be simply functionless end products of the metabolism, or metabolic wastes. More recently, many BVOC were suggested to have important ecological functions in plants. Chief among these functions is protection against being eaten by hervibores and infection by microbial pathogens, as found for monoterpenes, sesquiterpenes, diterpenes, triterpenes and

polyterpenes (Croft *et al.*, 1993; De Moraes *et al.*, 2001; Kessler and Baldwin, 2001; Picman, 1986; Vancanneyt *et al.*, 2001). These compounds have also been shown to serve as attractants for pollinators and seed-dispersing animals and as agents of plant-plant competition (Taiz and Zeiger, 1998). Furthermore, in corn, cotton and other species, certain monoterpene and sesquiterpenes are produced and emitted only after insect feeding has already begun. These substances attract natural enemies, including predatory and parasitic insects, that kill plant-feeding insects and so help minimize further damage (Turlings *et al.*, 1995). Recently, it was demonstrated that isoprene influences plant–herbivore interactions by deterring herbivores from feeding (Laothawornkitkul *et al.*, 2008b) and by interfering in tritrophic interactions (Loivamaki *et al.*, 2008). For isoprene thermotolerance has been the most often discussed function. This compound might confer stability to photosynthetic membranes at high temperatures (Sharkey and Singsaas, 1995). Some monoterpenes can also provide thermoprotection (Copolovici *et al.*, 2005; Delfine *et al.*, 2000; Loreto *et al.*, 1998; Penuelas and Llusia, 2002). A second role for isoprene is the tolerance of ozone and other reactive oxygen species (ROS). Isoprene can prevent visible damage caused by ozone exposure (Loreto and Velikova, 2001) and measurable loss in photosynthetic capacity by ROS (Affek and Yakir, 2002). The mechanism by which isoprene protects against heat flecks and ROS is unknown. Only the study performed by Siwko *et al.* (2007) showed that isoprene can directly stabilize lipid membranes.

1.4.3 INFLUENCE OF ENVIRONMENTAL FACTORS ON VOC EMISSION

In the chapter 1.4.2 we have seen some of the biotic factors that can influence BVOCs emissions, such as herbivores attack, allosteric effects, pollinators effects, etc. However there is a variety of abiotic factors that can also influence the BVOCs emissions. The main and best studied factors are light intensity and temperature. Direct emissions of isoprene and monoterpenes follow a saturation curve similar to the light curve of photosynthesis (see figure 1.5) (Kuhn *et al.*, 2002b). Contrasting, plants with stored VOCs exhibit an exponentially rising curve (see figure 1.6) (Schuh *et al.*, 1997). CO_2 is another environmental factor that can affect isoprene emissions. Many laboratory experiments indicate that emissions increase significantly at below-ambient CO_2 conditions and decrease when concentrations are raised to above-ambient (Arneth *et al.*, 2007). Plant damage can also lead to the increase of emissions of some BVOC or to produce other, so-called, green leaf volatiles (C6 aldehydes and ketones) (Fall *et al.*, 1999; Laothawornkitkul *et al.*, 2008a). Biotic and abiotic stresses may also induce the production of several BVOCs, such as terpenes, methyl jasmonate (MeJA) and methyl

salicylate (MeSA), from leaves, the magnitude and quality of which depend on the type of damage (Laothawornkitkul *et al.*, 2008a; Martin *et al.*, 2003; Mithofer *et al.*, 2005; Takabayashi *et al.*, 1994). Not only the stress produced by injury, but also drought or flooding stress, can lead to an induction or reduction of biogenic VOCs.

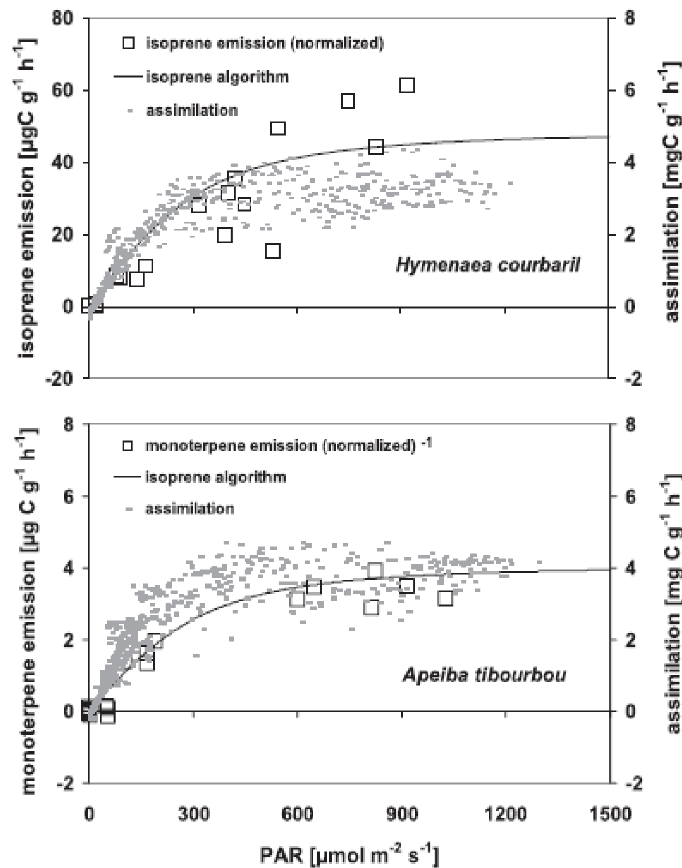


Figure 1.5 Response of photosynthesis and isoprene emission from *H. courbaril* (upper diagram) and monoterpene emission from A. tibourbou (lower diagram) to the corresponding light intensities (PAR). The solid lines indicate the dependence on light predicted by the Guenther algorithm for isoprene (1993). The measured emission rates were normalized to standard temperature (30°C) by dividing by the temperature correction factor C_T (see chapter 4.2.4) (Kuhn *et al.*, 2002b)

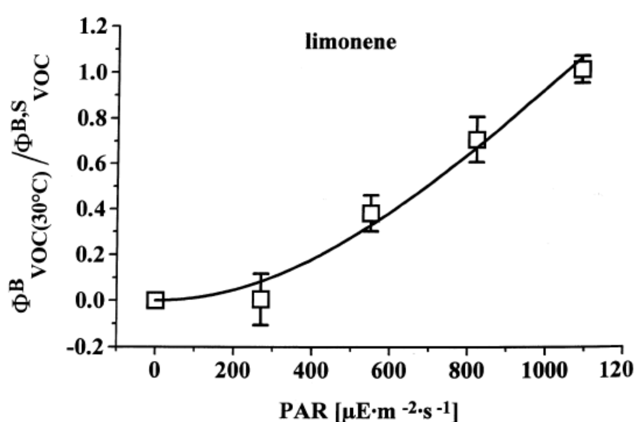


Figure 1.6 Plot of $\phi^{B}_{limonene}/\phi^{B,S}_{limonene}$ normalized to 30°C versus light intensity. Data points represent the arithmetic means of the data measured by sunflower plants at 19, 23.3, and 27.5 °C extrapolated to 30 °C. Error bars are the 1σ standard errors. The line is the simulation of: C_L (see chapter 4.2.4). In darkness $\phi^{B}_{limonene}$ is zero (Schuh *et al.*, 1997).

As mentioned above, flooding can induce a variety of oxygenated VOC (Kreuzwieser *et al.*, 1999b; Rottenberger *et al.*, 2008), whereas severe drought stress, that is typical in

Mediterranean areas during the summer (LeHouerou, 1996) significantly reduces biogenic VOCs emissions. In contrast, moderate drought can decrease, enhance or have no effect on isoprene and monoterpene emissions (Laothawornkitkul *et al.*, 2009).

1.5 OBJECTIVES AND THESIS STRUCTURE

The aim of this work was to achieve a description and comparison of VOC emission from tropical and Mediterranean vegetation. For this task common plant species of both ecosystems were chosen. The Mediterranean area is known for its special diversity in VOC emitting plant species, whereas little information is currently available regarding emissions of VOC from tropical tree species at leaf level (Geron *et al.*, 2002; Kuhn *et al.*, 2002b). Tropical forest comprise roughly 7% of global terrestrial land area, but because of large amounts of biomass, high insolation, warm temperatures and high rates of biological productivity, tropical forest ecosystems are estimated to emit a disproportionately high 30% of global VOCs (Guenther *et al.*, 1995). Therefore, there is a high interest to investigate tropical vegetation for VOC emission. Not only a qualitative study but also a quantitative characterization is pretended, in order to provide useful data for the performance of prediction models.

VOC emission rates normalized with the help of the G93-algorithm (Guenther *et al.*, 1993), which is based on temperature and light intensity, will be compared with the measured values. A further objective is to accomplish a characterization of the plant physiological state of the plants, describing the photosynthesis, transpiration and stomatal conductance.

In addition, different analytical techniques were compared in order to reach a more complete view on the emitted VOCs. The limitations of some techniques can be restricted by additional use of other systems. A PTR-MS (Proton Transfer Reaction-Mass Spectrometer), a GC-FID (Gas Chromatograph with Flame Ionization Detector) and a GC-MS (Gas Chromatograph with Mass Spectrometer) were available for this study. While PTR-MS is an excellent technique for fast chemical measurements, it lacks specificity and compounds with the same mass can not be distinguished. In contrast, slower offline techniques, such as GC-FID and GC-MS, where cartridge samples must be collected and analyzed afterwards with the GC, enabled assignment of some of the observed PTR-MS masses to charge ratios (m/z) which could be related to specific VOC species. Thus, combining the PTR-MS and GC analyses enabled accurate and online identification of several VOC species emitted.

Since the reason for a release of VOC from vegetation is still a matter of debate, several studies have tried to investigate influencing factors that could initiate or control these emissions (e.g. Laothawornkitkul *et al.* (2008b), Arneth *et al.* (2007), Loreto *et al.* (2004)). As mentioned above a variety of biotic and abiotic factors affect VOC emissions, such as light intensity, temperature, CO_2 and drought. Another, usually overlooked stress factor, and very important for the Amazon region, is flooding. In the Amazon region an area as big as Germany (300.000 km²) is flooded during a mean of 210 days a^{-1} (Junk, 1989; Junk, 1997). The anoxia produced by waterlogging has already shown to provoke emissions of several oxygenated VOCs as ethanol and acetaldehyde (Rottenberger *et al.*, 2008), but the effect of other VOC such as the common emitted terpenoids has still not been investigated. In this study, we aim to investigate the possible effects of flooding on VOC emissions, such as terpenoids and oxygenated VOCs as well.

Plant ontogeny also seems to be of importance for the VOC emission in terms of quality and quantity. A substantial number of studies are available dealing with the effect of plant ontogeny to VOC emission (Batten *et al.*, 1995; Centritto *et al.*, 2004; Geron *et al.*, 2000; Grinspoon *et al.*, 1991; Guenther *et al.*, 1991; Hakola *et al.*, 1998; Harley *et al.*, 1994; Kuhn *et al.*, 2004b; Kuzma and Fall, 1993; Lehning *et al.*, 2001; Lerdau *et al.*, 1995; Loreto *et al.*, 2007; Mayrhofer *et al.*, 2005; McConkey *et al.*, 2000; Monson *et al.*, 1994; Wiberley *et al.*, 2005; Zhang *et al.*, 2008), demonstrating that the growth development of the plant may affect VOC emission. Therefore, VOC emissions of young and mature leaves of principally already well characterized plant species from the Mediterranean area were studied in detail for VOCs emissions.

CHAPTER 2: SCREENING OF TROPICAL AMAZON VEGETATION TO VOC EMISSIONS

2.1 INTRODUCTION

Vegetation is one of the main producers of Volatile Organic Compounds (VOCs) and the release of this organic matter affects the chemical and physical properties of the atmosphere (Fehsenfeld *et al.*, 1992), playing a significant role in the global climate system. The effect of biogenic VOCs on global OH distributions in the atmosphere leads to a significant impact on the lifetimes of important greenhouse gases (Collins *et al.*, 2002). Their release also contributes to the formation and secondary growth of organic aerosol particles, which exert control over cloud development and precipitation (Andreae and Crutzen, 1997).

As VOCs can induce significant changes in the atmosphere, therefore, emission inventories mostly from temperate areas of North America and Europe, have been produced to define regional and global biogenic VOC emission strength (Guenther *et al.*, 1995; Guenther *et al.*, 2006; Kinnee *et al.*, 1997; Simpson D, 1999) and are being used for model calculations. Because of the variety of ecoregions, species diversity, inaccessibility, logistical difficulties etc., the numbers of investigations from tropical regions are limited (Geron *et al.*, 2002; 2006b; Harley *et al.*, 2004; Kesselmeier *et al.*, 2002b; Kuhn *et al.*, 2002b, 2004a). However, VOC emissions from tropical forests, which comprise approximately 7% of the global terrestrial land area, are of enormous interest because they are assumed to be responsible for a disproportional 30% of the global emissions due to the large amounts of biomass, high insolation, warm temperatures and high rates of biological productivity in these forests (Guenther *et al.*, 1995).

Emission of VOCs by plants accounts for a significant amount of the photosynthetically fixed carbon (Loreto *et al.*, 2008), a loss which results in a reduction of carbon available for plant metabolism (Sharkey and Yeh, 2001). Furthermore, the loss is significantly affecting numbers for net ecosystem productivity (NEP) and net biome productivity (NBP) (Kesselmeier *et al.*, 2002a). Reasons for such a significant release of resources are numerous and still a matter of discussion (see Kesselmeier and Staudt, 1999). The variability of VOC emissions from plants is the result of complex interactions with the environment; to date, it is poorly understood which factors control and influence the emissions (Monson *et al.*, 1995). Important triggers for VOC emissions are light and temperature (Kesselmeier and Staudt, 1999). The gaps in our knowledge are demonstrated by recent reports about light dependent monoterpene emission from plants which were regarded as low or non monoterpene emitters (Dindorf *et al.*, 2006; Folkers *et al.*, 2008; Fuentes *et al.*, 2000).

Until now, mostly terra firme areas in tropical areas and their plant species have been studied for VOC emissions (Geron *et al.*, 2002; 2006b; Harley *et al.*, 2004; Kesselmeier *et al.*, 2002b; Kuhn *et al.*, 2002b, 2004a), whereas almost no information is available about VOC emissions from tropical plant species in floodplain areas. An area of 300.000 km² in the Amazon basin is subjected to a flood pulse with a flood period lasting up to 210 days a^{-1} on an average (Junk, 1989). According to their geological origin floodplains associated to different water types may be found. Their particular physico-chemical characteristics of the water may affect the physiology of plant species of each typology. For example, the sediment and nutrient rich white water (várzea) with its high fertility supports high rates of primary production whereas the black water regions with their nutrient poor waters are less productive (Piedade, 2001). A recent paper reports about laboratory studies of several floodplain tree species and the emission of considerable quantities of ethanol, acetaldehyde and acetic acid as a response to root anoxia (Rottenberger *et al.*, 2008) giving importance to the contribution of floodplain areas to atmospheric VOC concentrations.

During this study, VOC emissions from plant species from three different very heterogeneous ecosystems from the Amazon rain forest were measured using PTR-MS (Proton Transfer Reaction Mass Spectrometer). This online method allows measurements of a high variety of volatile organic compounds with high temporal resolution and sensitivity (Lindinger *et al.*, 1993), but different VOCs with the same mass can not be identified. Therefore two offline methods: a gas chromatograph with flame ionization detection (GC-FID) and a gas chromatograph with a mass spectrometer (GC-MS) were used to identify, for example, the different monoterpene species and also to validate the PTR-MS measurements. Species from the upland evergreen forest and from the seasonally flooded forest, i.e. várzea as well as igapó were investigated.

2.2 MATERIAL AND METHODS

2.2.1 PLANT MATERIAL AND AMBIENT CONDITIONS

Screening of VOC emissions were carried out with a total of twenty four plant species common for terra firme and floodplain areas in the Amazonas region (see table 2.2.1.1). Three representative trees from the upland forest (terra firme) ecosystem, twenty from the floodplain region inundated with white-water during the flooding period (seasonal várzea) and three from the floodplain region inundated with black or clear-water (seasonal igapó) were studied. The species *Vatairea guianensis* and *Hevea spruceana* from várzea as well as from igapó were compared.

The terra firme species *Scleronema micranthum, Hevea brasiliensis* and *Hevea guianensis* were chosen for our study. *Scleronema micranthum* (Malvaceae) is mainly distributed in the area of central Amazonia in Brazil, Colombia and Venezuela and is used for the timber industry. *Hevea* species belong to one of the leading families of the Amazonian terra firme, the Euphorbiaceae and all of this genus have latex secreting ducts throughout the plant body. Outflow and coagulation of the latex presumably serve as a defense against boring insects and injury (Agrawal and Konno, 2009). Latex is a watery suspension whose main organic component is natural rubber. A natural rubber molecule is an enormous branching polymer of thousands of isoprene (C_5H_8) building blocks (Sauer, 1993). *Hevea* species are original from the Amazon basin but at the beginning of the twentieth century the most economically important member of this genus, *Hevea brasiliensis,* was spread in Southeast Asia. Since 1950, fresh introductions of *H. brasiliensis* and other *Hevea* species have been sent from Brazil to Asia and West Africa (Sauer, 1993). *H. brasiliensis* trees grow mainly in terra firme forest but can also be found in floodplain areas, though being poorly adapted to flood conditions (Goulding, 1980).

Vegetation of white-water (*seasonal várzea*) and black-water (*seasonal igapó*) floodplain areas differentiate not only in their hydro chemical characteristics (Prance, 1979), but also in their taxonomy (Kubitzki, 1989). Várzea water areas are nutrient rich and sediment loaded rivers containing high plant biodiversity (Worbes, 1997) and biomass with many large trees and lianas, whereas black water rivers have clear water which is stained dark brown by a colloidal suspension of plant compounds (humic matter) and are more acid. Igapó usually has a sandy soil which sustains much poorer vegetation than várzea forest of white-water rivers.

Consequently there is much less species diversity and the trees tend to be low and tortuous (Prance, 1979).

The chosen species from várzea and igapó are widely distributed in the Central Amazonian floodplain forest (Prance, 1979; Wittmann *et al.*, 2006; Worbes *et al.*, 1992) and are in many cases of commercial importance like *Erythrina fusca*, *Hevea brasiliensis*, *Hura crepitans*, *Macrolobium acacciifolium, Nectandra amazonum, Ocotea cymbarum, Apeiba sp., Vatairea guianensis, Pachira insignis, Pseudobombax munguba, Laetia corymbulosa* and *Garcinia macrophylla* for wood industry (Duke and Vasquez Martinez, 1994; Wittmann and De Oliveira Wittmann, in press). But also important non-timber product species were found among the investigated plants like *Erythrina fusca, Ocotea cymbarum, Tabernaemontan siphilitca, Vatairea guianensis, Laetia corymbulosa, Salix martiana* that are used in traditional medicines for the treatment of a variety of diseases or *Crescentia amazonica, Hevea spruceana, Pseudobombax munguba* used as handycrafts, *Crataeva benthamii* and *Vitex cymosa* used for fish bait, *Vitex cymosa* used as ornamental tree and *Annona hypoglauca, Pachira insignis, Psidium acutangulum* that have edible fruits (Payne, 1991; Pilar Rauter *et al.*, 2002; Wittmann and De Oliveira Wittmann, in press). In their natural habitat plants have different survival strategies to the stress caused by flooding, resulting in different functional types (see table 2.2.1.1) (Schöngart *et al.*, 2002). Some várzea and most igapó trees are evergreen species (Junk, 1997), developing sclerophyllous foliage which reduces water loss by transpiration (Waldhoff, 2003; Waldhoff and Furch, 2002), but a more detailed analysis shows that evergreen and deciduous species exist in both habitats (Junk, 1997). In evergreen species like the *Pouteria glomerata* and *Tabernaemontana juruana* leaves may be kept below water for several months without apparent damage (Waldhoff and Furch, 2002), whereas brevi-deciduous tree species shed their leaves synchronously and after remaining bare for a short period, leaf flush follows immediately (Schöngart *et al.*, 2002). Moreover many of the species that are deciduous survive most of the aquatic phase by down-regulation of their metabolism resulting in dormancy (Parolin, 2000a). Physiology can also be affected during the flooding period. Most trees have photosynthetic activity reduction for weeks to months (Parolin *et al.*, 2004).

Some of the investigated species are widespread in both ecosystems of the floodplain areas expecting different adaptation strategies for these species. *Hevea spruceana* and *Vatairea*

guianensis were chosen as representative species achieving high abundances in várzea as well as in igapó.

Table 2.2.1.1 Plant species, family, ecosystem, functional type and occurrence of the 22 tropical plant species investigated.

Plant species	Synonyms	Common names	Family	Ecosystem[1]	Functional[2] Type	Country occurences[3]
Annona hypoglauca Mart.	*Annona tessmanii*	Graviola	Annonaceae	a*, c, d	brevi-deciduous	Argenitna, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela
Apeiba sp.		Pojo	Tiliaceae	a*, c	evergreen	Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Honduras, Jamaica, Martinique, Mexico, Nicaragua, Panamá, Perú, Suriname, El Salvador, Trinidad and Tobago, United States, Venezuela
Crescentia amazonica Ducke		Wild calabash, Cuia	Bignoniaceae	a*, d	n.d.	Argentina, Brazil, Colombia, Guyana, Peru, Venezuela
Crataeva benthamii		Catoré	Capparidaceae	a*, e, f, g	deciduous	Brazil
Erythrina fusca Lour.	*Gelala aquatica, Erythrina picta, Erythrina patens, Erythrina ovalifolia, Erythrina moelebei, Erythrina glauca, Erythrina caffra, Erythrina atrosanguinea, Duchassaingia ovalifolia, Duchassaingia glauca, Corallodendron patens, Corallodendron fuscum, Corallodendron glaucum, Erythrina fusca var. inermis*	Ahuijot,, Amapola, Bucare, Bucayo, Eritrina-da-baixa, Eritrina-do-alto, Guiliqueme, Monongondrano, Swamp Imortelle, Yak-erabadu (Sinh), Yak-errabadoogas (Sinh), lucky beantree, Cape Kaffirboom, Kaffirboom , Molongó	Fabaceae	a*, b, c, e, i	n.d.	Australia, Bolivia, Brazil, Belize, China, Colombia, Costa Rica, Ecuador, Spain, French Guiana, Equatorial Guinea, Guatemala, Guyana, Honduras, Comoros, Lao Peoples Democratic Republic, Madagascar, Mexico, Namibia, Nicaragua, New Zealand, Panama, Peru, Papua New Guinea, Puerto Rico, Solomon Island, El Salvador, United States, Venezuela, Viet Nam, Vanuatu, Mayotte, South Africa
Garcinia macrophylla (Mart.) Planch. & Triana	*Garcinia gardneriana, Rheedia benthamiana, Rheedia gardneriana, Rheedia macrophylla*	charichuelo hoja grande, bacuriparí	Clusiaceae	a, b*, c, d, i	evergreen	Bolivia, Brazil, Ecuador, French Guiana, Guyana, Peru, Suriname, United States, South-East Asia
Hevea brasiliensis (Willd. ex A.Juss.) Müll.Arg.	*Siphonia ridleyana, Siphonia brasiliensis, Hevea granthamii, Hevea paludosa, Hevea sieberi, Hevea janeirensis, Siphonia janeirensis, Hevea randiana, Hevea camargoana, Hevea brasiliensis var. randiana, Hevea brasiliensis forma randiana, Hevea brasiliensis var. stylosa, Hevea brasiliensis var. acreana, Hevea brasiliensis forma angustifolia, Hevea brasiliensis var. angustifolia, Hevea brasiliensis granthamii, Hevea brasiliensis var. janeirensis, Hevea brasiliensis forma latifolia, Hevea brasiliensis var. latifolia, Hevea brasiliensis forma acreana*	Seringueira verdadeira	Euphorbiaceae	b, c*, i, j	---	Benin, Bolivia, Brazil, Congo, Cote Divoire, Cameroon, Colombia, Costa Rica, Dominican Republic, Ecuador, Fiji, Gabon, Fench Guiana, Ghana, Ecuatorial Guinea, Guatemala, Honduras, Haiti, Indonesia, India, Sri Lanka, Liberia, Myanmar, Mexico, Malysia, Nigeria, Nicaragua, Panama, Peru, Papua New Guinea, Philippines, Singapore, Thailand, Trinidad and Tobago, Chinese Taipei, United States, Venezuela, Viet Nam
Hevea guianensis Aubl.	*Siphonanthus elasticus, Siphonia cahuchu, Siphonia guianensis, Caoutchoua elastica, Caoutchoua guianensis, Siphonia elastica, Hevea guianensis subsp. typica, Hevea guianensis forma typica*		Euphorbiaceae	b, c*, d	---	Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Indonesia, Peru, Suriname, Venezuela

Plant species	Synonyms	Common names	Family	Ecosystem[1]	Functional[2] Type	Country occurences[3]
Hevea spruceana(Benth.) Müll.Arg.	*Siphonia discolor, Siphonia spruceana, Micrandra ternata, Hevea discolor, Hevea paraensis, Hevea similis, Hevea spruceana var. discolor, Hevea spruceana forma discolor, Hevea spruceana var. similis, Hevea spruceana forma similis, Hevea spruceana var. tridentata, Hevea spruceana forma tridentate*	Seringa barriguda	Euphorbiaceae	a*, b*, d	deciduous/brevi-deciduous	Bolivia, Brazil, Colombia, Costa Rica, Peru
Hura crepitans L.	*Hura brasiliensis, Hura strepens, Hura senegalensis, Hura crepitans var. oblongifolia, Hura crepitans var. orbicularis, Hura crepitans forma oblongifolia, Hura crepitans forma ovata, Hura crepitans var. senegalensis, Hura crepitans var. senegalensis, Hura crepitans var. strepens, Hura crepitans var. membranacea, Hura crepitans forma orbicularis, Hura crepitans var. ovata, Hura crepitans var. genuina*	Sandbox tree, areeiro, assacuzeiro, assacu, caçacu, guaçacu	Euphorbiaceae	a*, c, i	brevi-deciduous	Netherlands Antilles, Benin, Bolivia, Brazil, Bahamas, Belize, Central African Republic, Cote Divoire, Colombia, Costa Rica, Cuba, Domincan Republic, Ecuador, French Guiana, Guatemala, Indonesia, Lao Peoples Democratic Republic, Madagascar, Martinique, Mexico, Nicaragua, Panama, Suriname, El Salvador, Togo, Thailand, Trinidad and Tobago, Chinese Taipei, Tanzania, United States, Venezuela, Vietnam
Laetia corymbulosa Spruce ex Benth.		Sardinheira	Flacourtiaceae	a*	brevi-deciduous	Bolovia, Brazil, Colombia, Ecuador, Perú
Macrolobium acaciifolium Benth.	*Vouapa acaciaefolia, Outea acaciifolia, Macrolobium acaciifolium var. vestitum*	Arapari, Arapary, Arepillo, Arepito, Faveira Arapary, Parapari, Pashaquilla	Fabaceae	a*, b, c	brevi-deciduous	Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela
Nectandra amazonum Nees		Louro chumbo	Lauraceae	a*, e, f, g	evergreen	Bolvia, Brazil, Colombia, Guyana, Peru, Venezuela
Ocotea cymbarum Kunth		Louro inhamui	Lauraceae	a*, d	brevi-deciduous /evergreen	Brazil, Colombia, Ecuador, Guyana, Venezuela
Pachira insignis (Sw.) Sw. ex Savigny		Castanha silvestre	Malvaceae	a*, c	brevi-deciduous	Bolivia, Brazil, Cote Divoire, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, United States, Venezuela
Pouteria glomerata (Miq.) Radlk.	*Lucuma glomerata, Guapeba glomerata, Neolabatia glomerata, Richardella glomerata, Labatia glomorata, Pouteria glomerata var. typica*	Abiurana	Sapotaceae	a*, c, d, e, f, g, h	evergreen	Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Panama, Peru, Paraguay, Suriname, El Salvador, United States, Venezuela
Pseudobombax munguba (Mart. & Zucc.) Dugand		Munguba	Malvaceae	a*, b, d	deciduous	Brazil, Colombia, Ecuador, Peru
Psidium acutangulum		Goiaba	Myrtaceae	a*, c, d, h	deciduous	Bolivia, Brasil, Colombia, Costa Rica, Ecuador, Guayana, Perú, Surinam, United States, Venezuela
Salix martiana (Leyb)		Oeirana	Salicaceae	a*	evergreen	Perú, Brazil
Scleronema micranthum Ducke		Cardeiro, Castanha de paca, Cedrinho, Cedro bravo	Malvaceae	c*	---	Brazil, Colombia, Venezuela
Tabaernamontana siphilitica (L.f.) Leeuwenb.		Bonafusia	Apocynaceae	a*, d, f, g	evergreen	Brazil, Bolivia; Colombia; Ecuador, French Guiana; Guyana; Peru, Suriname; Venezuela

Plant species	Synonyms	Common names	Family	Ecosystem[1]	Functional[2] Type	Country occurences[3]
Vatairea guianensis Aubl..	*Vuacapua amazonum, Vatairea surinamensis, Ormosia pacimonensis, Andira bracteosa, Andira amazonum*	Ana Caspi, Andira De Varzea, Angelim-do-igapo, Arisauroe, Arisauru, Bois Dartres, Coumate, Fava Bolacha, Fava De Bolacha, Fava De Empilhagem, Faveira, Faveira Amarela, Faveira De Empigem, Faveira Do Igapo, Faveira Grande, Faveira Grande Do Igapo, Gaine Dartres, Guaboa, Lombrigueira, Maria Congo, Marupa Del Bajo	Fabaceae	a*, b*, c, d, e	deciduous	Brazil, Colombia, French Guiana, Guyana, Peru, Venezuela
Vitex cymosa Bert. Ex Spreng		Tarumã	Verbenaceae	a*, c, e, g, h	deciduous	Netherland Antilles, Argentina, Bolivia, Brazil, Colombia, Ecuador, Panama, Peru, Paraguay, United States, Venezuela
Zygia juruana (Harms)L.Rico	*Pithecolobium juruanum, Pithecellobium juruanum*	Jarandeua Da Folha Grande	Fabaceae	a*, c	evergreen	Brazil, Colombia, Ecuador, Guyana, Peru

[1] Missouri Bot Garden, New Bot. Garden, Royal Bot. Gardens Kew, INPA-Herbarium and Wittmann pers. com.
[2](Schöngart *et al.*, 2002)
[3]Global Biodiversity Informations Facility: http://data.gbif.org
n.d. not defined
* Indicates the plant's environment selected for the measurement
a) Várzea of Central Amazonian
b) Igapó of Central Amazonian
c) Amazonian Terra Firme
d) Orinoco basin
e) Atlantic rainforest (non-flooded)
f) Brazilian Pantanal (non-flooded)
g) Brazilian Pantanal (flooded)
h) Cerrado
i) Central America
j) West Africa, Asia

Two series of measurements were performed in different emplacements and using each time different enclosure systems (see chapter 2.2.2). The first experiment took place from the 18th of August to the 21st of September 2005 in the greenhouse of the botanical garden at Kiel´s University, Germany (see table 2.2.1.2). Ten plants from várzea were used for this experiment. Seeds of these plants were transported from the Island Ilha da Marchantaria near Manaus, Brazil, to Kiel, Germany, with the permission of the Brazilian Government and planted in pots.

During the VOC measurements, plants were around 1 ½ years old. Experiments took place in the greenhouse, where the plants were kept and grown. High temperature and high relative humidity conditions were provided in the greenhouse. Day time ambient temperatures ($T_{ambient}$) of 30 °C and relative humidity means of 60 % were monitored until the 7th of September. From the 8th of September mean temperatures in the greenhouse decreased to 25 °C and relative humidity increased to around 78 %. Relative humidity reached maximal values that are typical for the tropics during the whole experiment (69 – 96%). Ambient CO_2 concentrations were in the range of 310-371 ppm, reflecting concentrations typical from the present days (Prentice and Harrison, 2009). Plants were grown under low light conditions in the greenhouse (33-255 µmol m^{-2} s^{-1}), reaching values regarded as light saturation points of shade leaves of well irrigated tropical forest at optimal temperature and ambient CO_2 concentrations (Larcher, 2003). For this reason it was supposed that the high light intensity (1000 µmol m^{-2} s^{-1}) used during the measurements might be enough to reach a non-limiting photosynthetic photon flux.

The second screening experiment was performed outside at the main office of the Max Planck Project at the INPA, Manaus, Brazil during the dry season in the months of July, August and October 2006 and during the wet season in the months of November and December of 2006 and March-May 2007. Table 2.2.1.2 shows the plant age and the measurement period of each plant species. Seedlings were collected from the three mentioned environments of the Amazon region. The terra firme species *Hevea brasiliensis, Hevea guianensis* and *Scleronema micranthum* were collected at the Reserva Ducke (see figure 2.2.1.1), that is situated 40 km from Manaus; 90 % of the Reserva's area is covered by primary vegetation characteristic of the Central Amazon terra firme (Gomes and Mello-silva, 2006). The várzea species *Hevea spruceana, Hura crepitans, Macrolobium acaciifolium, Ocotea cymbarum, Pachira insignis, Pouteria glomerata, Pseudobombax munguba, Tabaernamontana siphilitaca, Vatairea*

guianensis and *Zygia juruana* were collected at the bank of the Ilha da Marchantaria (03°15´S, 59°58´W) an island located in the Solimões River and the igapó species *Garcinia macrophylla* and *Hevea spruceana* at the bank of the Tarumã Mirim (03°08´S, 60°01´W) an affluent of the Rio Negro (see figure 2.2.1.2). The different floodplain environments were compared collecting plants that occur in várzea and igapó like *Hevea spruceana* and *Vatairea guianensis*. After collection plants were potted in earth obtained in the original emplacement of the plant and they were let adapt at least one month to the new conditions before measurements. Plants were kept in the sun in a place protected from insects with mosquito nets and were irrigated daily. Mean temperature conditions of 30°C and relative humidity values of 78% ± 9% and 91 ± 7% during the dry and wet season respectively were recorded. Ambient CO_2 concentrations were in the range of 335-408 ppm.

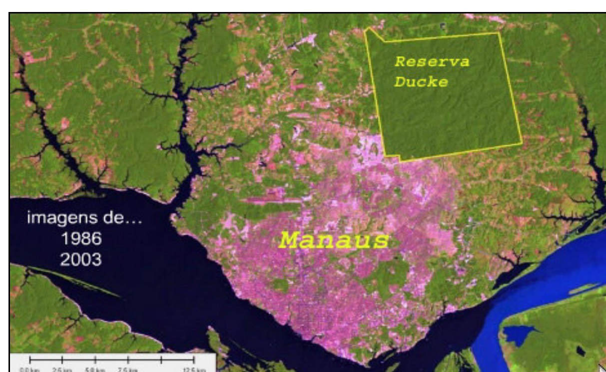


Figure 2.2.1.1 Satellite image of the Reserva Ducke Copyright © 2002-2009 authorSTREAM

Figure 2.2.1.2 Satellite image of the Ilha da Marchantaria and the Tarumã-mirim

Table 2.2.1.2 Age and measurement period of each investigated plant species

Plant species (environment)	n	Place of experiment	Age [year]	Measurement Period [mm/yyyy]
Annona hypoglauca (v)	3	K	1 ½	08/2005
Apeiba sp. (v)	3	K	1 ½	09/2005
Crescentia amazonica (v)	3	K	1 ½	08/2005
Crataeva benthamii (v)	3	K	1 ½	09/2005
Erythrina fusca (v)	3	K	1 ½	08/2005
Garcinia macrophylla (i)	3	M	< 1	07/2006
Hevea brasiliensis (tf)	3	M	1-2	04/2007
Hevea guianensis (tf)	2	M	1-2	05/2007
Hevea spruceana (i)	3	M	< 1	08/2006
Hevea spruceana (v)	2	M	1-2	03/2007
Hura crepitans (v)	3	M	<1	08/2006
Macrolobium acaciifolium (v)	3	K	1 ½	09/2005
Macrolobium acaciifolium (v)	1	M	< 1	11/2006
Nectandra amazonum (v)	3	K	1 ½	08/2005
Ocotea cymbarum	3	M	< 1	11/2006
Pachira insignis (v)	3	M	< 1	11/2006
Pouteria glomerata (v)	3	M	< 1	08/2006

Plant species (environment)	n	Place of experiment	Age [year]	Measurement Period [mm/yyyy]
Pseudobombax munguba (v)	3	M	< 1	07/2006
Psidium acutangulum (v)	3	K	1 ½	09/2005
Scleronema micranthum (tf)	3	M	< 1	12/2006
Tabaernamontana siphilitica (v)	1	M	< 1	12/2006
Tabaernamontana siphilitica (v)	3	K	1 ½	09/2005
Vatairea guianensis (i)	2	M	1-2	04/2007
Vatairea guianensis (v)	2	M	1-2	04/2007
Vitex cymosa (v)	3	K	1 ½	09/2005
Zygia juruana (v)	3	M	< 1	12/2006

(i) igapó
(v) várzea
(tf) terra firme
n: number of replicates
M: Manaus
K: Kiel

The results of the species *Salix martiana* and *Laetia corymbulosa* presented here belong to an experiment carried out at the Oldenburg University in 2000 by the Dr. Rottenberger and Dr. Kleiss. Details of the experimental setup can be obtained in Rottenberger *et al.* 2008.

In order to assure reliability on the data presented in this study a total of three replicates of each species were measured with some exceptions (see table 2.2.1.2).

2.2.2 GAS EXCHANGE MEASUREMENTS

A new dynamic enclosure system (GFS-3000 Portable Gas Exchange Fluorescence System, see figure 2.2.2.1) was used to determine VOC exchange rates during the first screening experiments carried out at Kiel´s botanical garden. This system was developed by the German manufacturer Walz GmbH for CO_2/H_2O exchange and fluorescence measurements under controlled conditions. VOC were measured by drawing a constant portion of the inlet and outlet air through the different equipments used for VOC measurements. Due to absorptions problems and high uncertainties detected by measuring VOC with this enclosure system, VOC data obtained with the GFS-3000 must be considered for a first orientation only. Further information about the validation of this system for VOC measurements are explained in appendix 1. The cuvette was constantly flushed with ozonfree scrubbed air (filter of copper nets covered with MnO_2) at 300-400 µmol s^{-1} allowing a total exchange of the cuvette volume every 5.9-10.5 s. In addition, a fan in the cuvette allowed homogenous mixing of the air. A small area (8 cm²) of the leaf was enclosed by the cuvette window of the Walz cuvette measuring head. Light and leaf temperatures were maintained constant at standard conditions (1000 µmol m^{-2} s^{-1} and 30°C). Light was produced by a LED Light Source 3040-L consisting

of 24 red and 2 blue LEDs placed around one centimetre above the measured leaf. Temperature, relative humidity and CO_2 concentration in the cuvette were followed and monitored. The plants were maintained at least one hour in the enclosure before the measurement started in order to adapt the leaf to the enclosure conditions. CO_2-Exchange and transpiration measurements were carried out with an integrated CO_2/H_2O gas analyzer in the Walz system (see figure 2.2.2.1). Stomatal conductance was provided by the system calculated according to Pearcy *et al.* (1989). At the end of each measurement the studied leaves were harvested and leaf area and dry weight were determined.

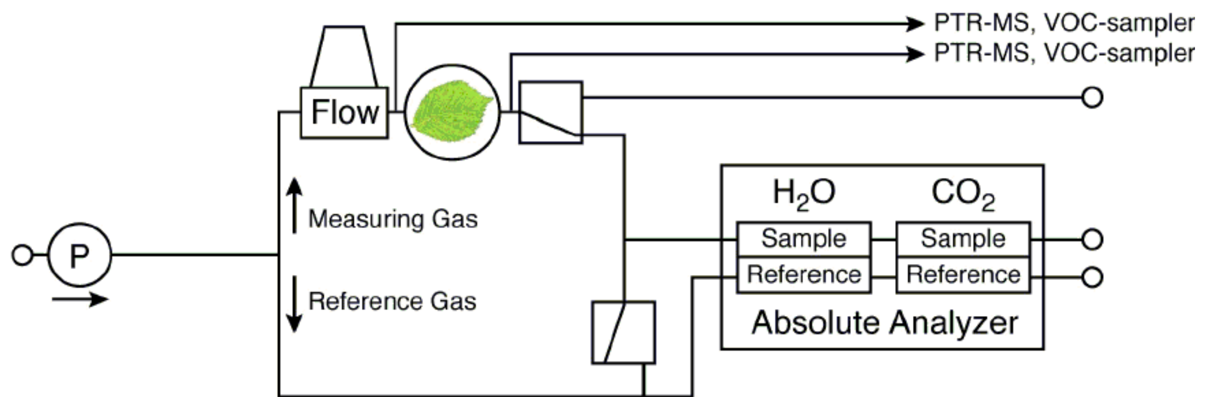


Figure 2.2.2.1 Experimental set-up of the portable Gas Exchange Fluorescence System GFS-3000 (© Heinz Walz GmbH 2007)

During the second screening experiment series performed at the INPA in Manaus, Brazil an enclosure system developed at the Max-Plank institute for Chemistry in Mainz, Germany was used. This enclosure system has been described in detail elsewhere (Kesselmeier *et al.*, 1996; Kuhn *et al.*, 2002a). Two identical branch cuvettes made of FEP Teflon foil (Norton, 50 µm thickness, Saint-Gobain Performance Plastics, Germany), that were fully light permeable (Schäfer *et al.*, 1992) were flushed with ozone free ambient air. One cuvette was used as reference (empty cuvette) and other cuvette enclosed a branch or, if possible, the complete above ground plant. As shown in figure 2.2.2.2 ambient air was scrubbed from small particles with Teflon filters (Zefluor Teflon filters, 2 µm pore size, Gelman Science, USA) and from ozone with an ozone scrubber composed by ten copper nets covered with MnO_2 (Ansyco, Germany) placed in a Teflon tube to prevent oxidant interferences inside the enclosure. A combination of three Teflon membrane pumps (Vacuubrand, Germany) was used to pump the filtered ambient air to both cuvettes passing through a flow meter (EL-Flow, 50 l min^{-1}, Bronkhorst Hi-Tec, Germany) monitoring the flow rate of each cuvette. Two different cuvette sizes were used depending on the size of the measured plant. For the small plants a 9 l cuvette was built and for the big plants a 100 l cuvette. The flow controlled by a needle valve was

adjusted to 10 l min^{-1} for the small cuvette and to 20-40 l min^{-1} for the larger cuvette. Table 2.2.2.1 gives an overview about cuvette size, flow parameters and air exchange times for each plant species investigated.

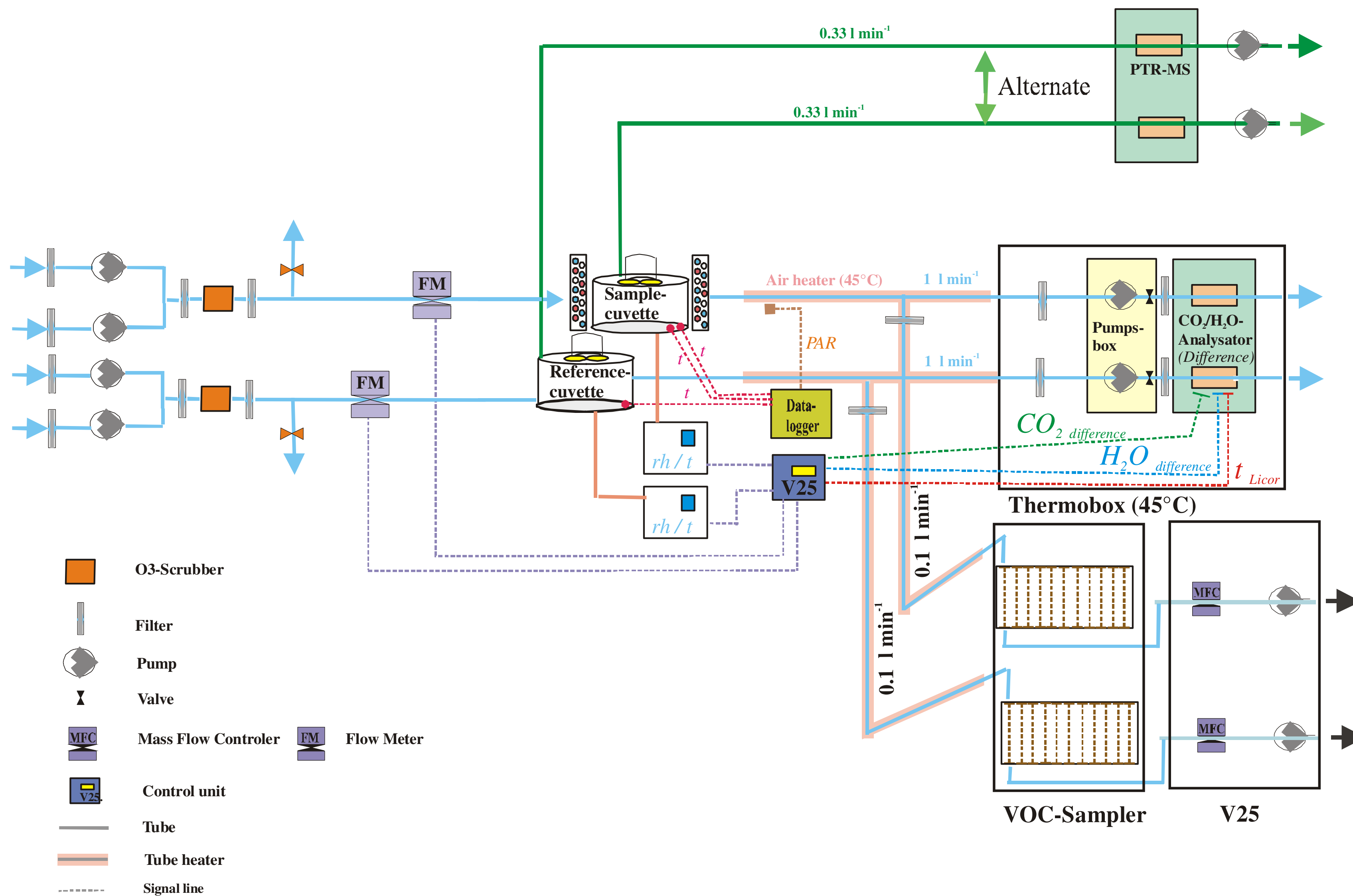


Figure 2.2.2.2 Experimental set-up for branch cuvette measurements used during the experiments at the INPA site in Manaus, Brazil

Table 2.2.2.1 Cuvette size, flow and time exchange of a cuvette volume in the branch cuvette system

Plant species	Cuvette Size [l]	Air Flow [l min^{-1}]	Exchange time of a cuvette volume [min]
Garcinia macrophylla	9	10	0.9
Hevea brasiliensis	100	20	4.9
Hevea guianensis	100	20	4.9
Hevea spruceana (v)	100	20	4.9
Hevea spruceana (i)	9	10	0.9
Hura crepitans	9	10	0.9
Macrolobium acaciifolium	100	40	2.5
Ocotea cymbarum	100	40	2.5
Pachira insignis	100	40	2.5
Pseudobombax munguba	9	10	0.9
Pouteria glomerata	9	10	0.9
Scleronema micranthum	100	40	2.5
Tabernaemontana siphilitica	100	40	2.5
Vatairea guianensis (v)	100	20	4.9
Vatairea guianensis (i)	100	20	4.9
Zygia juruana	100	40	2.5

A Teflon fan installed in the cuvette allowed a homogenous mixing of the air in the cuvette. A constant part of the air entering the sample and reference cuvette was bypassed to measure VOCs- CO_2 and H_2O-exchange and micrometeorological parameters (temperature and relative humidity in the cuvette measured by a temperature/relative humidity sensor, model Rotronics YA-100F, Walz, Germany). All tubing was made of Teflon and maintained at a constant temperature of 45°C, which was always higher than the cuvette temperature in order to avoid condensation in the lines.

CO_2 and H_2O exchange were measured by using a CO_2/H_2O infrared gas analyzer (LI-COR inc. 7000, Lincoln, Nebraska, USA). This equipment was operated in differential mode receiving an analog signal of the absolute concentration measured by a second CO_2/H_2O infrared gas analyzer (LI-COR inc. 7000, Lincoln, Nebraska, USA) as reference. A pressurized bottle of nitrogen (N_2 5.0, Messer Griesheim, Germany) was used as reference for CO_2 and H_2O zero point calibration of the infrared gas analyzers. The gas flow to the instrument was supplied by a custom made pump unit (membrane pump, 12 Volt; KNF-NEUBERGER) passing through a rotameter (Omega, USA) that adjusted the flow to 0.5 l min^{-1} and was placed in front of the analyzer inlet. Calibration of the analyzer was

accomplished prior to the experiments by use of a calibration gas standard for the calibration of CO_2 (512 ± 2 ppm CO_2 in synthetic air, LI-COR, Lincoln, Nebraska, USA) and a dew point generator for the calibration of water vapor (Li 610; LI-COR, Lincoln, Nebraska, USA). At the end of each experiment the calibration of the analyzer was checked and the signal response was corrected for sensitivity and zero drifts as a function of time. Furthermore, the signal response of the instrument was corrected for temperature effects and with regard to the offset of specified and measured reference concentrations. Assimilation, transpiration and stomatal conductance were calculated according to Pearcy, Schulze & Zimmermann (1989).

Measurements were performed under semi-controlled conditions following ambient relative humidity and temperature. In most cases photosynthetic active radiation (PAR) was constantly provided by a LED system consistent on four double chains of a mixture of red, blue and white light, constructed and designed by the electronic department of the MPI for Chemistry in Mainz, Germany. This LED system was placed perpendicular to the cuvette as shown in figure 2.2.2.2 and the gaps between the LED groups were closed reflecting film in order to obtain a homogenous distribution of the light in the cuvette. PAR was measured with a quantum sensor (Model SB 190, Licor, USA) inside the cuvette at different heights before and after the measurements. Except during the measurement of the plant species *Garcinia macrophylla, Hevea spruceana* (igapó), *Hura crepitans, Pouteria glomerata, Pseudobombax munguba* ambient light conditions were followed. The light intensity and the temperature of the cuvette and the leaf measured with thermocouples of type E (Chrom-Constantan, OMEGA) and were recorded with a datalogger CR23X (Campbell Scientific Ltd. Shepsherd, UK). All other micrometeorological and physiological parameters were monitored and recorded by a control unit (V25).

2.2.3 VOC MEASUREMENTS

There are different methods for determining VOC emissions. Chromatographic methods are still the most effective instrument in the gas analysis. However, these methods are unable to perform online analysis. Therefore, in recent years have been developed efficient methods for the systematic online analysis of various gases, such as the proton-transfer mass spectrometry (PTR-MS). As the name indicates this method is a mass spectrometry method with chemical ionisation.

During this work, the emissions of volatile organic compounds (VOCs) were measured by three different methods: by collection of cartridges and afterwards analysis of the samples

with two offline gas chromatographic (GC-) methods, one with a flame ionisation detector (FID) and another with a mass spectrometer (MS) and as the third method with online PTR-MS

2.2.3.1 SAMPLE TECHNIQUE

Silicosteel Cartridges filled with Carbograph 5 and 1 were used to collect VOCs using an automated cartridge sampling device (see figure 2.2.3.1) (Kuhn *et al.*, 2005). This sampling device has capacity for 20 cartridges containing a separate air input for each group of 10 cartridges and allowing a synchronous collection of the reference and the sample cuvette. A 12 volt membrane pump (KNF-NEUBERGER) sucked the filtered air (Zefluor Teflon filters, 2 µm pore size, Gelman Science, USA) from the cuvette. The flow was regulated with a mass flow controller (size 500 sccm, MKS Instruments, USA). Automatic sampling at preset times was achieved by a control unit (V25). A total of four litres of air were collected in each cartridge sampling during 40 minutes with a flow of 100 ml min^{-1}.

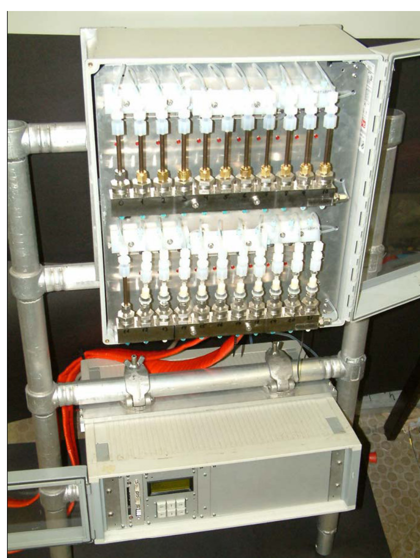


Figure 2.2.3.1 Automated cartridge sampling device with control unit

2.2.3.2 GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR (GC-FID)

VOC species collected on the cartridges were analyzed in the GC-laboratory of the MPI for Chemistry in Mainz, Germany, using a GC coupled to a FID (Model AutoSystem XL, Perkin Elmer, Germany). Further cartridge samples were analysed by GC-MS in the CNR laboratory Montelibretti (Ciccioli et al. 2002).

For the Mainz analyses helium was used as the mobile carrier gas. A 100 meter HP-1 column with a 0.22 mm inner diameter and coated with the non-polar substance methyl silicone served as stationary phase.

The air mixture collected in the cartridges was discharged into the gas stream with the help of a two step desorption system (Model ATD400, Perkin Elmer, Germany) as described by Kesselmeier *et al.* (2002a). Air samples were cryfocused in a cold trap at -30 °C filled with Carbograph 5 allowing better defined peaks in the chromatograms. Afterwards the cold trap was rapidly heated for 2-3 seconds to 280°C and the pre-concentrated air injected onto the column.

Following temperature programme was used: (-10 to 40°C at 20°C min^{-1}, 40 to 145°C at 1.5°C min^{-1}, and 145 to 220°C at 30°C min^{-1})

At the end of the column the compounds were detected with a very sensitive Flame ionization detector (FID) (Gottwald, 1995). Calibration for VOCs containing no heteroatoms was achieved by using standard gas mixture of isoprene and several n-alkanes (n-pentane, n-hexane, n-heptane, n-octane, n-nonane, and n-decane) (Apel-Riemer). In this case it is assumed that the "effective carbon number" (Sternberg *et al.*, 1962) is equal to the real carbon number of the molecules (Komenda, 2001), yielding a signal response that is proportional to the real carbon number. Another standard gas performed also by Apel-Riemer (USA) containing toluene was used for the calibration of this compound.
Based on the sequence (retention time) and the height of the peaks, the chemical components were identified and quantified. Table 2.2.3.2 shows the evaluated VOCs and their retention times.

Table 2.2.3.2 VOCs evaluated by GC-FID analysis.

VOC		Retention time [min]
Alkanes	n-pentane	~14.5
	n-hexane	~18.8
	n-heptane	~26.1
	n-octane	~36.2
	n-nonane	~47.9
	n-decane	~59.9
Hemiterpenes	isoprene	~14.8
Monoterpenes	camphene	~54.3
	Δ-3-carene	~61.4
	p-cymene	~62.1
	limonene	~63.3
	myrcene	~58.2
	alpha-pinene	~52.5
	ß-pinene	~57.5
	sabinene	~56.6
	alpha-terpinene	~61.8
	γ-terpinene	~66.4
Aromatic compounds	toluene	~31.8

2.2.3.3 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

In addition to the GC-FID analysis carried out at the MPI for Chemistry, samples for GC-MS analysis were collected occasionally on glass tubes (6 mm OD, 160 mm length) filled sequentially with 130 g Tenax GC, 20–35 mesh, (Alldrich, USA) and 115 g Carbograph 1, 20–40 mesh, (LARA S.p.A., Rome, Italy). Analyses of these cartridges were performed with a GC-MS at the Italian Research Council (CNR) in Rome, Italy. A detailed description of the GC-MS analysis applied by CNR is given by Ciccioli *et al.* (2002) and Fares *et al.* (2009). The terpenes evaluated from these analyses were isoprene, thujene, alpha-pinene, ß-pinene, camphene, p-cymene, sabinene, myrcene, alpha-phellandrene, ß-phellandrene, Δ-3-carene, limonene, cis-ß-ocimene, tans-ß-ocimene, gamma-terpinene and linalool.

2.2.3.4 PROTON TRANSFER REACTION-MASS SPECTROMETRY (PTR-MS)

An online gas analysis of VOCs was performed by a proton transfer reaction mass spectrometer.

The PTR-MS consists of three main components (see figure 2.2.3.4): an ion source, a reaction chamber (drift tube) and a detection system.

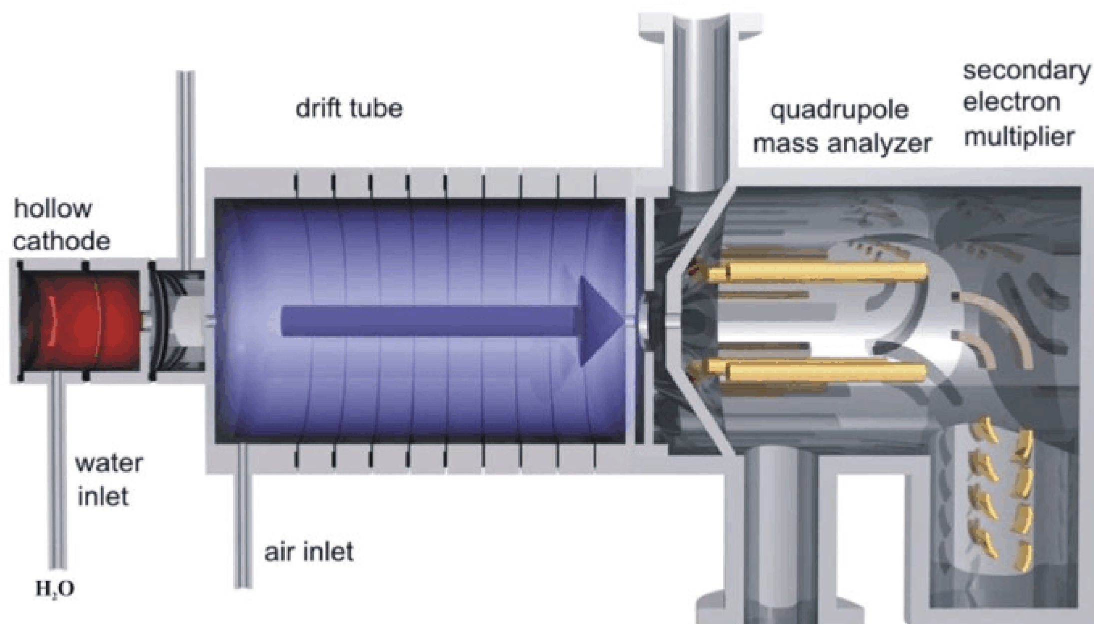
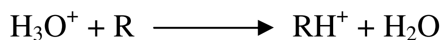


Figure 2.2.3.4 Schematic representation of the PTR-MS system[1]

This method uses the principle of the chemical ionization to detect gas mixtures (Munson and Field, 1966). Hereby the gas to analyze is ionised through proton exchange reactions with H_3O^+ (primary ions). The primary ions are produced in the ion source and transported together with the buffer gas (air) with the density [B] through the influence of an electric field in the drift tube. The density of the buffer gas [B] must be much greater than the density of the reactant gas [R] in order to ensure that the primary ions do not react completely in the front part of the drift tube making quantification impossible. In the drift tube the following proton exchange reactions take place:

$H_3O^+ + R$  $RH^+ + H_2O$

R is the reactant and RH^+ the product ion. With this method, all neutral molecules that have a higher proton affinity than water (166.5 kcal mol^{-1}) can be detected, which includes a great number of VOCs. The proton transfer reaction is not energetically possible for the main components of the air since they have lower proton affinity than water.

[1] http://www.ptrms.com/technology/technology.html

The mass selection is performed by a conventional quadrupole mass analyzer (QMG 422) and the detection of the ions by a Secondary Electron Multiplayer (SEM).
The instrument was operated in selected ion-monitoring mode at standard operational settings (E/N = 130 Td; E electric field strength, N buffer gas number density, 1 Td = 10^{-17} cm² V molecule^{-1}) by a drift tube pressure of 2.1 mbar and a voltage of 600 V, measuring the sample cuvette and the reference cuvette for eleven cycles, each (dwell time of 1s per mass). Each of the twenty-two measurement cycles were interspersed with seven cycles of instrumental background measurements by passing the reference air over a heated platinum catalyst maintained at 350 °C (Parker co., USA). This measurement sequence was repeated during twenty four hours of measurement for each experiment. The background signals were subtracted from the sample and reference air measurements.

The main compounds (protonated masses (Molecular Weight + 1)) detected by the PTR-MS and given as atomic mass per unit (amu) were methanol (33), acetone (59) isoprene (69), monoterpenes (137, fragment on 81) and a non identified compound (73 amu). Note that all identifications are tentative and different isomers of monoterpenes cannot be distinguished with a PTR-MS. Therefore, standard emissions factors for monoterpenes calculated as based on PTR-MS data in this study, always refer to the sum of all monoterpenes, respectively. The PTR-MS instrument was calibrated using a gas standard (Apel-Riemer, USA/Deuste Steininger GmbH, Germany) containing this study's target VOCs mixed with nitrogen at concentrations of ~300 ppb (± 5% accuracy by Apel-Rimer and ± 10% accuracy by Deuste). The standard gas was diluted with synthetic air to final concentrations of 0.5 – 10 ppb.

2.2.4 CALCULATION OF VOC MIXING RATIONS AND STANDARD EMISSION RATES (E_s)

The emission rates F of each compound was then calculated as µg g^{-1} h^{-1} if data were based on leaf weight and as µg m^{-2} h^{-2} if data were based on leaf area according to (1a and b) from the measured concentration difference ($\Delta c = c_{\text{sample cuvette or cuvette outlet}} - c_{\text{reference cuvette or cuvette inlet}}$), the enclosure flush rate Q, and the leaf dry weight (dw) or leaf area (A).

$$F = \Delta c(Q/dw). \qquad (1a)$$

$$F = \Delta c(Q/A). \qquad (1b)$$

Mixing ratios in µg g^{-1} h^{-1} were standardized using a mathematical algorithm developed by Guenther *et al.* in 1993, 1995 and 1997 (in following referred to as G93). This algorithm was

developed to predict isoprene emissions, but it has been demonstrated that G93 can very well predict monoterpene emissions (Ciccioli *et al.*, 1997; Kuhn *et al.*, 2002b, 2004a). G93 describes the emission of volatile organics as a function of a basic emission strength (i.e. a standard emission factor or basal emission rate) combined with environmental parameters like ambient light and temperature conditions. Standard emission factors that describes the basal VOC emission at standard conditions (1000 µmol m^{-1} s^{-1} and 30°C) were calculated according to (2)

$$Es = \frac{F}{C_L \cdot C_T} \qquad (2)$$

$$C_L = \frac{\alpha \cdot C_{L1} \cdot L}{\sqrt{1 + \alpha^2 \cdot L}} \qquad (3)$$

$$C_T = \frac{\exp\left(\frac{C_{T1} \cdot (T - T_s)}{R \cdot T_s \cdot T}\right)}{C_{T3} + \exp\left(\frac{(C_{T2} \cdot (T - T_M)}{R \cdot T_s \cdot T}\right)} \qquad (4)$$

where α is the empirical coefficient 0.0027, C_{L1} the empirical coefficient 1.066, C_L the light dependent term of the G93 function, C_T the temperature dependent term of the G93 function, C_{T1} the empirical coefficient 95000 J mol^{-1}, C_{T2} the empirical coefficient 230000 J mol^{-1}, C_{T3} the empirical coefficient 0.961, L the photosynthetic active radiation (PAR) in µmol m^{-2} s^{-1}, R the universal gas constant 8.314 J K^{-1} mol^{-1}, E_s the standard emission factor in µg g^{-1} h^{-1}, T the leaf temperature in °K, T_M the empirical coefficient 314 °K, T_S the leaf temperature at standard condition 303 °K and F the VOC emission rate in µg g^{-1} h^{-1}.

2.2.5 APPLIED STATISTICS

2.2.5.1 AVERAGE AND STANDARD DEVIATION

The formula (5) and (6) were used to calculate the arithmetic average, that is the sum of all observations x divided by the number of observations n, and the standard deviation, that is a measure of the variability, in a normal distributed dataset, where x is the value of the mean, n is the sample size, and x_i represents each data value from i=1 to i=n

$$\bar{x} = \frac{\sum x_i}{n} \qquad (5)$$

$$s = \sqrt{\frac{n \cdot \sum x_i^2 - \left(\sum x_i\right)^2}{n \cdot (n-1)}} \qquad (6)$$

2.2.5.2 SLOPE; Y-INTERCEPT; CORRELATION COEFFICIENT AND STANDARD REGRESSION ERROR

By the linear relationship between two groups of datapairs ($x_{i,}\, y_i$) a linear regression line was draw through the data with n data points. This regression line was governed by the equation (7), with $\bar{x}$ and $\bar{y}$ representing the respective averages of all x and y values, m the slope and b the y-intercept for the data. The exact formula applied to calculate the slope and the y-intercept are represented in (8) and (9), respectively.

$$\bar{y} = m \cdot \bar{x} + b \qquad (7)$$

$$m = \frac{n\sum x_i y_i - \left(\sum x_i\right)\cdot\left(\sum y_i\right)}{n\sum x_i^2 - \left(\sum x_i\right)^2} \qquad (8)$$

$$b = \frac{\sum y_i - m\sum x_i}{n} \qquad (9)$$

The reliability for the linear relationship between the x_i and y_i values was given by the correlation coefficient r, yielding a coefficient ranging between +1 and -1, with +1 and -1 giving the best correlation between two datasets. Formula (10) was applied in order to calculate the correlation coefficient with n representing the number of data points.

$$r = \frac{n\left(\sum x_i \cdot y_i\right) - \left(\sum x_i\right)\cdot\left(\sum y_i\right)}{\sqrt{\left(n\sum x_i^2 - \left(\sum x_i\right)^2\right)\cdot\left(n\sum y_i^2 - \left(\sum y_i\right)^2\right)}} \qquad (10)$$

The residual sum of squares (RSS) *(*here called *"Standard regression error")* was used in order to identify the uncertainties provoked by the calculation of the standard emission factor with G93. RSS measures the discrepancy between the experimental data (VOC measurements in µg g^{-1} h^{-1} or µg m^{-2} h^{-1}) and the estimation model (C_L * C_T). In this statistics, a small RSS indicates a tight fit of the model to the data.

2.2.5.3 DIXON'S Q-TEST

The identification of outliers from a set of replicate measurements was tested using the Dixon's Q-test. By the application of this test a normal (Gaussian) distribution of the data is assumed. The test allows us to examine if one observation from a small set of replicate observations (typically 3 to 10) can be "legitimately" rejected or not.

By the application of the test the n values comprising the set of observations under examination should be arranged in ascending order($x_1 < x_2 < . . . < x_N$). Then the statistical experimental Q-value (Q_{exp}), that is a ratio defined as the difference of the suspect value from its nearest one, divided by the range of the values was calculated according to formula (11). The obtained Q_{exp} value was compared to a critical Q value (Q_{crit}) found in tables (Kennedy, 1990). This critical value should correspond to the confidence level we have decided to run the test (usually: CL=95%). If $Q_{exp} > Q_{crit}$, then the suspect value can be characterized as an outlier and it can be rejected, if not, the suspected value must be retained.

$$Q_{exp} = \frac{x_N - x_{N-1}}{x_N - x_1} \qquad (11)$$

2.2.5.4 ANOVA-TEST

One way anova tests were carried out in order to detect possible differences among two or more independent groups. This test can be used when the data are normally distributed, the samples are independent and the variances of the data are equal.

The statistically significant difference effect in ANOVA was followed up with the Tukey's test, which compares every group mean with every other group mean and typically incorporate some method of controlling for false positive. This is the error identified by rejecting a null hypothesis when it is acutally true.

2.2.5.5 PRECISION OF MIXING RATIOS AND EXCHANGE RATES

Propagation error (value of precision)

The precision of all mixing ratios was calculated by Gaussian error propagation and gives the sum of uncertainty of all components used for the calculation of these mixing ratios.

In the case of the mixing ratios measured with the GC systems, the propagation error was calculated by the sum of the uncertainties provoked by the calibration factor (precision of analysis), the sample flow and the blank values (=cartridges analyzed without being

collected). Details of the exact formula used to calculate this error are reported by Dindorf (2006).

The value of precision calculated for the mixing ratios measured with the PTR-MS system, was accomplished using also the Gaussian error propagation. The sum of the uncertainties originated by the calibration factor, the drift tube temperature, the drift tube pressure and the background signal were taken in to account in order to calculate the precision value. Formula (12) represents this calculation.

$$\text{PrecisionMR} = \text{MR}\left(\frac{\sqrt{\left(\left(RH^{+}-RH^{+}_{cat}\right)\cdot\sqrt{\left(\frac{P_{Analyt}\cdot CalFactor}{\left(RH^{+}-RH^{+}_{cat}\right)}\right)^{2}+P_{Tdrift}+P_{Pdrift}}\right)^{2}+SD_{RH^{+}}{}^{2}+SD_{RH^{+}_{cat}}{}^{2}}}{RH^{+}-RH^{+}_{cat}}\right) \quad (12)$$

Formula (12) MR = Mixing Ratio in [ppb], RH^{+} = mean sample signal of five minutes measurement (n=8) in [cps], RH^{+}_{cat} = mean background signal of five minutes measurement (n=3) in [cps], P_{Analyt} = precision of analysis in [ppb], CalFactor = calibrations factor in [ppb], P_{Tdrift} = relative uncertainty of the drift temperature in [%], P_{Pdrift} = relative uncertainty of the drift pressure in [%], SD_{RH+} = Standard deviation of sample signal measured for five minutes (n=8) in [cps], SD_{RH+cat} = Standard deviation of background signal measured for five minutes (n=8) in [cps]

2.3. RESULTS

2.3.1 MICROCLIMATIC CONDITIONS

- Greenhouse experiment at the botanical garden of Kiel's University, Germany:

During this experiment leaf temperature and light intensity in the enclosure system was maintained constant at standard conditions (30 °C leaf temperature and 1000 μmol m^{-2} s^{-1} light intensity). Leaf temperature was 1 - 2°C lower than the enclosure temperature due to the cooling effect produced by the transpiration effect (Cook and Dixon, 1964) (see table 2.3.1). Ambient CO_2 entering the enclosure system was monitored and a mean of 341 ± 13.5 ppm was registered. High relative humidity values typical from the tropics (88 ± 6 %) were maintained in the enclosure system.

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- Outdoor experiment at surroundings of the INPA/Max-Planck station in Manaus, Brazil:

The experiments carried out at the INPA were executed under semi controlled conditions (see table 2.3.1). The Photosynthetic Active Radiation (PAR) that is one of the main factors influencing photosynthesis as well as stomatal conductance, VOC emissions and other important physiological processes in the plant, was maintained constant during the measurement of each individual from the same species, with exception of four species (see table 2.3.1). In any case, PAR was high enough to assure a non-limiting photosynthetic photon flux. Light saturation curves performed for these species confirmed this assumption (see chapter 2.3.3). The rest of the micrometeorological parameters were not controlled. Even though the enclosure temperature was not controlled, temperature conditions during the day were quite constant throughout the whole experiments (32.7 ± 2 °C) and almost no difference between leaf and enclosure temperature was found (ΔT= ± 0.2 °C). The reason for this lack of temperature difference might be the efficient mixing of the air in the enclosure, which allows the leaf temperature range around ambient temperature (Cook and Dixon, 1964). Low relative humidity values were found in the measurements done during the dry season (July-August) by *Garcinia brasiliensis, Hevea spruceana* from igapó, *Hura crepitans, Pseudobombax munguba* and *Pouteria glomerata* (47 ± 12.8 %). Relative humidity means recorded during the rest of the measurements achieved values similar to those found during the experiment performed in Kiel (88.1 ± 8.4 %). CO_2 ambient concentrations recorded varied during the whole experiment (368.9 ± 21.6 ppm), but low differences were found between measurements of the same species.

Table 2.3.1 Microclimatic conditions during branch enclosure measurements of the screened plants in alphabetical order. Data are means of the measurement period by maximal photon active radiation (n = 120) ± SD. The sample enclosure and the leaf temperature (°C) is given along with the photosynthetic active radiation (µmol m^{-2} s^{-1}), CO_2 absolute concentration ($CO_{2\ abs}$) and relative humidity (RH) of the air entering the enclosure and the environment of each plant species (En), which can be igapó (i), várzea (v) and terra firme (tf). The place of experiment (p.e.) is identified as *K* for the greenhouse at the Botanical Garden in Kiel, Germany and *M* for the surrounding of the INPA/Max-Planck station in Manaus, Brazil.

Plant species	p.e.	En		T_{leaf} [°C]	PAR [µmol m^{-2} s^{-2}]	$T_{enclosure}$ [°C]	CO_2abs [ppm]	RH [%]
Annona hypoglauca	K	v	tree 1	30 ± 0.02	1000 ± 0.05	31.18 ± 0.15	349.94 ± 10.15	88.02 ± 0.43
			tree 2	30 ± 0.02	1000 ± 0.05	31.97 ± 0.05	345.44 ± 12.86	87.05 ± 0.20
			tree 3	30 ± 0.02	1000 ± 0.05	31.43 ± 0.06	329.56 ± 23.46	89.47 ± 0.40
Apeiba sp.	K	v	tree 1	30 ± 0.02	1000 ± 0.05	32.15 ± 0.11	335.25 ± 12.72	88.75 ± 1.64
			tree 2	30 ± 0.02	1000 ± 0.05	31.73 ± 0.11	339.43 ± 24.36	87.35 ± 1.62
			tree 3	30 ± 0.02	1000 ± 0.05	32.33 ± 0.14	339.25 ± 11.6	87.61 ± 0.71
Crescentia amazonica	K	v	tree 1	30 ± 0.02	1000 ± 0.05	32.20 ± 0.14	371.59 ± 43.05	75.35 ± 0.88
			tree 2	30 ± 0.02	1000 ± 0.05	31.10 ± 0.06	351.75 ± 36.78	75.89 ± 6.01
			tree 3	30 ± 0.02	1000 ± 0.05	32.03 ± 0.1	337.29 ± 5.08	76.89 ± 1.01
Crataeva benthamii	K	v	tree 1	30 ± 0.02	1000 ± 0.05	31.85 ± 0.06	350.70 ± 26.76	90.12 ± 1.57
			tree 2	30 ± 0.02	1000 ± 0.05	32.36 ± 0.06	335.34 ± 6.7	89.10 ± 1.05
			tree 3	30 ± 0.02	1000 ± 0.05	32.04 ± 0.05	355.25 ± 33.07	90.51 ± 1.17
Erythrina fusca	K	v	tree 1	30 ± 0.02	1000 ± 0.05	31.56 ± 0.04	310.23 ± 2.48	91.02 ± 0.25
			tree 2	30 ± 0.02	1000 ± 0.05	31.62 ± 0.29	329.30 ± 14.64	89.74 ± 2.73
			tree 3	30 ± 0.02	1000 ± 0.05	31.49 ± 0.07	326.89 ± 2.97	92.42 ± 0.38
Garcinia macrophylla	M	i	tree 1	35.4 ± 2.5	1211 ± 466*	34.2 ± 2.2	351.7 ± 8.5	40.2 ± 15.4
			tree 2	38.0 ± 1.7	1328 ± 368*	35.6 ± 1.0	347.1 ± 3.8	33.2 ± 8.8
			tree 3	34.8 ± 2.3	1238 ± 482*	34.9 ± 1.6	341.1 ± 5.3	36.1 ± 11.4
Hevea brasiliensis	M	tf	tree 1	33.9 ± 0.6	500 ± 7	33.3 ± 0.8	385.9 ± 4.3	67.3 ± 10.4
			tree 2	36.3 ± 1.3	506 ± 10	36.2 ± 1.3	373.9 ± 5.5	54.7 ± 12.3
			tree 3	31.4 ± 1.2	499 ± 6	31.5 ± 1.3	364.1 ± 8.8	79.6 ± 8.2
Hevea guianensis	M	tf	tree 1	33.6 ± 0.9	499 ± 6	33.3 ± 0.9	390.9 ± 9.4	68.7 ± 7.6
			tree 2	34.3 ± 1.2	499 ± 4	32.4 ± 1.2	380.8 ± 4.4	73.4 ± 6.1
Hevea spruceana	M	i	tree 1	33.8 ± 2	1170 ± 429*	34.7 ± 2	359.2 ± 6.2	38.1 ± 17.6
			tree 2	34.6 ± 1.4	1410 ± 372*	35.6 ± 1.4	350.4 ± 6.5	30.1 ± 11.3
			tree 3	37.4 ± 2.4	1407 ± 392*	35.8 ± 1.6	338.6 ± 4.7	32.4 ± 13.8
Hevea spruceana	M	v	tree 1	32.8 ± 1.4	496 ± 2	32.5 ± 1.5	394.8 ± 5.2	72.4 ± 2.6
			tree 2	33.3 ± 1.8	500 ± 7	32.4 ± 1.8	395.3 ± 5.2	63.8 ± 3.2
Hura crepitans	M	v	tree 1	31.9 ± 0.8	723 ± 254*	33.7 ± 1.1	348.3 ± 5.6	45.9 ± 9.8
			tree 2	32.8 ± 1.2	898 ± 361*	33.4 ± 1.2	335.8 ± 5.9	50.3 ± 9.7
			tree 3	31.0 ± 0.9	762 ± 386*	33.1 ± 1.2	334.6 ± 4.9	58.3 ± 6.0
Macrolobium acaciifolium	M	v	tree 1	30 ± 0.02	1000 ± 0.05	32.2 ± 2.1	375.72 ± 2.6	57.2 ± 2.2
			tree 2	30 ± 0.02	1000 ± 0.05	26.1 ± 0.4	381.8 ± 4.8	88.8 ± 0.8
Macrolobium acaciifolium	K	v	tree1	30 ± 0.02	1000 ± 0.05	31.15 ± 0.2	356.09 ± 5.45	77.08 ± 5.70
			tree2	30 ± 0.02	1000 ± 0.05	31.46 ± 0.18	362.45 ± 17.76	89.31 ± 1.87
			tree3	30 ± 0.02	1000 ± 0.05	31.27 ± 0.04	358.59 ± 16.03	89.79 ± 0.95
			tree4	30 ± 0.02	1000 ± 0.05	30.78 ± 0.06	328.39 ± 25	95.48 ± 1.42

Plant species	p.e.	En		T_{leaf} [°C]	PAR [µmol m-² s-²]	$T_{enclosure}$ [°C]	CO_2abs [ppm]	RH [%]
Nectandra amazonum	K	v	tree1	30 ± 0.02	1000 ± 0.05	31.56 ± 0.08	329.53 ± 12.59	90.64 ± 0.20
			tree2	30 ± 0.02	1000 ± 0.05	31.32 ± 0.06	343.14 ± 22.5	81.92 ± 0.60
			tree3	30 ± 0.02	1000 ± 0.05	32.10 ± 0.05	352.76 ± 15.06	86.59 ± 1.51
Ocotea cymbarum	M	v	tree 1	30.0 ± 1.3	591.7 ± 9	30.6 ± 1.5	388.4 ± 5.5	67.8 ± 1.5
			tree 2	32.6 ± 1.3	615.9 ± 46	32.6 ± 1.3	376.2 ± 5.0	59.3 ± 3.6
			tree 3	32.8 ± 1.8	614.1 ± 33	33.0 ± 1.8	353.7 ± 3.3	54.0 ± 1.9
Pachira insignis	M	v	tree 1	31.5 ± 0.8	608 ± 25	31.3 ± 0.9	386.67 ± 8.6	67.14 ± 3.8
			tree 2	31.9 ± 1.1	590 ± 11	32.0 ± 1.1	384.19 ± 9.9	67.27 ± 6.3
			tree 3	29.7 ± 1.1	587 ± 2	29.7 ± 1.1	363.07 ± 6.5	75.25 ± 4.3
Pouteria glomerata	M	v	tree 1	32.5 ± 1.5	575 ± 9	32.3 ± 1.5	352.9 ± 5.0	58.09 ± 12.7
			tree 2	32.1 ± 1.3	574 ± 7	31 ± 1.9	346.0 ± 34.6	70.93 ± 15.7
			tree 3	31.2 ± 0.7	570 ± 1.2	32.2 ± 1.2	349.8 ± 7.4	63.40 ± 7.5
Pseudobombax munguba	M	v	tree 1	33 ± 2.2	1078.9 ± 374*	32.5 ± 1.9	348.9 ± 4.1	47.6 ± 14.3
			tree 2	31.3 ± 2	918.6 ± 450*	31.4 ± 1.8	347.5 ± 9.7	58.1 ± 17.3
			tree 3	34.4 ± 1.2	1515.1 ± 214*	34.7 ± 1.2	337.1 ± 10.4	32.8 ± 7.8
Psidium acutangulum	K	v	tree1	30 ± 0.02	1000 ± 0.05	31.85 ± 0.06	328.82 ± 10.19	98.90 ± 0.56
			tree2	30 ± 0.02	1000 ± 0.05	31.80 ± 0.16	334.33 ± 9.99	97.11 ± 2.43
			tree3	30 ± 0.02	1000 ± 0.05	32.18 ± 0.09	321.10 ± 4.59	95.24 ± 3.01
Scleronema micranthum	M	v	tree 1	32.4 ± 1.7	616 ± 28	32.5 ± 1.7	389.4 ± 9.2	65.6 ± 6.5
			tree 2	33.5 ± 1.1	615 ± 33	33.4 ± 1.0	379.4 ± 8.5	63.6 ± 4.7
			tree 3	33.0 ± 1.5	590 ± 8	32.7 ± 1.4	353.3 ± 6.8	59.2 ± 5.8
Tabaernamontana siphilitica	K		tree1	30 ± 0.02	1000 ± 0.05	32.38 ± 0.05	333.74 ± 6.91	86.51 ± 0.77
			tree2	30 ± 0.02	1000 ± 0.05	32.18 ± 0.1	360.11 ± 42.70	84.84 ± 0.72
			tree3	30 ± 0.02	1000 ± 0.05	32.46 ± 0.17	333.22 ± 4.32	87.82 ± 1.42
Tabernaemontana siphilitica	M	v	tree 1	32.8 ± 2.2	612 ± 34	32.4 ± 2.3	381.0 ± 19.1	71.9 ± 15.6
Vatairea guianensis	M	i	tree 1	32.4 ± 1.7	497.6 ± 4.0	31.0 ± 1.9	402.0 ± 13.2	82.4 ± 8.0
			tree 2	33.1 ± 2.3	500.3 ± 6.9	32.6 ± 2.4	405.0 ± 16.1	76.4 ± 15.4
Vatairea guianensis	M	v	tree 1	31.4 ± 0.9	497.5 ± 3.7	30.5 ± 1.1	407.6 ± 17.3	83.8 ± 8.9
			tree 2	31.3 ± 0.7	497.3 ± 2.5	30.2 ± 1.0	400.6 ± 11.7	82.9 ± 7.1
Vitex cymosa	K	v	tree1	30 ± 0.02	1000 ± 0.05	32.72 ± 0.07	336.02 ± 3.95	74.55 ± 0.43
			tree2	30 ± 0.02	1000 ± 0.05	32.41 ± 0.08	345.32 ± 3.23	91.72 ± 0.63
Zygia juruana	M	v	tree 1	30.2 ± 0.7	608.5 ± 7	30.4 ± 0.8	389.7 ± 7.1	76.3 ± 3.0
			tree 2	32.8 ± 1.3	612.3 ± 6	32.5 ± 1.3	367.7 ± 6.0	66.5 ± 5.2
			tree 3	32.2 ± 1.5	614.2 ± 8	32.2 ± 1.5	364.3 ± 8.2	66.8 ± 7.7

*no control of PAR

2.3.2 PHYSIOLOGICAL PARAMETERS

Assimilation and transpiration were monitored and stomatal conductance and water use efficiency calculated (see table 2.3.2). The photosynthetic activity (assimilation) may be considered as a measurement of the "wellness" of the plant. Photosynthesis provides the plant with energy and organic compounds and it is directly related to the VOC production (Delwiche and Sharkey, 1993; Kuhn *et al.*, 2004b; Loreto *et al.*, 1996; Sharkey *et al.*, 1991). The assimilation data confirmed that all plants were photosynthetically active. Assimilation rates varied between 1.6 and 15 µmol m^{-2} s^{-1}. Such rates have been reported before for shade

leaves of tropical C3-plants (Da Matta *et al.*, 2001; Larcher, 2003). The pioneer tree *Erythrina fusca* and the non pioneer tree *Garcinia macrophylla* presented the highest and the lowest assimilations rate, respectively (see table 2.3.2). Assimilation rates in the range of 2.3-15.2 and 1.6-8.7 were detected for pioneer and non pioneer trees, respectively. Anova tests confirmed that there was a significant difference in assimilation between these two successional stages ($p<0.05$), observing lower assimilation values in the non pioneer trees.

For most of the measured species transpiration rates were ≤ 3 mmol m^{-2} s^{-1} except for *H. Guianensis* and one individual of *M. acaciifolium* (tree 4) exhibiting rates slightly above this value. Extremely high transpiration rates accompanied by corresponding high stomatal conductances was found for *Hevea spruceana* (igapó) (~20 mmol m^{-1} s^{-1}) and two individuals of *Pouteria glomerata* (tree 2 and 3) (~12 mmol m^{-1} s^{-1}) (see table 2.3.2).

Stomatal conductance was found to be very heterogeneous in the measured tree species and even within trees of the same species (see table 2.3.2). Several species from várzea, igapó as well as from terra firme forest, like *Apeiba sp.* (tree 3), *Crescentia amazonica, Garcinia macrophylla, Hevea brasiliensis* (tree 1 and 2), *Hevea spruceana* (várzea), *Laetia corymbulosa*, *Macrolobium acaciifolium* (only two individuals), *Ocotea cymbarum* (tree 2), *Pachira insignis, Psedobombax munguba, Scleronema micranthum, Salix martiana, Tabaernamontana siphilitica, Vatairea guianensis* (igapó) (tree 1), *Vitex cymosa* and *Zygia jurana* (tree 2) showed quite low stomatal conductance (< 3 mm s^{-1}). The rest of the species showed stomatal conductances from 3 to 10.9 mm s^{-1}. These values have been already found for other tropical tree species from the study areas (Parolin *et al.*, 2001). Exceptionally, *Pouteria glomerata* (tree 2 and 3), *Hevea spruceana* (igapó) and one individual of *M. acaciifolium* (tree 4) showed extremely high stomatal conductance (16.5-29 mm s^{-1}) and consequently high transpiration rates and low water use efficiency (<1 mmol m^{-1}).

The commonly observed correlation between photosynthetic capacity (assimilation) and stomatal conductance is demonstrated for all plant species except for all individuals of *Hevea spruceana* from igapó, the tree 2 and 3 from *Pouteria glomerata*, the tree 2 of *Hevea guianensis*, the last individual of *Macrolobium acaciifolium* (tree 4), the first individual of *Necandra amazonum* (tree1) and of *Psidium acutangulum* (tree1), whose stomatal conductance is disproportionally high (see figure 2.3.2). These data demonstrate that in these

special cases the stomatal conductance is not directly determined by the photosynthetic capacity of the leaf.

The water use efficiency (WUE) of the studied plants was in the range of 9.4 to 0.3 mmol mol^{-1}. Especially low WUE (< 1) was found in the species *Hevea spruceana* (igapó), *Hevea guianensis*, *Nectandra amazonum* (only first individual) and *Pouteria glomerata* (except for the first individual) coinciding with higher specific hydraulic conductance that allowed them to maintain probably low water potentials in the leaves and keep up high rates of transpiration and hence carbon fixation.

Possible differences between várzea and Igapó species were studied investigating the physiological parameters of the trees *Hevea spruceana* and *Vatairea guianensis* that occur in both environments. All physiological parameters of *Hevea spruceana* trees from várzea were significantly different to those of igapó. Assimilation, transpiration and stomatal conductance values of the várzea tree were significantly lower. On the other hand, physiology of *Vatairea guianensis* was similar in both environments, várzea and igapó, except for the assimilation rate that was slightly higher in várzea trees.

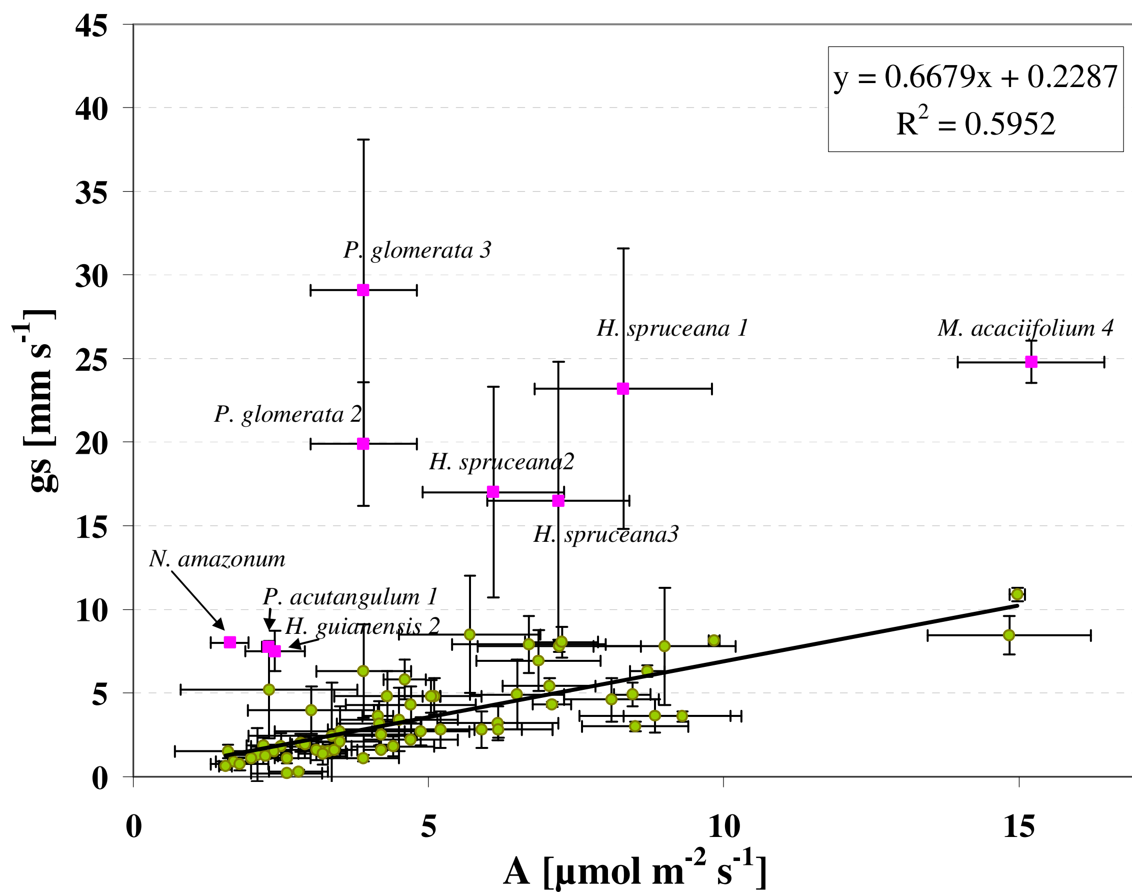
Table 2.3.2 Plant physiological parameters of screened plant species in alphabetical order. Data are means of measured periods at maximum quantum flux density (n = 120) ± standard deviation (SD). Given is the successional stage (NP: non pioneer, P: pioneer), Assimilation (A) in µmol m^{-2} s^{-1}, Transpiration (Tr) in mmol m^{-2} s^{-1}, stomatal conductance (gs) in mm s^{-1} and water use efficiency (WUE) in mmol mol^{-1} , the place of experiment (p.e.) (K= Kiel, M= Manaus) and the environment of each plant species (En), which can be Igapó (i), Várzea (v) and Terra Firme (tf).

Plant species	En	p.e.	Successional stage		A [µmol m^{-2} s^{-1}]	Tr [mmol m^{-2} s^{-1}]	Gs [mm s^{-1}]	WUE [mmol mol^{-1}]
Annona hypoglauca	v	K	NP	tree 1	8.70 ± 0.29	2.02 ± 0.10	6.31 ± 0.35	4.32 ± 0.22
				tree 2	7.09 ± 0.32	1.81 ±0.04	4.33 ± 0.16	3.92 ± 0.22
				tree 3	7.22 ± 1.38	2.50 ±0.09	7.80 ± 0.32	2.90 ± 0.64
Apeiba sp.	v	K	P	tree 1	8.83 ±0.28	1.49 ±0.27	3.63 ± 1.01	6.00 ± 0.43
				tree 2	6.17 ±1.02	1.24 ±0.27	3.19 ± 1.01	5.08 ± 0.61
				tree 3	6.18 ±0.40	1.25 ±0.13	2.80 ± 0.46	4.97 ± 0.40
Crescentia amazonica	v	K	NP	tree 1	3.21 ± 0.41	0.88 ±0.18	1.37 ± 0.67	3.80 ± 0.82
				tree 2	2.20 ± 0.29	0.96 ±0.13	1.86 ± 0.47	2.32 ± 0.31
				tree 3	2.83 ± 0.18	1.21 ±0.14	2.03 ± 0.54	2.37 ± 0.26
Crataeva benthamii	v	K	NP	tree 1	5.05 ± 0.14	1.70 ±0.27	4.80 ± 0.98	3.09 ± 0.78
				tree 2	4.15 ± 0.26	1.54 ±0.18	3.62 ± 0.67	2.71 ± 0.30
				tree 3	8.45 ± 0.31	1.80 ±0.19	4.90 ± 0.72	4.76 ± 0.72
Erythrina fusca	v	K	P	tree 1	9.84 ± 0.09	2.51 ±0.07	8.15 ± 0.24	3.92 ± 0.11
				tree 2	14.84 ± 1.39	2.84 ±0.31	8.46 ± 1.14	5.27 ± 0.65
				tree 3	14.98 ± 0.13	3.02 ±0.11	10.89 ± 0.40	4.96 ± 0.18

Plant species	En	p.e.	Successional stage		A [µmol m^{-2} s^{-1}]	Tr [mmol m^{-2} s^{-1}]	Gs [mm s^{-1}]	WUE [mmol mol^{-1}]
Garcinia macrophylla	i	M	NP	tree 1	2 ± 0.4	1.3 ± 0.3	1.1 ± 0.3	1.6 ± 0.8
				tree 2	1.7 ± 0.3	1.5 ± 0.2	0.9 ± 0.1	1.1 ± 0.2
				tree 3	1.6 ± 0.9	1.8 ± 0.3	1.5 ± 0.4	0.9 ± 0.5
Hevea brasiliensis	tf	M	P	tree 1	4.2 ± 0.6	1.4 ± 0.5	2.5 ± 0.6	3.4 ± 0.9
				tree 2	3.4 ± 0.3	1.3 ± 0.3	1.6 ± 0.4	2.7 ± 0.6
				tree 3	9.0 ± 1.2	1.9 ± 0.7	7.8 ± 3.5	5.3 ± 2.1
Hevea guianensis	tf	M	NP	tree 1	2.3 ± 1.5	3.1 ± 1.9	5.2 ± 2.9	0.7 ± 0.3
				tree 2	2.4 ± 0.5	4.9 ± 1.1	7.5 ± 1.2	0.5 ± 0.2
Hevea spruceana	i	M	NP	tree 1	8.3 ± 1.5	22.3 ± 6	23.2 ± 8.4	0.4 ± 0.1
				tree 2	6.1 ± 1.2	19.7 ± 3.4	17 ± 6.3	0.3 ± 0.1
				tree 3	7.2 ± 1.2	21.5 ± 3	16.5 ± 8.3	0.3 ± 0.1
Hevea spruceana	v	M	NP	tree 1	4.2 ± 0.3	0.8 ± 0.2	1.6 ± 0.1	5.7 ± 1.2
				tree 2	4.4 ± 0.7	0.8 ± 0.2	1.8 ± 0.6	6.1 ± 2.9
Hura crepitans	v	M	P	tree 1	9.3 ± 1	2.9 ± 0.5	3.6 ± 0.3	3.2 ± 0.5
				tree 2	8.5 ± 0.9	2.4 ± 0.5	3.0 ± 0.3	1.5 ± 1.9
				tree 3	6.7 ± 1.3	1.8 ± 0.4	7.9 ± 1.7	3.8 ± 0.2
Laetia corymbulosa	v	O	NP	tree 1	1.9 ± 0.4	0.6 ± 0.2	1.4 ± 0.3	3.2 ± 0.7
Macrolobium acaciifolium	v	M	P	tree 1	5.2 ± 1.9	1.7 ± 0.8	2.8 ± 1.1	3.6 ± 1.8
				tree 2	3.3 ± 1.0	-	-	-
Macrolobium acaciifolium	v	M	P	tree1	3.36 ± 1.41	1.22 ±0.87	2.44 ± 3.19	3.88 ± 2.85
				tree2	6.86 ± 1.05	2.27 ±0.49	6.93 ± 1.81	3.11 ± 0.57
				tree3	7.26± 0.62	2.40 ±0.25	8.04 ± 0.91	3.07 ± 0.65
				tree4	15.21 ± 1.24	3.81 ±0.34	24.79 ± 1.26	4.01 ± 0.39
Nectandra amazonum	v	K	NP	tree1	1.63 ± 0.32	2.52 ±0.04	8.00 ± 0.16	0.65 ± 0.13
				tree2	7.05 ± 0.79	2.36 ±0.13	5.41 ± 0.48	2.97 ± 0.22
				tree3	4.16 ± 0.72	1.40 ±0.35	3.18 ± 1.31	3.05 ± 0.38
Ocotea cymbarum	v	M	NP	tree 1	5.7 ± 1.2	2.8 ± 1.3	8.5 ± 3.5	2.4 ± 1.3
				tree 2	3.5 ± 1.1	1.8 ± 1.3	2.7 ± 1.5	2.6 ± 1.0
				tree 3	4.5 ± 1.0	2.1 ± 0.8	3.4 ± 1.9	2.3 ± 0.5
Pachira insignis	v	M	NP	tree 1	2.5 ± 0.5	0.9 ± 0.5	1.8 ± 0.8	3.2 ± 1.2
				tree 2	2.6 ± 0.6	1.2 ± 0.7	0.2 ± 0.1	2.9 ± 1.3
				tree 3	2.8 ± 0.5	1 ± 0.4	0.3 ± 0.1	3.1 ± 1.1
Pouteria glomerata	v	M	NP	tree 1	6.5 ± 0.8	2.4 ± 0.4	4.9 ± 1.6	2.76 ± 0.4
				tree 2	3.9 ± 0.9	11.7 ± 4.1	19.8 ± 3.7	0.34 ± 0.2
				tree 3	3.9 ± 0.9	12.7 ± 4.0	29.1 ± 9.0	0.31 ± 0.05
Pseudobombax munguba	v	M	P	tree 1	4.7 ± 0.8	1.9 ± 0.6	2.2 ± 0.4	2.6 ± 0.8
				tree 2	5.9 ± 1.2	1.6 ± 0.4	2.8 ± 1.1	3.9 ± 1.2
				tree 3	3.5 ± 0.7	2.7 ± 0.8	2.1 ± 0.7	1.4 ± 0.3
Psidium acutangulum	v	K	P	tree1	2.29 ± 0.10	1.60 ±0.07	7.76 ± 0.27	1.43 ±0.08
				tree2	3.01 ± 1.07	0.92 ±0.39	3.96 ± 1.43	3.39 ± 0.41
				tree3	4.60 ± 0.36	1.75 ±0.32	5.79 ± 1.19	2.72 ± 0.61
Salix martiana	v	O	P	tree 1	2.1 ± 0.75	0.7 ± 0.2	1.5 ± 0.4	3.3 ± 0.7
Scleronema micranthum	v	M	NP	tree 1	3.2 ± 0.3	0.8 ± 0.3	1.5 ± 0.2	4.3 ± 1.3
				tree 2	2.6 ± 0.4	0.7 ± 0.2	1.1 ± 0.3	4.0 ± 1.0
				tree 3	3.3 ± 0.3	1.1 ± 0.2	1.5 ± 0.4	3.1 ± 0.4
Tabernaemontana siphilitica	v	M	P	tree 1	3.1 ± 0.7	1.1 ± 0.4	1.6 ± 0.6	3.0 ± 1.0
Tabernaemontana siphilitica	v		P	tree1	2.91 ± 0.22	0.91 ±0.11	1.94 ± 0.42	3.22 ± 0.31
				tree2	2.38 ± 0.30	0.73 ±0.11	1.52 ± 0.39	3.37 ± 0.96
				tree3	4.87 ± 0.83	1.24 ±0.23	2.70 ± 0.86	3.96 ± 0.21

Plant species	En	p.e.	Successional stage		A [µmol m^{-2} s^{-1}]	Tr [mmol m^{-2} s^{-1}]	Gs [mm s^{-1}]	WUE [mmol mol^{-1}]
Vatairea guianensis	i	M	NP	tree 1	3.9 ± 0.6	0.4 ± 0.1	1.1 ± 0.2	7.4 ± 2.7
				tree 2	3.9 ± 0.8	2.4 ± 1.1	6.3 ± 2.8	2.2 ± 1.3
Vatairea guianensis	v	M	NP	tree 1	5.1 ± 0.8	1.1 ± 0.5	4.8 ± 1.1	5.9 ± 3.4
				tree 2	8.1 ± 0.8	1.1 ± 0.5	4.6 ± 1.3	9.4 ± 5.6
Vitex cymosa	v	K	NP	tree1	1.80 ± 0.49	0.29 ±0.10	0.75 ± 0.38	6.56 ± 1.19
				tree2	2.24 ± 0.21	0.41 ±0.05	1.24 ± 0.19	5.56 ± 0.72
Zygia juruana	v	M	NP	tree 1	4.3 ± 0.9	1.3 ± 0.3	4.8 ± 1.5	3.4 ± 0.7
				tree 2	2.1 ± 0.5	0.8 ± 0.4	1.3 ± 1.6	3.3 ± 1.2
				tree 3	4.7 ± 1.1	2.1 ± 0.9	4.3 ± 1.1	2.8 ± 1.4

Figure 2.3.2 Correlation of CO_2 assimilation (A) with stomatal conductance (gs). Pink squares are outliers found with a Q-test with a confidence level of 80-99%.



2.3.3 PHOTOSYNTHETIC LIGHT RESPONSE CURVES OF SOME SCREENED SPECIES

The photosynthetic light response curves of some screened plant species were detrmined using the GFS-3000 enclosure system. By illuminating the adaxial side of a leaf sector of the measured plants with different light intensities important physiological parameters, like the light compensation point (LCP) and the light saturation point (LSP), were characterized for the different species. The species chosen for this study were *Garcinia macrophylla, Hevea spruceana* (igapó)*, Hura crepitans, Macrolobium acaciifolium, Ocotea cymbarum, Pachira insignis, Pouteria glomerata* and *Pseudobombax munguba.* The photosynthetic light saturation point was reached at a photon flux density of 300 µmol m^{-2} s^{-1} in all measured tree species (see figure 2.3.3 a-h). Measured photosynthetic light saturation points are relatively high for young plants. Such a LSP has been found before in tropical tree's shade leaves (Larcher, 2003). Since the light could not be maintained constant in all performed experiments under ambient conditions it may, in some cases, be a limiting factor (in the case of *Garcinia macrophylla*, *Hevea spruceana* (igapó), *Hura crepitans* and *Pseudobombax munguba*). For this reason, we decided (after considering the results of the photosynthetic light curves) to take into account only the values of physiology or VOC measured with a light intensity higher than 300 µmol m^{-2} s^{-1}. At this light intensity, light is at least not a limiting factor for physiology.

The light curves of the studied plants (except for *Pachira insignis*) followed the typical pattern of a high light leaf, which assimilation continues increasing slightly even after saturation. Only the late succession tree *Pachira insignis* that is less light tolerant showed photosynthesis inhibition by the high PAR of 2000 µmol m^{-2} s^{-1}. However, the maximal assimilation rates of all trees individuals investigated were found to be quite low between 0.5 and 7 µmol m^{-2} s^{-1}. Light compensation points were observed at values typical for C3 plants with some species exhibiting very low values (see Table 2.3.3).

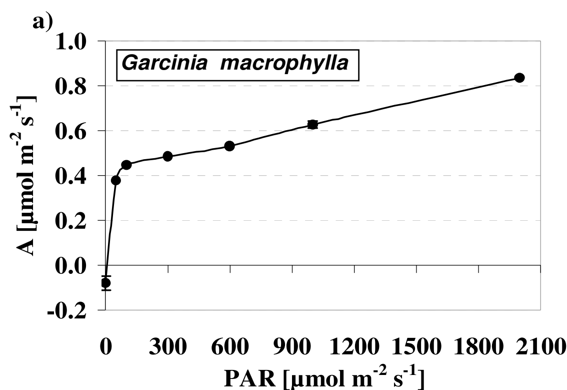
A_{max} for *Garcinia macrophylla* and *Pouteria glomerata* was extremely low during this experiment compared to the values measured during the screening experiments, showing that assimilation rates can be very variable even between leaves of the same individual. This difference in maximal assimilation may be due to differences in CO_2 transfer inside the leaf. As in other studies reported, mean maximum values of net photosynthesis under conditions of natural CO_2 concentrations, optimal temperature and good water supply, are usually in the

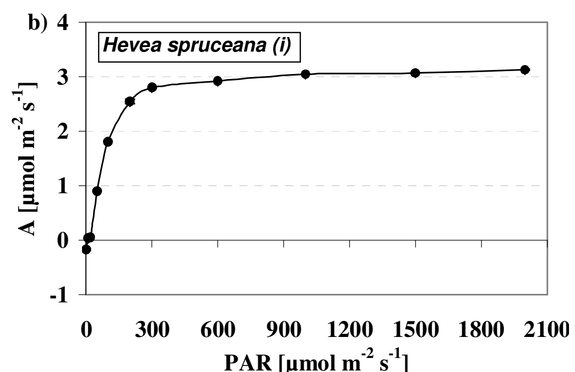
range of 5 to 7 µmol m^{-2} s^{-1} for shade leaves and 1.5 to 5 µmol m^{-2} s^{-1} for saplings of tropical trees (Larcher, 2003). Values in this order of magnitude were observed for the rest of the species investigated (see table 2.3.3). The highest A_{max} were found in the pioneer species *Hura crepitans* and *Pseudobombax munguba.*

Light compensation points typical of shade leaves of tropical trees were found for the majority of the studied species except for *Garcinia macrophylla, Ocotea cymbarum* and *Pseudobombax munguba,* which showed LCP values like those classically found in young tropical plants (Larcher, 2003). Usually pioneer trees have higher LCP than late successional trees (Timm *et al.*, 2002), but in this study no relationship between successional stage of the plant and LCP was found. The late successional tree *Pouteria glomerata* and *Ocotea cymbarum* showed the highest and the lowest LCP respectively.

Table 2.3.3 Maximal assimilation rate in µmol m^{-2} s^{-1} (A_{max}) and light compensation point in µmol m^{-2} s^{-1} (LSP).

	A_{max} [µmol m^{-2} s^{-1}]	LCP [µmol m^{-2} s^{-1}]
Garcinia macrophylla	0.5	3.3
Hevea spruceana (i)	2.8	10.3
Hura crepitans	5.0	13.2
Macrolobium acaciifolium	2.0	8.6
Ocotea cymbarum	3.0	2.2
Pachira insignis	2.0	12.6
Pouteria glomerata	0.6	19.0
Pseudobombax munguba	6.6	4.1





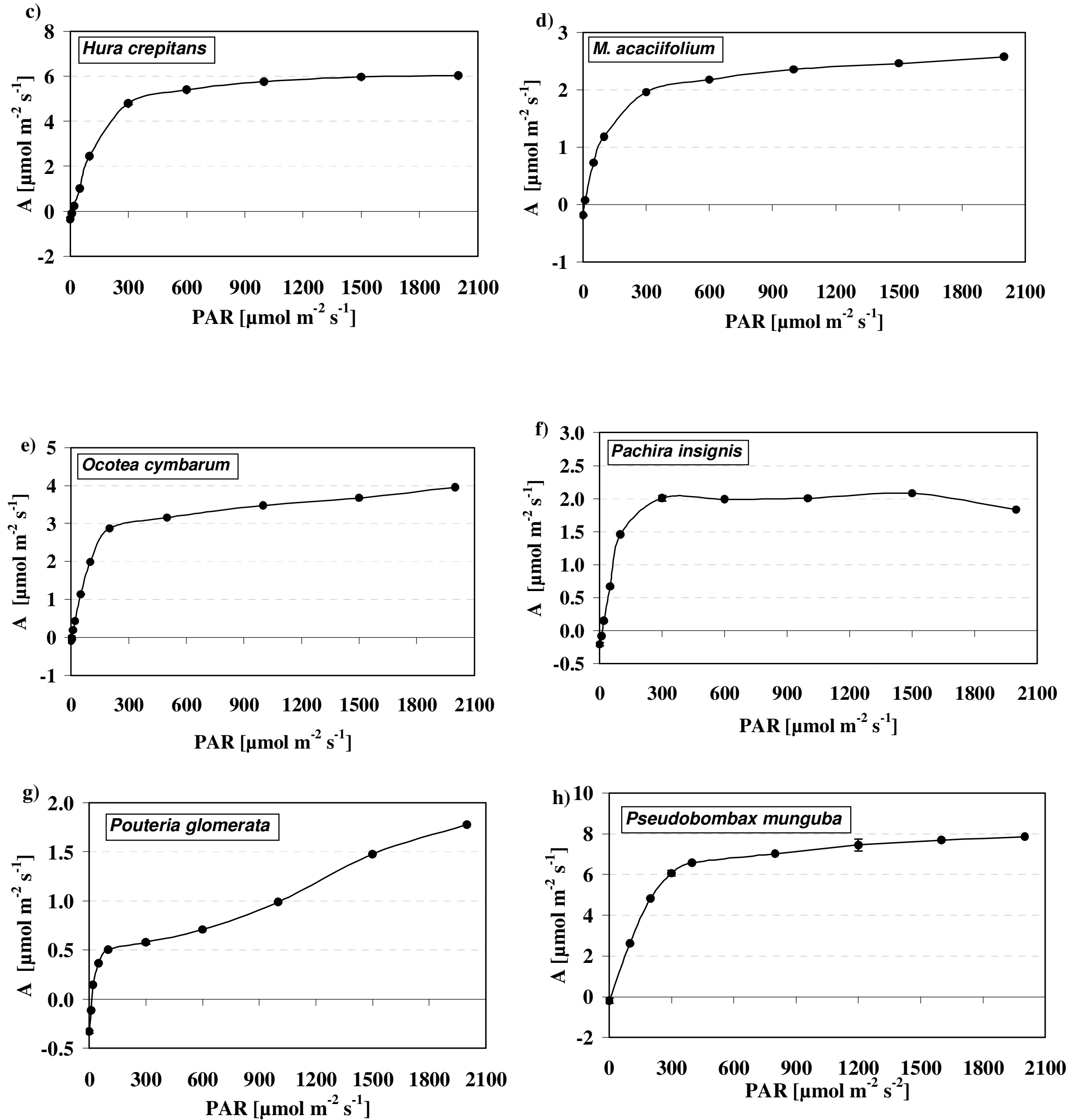


Figure 2.3.3 a-h Photosynthetic light curve of *Garcinia macrophylla, Hevea spruceana(i), Hura crepitans, Macrolobium acaciifolium, Ocotea cymbarum, Pachira insignis, Pouteria glomerata and Pseudobombax munguba.* Data points represents 5 minutes average (n=5). Standard deviations are indicated but smaller than symbols for assimilation rates.

2.3.4 EXCHANGE OF VOC

2.3.4.1 DETERMINATION OF THE VOC STANDARD EMISSIONS FACTORS OF THE SCREENED PLANT SPECIES

Every tree was measured for at least one day using the measured diurnal VOC emission for the estimation of standard emission factors (E_S), except for the measurement of *Garcinia macrophylla, Hevea spruceana (igapó), Hura crepitans, Pouteria glomerata* and

Pseudobombax munguba, where half day measurements were performed. As shown above, micrometeorological and physiological parameters were monitored during the one day measurements. Figure 2.3.4.1 a)-c) shows an example of a diurnal cycle of all micrometeorological and physiological monitored parameters.

During the experiments performed at Kiel's Botanical garden, measurements were realized at standard conditions. For this reason emission rates in this study were considered as standard emission rates. Experiments carried out at the INPA/Max-Planck Station in Manaus were not performed at standard conditions but at ambient conditions with additional light support. The phenomenological algorithm G93 described in 2.2.4 was used to standardize the constitutive VOC emissions. The terms C_T and C_L from G93 describes the dependence of emission rates on temperature and PAR, respectively. The factors C_L and C_T can be calculated using the measured light intensities and leaf temperatures and describe the relative emission performance under the given ambient conditions (see figure 2.3.4.1 d)). Plotting $C_L * C_T$ against the measured VOC emissions, the slope of this linear regression line gives the standard emission rate of VOC (E_s) often termed the basal emissions or emission factor at standard conditions (1000 µmol m^{-2} s^{-1} and 30°C) (example see figure 2.3.4.2). Light dependency curves of diurnal cycles of isoprene and monoterpene emissions were reasonably represented with the G93 algorithm (see figure 2.3.4.3-2.3.4.4).

Phenological algorithms can be successfully used to describe light and temperature dependencies for VOC originating from DMAPP (Dimethylallyl pyrophosphate), such as isoprene and monoterpenes. But since acetone, methanol and compounds with m/z 73 showed mostly light and temperature dependency, emission rates of these compounds could also be described with G93 (see figure 2.3.4.5-2.3.4.7). Methanol emission depends on the stomatal conductance and methanol is accumulated during the night in the stomatal cavities. Thus, in the morning a methanol peak was detected in some occasions, due to the release of the nocturnally accumulated methanol by the stomatal opening. This morning peak observed for methanol was excluded for the calculation of E_s.

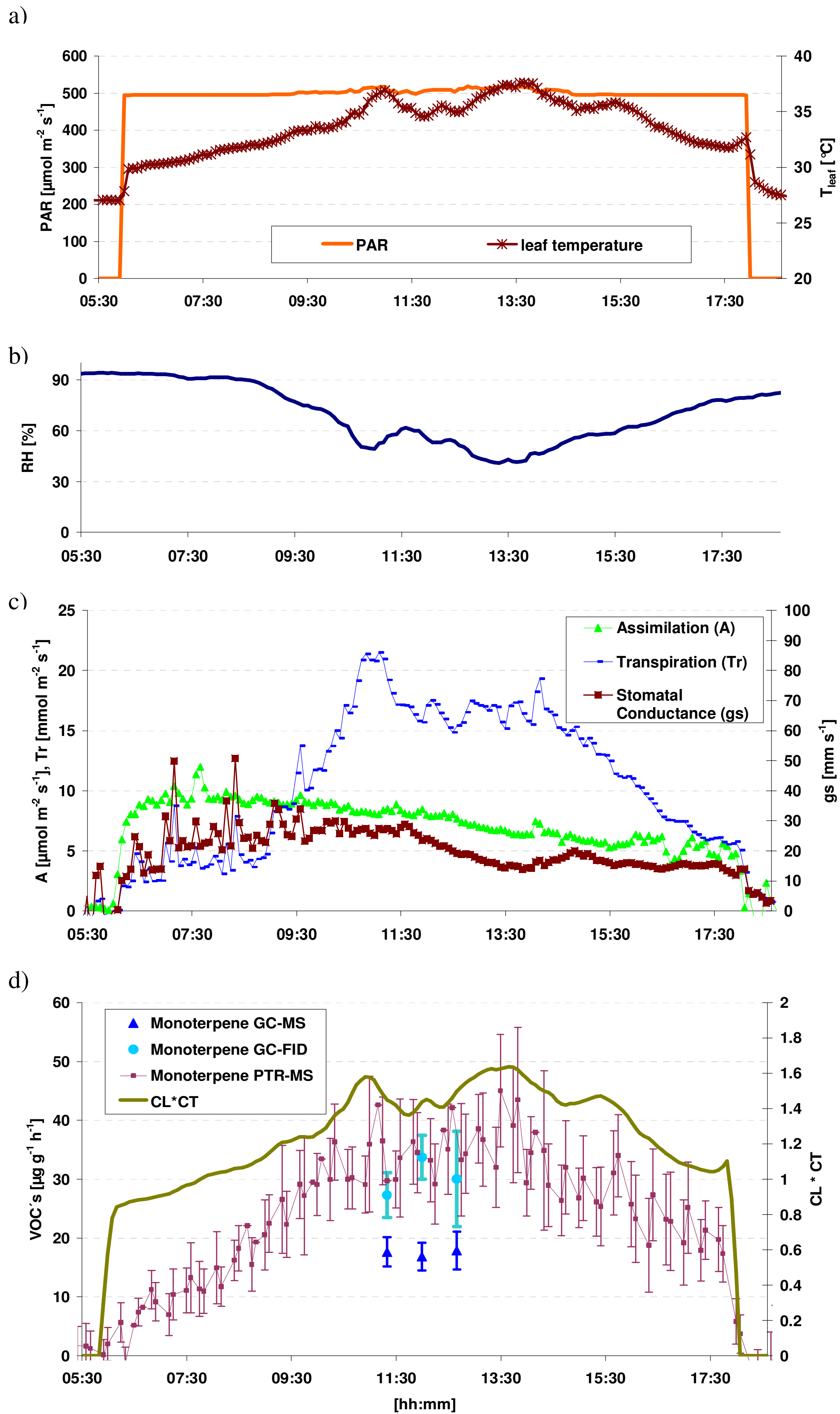


Figure 2.3.4.1 Example of a diurnal cycle of micrometeorological, physiological parameters and monoterpene emissions by *Hevea spruceana* (igapó). Plots show both the measured and the relative monoterpene emissions

(C_L * C_T). Three different methods for the monoterpene measurement are shown: GC-FID, GC-MS and PTR-MS. Assimilation (A) in µmol m^{-2} s^{-1}, transpiration (Tr) in mmol m^{-2} s^{-1}, stomatal conductance (gs) in mm s^{-1}, photosynthetic active radiation (PAR) in µmol m^{-2} s^{-1}, leaf temperature in °C and relative humidity in % are given. Each represented data point is a mean of 300 second measurements (n ~ 300) ± propagation error

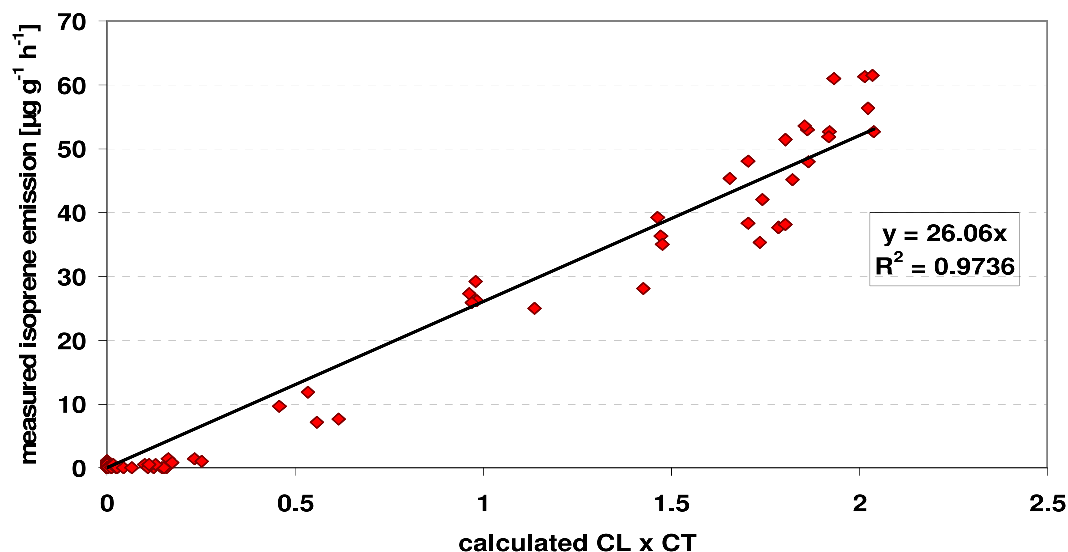


Figure 2.3.4.2 Example of standard emission factor (E_S) estimation for isoprene by *Garcinia macrophylla* from the slope of the regression line. Each represented data point is a mean of 300 second measurements (n ~ 300).

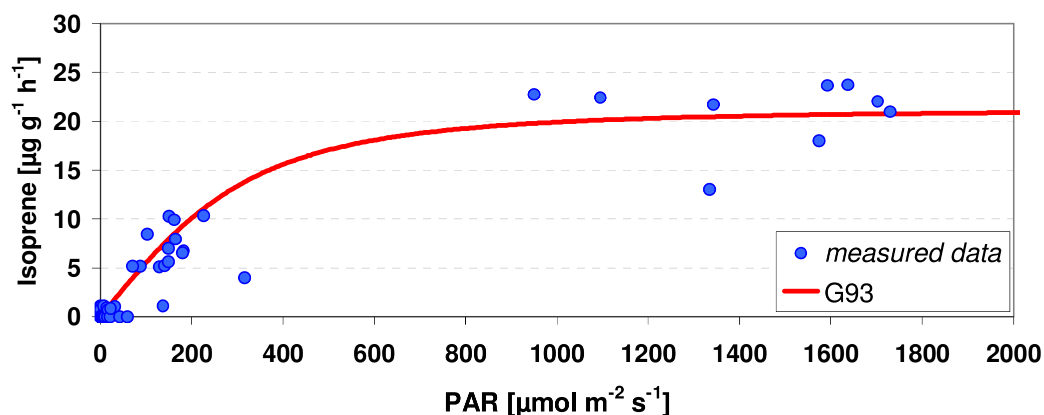


Figure 2.3.4.3 Isoprene emissions in relation to photosynthetic active radiation (PAR) compared with the G93. Measured data are normalized with G93 to 30°C. Each represented data point is a mean of 300 second measurements (n ~ 300)

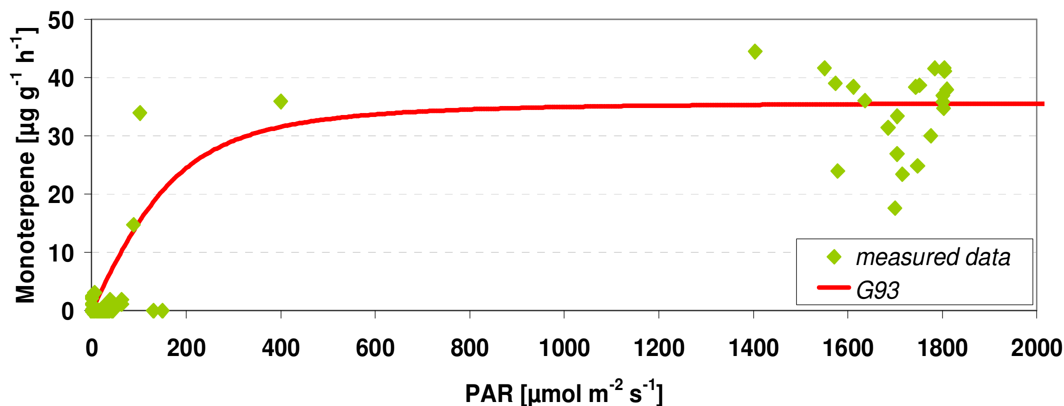


Figure 2.3.4.4 Monoterpene emissions in relation to photosynthetic active radiation (PAR) compared with the G93. Measured data are normalized with G93 to 30°C. Each represented data point is a mean of 300 second measurements (n ~ 300).

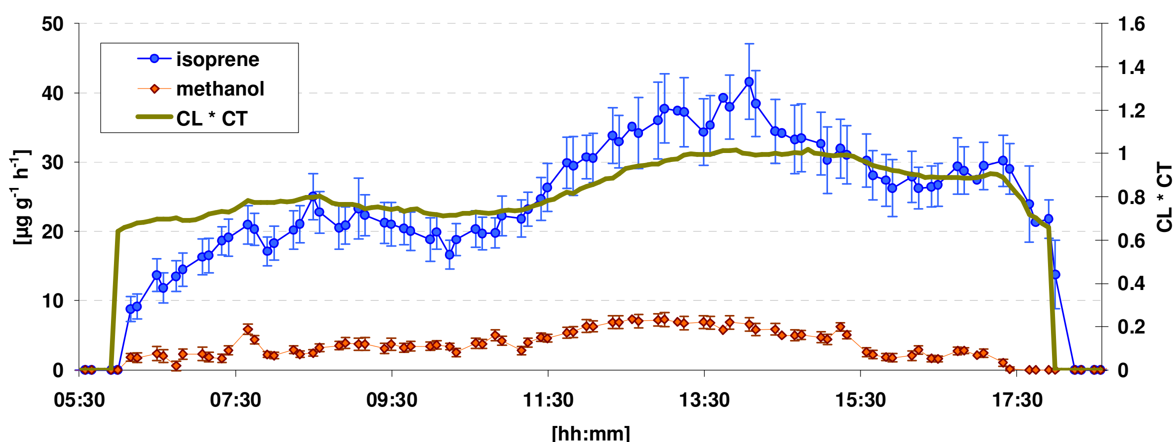


Figure 2.3.4.5 Example of a diurnal cycle of methanol and isoprene emissions measured and relative prediction by G93 (C_L * C_T) for *Pachira insignis*. Each represented data point is a mean of 300 second measurements (n ~ 300) ± propagation error

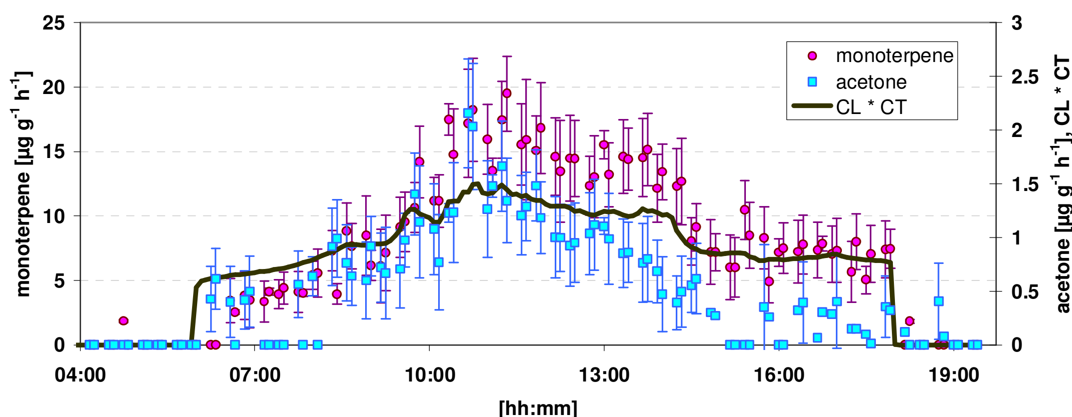


Figure 2.3.4.6 Example of a diurnal cycle of monoterpene and acetone emission measured and relative prediction by G93 (C_L * C_T) for *Hevea spruceana* (igapó). Each represented data point is a mean of 300 second measurements (n ~ 300) ± propagation error

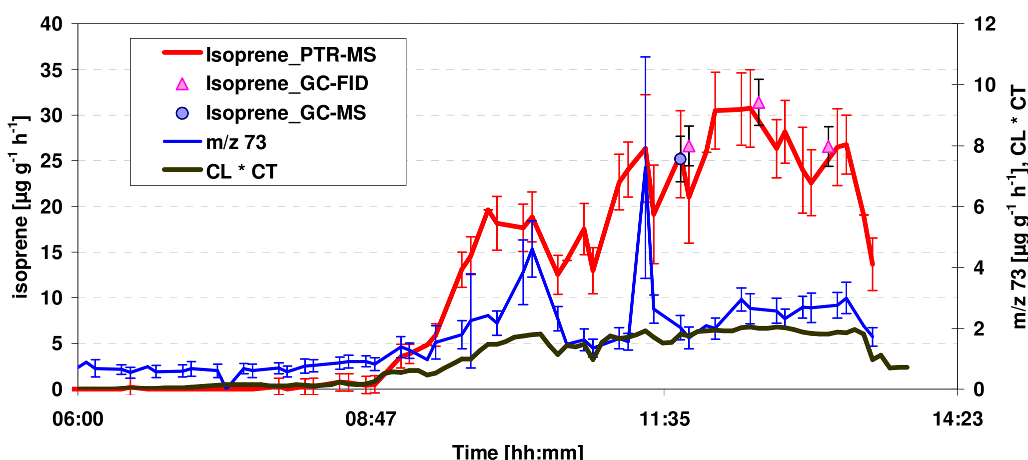


Figure 2.3.4.7 Example of a diurnal cycle of m/z 73 emission measured and relative prediction by G93 (C_L * C_T) for *Garcinia macrophylla*. Each represented data point is a mean of 300 second measurements (n ~ 300) ± propagation error

Emissions of all measured VOC during the day and the night were light dependent, following a diurnal cycle (see figure 2.3.4.1, 2.3.4.5-2.3.4.7) with emissions around zero during the night. Calculated standard emission factors (E_s) based on leaf area as well as on leaf dry weight for methanol, isoprene, total monoterpenes, acetone and m/z 73 are listed in table 2.3.4.1. The lowest estimated error for E_s was found for isoprene (2.9 %) followed by acetone, methanol and monoterpene with 3.2, 4.5 and 4.6 % respectively. Emissions of the compounds with m/z 73 were very low and showed higher uncertainties in the calculation method with an estimated error of 10.3 %.

By observing the contribution of the mean E_s of each VOC species (mean of all E_s of the measured plant species) to the mean total VOC emission (addition of every VOC species mean = 100%), highest E_s were found for isoprene comprising 43.1 % of the total mean VOC emitted from all measured plant species. Monoterpene emissions constituted 32.9% and the rest of VOC (methanol (11.6%), acetone (5.1%) and m/z 73 (7.2%)) 24 % reflecting the proportion of VOC usually found in the atmosphere (except for monoterpenes), where the flux of NMVOC from the vegetation is comprised primarily of isoprene followed by other reactive compounds (alkenes, aldehydes and organic acids) and monoterpenes (Guenther *et al.*, 2000).

Twenty-two species, out of the 24-plant species screened, were found to emit VOC, and in the rest two plant species, no emission was detected or the level of emission was below detection limit (<d.l.) (Table 2.3.4.1). If we consider the sum of VOC emitted, from the twenty-two plant species that show emission of VOC, seventeen species were high emitter (emission above 10 µg g^{-1} h^{-1}) and five species were moderate-emitter (emission ranged between 1 and 10 µg g^{-1} h^{-1}). No low-emitters were found (emission less than 1 µg g^{-1} h^{-1}) (Table 2.3.4.1).

Standard emission factors (E_s) for isoprene were detected in the range of 1.5 to 93.5 µg g^{-1} h^{-1}. Most of the isoprene emitting tree species were high emitters (>10 µg g-1 h-1) except for *Psidium acutangulum* that E_s in the range of 1.5-6.9 µg g^{-1} h^{-1} were measured.

Only four tree species (*Hevea brasiliensis, Hevea guianensis, Hevea spruceana from várzea and igapó* and *Scleronema micranthum*) were found to emit monoterpenes. No monoterpene emission was detected in the other twenty species. Monoterpene E_s varied from 0.3 to 67.1 µg g^{-1} h^{-1} (see table 2.3.4.1). The highest monoterpene emitting tree was *H. spruceana* from igapó followed by *H. brasiliensis*, *H. spruceana* from várzea, *H. guianensis* and *S. micranthum*, the latter one a low monoterpene emitter (<1 µg g^{-1} h^{-1}) and the other ones moderate emitters (>1 µg g^{-1} h^{-1}). Monoterpene E_s of *H. spruceana* from two floodplain ecosystems (várzea and igapó) exhibited significant quantitatively differences between E_s of the same species (see table 2.3.4.1). On the other hand, no significant differences were found for the monoterpene species composition by *H. spruceana* from both environments; α-pinene (63%) was the most abundant monoterpene followed by limonene (14%), sabinene (7.5%), p-cymene (5.4%) and ß-pinene (2.8%) (see figure 2.3.4.8). α-Pinene was also the main monoterpene emitted by the rest of monoterpene emitting tree species (35-90%). Confirming the "*Hevea* monoterpene emission pattern" *H. brasiliensis* emitted a-pinene (57%) followed by limonene (17%), sabinene (7.4%) and p-cymene (5.9%), but in this case γ-terpinene (6.3%) exceeded the occurrence of ß-pinene (1.8%). In *Scleronema micranthum* monoterpene species composition was similar to those of the *Hevea* species: α -pinene (35%) again as main monoterpene followed by limonene (26.6%) and in this case ß-pinene (15.9%) and sabinene (9.1%). However, emissions of *H. guianensis* did not confirm the supposed "*Hevea* monoterpene emission pattern" showing only α –pinene (90.2%) and camphene (9.8%).

The oxygenated VOCs, methanol and acetone, were emitted from a variety of plant species. Methanol emission was detected in almost all measured plant species. Eighteen trees were

methanol emitters (see table 2.3.4.1). Emission rates varied between 1.3 to 33.9 µg g^{-1} h^{-1}. For twelve species (*Annona hypoglauca, Crescentia amazonica, Garcinia macrophylla, Hevea brasiliensis, Macrolobium acaciifolium, Nectandra amazonum, Pachira insignis, Psidium acutangulum, Scleronema micranthum, Tabaernamontana siphilitica, Vatairea guianensis* and *Vitex cymosa*) emissions were in the range of 1-10 µg g^{-1} h^{-1} and the other five emitted <10 µg g^{-1} h^{-1} (*Apeiba sp., Crataeva benthamii, Erythrina fusca, Hura crepitans* and *Pseudobombax munguba*). In addition, acetone was emitted from *Apeiba sp., Crescentia amazonica, Psidium acutangulum,* and *Vitex cymosa* in the range of 2.6 -13.6 µg g^{-1} h^{-1}. Furthermore low emissions of acetone could be detected in case of *Hevea spruceana* (<1.5 µg g^{-1} h^{-1}).

Emissions of an unknown compound with m/z 73 following a light dependency were observed during the measurements of *Garcinia macrophylla* (see figure 2.3.4.7), showing E_s values from 1 to 2.8 µg g^{-1} h^{-1}. These ranges are comparable with those measured for methanol by the same plant species.

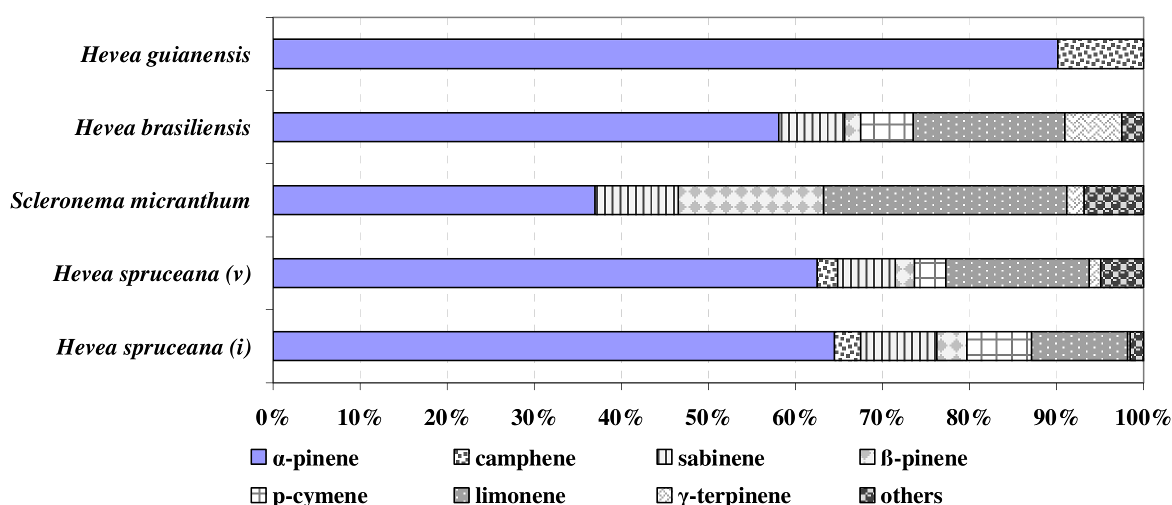


Figure 2.3.4.8 Species composition (%) of monoterpenes emitted by *Scleronema micranthum*, *Hevea guianensis*, *Hevea brasiliensis* and *Hevea spruceana,* the latter from várzea (v) and igapó (i).

Table 2.3.4.1 VOC standard emission factors (E_S) for tropical plant species based on leaf area and leaf dry weight (SLA: specific leaf area). Species from the environments (En) várzea (v), igapó (i) and terra firme (tf) were characterised. Measurements were performed at three different places (p.e.= place of experiment): Manaus (M), Kiel (K) and Oldenburg (o), and with three different enclosure systems (see chapter 2.2.2), but with the same VOC analyses technique: PTR-MS. The E_s from each replicate (n = 104-344) ± estimated error calculated from the standard error of the regression line is given. No data are given in case of below detection limit (<d.l) or if not measured (---).

Plant Species	En		p.e.	SLA	Total VOC		Methanol		Monoterpene		Isoprene		Acetone		m/z 73	
				[m² g⁻¹]	[µg m⁻² h⁻¹]	[µg g⁻¹ h⁻¹]	[µg m⁻² h⁻¹]	[µg g⁻¹ h⁻¹]	[µg m⁻² h⁻¹]	[µg g⁻¹ h⁻¹]	[µg m⁻² h⁻¹]	[µg g⁻¹ h⁻¹]	[µg m⁻² h⁻¹]	[µg g⁻¹ h⁻¹]	[µg m⁻² h⁻¹]	[µg g⁻¹ h⁻¹]
Annona hypoglauca	v	tree1	K	265.2	303.9	8.1	303.9 ± 23.9	8.1 ± 0.6	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	K	265.2	126.1	3.3	126.1 ± 10.7	3.3 ± 0.3	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	K	265.2	519.9	13.8	519.9 ± 8.0	13.8 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Apeiba sp.	v	tree1	K	311.3	468.3	14.5	258.2 ± 3.6	8.0 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	210.3 ± 7.3	6.5 ± 0.2	<d.l.	<d.l.
		tree2	K	311.3	759.6	23.7	628.6 ± 12.8	19.6 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.	131.0 ± 1.7	4.1 ± 0.05	<d.l.	<d.l.
		tree3	K	311.3	745.5	23.1	602.3 ± 26.6	18.7 ± 0.8	<d.l.	<d.l.	<d.l.	<d.l.	143.2 ± 4.1	4.4 ± 0.1	<d.l.	<d.l.
Crescentia amazonica	v	tree1	K	414.4	349.4	14.4	211.3 ± 11.7	8.7 ± 0.5	<d.l.	<d.l.	<d.l.	<d.l.	138.1 ± 5.6	5.7 ± 0.2	<d.l.	<d.l.
		tree2	K	414.4	520.6	21.6	200.5 ± 8.5	8.3 ± 0.3	<d.l.	<d.l.	<d.l.	<d.l.	320.1 ± 7.8	13.3 ± 0.3	<d.l.	<d.l.
		tree3	K	414.4	555.5	23.0	306.1 ± 8.8	12.7 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.	249.4 ± 4.9	10.3 ± 0.2	<d.l.	<d.l.
Crataeva benthamii	v	tree1	K	337.8	360.8	12.2	360.8 ± 9.2	12.2 ± 0.3	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	K	337.8	737.7	24.9	737.7 ± 12.5	24.9 ± 0.5	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	K	337.8	668.0	22.6	668.0 ± 20.6	22.6 ± 0.7	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Erythrina fusca	v	tree1	K	551.9	391.5	21.6	391.5 ± 7.6	21.6 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	K	551.9	358.6	19.8	358.6 ± 10.0	19.8 ± 0.5	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	K	551.9	333.0	18.4	333.0 ± 7.2	18.4 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Garcinia macrophylla	i	tree1	M	70.5	2315.9	16.4	250.4 ± 8.8	1.8 ± 0.1	<d.l.	<d.l.	1847.8 ± 24.4	13.1 ± 0.2	<d.l.	<d.l.	217.7 ± 20	1.5 ± 0.1
		tree2	M	70.5	2824.8	19.9	203.1 ± 10.9	1.4 ± 0.1	<d.l.	<d.l.	2481.1 ± 32.2	17.5 ± 0.2	<d.l.	<d.l.	140.6 ± 16	1.0 ± 0.1
		tree3	M	70.5	1973.8	25.1	189.2 ± 15.4	2.4 ± 0.2	<d.l.	<d.l.	1565.8 ± 27.2	19.9 ± 0.3	<d.l.	<d.l.	218.8 ± 34	2.8 ± 0.4
Hevea brasiliensis	tr	tree1	M	299.5	423.91	12.7	58.42 ± 5.3	1.75 ± 0.2	365.49 ± 5.6	10.95 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	M	299.5	1358.1	40.7	85.9 ± 2.7	2.57 ± 0.1	1272.2 ± 20.8	38.10 ± 0.6	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	M	299.5	574.3	17.2	75.8 ± 14.2	2.27 ± 0.4	498.49 ± 19.1	14.93 ± 0.6	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Hevea guianensis	tr	tree1	M	252.6	421.1	10.6	<d.l.	<d.l.	421.1 ± 19.1	10.6 ± 0.6	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	M	252.6	328.6	8.3	<d.l.	<d.l.	328.6 ± 76.7	8.3 ± 1.9	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.

Plant Species	En		p.e.	SLA	Total VOC		Methanol		Monoterpene		Isoprene		Acetone		m/z 73	
				[m² g-1]	[µg m-2 h-1]	[µg g-1 h-1]	[µg m-2 h-1]	[µg g-1 h-1]	[µg m-2 h-1]	[µg g-1 h-1]	[µg m-2 h-1]	[µg g-1 h-1]	[µg m-2 h-1]	[µg g-1 h-1]	[µg m-2 h-1]	[µg g-1 h-1]
Hevea spruceana	v	tree1	M	423.0	208.1	8.8	<d.l.	<d.l.	174.9 ± 9.4	7.4 ± 0.4	<d.l.	<d.l.	33.2 ± 2.3	1.4 ± 0.1	<d.l.	<d.l.
		tree2	M	423.0	719.6	30.4	<d.l.	<d.l.	719.6 ± 28.2	30.4 ± 1.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Hevea spruceana	i	tree1	M	232.9	2163.4	50.3	<d.l.	<d.l.	2126.7 ± 37.1	49.5 ± 0.9	<d.l.	<d.l.	36.7 ± 3.6	0.8 ± 0.1	<d.l.	<d.l.
		tree2	M	232.9	1795.2	41.8	<d.l.	<d.l.	1757.7 ± 32.1	40.9 ± 0.7	<d.l.	<d.l.	37.5 ± 3.5	0.9 ± 0.1	<d.l.	<d.l.
		tree3	M	232.9	2893.7	67.4	<d.l.	<d.l.	2880.8 ± 67.7	67.1 ± 1.6	<d.l.	<d.l.	13.5 ± 3.7	0.3 ± 0.1	<d.l.	<d.l.
Hura crepitans	v	tree 1	M	221.2	1534.7	33.9	1534.7 ± 46.5	33.9 ± 1.0	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 2	M	221.2	374.9	8.3	374.9 ± 19.1	8.3 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 3	M	221.2	602.6	18.3	602.6 ± 46.7	18.3 ± 1.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Laetia corymbulosa	v	tree 1	O	171.9	3656.5	62.9	---	---	---	---	1387.5 ± 407.8	23.85 ± 7.0	---	---	---	---
Macrolobium acaciifolium	v	tree1-2	M	258.5	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Macrolobium acaciifolium	v	tree1	K	237.4	143.2	3.4	143.2 ± 7.9	3.4 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	K	237.4	192.2	4.6	192.2 ± 6.0	4.6 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	K	237.4	191.3	7.9	191.3 ± 15.3	7.9 ± 0.6	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 4	K	237.4	346.1	8.2	346.1 ± 16.2	8.2 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Nectandra amazonum	v	tree1	K	237.6	237.6	5.6	237.6 ± 9.5	5.6 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	K	237.6	228.8	5.4	228.8 ± 8.3	5.4 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	K	237.6	120.2	2.8	120.2 ± 4.6	2.8 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Ocotea cymbarum	v	tree1-3	M	131.6	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Pachira insignis	v	tree1	M	217.6	529.5	11.5	164.3 ± 8.9	3.6 ± 0.2	<d.l.	<d.l.	365.2 ± 5.0	7.9 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	M	217.6	771.1	16.8	169.0 ± 4.9	3.7 ± 0.1	<d.l.	<d.l.	602.1 ± 18.0	13.1 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	M	217.6	925.9	20.1	217.9 ± 9.7	4.7 ± 0.2	<d.l.	<d.l.	708.0 ± 12.3	15.4 ± 0.3	<d.l.	<d.l.	<d.l.	<d.l.
Pouteria glomerata	v	tree 1-3	M	147.7	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Pseudobombax munguba	v	tree 1	M	153.7	1430.5	12.6	621.0 ± 32.8	9.7 ± 0.5	809.5 ± 38.3	2.9 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 2	M	153.7	816.5	13.1	816.5 ± 56.5	13.1 ± 0.9	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 3	M	153.7	1154.4	17.8	1154.4 ± 45.9	17.8 ± 0.7	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.

Plant Species	En		p.e.	SLA	Total VOC		Methanol		Monoterpene		Isoprene		Acetone		m/z 73	
				[m² g^{-1}]	[µg m^{-2} h^{-1}]	[µg g^{-1} h^{-1}]	[µg m^{-2} h^{-1}]	[µg g^{-1} h^{-1}]	[µg m^{-2} h^{-1}]	[µg g^{-1} h^{-1}]	[µg m^{-2} h^{-1}]	[µg g^{-1} h^{-1}]	[µg m^{-2} h^{-1}]	[µg g^{-1} h^{-1}]	[µg m^{-2} h^{-1}]	[µg g^{-1} h^{-1}]
Psidium acutangulum	v	tree1	K	263.4	356.1	9.4	201.4 ± 9.9	5.3 ± 0.3	<d.l.	<d.l.	56.5 ± 6.9	1.5 ± 0.2	98.2 ± 3.1	2.6 ± 0.1	<d.l.	<d.l.
		tree2	K	263.4	325.1	13.8	225.2 ± 5.6	5.9 ± 0.1	<d.l.	<d.l.	115.4 ± 4.2	3.0 ± 0.1	184.5 ± 6.2	4.9 ± 0.2	<d.l.	<d.l.
		tree3	K	263.4	676	17.7	266.2 ± 8.7	7.0 ± 0.2	<d.l.	<d.l.	263.9 ± 7.1	6.9 ± 0.2	145.9 ± 2.6	3.8 ± 0.1	<d.l.	<d.l.
Salix martiana	v	tree1	O	426.8	546.7	24.6	---	---	---	---	546.72 ± 14.2	24.6 ± 0.6	---	---	---	---
Scleronema micranthum	tr	tree1	M	130.1	156.8	2.1	129.6 ± 5.6	1.7 ± 0.1	27.2 ± 0.7	0.4 ± 0.01	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	M	130.1	142.5	1.9	120.3 ± 6.6	1.6 ± 0.1	22.2 ± 1.1	0.3 ± 0.01	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree3	M	130.1	103.7	1.3	103.7 ± 3.3	1.3 ± 0.04	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Tabaernamontana siphilitica	v	tree 1	M	136.2	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Tabaernamontana siphilitica	v	tree 1	K	187.3	175.3	3.3	175.3 ± 5.1	3.3 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 2	K	187.3	170.0	3.2	170.0 ± 7.4	3.2 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
		tree 3	K	187.3	179.9	3.4	179.9 ± 4.9	3.4 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Vatairea guianensis	v	tree1	M	383.3	987.7	37.9	129.8 ± 3.1	5.0 ± 0.1	<d.l.	<d.l.	857.9 ± 10.6	32.9 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	M	383.3	3652.2	100.6	186.0 ± 4.9	7.1 ± 0.2	<d.l.	<d.l.	2439.2 ± 33.5	93.5 ± 1.3	<d.l.	<d.l.	<d.l.	<d.l.
Vatairea guianensis	i	tree1	M	275.0	1677.7	46.1	51.3 ± 2.9	1.4 ± 0.1	<d.l.	<d.l.	1626.4 ± 13.9	44.7 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.
		tree2	M	275.0	1924	52.9	116.9 ± 2.3	3.2 ± 0.1	<d.l.	<d.l.	1807.1 ± 16.3	49.7 ± 0.4	<d.l.	<d.l.	<d.l.	<d.l.
Vitex cymosa	v	tree 1	K	352.3	320.9	11.3	230.9 ± 4.9	8.1 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	90.0 ± 3.0	3.2 ± 0.1	<d.l.	<d.l.
		tree 2	K	352.3	516.0	18.2	287.3 ± 6.6	10.1 ± 0.2	<d.l.	<d.l.	<d.l.	<d.l.	228.7 ± 8.6	8.1 ± 0.3	<d.l.	<d.l.
Zygia juruana	v	tree 1	M	238.8	175.9	4.2	<d.l.	<d.l.	<d.l.	<d.l.	175.9 ± 6.7	4.2 ± 0.1	<d.l.	<d.l.	<d.l.	<d.l.
		tree 2	M	238.8	651.8	15.5	<d.l.	<d.l.	<d.l.	<d.l.	651.8 ± 22.2	15.5 ± 0.5	<d.l.	<d.l.	<d.l.	<d.l.
		tree 3	M	238.8	900.8	21.5	<d.l.	<d.l.	<d.l.	<d.l.	900.8 ± 24.7	21.5 ± 0.6	<d.l.	<d.l.	<d.l.	<d.l.

2.3.4.2 CARBON LOSS DUE TO VOC EMISSION

The carbon loss of photosynthetically fixed carbon in form of VOC was calculated for each emitted compound. During this study carbon loss due to isoprene emission and monoterpene emission was of similar proportion (see table 2.3.4.2). Isoprene emission contributed for a carbon loss of 0.2-8.14 % for the plant species *Laetia corymbulosa, Pachira insignis, Salix martiana, Vatairea guianensis, Zygia jurana* and *Psidium acutangum*, whereas monoterpene emission implied a carbon loss of 0.1-8.84 % in all monoterpene emitting trees. The carbon loss due to monoterpene emission detected for *H. spruceana* varied between the different studied ecosystems, showing the highest carbon loss of all monoterpene emitting plants by *H. spruceana* from igapó (4.4 – 8.8 %), whereas the same species from várzea revealed lower carbon loss due to monoterpene emission (0.9-2.2%). It is also worth noting the extremely high carbon loss due to isoprene emission observed in *Garcinia macrophylla*, with values varying from 17.4 to 34.1 %.

The carbon loss due to emissions of oxygenated VOC, methanol and acetone, was very low in comparison with loss provoked by isoprenoid emissions. Mean carbon loss of 0.2 and 0.1 for methanol and acetone were detected. The contribution to the carbon loss due to methanol emission was observed to be highly variable. Maximum losses were detected in case of *Garcinia macrophylla*, *Hura crepitans* and *Pseudobombax munguba* with 0.4-1.2, 0.1-0.3 and 0.2-0.4 % of carbon loss respectively. The other plant species showed lower values of carbon loss by methanol emissions, varying between 0.06 and 0.02 % (see table 2.3.4.2). The carbon loss due to the emission of acetone was in the range of 0.02 to 0.38 % and thus relatively low in comparison with the other VOC species.

The emission of the unknown compound with m/z 73 was considerable, resulting in a potential carbon loss for the plant between 0.71 – 1.32 %.

Table 2.3.4.2 Carbon loss in the form of total volatile organic compounds (VOC) measured as methanol (meth), monoterpene (mono), isoprene (iso), acetone (ace) and m/z 73) in µg g^{-1} h^{-1} ± standard deviation calculated in relation to CO_2 assimilation rates (A; ng g^{-1} h^{-1}) under maximal photon flux (5 minute averages; n~300).
All data are given as % weight of carbon assimilated during the measurements. Tree species from várzea (v), igapó (i) and terra firme (tf) were investigated.

Plant species	En	VOC species		Carbon-A [mg g^{-1} h^{-1}]	VOC Carbon emitted [µg g^{-1} h^{-1}]	VOC Carbon loss [%]
Annona hypoglauca	v	meth	tree1	10.0 ± 0.3	11.3 ± 6.8	0.11
		meth	tree2	8.1 ± 0.4	4.5 ± 2.5	0.06
		meth	tree3	8.3 ± 1.3	16.1 ± 2.6	0.19
Apeiba sp.	v	meth	tree1	11.9 ± 1.7	10.1 ± 1.4	0.09
		meth	tree2	8.3 ± 1.4	23.6 ± 5.2	0.28
		meth	tree3	8.3 ± 0.5	20.5 ± 9.5	0.25
Crescentia amazonica	v	meth	tree1	5.7 ± 0.7	11.2 ± 3.4	0.20
		meth	tree2	3.9 ± 0.5	9.4 ± 3.7	0.24
		meth	tree3	5.1 ± 0.3	16.1 ± 4.4	0.32
Crataeva benthamii	v	meth	tree1	7.1 ± 0.2	15.0 ± 2.9	0.20
		meth	tree2	6.1 ± 0.4	32.3 ± 2.7	0.53
		meth	tree3	12.3 ± 0.4	28.1 ± 6.4	0.23
Erythrina fusca	v	meth	tree1	23.4 ± 0.2	25.8 ± 4.4	0.11
		meth	tree2	35.4 ± 3.3	23.6 ± 7.7	0.07
		meth	tree3	35.7 ± 0.3	21.7 ± 3.8	0.06
Garcinia macrophylla	i	meth	tree1	0.6 ± 0.1	2.8 ± 1.2	0.47
		meth	tree2	0.5 ± 0.1	2.7 ± 0.8	0.54
		meth	tree3	0.5 ± 0.2	4.0 ± 2.0	0.80
Hevea brasiliensis	tf	meth	tree1	12 ± 2.2	3.8 ± 1.4	0.03
		meth	tree2	9.8 ± 2	3.8 ± 1.2	0.04
		meth	tree3	23.8 ± 3.6	8.6 ± 0.8	0.04
Hura crepitans	v	meth	tree 1	13.8 ± 2	36.4 ± 10	0.26
		meth	tree 2	16.2 ± 1.8	11.2 ± 2.6	0.07
		meth	tree 3	12.8 ± 2.6	17.0 ± 2.2	0.13
Macrolobium acaciifolium	v	meth	tree 1	3.4 ± 1.4	3.9 ± 2.0	0.11
		meth	tree 2	7.0 ± 1.1	5.4 ± 1.6	0.08
		meth	tree 3	7.4 ± 0.6	6.5 ± 1.8	0.09
		meth	tree 4	15.6 ± 1.3	8.2 ± 3.6	0.05
Nectandra amazonum	v	meth	tree 1	1.7 ± 0.3	6.7 ± 2.5	0.40
		meth	tree 2	7.2 ± 0.8	6.8 ± 2.7	0.09
		meth	tree 3	4.3 ± 0.7	3.6 ± 1.3	0.08
Pachira insignis	v	meth	tree1	4.4 ± 1	3.4 ± 2.0	0.08
		meth	tree2	4.6 ± 1.2	3.6 ± 1.8	0.08
		meth	tree3	5.2 ± 1	4 ± 1.8	0.08
Pseudobombax munguba	v	meth	tree 1	6.4 ± 1	25.5 ± 5.4	0.4
		meth	tree 2	7.8 ± 1.6	28.4 ± 7.8	0.36
		meth	tree 3	4.8 ± 0.8	56 ± 17.6	1.17
Psidium acutangulum	v	meth	tree 1	2.6 ± 0.1	6.5 ± 2.4	0.25
		meth	tree 2	3.4 ± 1.2	7.2 ± 1.4	0.21
		meth	tree 3	5.2 ± 0.4	8.9 ± 2.2	0.17

Plant species	En	VOC species		Carbon-A [mg g^{-1} h^{-1}]	VOC Carbon emitted [µg g^{-1} h^{-1}]	VOC Carbon loss [%]
Scleronema micranthum	tf	meth	tree1	3.6 ± 0.4	2.0 ± 0.6	0.06
		meth	tree2	3.2 ± 0.6	2.0 ± 1.0	0.06
		meth	tree3	3.8 ± 0.6	2.0 ± 0.8	0.05
Tabaernamontana siphilitica	v	meth	tree 1	2.3 ± 0.2	4.2 ± 0.9	0.18
		meth	tree 2	1.9 ± 0.2	4.0 ± 1.4	0.21
		meth	tree 3	3.9 ± 0.7	4.4 ± 0.9	0.11
Vatairea guianensis	v	meth	tree1	17.0 ± 2.6	5.0 ± 1.2	0.03
		meth	tree2	26.8 ± 3.4	6.8 ± 2.0	0.03
Vatairea guianensis	i	meth	tree1	10.2 ± 0.2	1.6 ± 1	0.02
		meth	tree2	13.0 ± 2.6	4 ± 0.6	0.03
Vitex cymosa	v	meth	tree 1	2.7 ± 0.7	10.5 ± 1.6	0.39
		meth	tree 2	3.4 ± 0.3	12.7 ± 2.2	0.37
Hevea brasiliensis	tf	mono	tree1	12 ± 2.2	124 ± 44	1.03
		mono	tree2	9.8 ± 2.0	504 ± 192	5.14
		mono	tree3	23.8 ± 3.6	136 ± 76	0.57
Hevea guianensis	tf	mono	tree1	7.8 ± 4.6	127 ± 58	1.63
		mono	tree2	4.8 ± 4.0	106 ± 22.8	2.21
Hevea spruceana	v	mono	tree1	15.4 ± 1.0	146 ± 20	0.95
		mono	tree2	16.2 ± 2.4	362 ± 128	2.23
Hevea spruceana	i	mono	tree1	17.8 ± 1.2	792 ± 176	4.44
		mono	tree2	12.8 ± 1.0	681 ± 167	5.32
		mono	tree3	14.6 ± 0.6	1298 ± 267	8.89
Scleronema micranthum	tf	mono	tree1	3.6 ± 0.4	3.8 ± 1.6	0.11
		mono	tree2	3.2 ± 0.6	3.6 ± 2.8	0.11
		mono	tree3	3.8 ± 0.6	<d.l.	<d.l.
Garcinia macrophylla	i	iso	tree1	0.6 ± 0.1	106 ± 33	17.67
		iso	tree2	0.5 ± 0.1	167 ± 26	33.40
		iso	tree3	0.5 ± 0.2	178 ± 33	35.6
Laetia corymbulosa	v	iso	tree1	1.4 ± 0.3	119.3 ± 35	8.52
Pachira insignis	v	iso	tree1	2.2 ± 0.5	36.23 ± 13.8	1.65
		iso	tree2	2.3 ± 0.6	64.2 ± 36.9	2.79
		iso	tree3	2.6 ± 0.5	64 ± 19.8	2.46
Psidium acutangulum		iso	tree1	2.6 ± 0.1	26.4 ± 14.4	1.02
		iso	tree2	3.4 ± 1.2	37.1 ± 10.4	1.09
		iso	tree3	5.2 ± 0.4	88.3 ± 17.8	1.70
Salix martiana	v	iso	tree1	3.8 ± 1.4	110.4 ± 26.4	2.91
Vatairea guianensis	v	iso	tree1	8.5 ± 1.3	163 ± 45	1.92
		iso	tree2	13.4 ± 1.7	448 ± 113	3.34
Vatairea guianensis	i	iso	tree1	5.1 ± 0.1	244 ± 68	4.78
		iso	tree2	6.5 ± 1.3	290 ± 93	4.46
Zygia juruana	v	iso	tree 1	4.3 ± 0.9	17 ± 11	0.40
		iso	tree 2	2.2 ± 0.6	82 ± 54	3.73
		iso	tree 3	4.6 ± 1.1	108 ± 63	2.35
Apeiba sp.	v	ace	tree 1	11.9 ± 1.7	24.7 ± 8.8	0.21
		ace	tree 2	8.3 ± 1.4	14.7 ± 2.1	0.18
		ace	tree 3	8.3 ± 0.5	11.7 ± 4.5	0.14
Crescentia amazonica	v	ace	tree 1	5.7 ± 0.7	22.0 ± 4.9	0.39
		ace	tree 2	3.9 ± 0.5	45.0 ± 10.3	1.15

Plant species	En	VOC species		Carbon-A [mg g^{-1} h^{-1}]	VOC Carbon emitted [µg g^{-1} h^{-1}]	VOC Carbon loss [%]
		ace	tree 3	5.1 ± 0.3	39.4 ± 7.3	0.77
Hevea spruceana	v	ace	tree1	15.8 ± 1	7.4 ± 1.6	0.05
		ace	tree2	16.2 ± 2.4	<d.l.	<d.l.
Hevea spruceana	i	ace	tree 1	17.8 ± 1.2	4.4 ± 1.2	0.02
		ace	tree 2	12.8 ± 1.0	3.6 ± 1.8	0.03
		ace	tree 3	14.6 ± 0.6	2 ± 0.4	0.01
Psidium acutangulum	v	ace	tree 1	2.6 ± 0.1	9.5 ± 2.3	0.37
		ace	tree 2	3.4 ± 1.2	17.8 ± 4.5	0.52
		ace	tree 3	5.2 ± 0.4	14.6 ± 1.9	0.28
Garcinia macrophylla	i	m/z 73	tree1	0.6 ± 0.1	5.0 ± 2.4	0.83
		m/z 73	tree2	0.5 ± 0.1	3.6 ± 0.4	0.72
		m/z 73	tree3	0.5 ± 0.2	7.31 ± 1.5	1.46

2.3.5 INTERCOMPARISON OF THE DIFFERENT VOC MEASUREMENTS TECHNIQUES USED: A QUALITATIVE AND QUANTITATIVE STUDY

Intercomparison experiments were carried out during this study to demonstrate the quality of VOC analysis. GC-MS and GC-FID measurement of different monoterpene species were found to be well correlated, except for sabinene which showed higher values with GC-MS than GC-FID (see figure 2.3.5.1). It has been demonstrated that sabinene can decompose during the time required for storage in the carbograph cartridges (personal notification Prof. Dr. P. Ciccioli). The rate of decomposition was calculated to be 80% and the sabinene concentrations measured with GC-FID were corrected accordingly. The results are represented in figure 2.3.5.2 a) and b), which shows considerable effects for sabinene data with substantial improvements of the total monoterpene report.

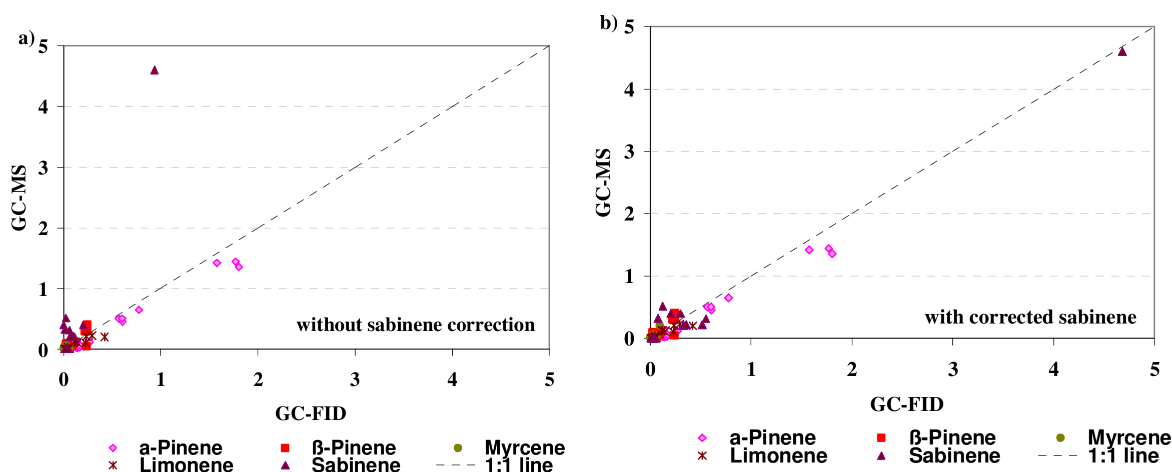


Figure 2.3.5.1 Comparison of the monoterpene species α-pinene, ß-pinene, myrcene, limonene and sabinene measured with GC-FID and GC-MS. All data are given in ppb. Dashed line represents the 1:1 line.

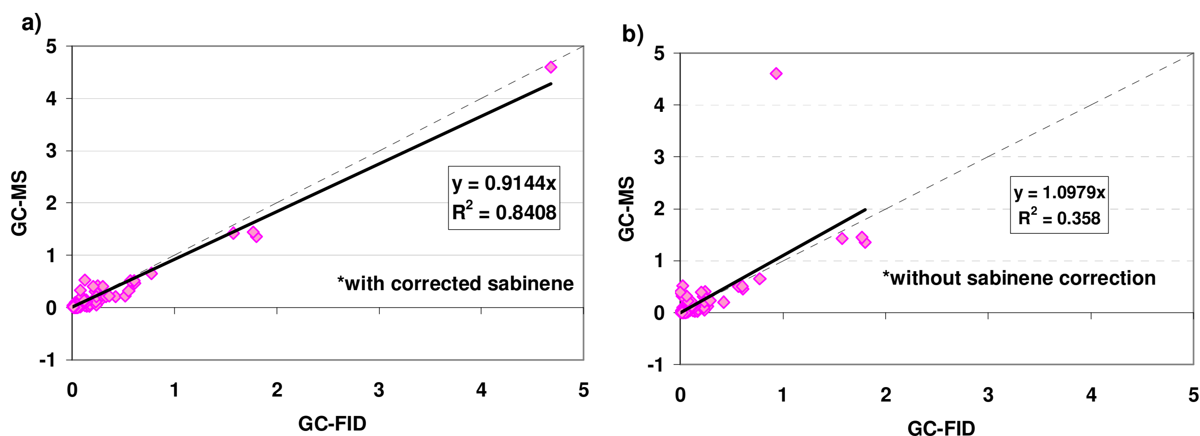


Figure 2.3.5.2 Comparison of GC-FID with GC-MS measurements of monoterpenes. All data are given in ppb. The correction of sabinene is represented in figure a) and the comparison without correction is shown in figure b). The solid line represents the linear regression, the numerical relationship and regression coefficient are also displayed. The dashed line represents the 1:1 line.

Monoterpene measurements made with the PTR-MS and the GC-FID/GC-MS data are compared in figure 2.3.5.3. The measurements correlate very well, with the data close to the 1:1 line. In case of isoprene, however, a systematic difference between PTR-MS measurements and those performed with GC-FID and GC-MS was found. PTR-MS values for isoprene were two times higher than the GC-FID and GC-MS values. Since PTR-MS measures compounds with one specified mass as a whole, different compounds with the same mass can interfere with identification and quantification. Hence, potential mass interference of unidentified compounds in ambient air might be the reason of the difference found in isoprene between GC-methods and PTR-MS measurements. Therefore, PTR-MS derived isoprene data within this study were divided by two and thus corrected on the GC basis.

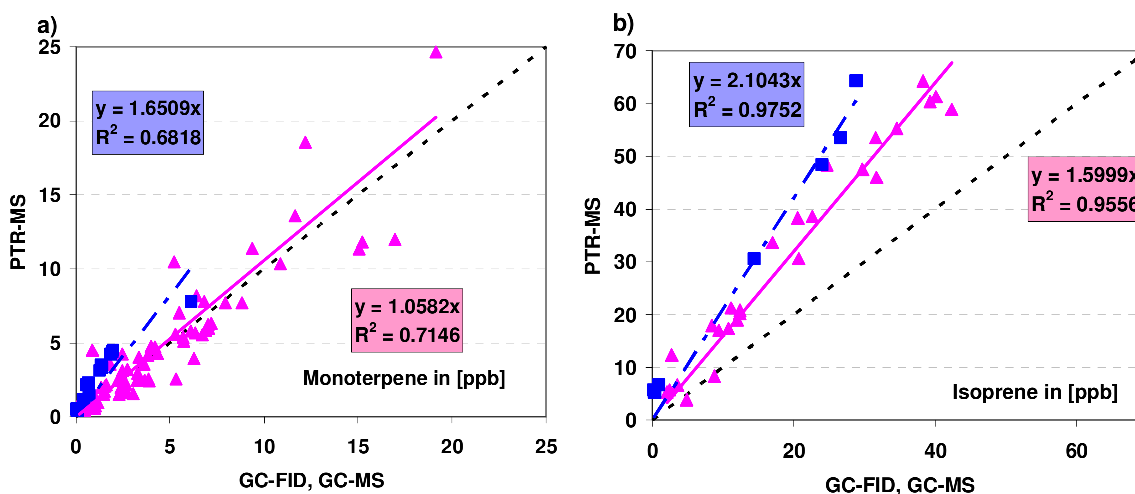


Figure 2.3.5.3 Comparison of GC-FID/GC-MS data with PTR-MS measurements of total monoterpenes (a) and isoprene (b). All data are given in ppb. The pink triangles represent the plot of GC-FID with PTR-MS and the pink line is the regression line with the numerical relationship and regression coefficient (pink right square) also

shown. The blue squares represent the plot of GC-MS with PTR-MS and the dashed blue line its regression line with the numerical relationship and regression coefficient (blue left square) also shown. The dashed black line represents the 1:1 line.

2.4 DISCUSSION

Information on foliar emission of VOC from tropical species of Amazon region is still poor, representing a major lacuna in the global database on VOC (http://bai.acd.ucar.edu/Data/BVOC/index.shtml). However, within the last decade some data on fluxes and primary emissions were reported for the Amazonian rainforest (Kesselmeier *et al.*, 2009). But especially primary emission data on a plant species level are rare. Such information is needed to understand processes for model evaluation. We have realized a VOC emission inventory of 24 tropical tree species with the following objectives (1) to scan qualitative and quantitative various tree species growing in three different ecotypes of the Amazon region (terra firme, várzea and igapó) for VOC emissions, complementing it with physiological data of the plants, (2) to determine the carbon loss in relation to the photosynthetic activity due to VOC emission, (3) to compare two offline (GC-FID, GC-MS) and one online (PTR-MS) VOC measured methods.

2.4.1 PHYSIOLOGY OF THE PLANTS

Tropical forest species tend to have high photosynthetic activity (Jayasekara and Jayasekara, 1995). In general assimilation is higher in pioneer tree species than in non-pioneer species (Ellsworth and Reich, 1996; Parolin *et al.*, 2001). This tendency could be observed during this study, detecting assimilation rates in the range of 2.3 to 15.2 and 1.6 to 8.7 for pioneer and non-pioneer tree species, respectively. *Erythrina fusca* achieved the highest photosynthetic activity, reaching rates already found in the pioneer trees from várzea *Cecropia latiloba* and *Senna reticulata* (Parolin, 2001b), whereas the lowest values of assimilation were observed for non-pioneer trees *Vitex cymosa* and *Garcinia macrophylla*. However, the low assimilation rate by *V. cymosa* (1.8-2.2 µmol m^{-2} s^{-1}) was not in accordance with Parolin (2001b) reporting a mean value of 8 µmol m^{-2} s^{-1}. This difference might be due to multiple environmental factors like ambient temperature differences of 3-5 °C, however only temperature optimum curves could confirm this assumption. Additionally developmental stages may have been of substantial importance. Other data such as assimilation rates of *Nectandra amazonum* and *Crataeva banthamii* as given in the study by Parolin (2001) coincided with the values found for these species in our study. No reports about physiological data of the rest of investigated plants were found in the literature.

Transpiration was controlled by stomatal conductance, finding rates usually recorded for tropical and temperate tree species (Bazzaz, 1980). Under these gas exchange conditions the

water use efficiency of photosynthesis reached values similar to those reported for sclerophyllous woody plants and deciduous trees (Larcher, 2003). Some exceptions were observed in case of *Pouteria glomerata* (except for one individual), *Hevea spruceana* (igapó), and some individuals of *H. guianensis* (tree2), *M. acaciifolium* (tree 4), *Nectandra amazonum* (tree1) and *Psidium acutangulum* (tree 1), where transpiration was extremely high, coinciding with high stomatal conductance. This phenomenon will be discussed below.

The physiological variability of the investigated plants was reflected in the values of stomatal conductance. In general, stomatal conductances were in the same order of magnitude or even lower for tropical tree species from the study areas (Larcher, 2003). The low stomatal conductance might have been caused by large humidity gradients between the leaf and the air, a phenomenon that occurs in the tropics due to the high leaf temperature (Tenhunen *et al.*, 1987). The low evaporative demand of water from these plants maintained low rates of stomatal conductance in most of the cases and thus transpiration. Exceptionally high stomatal conductance was found in case of *Pouteria glomerata* (except for one individual), *Hevea spruceana* (igapó), and one individual of *M. acaciifolium* (tree 4) and consequently high transpirations rates and low water use efficiency were observed for these trees. Figure 2.3.2 represents the commonly linear correlation between assimilation and stomatal conductance, finding some outliers in this diagram. The outliers observed in this figure correspond to plant species with elevated stomatal conductance. Probably the influence of an extremely high relative humidity (under optimal CO_2, light and temperature conditions) in the cases of *Macrolobium acaciifolium*, *Nectandra amazonum* and *Psidium acutangulum* has allowed a wide opening of the stomata (see table 2.3.2). This was not the case for *Hevea spruceana* from igapó, *Pouteria glomerata* and *Hevea guianensis*, when relative humidity was rather low, where we did not find any correlation between environmental parameters and such a behaviou.

2.4.2 VOC STANDARD EMISSIONS

Isoprene was the main emitted compound followed by monoterpenes, methanol and acetone. These compounds are known to be emitted in large quantities by tropical trees, and atmospheric chemists have previously detected them in high concentration in the atmosphere above Amazonian forest (Eerdekens *et al.*, 2009; Karl *et al.*, 2004). Furthermore, considerable emissions of an unknown compound of m/z 73 were observed.

The most prevalent compound, isoprene, is an hydrocarbon emitted by leaves of many tropical tree species and is the most important VOC in rural atmospheres (Harley *et al.*, 2004). The proportion of isoprene emitting species in the present study was 29 %. Though the number of tree species investigated was small, this was in surprising acccordance with the values reported by other studies for tropical and subtropical forest: 29 % for semi-deciduous forest in Panamá (Keller and Lerdau, 1999), 29 % for subtropical forest in Puerto Rico (Lerdau and Keller, 1997), 28% for subtropical forest in South Africa (Guenther *et al.*, 1996), but it disagreed with tropical forest of India (63%) (Padhy and Varshney, 2005). The most substantial emissions were found with *Vatairea guianensis* from várzea and igapó as well as *Laetia corymbulosa* and *Salix martiana* from várzea, which reached values similar to those measured for several *Quercus* species (Kesselmeier and Staudt, 1999). The results obtained here for the rest of the isoprene emitting species are consistent with E_s values reported for other tropical tree species (Harley *et al.*, 2004; Padhy and Varshney, 2005), but no report specific for these isoprene emitting plant species were found among the literature.

The family of monoterpenes makes one of largest contribution to global VOCs flux and the subsequent effect on atmospheric chemistry. Together, isoprene and monoterpenes make up 55% of the estimated global emissions (Guenther, A. *et al.* 1995). During this experiment, monoterpene emission dominated in the group of *Hevea* species. The economically important species *Hevea brasiliensis* has already been described as an isoprene and monoterpene emitting tree (Cronn and Nutmagul, 1982; Geron *et al.*, 2006b; Wang *et al.*, 2007). In this study no isoprene emission was detected from *Hevea brasiliensis,* but relatively high monoterpene emissions (see table 2.3.4.1). The total monoterpene E_s of *H. brasiliensis* varied from 10.9 to 38.1 µg g^{-1} s^{-1}, exceeding the emissions reported before (Cronn and Nutmagul, 1982; Geron *et al.*, 2006a; Klinger, 2002). No reports were found for the rest of the monoterpene emitting *Hevea* species. These species emitted monoterpenes in the order of magnitude of *H. brasiliensis* during this study except for *H. spruceana* from igapó that exceeded the emissions on 25-89 % in comparison with *H. spruceana* from várzea. These quantitative differences were not statistically significant ($p<0.15$) due to the small amount of samples measured and no qualitative differences were observed on the monoterpene species emitted by all *Hevea* species except for *H. guianensis*. Mostly all monoterpene emitting trees of this work emitted alpha-pinene followed by limonene and sabinene, in contrast with the emission pattern reported previously for *H. brasiliensis*, where sabinene emissions followed alpha-pinene and ß-pinene (Wang *et al.*, 2007). Further investigation is needed for a better

understanding of the monoterpene emission pattern of the dominant floodplain species *H. spruceana* and the terra firme species *H. guianensis*. Nevertheless, it is of special interest to note that all *Hevea* species investigated so far belong to the group of monoterpene emitters.

In addition, this study revealed that the terra firme species *Scleronema micranthum* was a low monoterpene emitting species (< 1 μg g^{-1} s^{-1}) with similar monoterpene emission pattern as *Hevea brasiliensis* and *Hevea spruceana* (see figure 2.3.4.8). No bibliography data were found about the VOC emission pattern of this important tree from the Central Amazonian forest.

Isoprene and monoterpene emissions from plants are dependent on light and temperature but also on other triggers such as leaf development, leaf age, phenology and immediate past weather conditions (Kesselmeier and Staudt, 1999). We measured the relationship between light and isoprene/monoterpene emission, observing a saturation curve for both compounds. It was already reported that light and temperature affect emission rates by altering enzymatic production in leaf tissue (Müller, 2003). The figure 2.3.4.3 and 2.3.4.4 show that isoprene and monoterpene emission of *Garcinia macrophylla* and *Hevea spruceana* (igapó) respectively are affected by light reaching a saturation at 300 μmol m^{-2} s^{-1}. These curves are very well represented with the G93 algorithm.

Like isoprene, methanol and acetone are also strongly emitted by terrestrial vegetation, but their contribution to global VOCs budgets has been poorly described (Eerdekens *et al.*, 2009). Methanol is the most abundant organic compound in the atmosphere after methane and acetone. The relative long lifetimes of several days allows a long-range transport of these oxVOC and hence influence the atmospheric chemistry of distant regions. Most of the studied plant species emitted high quantities of methanol that were comparable to those found in other studies (Holzinger *et al.*, 2000; Huve *et al.*, 2007; Macdonald and Fall, 1993a; Nemecek-Marshall *et al.*, 1995). Methanol is a product of demethylation of pectin during cell wall formation and is produced by leaf expansion (Galbally and Kirstine, 2002; Nemecek-Marshall *et al.*, 1995). Considering that the investigated plants were young plants (see table 2.2.1.2) and thus still in the growing period, a high methanol E_s was expected. However, the physiological background for methanol emission is still a matter of discussion and results may not be predictable (Folkers *et al.*, 2008). In the case of acetone, several studies have reported acetone emissions from vegetation at leaf level (Geron *et al.*, 2006a; Geron *et al.*, 2006b;

Grabmer *et al.*, 2006; Holzinger *et al.*, 2000; Macdonald and Fall, 1993b) as well as in the troposphere (Geron *et al.*, 2002; Helmig *et al.*, 1998; Karl *et al.*, 2003b; Müller *et al.*, 2006; Pöschl *et al.*, 2001). In this study low E_s of acetone (<1.5 µg g^{-1} h^{-1}) was found for *H. spruceana* from várzea and igapó, and no difference in this VOC emission pattern was observed between both environments. Acetone emissions above 1 µg C g^{-1} h^{-1} were observed for *H. brasiliensis* by Geron *et al.* (2006b), but no detectable acetone was found during this investigation. Higher E_s of acetone were detected in the várzea species *Apeiba sp., Crescentia amazonica, Psidium acutangulum* and *Vitex cymosa* varying between 2.6 and 13.3 µg g^{-1} s^{-1} (see table 2.3.4.1). The light dependent acetone emissions found in this study are supposed to be direct emission from the leaves. The metabolic pathway of acetone has been still not proven. However, it was hypothesised that acetone is produced in spruce needles by the decarboxylation of acetoacetate (Macdonald and Fall, 1993b), whereas a cyanohydrin-lyase catalysed reaction was found in cyanogenic plant species (Fall, 2003). No information about the cyanogenicity of the tree species measured in this study is available, however. On the other hand, it is known that the atmospheric OH-oxidation of several monoterpenes, like alpha- and ß-pinene, in the presence of NOx appears to be a potentially relevant source of acetone (Wisthaler *et al.*, 2001). A contribution of this oxidation product to the measured acetone concentrations could not be excluded, particularly by *Hevea spruceana* that emitted relevant quantities of alpha- and ß-pinene. But NO_x data for these measurements are not available.

Diurnal emissions of an unknown compound with m/z 73 has been detected. This mass might be considered as protonated methyl ethyl ketone (MEK), also known as 2-butanone. Not much is known about the biosynthetic pathways leading to the formation of MEK and its emission mechanisms, but it has been reported to be emitted by a variety of plants and grasses (De Gouw *et al.*, 1999; Isidorov *et al.*, 1985; Kirstine *et al.*, 1998). On the other hand, Holzinger *et al.* (2007) identified m/z 73 as protonated methylglyoxal, a secondary oxidation product of isoprene (Lee *et al.*, 2006). Since *G. macrophylla* is a high isoprene emitter, formation of such an oxidation product could be plausible. The detection of unknown compound makes clear that further investigation is needed in order to identify compounds that probably have been overseen until now due to technical limitations. The application of new techniques like the PTR-MS that allows the realisation of fast screenings of different masses give us the opportunity to identify possible missing compounds, as have been supposed by Di Carlo *et al*. (2004), but identification is not easy.

2.4.3 CARBON LOSS DUE TO VOC EMISSION

Emission of VOC in relation to the CO_2 taken up represents a considerable carbon loss for the plant and a substantial amount that contributes to the carbon cycle and the atmospheric chemistry. Why the plant emits such a high quantity of carbon in form of VOC is still under discussion (Penuelas and Llusia, 2004; Penuelas *et al.*, 1995; Rosenstiel *et al.*, 2004; Sharkey *et al.*, 2008). During this study the most substantial fraction of VOC was released in from of isoprenoids. A similar fraction of isoprene and monoterpenes was emitted, accounting for a mean carbon loss of 2.5 and 2.6 %, respectively and coinciding with values reported previously for both isoprenoids (Kesselmeier *et al.*, 2002a; Sharkey and Yeh, 2001). Exceptionally high carbon loss generated by isoprene emissions from *Garcinia macrophylla* coincided with values reported under stress situations, like those provoked through high temperatures or inundation (Sharkey and Loreto, 1993; Staudt and Bertin, 1998). Since no special treatment was applied to this tree species, a non stress situation was supposed during the measurements. The reason why this species released such a high quantity of isoprene remains unclear. Furthermore, carbon loss due to monoterpene emission was highly variable (see table 2.3.4.2). This variability makes difficult the consideration of including production and release of organic carbon by VOC emission in carbon budget calculations. This variability was also reflected in the percentage of carbon loss due to emission of methanol that varied between 0.02 and 1.2%. Similar values have been found in other studies performed with *Fagus sylvatica, Gosypium hirsutum* and *Populus deltoides* (Huve *et al.*, 2007). A considerable carbon loss in form of MEK/Methylglyoxal in the order of magnitude of the loss provoked by methanol emission was found, whereas much lower carbon loss in form of acetone was detected. The percentage of carbon loss due to acetone emission found in our study was very high in comparison with values of less than 0.02% as reported by Shao and Wildt (2002) and Singh *et al.* (1994).

2.4.4 COMPARISON OF DIFFERENT METHODS FOR VOC DETECTION (PTR-MS/GC-FID/GC-MS)

The application of different methods for VOC measurement led to a more reliable result and robust conclusions. In order to verify the mass-compound relation found with GC methods and to test the stability of the calculated PTR-MS results we compared them to the simultaneous GC-FID and GC-MS measurements. Accuracy was improved and uncertainties associated with e.g. reaction time and reagent ion distribution were avoided, using standard gases for the calibration of PTR-MS. A comparison was possible for monoterpenes and

isoprene that were successfully detected by both systems. The agreement of the PTR-MS concentrations and the GC concentrations (GC-FID and GC-MS) was fairly good for monoterpenes, but significant differences were found for isoprene. Field applications of PTR-MS always have an intrinsic identification problem due to potential mass interference of unidentified compounds in ambient air. For isoprene a systematic overestimation was observed. This overestimation can be caused by the interference of unexpected or unknown compounds on the detected ion mass, e.g. a contribution of 2-Methyl-3-butene-2-ol (MBO) that fragment to the mass of isoprene (Goldan *et al.*, 1993). However, MBO might additionally be released from vegetation. Furthermore, the zero-air signals of the PTR-MS determined empirically by the catalytic converter are relatively high, their origin is not well explained up to now and they show considerable variability. This background was measured every 18 minutes, but it still leaves significant uncertainty that might affect the measurement of low concentrations in particular (Ammann *et al.*, 2004).

CHAPTER 3: SCREENING OF MEDITERRANEAN VEGETATION TO VOC EMISSIONS

3.1 INTRODUCTION

In the last decades it has been demonstrated that the emission of volatile organic compounds (VOCs) can influence the oxidation capacity of the atmosphere (Atkinson and Arey, 2003). One of the most important sources of VOCs are forests, crops and grasslands. Biogenic VOCs are strong precursors of secondary pollutants and are implicated in the formation and growth of secondary organic aerosols (Claeys *et al.*, 2004b). VOC emission from Mediterranean vegetation, in particular, can form ozone together with anthropogenic VOC and NO_x that are abundant in this area. Therefore VOC fluxes have to be accounted for accurately when developing strategies to mitigate air pollution and global climate change hazards (Andreae and Crutzen, 1997; Atkinson, 2000; Fehsenfeld, 1992; Went, 1960). The other reason for such a study is that the emitted VOCs can contribute significantly to the global carbon budget of terrestrial ecosystems (Guenther, 2002; Kesselmeier *et al.*, 2002a), representing a substantial proportion of the carbon fixed by plants (Llusia and Penuelas, 2000; Penuelas and Llusia, 2004).

Emission of biogenic VOCs from Mediterranean plant species has already been addressed in a series of papers (Kesselmeier and Staudt, 1999; Llusia *et al.*, 2002; Owen *et al.*, 1997; Owen *et al.*, 2001; Owen *et al.*, 2002; Simon *et al.*, 2006). These studies concentrated on the emission of isoprene and monoterpenes. But less information is available on emission of short-chain oxygenated compounds (oxVOCs) such as formaldehyde, acetaldehyde, acetone, methanol, ethanol and formic and acetic acids. Emissions of these oxygenated compounds were identified only recently as a large source of carbon (150-500 Tg C y^{-1}) to the atmosphere (Singh *et al.*, 2001). Other compounds studied very intensively in the last years have been sesquiterpenes, but it has been very difficult to measure them because of the high reactivity of these compounds. Sesquiterpenes and other highly reactive compounds may represent a considerable amount of missing VOCs (Goldstein *et al.*, 2004) as evidenced by indirect approaches (Di Carlo *et al.*, 2004; Kuhn *et al.*, 2007).

The calculation of regional and global VOC budgets relies on standard emissions factors (E_s, emissions measured at standard conditions of 30°C and 1000 µmol m^{-2} s^{-1} PAR), which are plant-species specific. Therefore an exact knowledge of the quantity and quality of VOCs emitted by each species is needed. Several plant species of high abundance have not been sufficiently investigated, although they have been included in VOC budget calculations (Karlik and Winer, 2001).

The present study aims to present a more complete assessment of VOCs released at leaf level. Different methods for VOCs characterization and quantification were used: A proton-transfer-reaction mass spectrometer (PTR-MS) for detection of all VOCs with higher proton affinity than water; an online gas-chromatograph with flame ionization detector for the determination of isoprene emissions; an offline gas-chromatograph with flame ionization detection (GC-FID) together with an offline gas-chromatograph coupled to a mass spectrometer (GC-MS) for determination of higher VOC including sesquiterpenes.

3.2 MATERIAL AND METHOD

3.2.1 PLANT MATERIAL

Deciduous and non-deciduous trees, shrubs, grasses and palm trees typical for the Mediterranean area were analyzed for VOC emissions during the months of June to July 2008. Three replicates were taken for every species. Table 3.2.1 lists all 16 investigated plants, their functional type, family and origin.

Table 3.2.1 Name, functional type, family, origin and specific leaf weight of 16 investigated Mediterranean plant species

Species, trivial name	Functional type[a]	Family	Origin	Specific Leaf weight [g m-2]
Brachypodium retusum	1	Poaceae	mediterranean region	85
Buxus sempervirens, european box	2	Buxaceae	western and southern Europe, northwest Africa, southwest Asia	291
Ceratonia siliqua, carob tree	3	Fabaceae	mediterranean region	149
Chamaerops humilis, mediterranean farn palm	5	Arecaceae	western Mediterranean region, in southwestern Europe, northwest Africa	240
Cistus albidus, rock rose	2	Cistaceae	South west Europe to north Africa, Mediterranean region	91
Cistus monspenliensis, Montpelier rock rose	2	Cistaceae	south west Europe	110
Coronilla valentina	2	Fabaceae	mediterranean region	73
Ficus carica, common fig	4	Moraceae	southwest Asia and the eastern Mediterranean region	66
Olea europaea, olive tree	3	Oleaceae	coastal areas of eastern mediterranean region, Lebanon, Syria and the maritime parts of Asia Minor and northern Iran at the south end of the Caspian Sea	352
Pinus halepensis, aleppo pine	3	Pinaceae	mediterranean region	143
Prunus persica, peach tree	4	Rosaceae	China, Iran, mediterranean region	70
Quercus afares	4	Fagaceae	Algeria and Tunisia	114
Quercus coccifera, kermes oak	3	Fagaceae	western mediterranian region, Marocco, Portugal, east Greece	163
Quercus suber, cork oak	3	Fagaceae	southwest Europe, northwest Africa	121
Rosmarinus officinalis, rosemary	1	Lamiaceae	mediterranean region	215
Spartium junceum, spanish broom	2	Fabaceae	mediterranean region	135

[a]Functional type: 1 evergreen herb, 2 evergreen shrub, 3 evergreen tree, 4 deciduous tree

The plants were around 2-3 years old and were collected from the surroundings of Montpellier, France and potted and maintained in the greenhouse of the CEFE-CNRS Montpellier, France.

3.2.2 GAS EXCHANGE MEASUREMENTS

Gas exchange measurements using the enclosure system started earliest one hour after enclosure to admit an adaptation of the the enclosed leaf. Emission samples were taken from potted plants using a Teflon branch enclosure system developed at the CEFE-CNRS in Montpellier, France. This technique has been characterized and validated by Staudt *et al.* (2004). In order to standardize the measurements standard conditions for VOC measurement

were maintained (leaf temperature = 30°C ± 1 and PAR = 1055 ± 36 µmol m^{-2} s^{-1}), relative humidity was maintained also constant to 48 ± 13%. A CO_2/H_2O infrared gas analyzer (LI-COR inc. 7000, Lincoln, Nebraska, USA) was used to determine CO_2-assimilation rate and transpiration, connected to the in- and outlet of the enclosure by heated (45°C) Teflon tubes.

Purified air (AIRMOPURE, Chromatotec, France) was flushed through the enclosure at 650 ml min^{-1} resulting in an air exchange of the enclosure system on average every 14 s. A Teflon fan installed inside the enclosure allowed homogenous mixing of the air. One or several terminal leaves of an individual plant were placed horizontally to the light to ensure homogenous light repartition on the adaxial surface of the leaves. Most of the plants had to be prepared for the fixation in the enclosure by removing some leaves at least one week before the measurement. At the end of each measurement the studied leaves were harvested and leaf area and dry weight were determined.

3.2.3 VOC DETERMINATION

A comparison of online and offline methods for VOC determination was carried out. For the online detection a PTR-MS (Lindinger *et al.*, 1998a, b) was used. PTR-MS is a chemical ionization technique based on proton transfer reactions from H_3O^+ reagent ions to VOCs. In principle this instrument can measured each volatile organic compound that has a higher proton affinity than water. Since the major atmospheric components in air like N_2, O_2 and CO_2 as well as the lighter alkanes, methane, ethane and propane, have lower proton affinities than water, there is no proton transfer to theses species and they can not be detected. The instrument was operated in the full scan mode (from mass 20 to mass 206) with a dwell time of 1 s at standard operation conditions (E/N = 130 Td; E electric field strength, N buffer gas number density, 1 Td = 10^{-17} cm² V $molecule^{-1}$) and at reduced E/N of ~96 Td, where the fragmentation of e.g. protonated ethanol molecules is largely reduced. At least three scans from the outlet and the inlet of the enclosure and the background signal were measured. The background signal, adjusted by passing air over a heated platinum catalyst maintained at 350 °C (Parker Co., USA), was first subtracted from the outlet and the inlet signal. The emission rates E_s of each compound was then calculated according to (1) based on the measured concentration difference ($\Delta c = c_{outlet} - c_{inlet}$), the enclosure flush rate Q, and the leaf dry weight (dw).

$$E_s = \Delta c(Q/dw). \qquad (1)$$

Since measurements were performed at standard conditions, emission rates in this study are regarded as standard emission rates. The detection limit of this method was estimated as the greater of the variability levels in the difference between the outlet and the inlet concentrations in the empty enclosure (at the 95% confidence level). All data under the detection limit were disregarded from the analysis. A standard gas (Deuste Steininger GmbH, Germany (± 10% accuracy)) containing a mixture of VOCs was used to calibrate measurements of methanol, acetone, isoprene and monoterpenes. This gas was diluted with synthetic air to mixing ratios of 0.5 - 10 ppb, performing a calibration line. Due to the lack of standard gas for the calibration of the rest of the compounds found in this study like e.g. sesquiterpenes, the volume mixing ratio calculation from theses compounds were performed using simple ion-molecule reaction kinetics, where the count rate of RH^+ product ions, $i(RH^+)$, can be calculated from

$$i(RH^+) = i(H_3O^+)_0(1-e^{-k[R]t}) \sim i(H_3O^+)_0 k[R]t \qquad (2)$$

$i(H_3O^+)$ is the count rate of H_3O^+ ions, k is the proton transfer reaction rate coefficient, [R] is the number of density of trace molecules in the reactor and t is the transit time of H_3O^+.
A detailed description of this calculation method was explained by Hansel *et al.* (1995) and Wisthaler *et al.* (2001). It is important to note that different monoterpenes and sesquiterpenes cannot be distinguished separately with PTR-MS. Therefore, standard emissions factors calculated for monoterpenes and sesquiterpenes with the PTR-MS in this study always refer to the sum of all monoterpenes and sesquiterpenes.

An intercomparison of the isoprene exchange rate measured with PTR-MS was performed measuring this compound also online by a Chromatotec AirmoVOC C2-C6 Gas Chromatograph (GC) equipped with a Flame Ionisation Detector (FID). The instrument continuously sucks air from the chamber outlet via a 1/8 inch PTFE tubing at a flow of 11 ml min^{-1}. For this measurement, chamber air was directed for 20 minutes to the internal multi-phase VOC trap maintained at 10 °C, which was subsequently flush heated for 2 min to release isoprene on a fused silica PLOT Al2O3/KCl column (25 m x 0.32 mm). The temperature program of the analysis was 1 min at 40°C, 15°C min^{-1} to 180°C, and 20 min at 180°C. The Online GC was calibrated by the same calibration gas used for the calibration of the PTR-MS.

Monoterpenes and sesquiterpenes emissions measured with the online method PTR-MS were compared with VOC collection on cartridges and posterior offline analysis with a gas chromatograph (GC) with a flame ionization detector (FID) as well as a mass spectrometer (MS) detector. Samples were collected by pumping enclosure exhaust air (1 l at 0.1 l min^{-1}). For the GC-FID system, cartridges were sampled by drawing air through glass tubes containing a mixture of 200 mg of Tenax and 20-35 mesh, Chrompack. The adsorption tubes were analysed by a Chrompack CP9003 gas chromatograph equipped with a FID and a Chrompack TCT4002 thermo-disorber (all Varian Inc.). Furthermore, parallel VOC samples were taken for analyses with gas chromatography coupled with Mass Spectrometry (Varian CP3800/Saturn2000 MS plus a Perkin-Elmer Turbomatrix thermo-disrober) using the same analytical column and temperature program as in the GC-FID instrument: VOC were separated on a Chrompack Sil 8 CB low bleed capillary column (30m x 0.25 mm) using the following temperature program: 3 min at 40°C, 3°C min^{-1} to 100°C, 2.7°C min^{-1} to 140°C, 2.4°C min^{-1} to 180°C, 6°C min^{-1} to 250°C. Peaks were identified by comparing their mass spectra and retention times with those from authentic standards analysed under the same conditions. Offline GC systems were calibrated for monoterpenes using the same gas standard as used for the PTR-MS. For sesquiterpenes the offline GC systems were calibrated using authentic standards (Fluka Chemie, Buchs, Switzerland; Roth, Karlsruhe, Germany) diluted in methanol. 0.5 to 3µl of standard solution was injected on the head of the adsorption trap through a T-fitting equipped with a septum and purged with 300ml pure nitrogen at a flow rate of 50 ml min^{-1}. Average precision as determined by repeated measurements of standards at realistic concentrations was within 5% (relative coefficient of variation of n=7 repetitions).

3.3. RESULTS

3.3.1 SCREENING OF 16 MEDITERRANEAN PLANTS SPECIES FOR VOC EMISSION AT STANDARD CONDITIONS AND COMPARISON WITH LITERATURE

The variability of VOC emissions from 16 common Mediterranean plant species at standard conditions (~30°C and ~1000 µmol m^{-2} s^{-1}) was determined quantitatively and qualitatively. Table 3.3.1.1 to 3.3.1.4 gives an overview about standard emission factors (E_s) of VOC species detected, comparing results determined in this study with PTR-MS and GC techniques with the literature. The results obtained for isoprene, monoterpenes and sesquiterpenes emissions measured with PTR-MS and with GC methods were qualitatively and quantitatively comparable, although PTR-MS values were usually slightly higher than GC measurements (see Table 3.3.1.1 to 3.3.1.3). The reason for this discrepancy is most likely because the PTR-MS cannot distinguish different compounds with the same mass, thus contributions from other compounds and/or fragments at the same m/z ratio cannot be avoided. In the case of sesquiterpenes sometimes the opposite was observed, finding low standard emission factors with the GC system that were not confirmed by the PTR-MS.

Five species were found to be major isoprene emitters: *Brachypodium retusum, Buxus sempervirens, Chamaerops humilis, Ficus carica* and *Spartium junceum.* Emission rates ranged between 15 and 45.7 µg g^{-1} h^{-1} (GC-FID data) (see Table 3.3.1.1). On the other hand, *Ceratonia siliqua, Coronilla valentina, Olea europea, Pinus halepensis, Prunus persica, Quercus afares, Quercus coccifera* and *Quercus suber* could be classified as low isoprene emitters with E_s lower than 1 µg g^{-1} h^{-1} and *Cistus albidus, Cistus monspeliensis* and *Rosmarinus officinalis* as non isoprene emitters. Comparing these results with literature, we found that two of these species (*Quercus afares and Coronilla valentina*) have not been examined for isoprenoid emissions before and another two species (*Brachypodium retusum, Prunus persica*) showed different patterns of isoprene emission in this study as compared to those reported about before (Llusia *et al.*, 2002; Owen *et al.*, 2001; Simon *et al.*, 2006). In contrats to our study, the species *Prunus persica* and *Brachypodium retusum* were described as non isoprene emitters (Owen *et al.*, 2001; Simon *et al.*, 2006), whereas in this study low emissions of isoprene by *Prunus persica* and very high emissions by *Brachypodium retusum* were detected.

The monoterpene emission predominated in the investigated species, detecting nine monoterpene emitters confirmed by PTR-MS and GC systems. Low emissions found by *Brachipodium retusum, Chamaerops humilis, Cistus albidus, Coronilla valentina* and *Prunus persica* (< 1 µg g-1 h-1) with the PTR-MS, but could not be confirmed with the GC systems. This discrepancy could be the result of typical PTR-MS interferences of different VOCs or fragments of compounds with masses classified as monoterpenes (m/z 81, m/z 137). For example, interferences from fragments of sesquiterpenes like ß-caryophyllene and α-humulene with m/z 81 and 137 have been already reported contributing for 7.5 and 6 % in each m/z, respectively (Demarcke *et al.*, 2009).

Five of the sixteen analyzed species were identified as high monoterpene emitters (>2 µg g^{-1} h^{-1}): *Ceratonia siliqua, Pinus halepensis, Quercus afares, Quercus coccifera* and *Quercus suber.* All three *Quercus sp.* studied emitted high quantities of monoterpenes (between ~10-31 µg g^{-1} h^{-1} (GC-FID data)). *Quercus suber* has been identified previously as a non isoprenoid emitter tree (Seufert *et al.*, 1997; Steinbrecher *et al.*, 1997), but this study found an emission factor of 31.7 ± 4.2 µg g^{-1} h^{-1} (GC-FID data), which corroborates more recent literature, showing typical summer time values (Pio *et al.*, 2005; Staudt *et al.*, 2004).
No literature was found regarding VOCs emissions from *Quercus afares*. This species was found to emit high quantities of monoterpenes and low quantities of isoprene. Furthermore, monoterpene emission by *Quercus coccifera* in the range observed in this study has already been described (Llusia and Penuelas, 2000; Ormeno *et al.*, 2007a; Ormeno *et al.*, 2007c; Ormeno *et al.*, 2007d; Ormeno *et al.*, 2009; Owen *et al.*, 2001), but also higher ranges have been reported (Hansen and Seufert, 1996; Pio *et al.*, 1993; Schaab *et al.*, 2000). The high values found in the literature might be principally due to the fact that in these studies emission rates were not normalized to standard light and temperature conditions making the comparison very difficult (see Table 3.3.1.2).

Until now only low monoterpene emissions, compared with those measured in this study, have been reported for *Ceratonia siliqua* (Llusia *et al.,* 2002; Owen *et al.,* 2001*).* On the other hand, *Pinus halepensis* emitted monoterpenes in the range that has previously been observed (Llusia and Penuelas, 2000; Ormeno *et al.*, 2007a; Ormeno *et al.*, 2007b; Owen *et al.*, 2001), but also higher E_s has been reported (Blanch, 2007; Llusia and Penuelas, 1998; Ormeno *et al.*, 2007c; Penuelas and Llusia, 1999; Simon *et al.*, 2005).

Ficus carica and *Spartium junceum* were classified as non monoterpene emitters, whereas, as mentioned above, monoterpene emission by *Prunus persica, Brachipodium retusum, Chamaerops humilis, Cistus albidus, Coronilla valentina* were detected with PTR-MS only. In the case of *Prunus persica, Brachipodium retusum, Cistus albidus* similar, lower or even higher emission rates were referred in earlier studies (Arey *et al.*, 1991; Benjamin and Winer, 1998; Llusia and Penuelas, 2000; Ormeno *et al.*, 2007a; Ormeno *et al.*, 2007b; Ormeno *et al.*, 2007c; Ormeno *et al.*, 2007d; Owen *et al.*, 2001; Pio *et al.*, 1993; Winer *et al.*, 1992), but for *Chamaerops humilis* and *Brachipodium retusum* no monoterpene emission was reported previously. Low but still detectable emissions of monoterpenes with the PTR-MS and GC methods used (<2 µg g^{-1} h^{-1}) were detected on *Buxus sempervirens, Cistus monspeliensis, Olea europea,* and *Rosmarinus officinalis*. This study has found - for the first time - monoterpene emissions by *Buxus sempervirens*. The species *Cistus monspeliensis* and *Rosmarinus officinalis* are already described in the literature as monoterpene emitters. High, low or even non monoterpene emissions were described for *Cistus monspeliensis* (Alessio *et al.*, 2004; Llusia and Penuelas, 1998; Owen *et al.*, 2002; Schaab *et al.*, 2000), whereas emissions in the range of those reported here were found for *Rosmarinus officinalis* (see Table 3.3.1.2), except for twice the amount as found by Owen *et al.* (2002).

Monoterpene emission data may fluctuate significantly among different reports. Such variation is also found in our study and may be understood to result from a variety of influences, such as different methods, plant developmental stages and environmental conditions or even questionable plant identification, making it difficult to use such data for emission models. The monoterpene composition found here for *Ceratonia siliqua* (myrcene, *Z*-ocimene, *E*-ocimene) was completely different as compared to the composition found by Owen *et al.* (2001) or by Llusia *et al.* (2002), who reported emissions of α- pinene and limonene or only α- pinene, respectively.

In *Cistus monspeliensis* emissions of compounds like α-pinene, camphene and ß-pinene coincided with results reported by Owen *et al.* (2002) but not with those found by Llusia *et al.* (1998) reporting an emission of α-phellandrene only. Two further compounds, myrcene and E-ocimene were found in our study only.

For *Olea europea* we found *Z*-ocimene and *E*-ocimene, the latter compound also reported by Arey et al. (1991) but completely disagreeing with the emission pattern reported in several

other reports (Benjamin and Winer, 1998; Llusia *et al.*, 2002; Owen *et al.*, 2001; Pio *et al.*, 1993; Winer, 1998).

Pinus halepensis is one of the most intensively investigated conifers of the Mediterranean area with a large variety of emission patterns (see Table 3.3.1.2). The compounds myrcene, *Z*-ocimene and *E*-ocimene were also observed in previous studies (for references see Table 3.3.1.2), but the monoterpene terpinen-2-ol was found for the first time in this investigation. Also a variety of compounds that were not detected in our study were reported before, like i. e. α-pinene, ß-pinene, limonene, sabinene, etc. (see Table 3.3.1.2).

Most of the monoterpene species emissions as found for *Quercus coccifera* were in close accordance with reports by Owen *et al.* (2001), Hansen and Seufert (1996), Llusia and Penuelas (2000) and Ormeno *et al*. (2007a, c, d, 2009) except for *Z*-3-hexenol, o-cymene, terpinolene. However, a variety of monoterpene Emissions were reported by these authors but could not be confirmed in our study. *Quercus suber* emission pattern can be very variable among the literature (see Table 3.3.1.2). Whereas α-pinene, camphene, ß-pinene, myrcene, limonene, borneol, eucalyptol emissions were commonly found in literature reports (Hansen *et al.*, 1997; Lenz *et al.*, 1997; Ormeno *et al.*, 2007b; Owen *et al.*, 2002; Seufert *et al.*, 1997) and were also found in this study, Camphor was detected only in this investigation.

Low emissions of sesquiterpenes ($< 1< 0.1 \mu g\ g^{-1}\ h^{-1}$) were observed in case of *Cistus albidus, Cistus monspeliensis Quercus coccifera*, and traces of this terpenoid ($<0.1\ \mu g\ g^{-1}\ h^{-1}$) were identified, but only by GC-FID, for *Buxus sempervirens, Ceratonia siliqua* and *Olea europea,* indicating that the GC-FID method may be more sensitive for these compounds than the PTR-MS system.

Little is known about qualitative and quantitative sesquiterpene emissions from vegetation. Technical limitations make it difficult to measure these reactive compounds. Thus, only few studies were found to describe sesquiterpene emission by the species chosen for this investigation (Llusia and Penuelas, 1998; Ormeno *et al.*, 2007a; Ormeno *et al.*, 2007b; Ormeno *et al.*, 2007c; Ormeno *et al.*, 2007d; Ormeno *et al.*, 2009). Sesquiterpene emissions from *Cistus albidus, Cistus monspeliensis, Pinus halepensis, Quercus coccifera* and *Rosmarinus officinalis* have been already described, but not from *Buxus sempervirens, Ceratonia siliqua* and *Olea europea*, being reported for the first time here. Quantitavely,

sesquiterpene emissions from *Quercus coccifera* were comparable, but not qualitatively (see Table 3.3.1.3). We found only ß-caryophyllene emissions, whereas a variety of sesquiterpene species were reported in other studies (see Table 3.3.1.3). As for monoterpenes, sesquiterpenes species pattern showed a great degree of variation among the different studies (see Table 3.3.1.3). It is important to note that for *Cistus albidus* most of the sesquiterpene species identified here were partially confirmed by other studies realized in the Mediterranean region (Llusia and Penuelas, 1998; Ormeno *et al.*, 2007a; Ormeno *et al.*, 2007b; Ormeno *et al.*, 2007c; Ormeno *et al.*, 2007d) except for emissions of α- and ß-cubebene that were measured only here. Furthermore, sesquiterpene E_s for *Cistus albidus* of 0.31 ± 0.19 µg g^{-1} h^{-1} (GC-FID data) were in concordance with the value reported by Ormeno *et al.* (2007a; 2007d). However, Sesquiterpene E_s identified by other authors are significantly higher (Llusia and Penuelas, 1998; Ormeno *et al.*, 2007b; Ormeno *et al.*, 2007c). Interestingly, no sesquiterpene emissions were detected during our investigation from *Pinus halepensis* and *Rosmarinus officinalis*, in contrast with the observation reported by several authors (see Table 3.3.1.3). In the particular case of *Cistus monspeliensis* ß-caryophyllene emissions of 0.2 ± 0.2 µg g^{-1} h^{-1} (GC-FID data) were observed, whereas no emissions were found by Llusia *et al.*, (1998).

Not only isoprenoids are emitted from plants. Oxygenated VOC have been found to be emitted in considerable quantities by vegetation (Seco *et al.*, 2007), especially methanol and acetone. Fifteen of the sixteen plant species investigated in this study emitted methanol. Only *Chamaerops humilis* was a non emitter. The range of emission rates was very large (between 1.04 and 13.5 µg g^{-1} h^{-1}). Acetone emissions were also detected for some species but at lower rates (see Table 3.3.1.4). Most of the emission rates were <1 µg g^{-1} h^{-1}. The highest acetone emission was found in case of *Ficus carica* with rates of 4.17 µg g^{-1} h^{-1}. To our knowledge methanol and acetone emissions as shown in table 3.3.1.4, are not reported by other studies, except for *Pinus halepensis*, that was measured by Filella *et al.* 2009 in the Spanish Mediterranean forest.

Table 3.3.1.1 Isoprene emission at standard conditions (E_s) of 17 Mediterranean plant species in µg g^{-1} h^{-1}: comparison of PTR-MS and GC-FID Method and with literature

Plant species	n	PTR-MS [µg g^{-1} h^{-1}]	Online GC [µg g^{-1} h^{-1}]	other studies [µg g^{-1} h^{-1}]	references
Brachypodium retusum	3	56.18 ± 20.0	38.0 ± 19.29	0 (sept.)	[7]
Buxus sempervirens	3	21.46 ± 5.21	17.72 ± 2.89	6±1 (june), 17 ± 15 (sept.)*	[7]
				11*	[10]
				11.5*	[8]
Ceratonia siliqua	3	0.52 ± 0.17#	0.02 ± 0.01	0	[7]
Chamaerops humilis	3	18.93 ± 3.44	14.96 ± 4.03	0	[18]
Cistus albidus	3	< d.l.	0	0	[7]
Cistus monspeliensis	3	< d.l.	0	0	[10]
Coronilla valentina	3	0.43 ± 0.08	0.14 ± 0.12	n.f.	
Ficus carica	3	60.75 ± 4.79	45.75 ± 3.5	0.71***	[9]
				8.61*	[18]
Olea europea	3	0.12 ± 0.09	0.08 ± 0.05	0	[7], [12], [16], [17]
Pinus halepensis	3	0.10 ± 0.07#	0.07 ± 0.02	0	[7], [16]
				0.05*	[12]
				0.1*	[10]
Prunus persica	3	0.04 ± 0.03	0	0	[17], [9]
Quercus afares	2	0.88 ± 0.61	0.38 ± 0.48	n.f.	
Quercus coccifera	3	0.64 ± 0.23	n.m.	0	[7]
				0.1	[3]
				0.1-25.31	[13]
				0.9***	[9]
				0.1*	[10]
Quercus suber	3	0.26 ± 0.09	0.16 ± 0.04	<0.15***	[14]
				<0.2*	[5]
Rosmarinus officinalis	3	< d.l.	0	0	[11]
				<0.01*	[4], [5]
Spartium junceum	3	28.28 ± 6.13	28.08 ± 11.91	6 ± 2 (may), 1.8 ± 1.6 (oct.)*	[7]
				6.38*	[6]
				5-6.4*	[11]

#one tree was not emitter

* normalized to 30°C and 1000°C

** normalized to 30°C

*** not normalized

[1] Arey *et al*. 1991, [2] Corchnoy *et al.* 1992, [3] Hansen and Seufert, 1996 [4] Hansen *et al.* 1997, [5] Lenz *et al.* 1997, [6] Owen *et al.* 1997, [7] Owen *et al.* 2001, [8] Parra *et al.* 2004, [9] Pio *et al.* 1993, [10] Schaab *et al.* 2000, [11] Seufert *et al.* 1997, [12] Simon *et al.* 2006, [13] Steinbrecher *et al*. 1996, [14] Steinbrecher *et al.* 1997, [15] Street *et al.* 1997, [16] Winer *et al.* 1983, [17] Winer *et al.* 1992, [18] Benjamin and Winer, 1996

Table 3.3.1.2 Monoterpene (Mtp.) standard emission factors (E_s) of 16 Mediterranean plant species in µg g^{-1} h^{-1}: comparison of PTR-MS and GC-FID Method and with literature

Plant species	n	Total Mtp. E_s (PTR-MS) [µg g^{-1} h^{-1}]	Total Mtp. E_s (GC-FID) [µg g^{-1} h^{-1}]	Mtp. species (this study)	Emission rates of other studies [µg g^{-1} h^{-1}]	Mtp. species (other studies)	ref.
Brachypodium retusum	3	1.1 ± 0.71	< d.l.		0.2**	1,2,10	[14]
Buxus sempervirens	3	0.13 ± 0.06#	0.08 ± 0.03#	6,12	0**		[14], [18]
Ceratonia siliqua	3	7.93 ± 5.41	6.03 ± 4.87	5,12,13	1.0 ± 1.7**	1,10	[14]
					0.19 ± 0.1 (spring), 0.75 ± 0.53 (summer), 1.2 ± 0.38 (winter)**	1	[11]
Chamaerops humilis	3	0.14 ± 0.03#	< d.l.		n.f.		
Cistus albidus	3	0.66 ± 0.07	< d.l.		0.2 ± 0.1**	1,10	[14]
					32.8*	1,4,5,7,6,10	[10]
					1.1-3.5**	1,2,3, 4,5,7, 9, 11, 14,19,13	[12]
					1.8**	17,1,2,3,4,5,6,7,8,9,10,33,25, 30,34,35,37,38,39	[27]
					~0.5**	1,4,30,14	[28]
					~0.7-1.9**	1,3,4,5,7,10,34,14,22,27	[29]
Cistus monspeliensis	3	1.03 ± 0.49	0.23 ± 0.17	1,2,4,5,13	0*		[1]
					3.59***	6	[9]
					11.7**	1,2,3,4,10	[15]
					0.3*	n.d.	[18]
Coronilla valentina	3	0.75 ± 0.45	< d.l.		n.f.		
Ficus carica	3	< d.l.	< d.l.		0.29***	25, 31	[16]
					0.07**	n.d.	[4]
Olea europea	3	1.21 ± 0.95	1.12 ± 1.77#	12,13	0.1 ± 0.1**	1	[14]
					0.4 ± 0.3**	1	[25]
					0.05***	n.d.	[26]
					0.05***	13	[2]
					0.08-3.9***	1,10	[11]
					0.36***	25,31	[16]
					0.11**	n.d.	[4]
Pinus halepensis	3	2.74 ± 0.93	1.65 ± 1.41	5, 12, 13,24	1.1-2.0 ± 1.7-0.6**	1,2,3,4,5,7,9,10,13,11	[14]

Plant species	n	Total Mtp. E_s (PTR-MS) [µg g^{-1} h^{-1}]	Total Mtp. E_s (GC-FID) [µg g^{-1} h^{-1}]	Mtp. species (this study)	Emission rates of other studies [µg g^{-1} h^{-1}]	Mtp. species (other studies)	ref.
					0.6 ± 0.4**	1,5,10	[25]
					2-3.5 (spring and fall) and 7-15 (summer)**	10,13,14, 1, 5, 25,12,27,29,30	[22]
					2.3-11.4**	1,4,5,7,6,10	[10]
					1.54**	1, 2, 3, 4, 5, 7, 9, 10, 25, 11, 14, 26, 22, 27, 19, 13	[12]
					14.76*/**	13,14, 5, 1,11,10,3,7,4,25,9,2	[20]
					43.9 ± 7.41*	1,4,5,7	[3]
					20**	1,4,5,7	[21]
					0.13**	9,10,4,3,2	[15]
					86***	1,4,7,5,6,2	[9]
					0.15 ± 0.15**	13,1	[5]
					0.11**		[4]
					9.6**	20,17,1,2,3,4,5,6,7,8,9,10,33, 25,13,11,30,34,35,14, 26,36, 39,27	[27]
					~0.2**	1, 7,4,5,14,	[28]
					3.12 ± 0.75**	17,1,2,3,4,5,7,8,9,10,13,11,34,14,26,22,27	[29]
Prunus persica	3	0.36 ± 0.24	< d.l.		0.1***		[26]
					0.3***	13	[2]
					1.0***	25,31	[16]
					0.9**		[4]
Quercus afares	2	16.18 ± 10.13	10.95 ± 0.71	1,3,4,5,10,12,13,14,15	n.f.		
Quercus coccifera	4	5.77 ± 6.42	10.19 ± 4.91	16,17,1,3-5,8,18,10,19	5.5-5.7 ± 2.3-3.0*	1,3,4,10,17,2,5,9,25,13,11	[14]

Plant species	n	Total Mtp. E_s (PTR-MS) [μg g^{-1} h^{-1}]	Total Mtp. E_s (GC-FID) [μg g^{-1} h^{-1}]	Mtp. species (this study)	Emission rates of other studies [μg g^{-1} h^{-1}]	Mtp. species (other studies)	ref.
					17.5*	10, 2, 5, 9, 17, 11, 20, 8, 6	[6]
					1-5.5*	1,4,5,7,10	[10]
					48*		[18]
					12.7***		[16]
					2.8*	20,17,1,2,3,4,5,6,7,8,9,10,33, 25,30,34,35,14, 26, 27,39	[27]
					~1.5*	1,3,4,5,	[28}
					~0.1-0.6*	1,3,4,5,7,34,14,22,27	[29]
					~1.4*	1,4,5	[30]
Quercus suber	3	35.58 ± 5.46	31.75 ± 4.17	20, 17, 1-5, 8,18,11,19,21-24	0.2 (winter) and 20-30 (summer)*	1,4,10,3	[17]
					1.3-10 (fall), 39.2-43.2 (summer)***	1,4,3,5,10	[23]
					0***		[24]
					22.2***	1,4,10,2,5,25,31	[16]
					0*		[19]
Rosmarinus officinalis	2	1.97 ± 1.36	0.35##	1,2,4,5,10,22,25,26	2.2**	1,4,25	[7]
					2.38**	1,2,3,4,5,7,9,10,25,11,14,26, 22,27,19	[12]
					2.2*		[19]
					2.1*		[8]
					4.8**	1,2,3,4,9,12,11,19	[15]
					2.85**	20,17,1,2,3,4,5,6,7,8,9,10,33, 25,13,11,30,34,35,14, 26,22	[27]
					~2**	1,3,5,25,30,	[28]
					~1.3**	1,4,5,10	[30]
Spartium junceum	3	< d.l.	< d.l.		0.5-0.6 ± 0.4-0.2**	1,2,3,4,5,6,7,8,9,10,12,11,14, 27	[14]
					0.53**	17,2,3,4,5,6,7,8,9,10,12,11,2 8,14,27	[13]
					0.2-0.53*	1,10,8,11,2,7,5,9,12,6,3,17,1 4	[19]

#one tree was a non emitter. Zero value was included to calculate the mean,## one sample, * normalized to 30°C and 1000°C, ** normalized to 30°C, *** not normalized, n.f. other studies not found, < d.l. under the detection limit, n.d. not defined

(1) α-pinene,(2) camphene, (3) sabinene, (4) ß-pinene, (5) myrcene, (6) α-phellandrene, (7) 3-carene, (8) α-terpinene, (9) p-cymene, (10) limonene, (11) g-terpinene, (12) z-ocimene, (13) e-ocimene, (14) linalool, (15) alloocimene, (16) z-3-Hexenol, (17) alpha-Tujene, (18) o-cymene, (19) Terpinolene, (20) Tricyclene, (21) cis-sabienehydrat, (22) Borneol, (23) Menthol, (24) Terpinen-2-ol, (25) Eucalyptol, cineole (26) Camphor, (27) α-Terpineol, (29) trans-linalool oxide, (30) cis-linalool oxide, (31) unknown, (32) 2-Methy-1propene, (33) ß-phellandrene, (34) delta-terpinene, (35) Fenchone, (36) Thujone trans, (37) Thujone cis, (38) Terpineol-4-ol, (39) Verbenone

[1] Alessio *et al.* 2004, [2] Arey *et al.* 1991, [3] Blanch *et al.* 2008, [4] Benjamin and Winer *et al.* 1998 [5] Corchnoy *et al.* 1992, [6] Hansen and Seufert, 1996 [7] Hansen *et al.* 1997, [8] Lenz *et al*. 1997, [9] Llusia *et al.* 1998, [10] Llusia *et al.* 2000, [11] Llusia *et al.* 2002, [12] Ormeno *et al.* 2007b, [13] Owen *et al.* 1997, [14] Owen *et al*. 2001, [15] Owen *et al.* 2002, [16] Pio *et al.* 1993, [17] Pio *et al.* 2005, [18] Schaab *et al.* 2000, [19] Seufert *et al.* 1997, [20] Simon *et al.* 2005, [21] Penuelas *et al.* 1999 [22] Staudt, PhD 1997, [23] Staudt *et al.* 2004, [24] Steinbrecher *et al.* 1997 [25] Winer *et al.* 1983, [26] Winer *et al.* 1992, [27] Ormeno *et al.* 2007c, [28] Ormeno *et al.* 2007d, [29] Ormeno *et al.* 2007a, [30] Ormeno *et al.* 2009

Table 3.3.1.3 Sesquiterpenes (Sqt.) standard emission factors (E_s) of 16 Mediterranean plant species in µg g^{-1} h^{-1}: comparison of PTR-MS and GC-FID Method and with literature

Plant species	n	Total Sqt. E_s (PTR-MS) [µg g^{-1} h^{-1}]	Total Sqt. E_s (GC-FID) [µg g^{-1} h^{-1}]	Sqt. species (this study)	Emission rates of other studies [µg g^{-1} h^{-1}]	Sqt. species (other studies)	ref.
Brachypodium retusum	3	<d.l.	<d.l.		n.f.		
Buxus sempervirens	3	<d.l.	0.07 ± 0.05	a,b	n.f.		
Ceratonia siliqua	3	<d.l.	0.02 ± 0.04	a	n.f.		
Chamaerops humilis	3	<d.l.	<d.l.		n.f.		
Cistus albidus	3	0.63 ± 0.32	0.31 ± 0.19	a, d, e, l,m,n, k	~2.1-2.9	a-i	[2]
					11	a,c-k,n-t	[3]
					~1	a,e,f-h,j,o	[4]
					~0.8-3.8	a,c-i,k,o,p	[1]
					~30	a	[6]
Cistus monspeliensis	3	0.60 ± 0.18	0.20 ± 0.21	a	0		[6]
Coronilla valentina	3	<d.l.	<d.l.		n.f.		
Ficus carica	3	<d.l.	<d.l.		n.f.		
Olea europea	3	<d.l.	0.09 ± 0.01	a,j	n.f.		
Pinus halepensis	3	<d.l.	<d.l.		~0.2-0.5	a-e,i	[2]
					0.7	a,c-e,g-j, n-s	[3]
					~0.1	a, f, j, k	[4]
					~1.8	a,d-f, h,i,o	[1]
					0		[6]
Prunus persica	3	<d.l.	<d.l.		n.f.		
Quercus afares	2	<d.l.	<d.l.		n.f.		
Quercus coccifera	3	0.24 ± 0.13	0.13 ± 0.09	a	0.66	a,c-e,g-k,n-p,r	[3]
					~0.3	f, j, k	[4]
					~0.1-0.5	c,d, f,g,i,o	[1]
					~0.2	a,b,d	[5]
Quercus suber	3	<d.l.	<d.l.		n.f.		
Rosmarinus officinalis	2	<d.l.	<d.l.		0.3-0.4**	a,b,c,i	[2]
					0.51**	a,d,e,g-j, n-s	[3]
					~1.9**	f,h,j.k	[4]
					~0.1**	a,b,d	[5]
Spartium junceum	3	<d.l.	<d.l.		n.f.		

one sample

<d.l. under the detection limit

n.f. other studies not found

(a) ß-caryophyllene, (b) alpha-humulene, (c) alpha-muurolene, (d) copaene, (e) ß-bourbonene, (f) allo-aromadendrene, (g) AR-curcumene, (h) alpha-zingiberene, (i) unkown sesquiterpenes, (j) germacrene-D, (k) delta-cadinene, (l) alpha-cubebene, (m) ß-cubebene, (n) ß-farnesene, (o) alpha- caryophyllene, (p) γ-cardinene, (q) Isocaryophyllene,(r) ß-Gurjunene,(s) ß-sesquiphellandrene, (t) ß-bisabolene

[1] Ormeno *et al.*2007a, [2] Ormeno *et al.*2007b,[3] Ormeno *et al.*2007c, [4] Ormeno *et al.*2007d, [5] Ormeno *et al.* 2009, [6] Llusia and Penuelas, 1998

Table 3.3.1.4 oxygenated VOC standard emission factors (E_s) of 17 Mediterranean plant species in µg g^{-1} h^{-1}: comparison of PTR-MS and GC-FID Method and with literature

Plant species	n	methanol E_s				acetone E_s			
		(this study) [µg g^{-1} h^{-1}]	(this study) [nmol m^{-2} s^{-1}]	(other studies) [nmol m^{-2} s^{-1}]	ref	(this study) [µg g^{-1} h^{-1}]	(this study) [nmol m^{-2} s^{-1}]	(other studies) [nmol m^{-2} s^{-1}]	ref
Brachypodium retusum	3	5.48 ± 1.99	4.06 ± 2.07	n.f.		1.77 ± 1.62	0.54 ± 0.31	n.f.	
Buxus sempervirens	3	1.04* ± 0.63	2.63 ± 0.89	n.f.		< d.l.	< d.l.	n.f.	
Ceratonia siliqua	3	2.66 ± 2.43	3.44 ± 2.41	n.f.		< d.l.	< d.l.	n.f.	
Chamaerops humilis	3	< d.l.	< d.l.	n.f.		< d.l.	< d.l.	n.f.	
Cistus albidus	3	8.20 ± 4.61	6.49 ± 3.63	n.f.		< d.l.	< d.l.	n.f.	
Cistus monspeliensis	3	7.46 ± 5.2	7.11 ± 5.69	n.f.		< d.l.	< d.l.	n.f.	
Coronilla valentina	3	13.48 ± 7.84	8.57 ± 3.85	n.f.		< d.l.	< d.l.	n.f.	
Ficus carica	3	4.22 ± 2.15	2.4 ± 0.59	n.f.		4.17 ± 1.18	1.42 ± 0.47	n.f.	
Olea europea	3	1.03 ± 0.20	3.15 ± 1.25	n.f.		0.11* ± 0.02	0.11* ± 0.02	n.f.	
Pinus halepensis	3	2.95* ± 0.09	3.67* ± 0.12	2.9 ± 0.43	[1]	0.12*± 0.01	0.09 ± 0.01	0.48 ± 0.08	[1]
Prunus persica	3	4.01 ± 1.30	2.44 ± 0.83	n.f.		0.32 ± 0.18	1.18 ± 0.09	n.f.	
Quercus afares	2	1.27 ± 0.4	1.26 ± 0.71	n.f.		< d.l.	< d.l.	n.f.	
Quercus coccifera	3	4.11 ± 3.9	5.8 ± 3.82	n.f.		0.25* ± 0.09	0.16 ± 0.03	n.f.	
Quercus suber	3	1.83 ± 1.10	1.92 ± 0.85	n.f.		< d.l.	< d.l.	n.f.	
Rosmarinus officinalis	3	2.03 ± 0.61	3.79 ± 0.65	n.f.		< d.l.	< d.l.	n.f.	
Spartium junceum	3	3.74 ± 0.72	4.38 ± 0.65	n.f.		< d.l.	< d.l.	n.f.	

*one tree was not emitter

< d.l. under the detection limit

n.f. other studies not found

[1] Filella *et al.*, 2009

3.3.2 INTERCOMPARISON OF DIFFERENT METHODS FOR VOCs MEASUREMENTS: A QUALITATIVE AND QUANTITATIVE STUDY

Intercomparison experiments were carried out during this study to demonstrate the quality of VOC analysis. Correlation of PTR-MS data with a C_2-C_6 online GC (for isoprene measurements) and a GC-FID (for monoterpene measurements) was reasonably good showing linear regression factors (R^2) of 0.77, 0.89, respectively (see Figure 3.3.2.1 and 3.3.2.2). The correlation between PTR-MS and GC-FID for the measurement of monoterpenes confirms the suitability of the PTR-MS for monoterpene measurements even though single species cannot be resolved. In some cases PTR-MS values were higher than online GC and GC-FID values (absolute error of ± 0.1 ppb for isoprene and monoterpenes), supposing and interference of additional compounds. However, we have to note that for technical reasons VOC measurements with online equipments and collection of cartridges were not carried out simultaneously, which also might contribute to such differences.

As shown in figure 3.3.2.1 and 3.3.2.2, a slightly general tendency of overestimation by PTR-MS was observed. However, it should be noted that isoprene emission values for *Spartium junceum* and *Buxus sempervirens* were higher as measured with the online GC (see Figure 3.3.2.1). Contrasting, the distribution of values for monoterpene emissions did not present any plant specific tendency (see Figure 3.3.2.2).

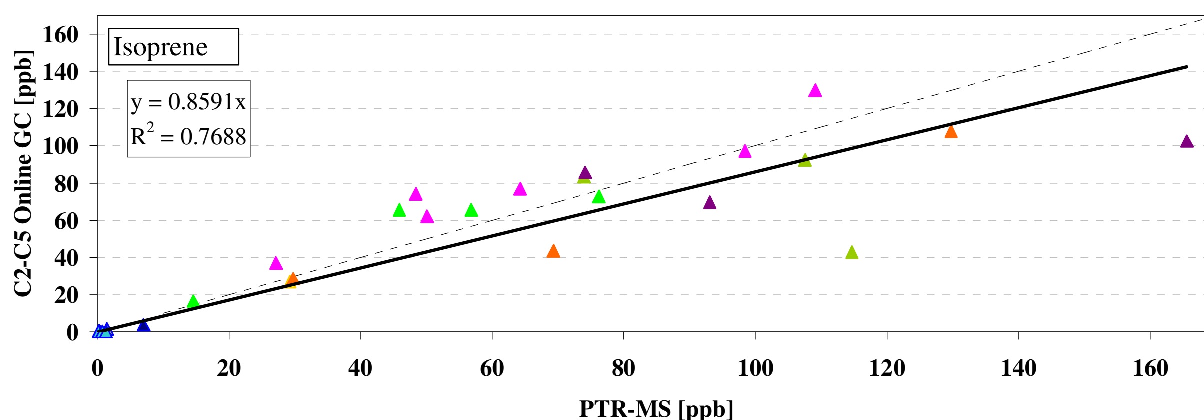


Figure 3.3.2.1 Intercomparison of C2-C5 online GC and PTR-MS for Isoprene measurement in ppb.

▲ *Buxus sempervierens*, ▲ *Chamaerops humilis*, ▲ *Coronilla glauca*, ▲ *Ficus carica*, ▲ *Prunus persica*
△ *Quercus suber*, ▲ *Spartium junceum*,

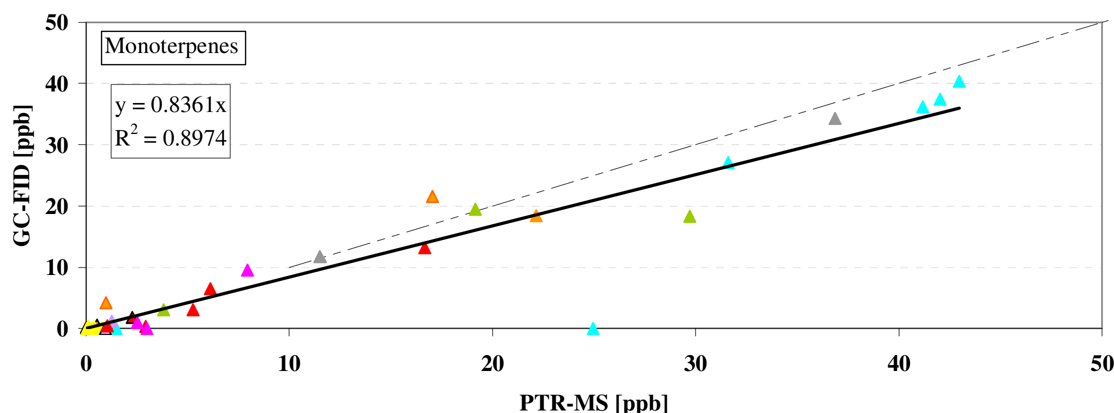


Figure 3.3.2.2 Intercomparison of GC-FID and PTR-MS for Monoterpene measurement in ppb.

▲ *Buxus sempervierens,* ▲ *Ceratonia siliqua,* ▲ *Cistus albidus,* ▲ *Olea europea,* ▲ *Pinus halepensis* ▲ *Quercus affares,* ▲ *Quercus coccifera,* △ *Quercus suber,* △ *Rosmarinus officinalis*

3.3.4 MASS SCAN OF EMISSIONS TO IDENTIFY UNKNOWN VOC SPECIES

The PTR-MS measurements were carried out in Mass Scan Mode. Scans from m/z 20 to m/z 206 were performed for each plant species by an E/N of 96 Td and 130 Td. As final result for each species the mean of the scans obtained from each of the 3 individuals was taken into account. Table 3.3.4 presents those masses (m/z), which could not be identified though repeatedly found in several plant species. However, a tentative identification of the m/z is given.

A variety of plant species emitted considerable quantities of compounds with m/z 41, 43, 51, 57, 61, 75, 79 and 93. The compound with m/z 41, 43 and 57 were detected in higher quantities by an E/N of 96 Td (see Table 3.3.4), whereas detection of m/z 51, 57, 61, 75, 79 and 93 was improved with an E/N of 130 Td. There are several potential candidates for each m/z. The most probable candidate according to literature is indicated in bold, if available.

The compound with m/z 41 may be addressed as a fragment of isoprene, since all plant species that emitted this compound were high isoprene emitters. Occurrence of m/z 43 was often accompanied by m/z 61, giving reasons to regard these masses as fragments of acetic acid. In case of *Olea europea, Prunus persica, Quercus afares* and *Rosmarinus officinalis* only m/z 61 was detected, but not m/z 43, a results which does not support an identification. Similarly, no reports for compounds with m/z 51 were found. In case of m/z 57 and 79 several possibilities are given in Table 3.3.4, but no justification was found to assign one of them as a possible candidate (see discussion). Two possible compounds for m/z 73 were reported by

several authors: methyl ethyl ketone (MEK) (De Gouw *et al.*, 1999; Isidorov *et al.*, 1985; Kirstine *et al.*, 1998) and protonated methylglyoxal (Holzinger *et al.*, 2007). Compounds with m/z 75 may correspond to hydroxyacetone, but only in the case of *Brachipodium retusum*. Hydroxyacetone is a known oxidation product of isoprene, which was highly emitted by *Brachipodium retusum,* but not by *Cistus albidus* and *Cistus monspeliensis*, where m/z 75 was also found. Therefore, this mass remains unclear. High quantities of m/z 79 (1.2-2.9 ppb) were observed in case of *Brachipodium retusum* and *Ceratonia siliqua*, but a reasonable identification was impossible, since m/z is regarded as benzene or fragments of ethylbenzene, iso- and n-propylbenzene, all with anthropogenic origin. Finally in five of the plant species considerable emissions of a compound with m/z 93 were identified. Firstly, toluene emissions were taken into account as reported for *Helianthus annuus* and *Pinus sylvestris* (Heiden *et al.*, 1999b). But this assumption was not confirmed by GC-FID measurements. On the other hand, it is known that the monoterpene p-cymene fragments with 70% to m/z 93 (Tani *et al.*, 2003). But this explanation must be also excluded as no p-cymene emission was detected in these plant species. Potential assignment of other fragments to α- and ß-pinene have been proposed by Warneke *et al.* (2003). Both monoterpene species were detected with GC-FID in case of *Quercus suber* and *Rosmarinus officinalis*, being possible candidates for m/z 93. But this assumption failed in case of *Buxus sempervirens, Chamaerops humilis* and *Pinus halepensis*, all three species without the emission of α- and ß-pinene. Thus identification of m/z 93 for these plants remains open.

These results confirm the necessity of using the PTR-MS in combination with GC techniques in order to confirm or exclude mass assignments.

Table 3.3.4: Unidentifiable masses (m/z) detected with PTR-MS in mass scan mode

m/z [amu]	**[ppb] E/N = 96 Td/130 Td**	**plant species**	**possible compound**	**references**
41	4.24 ± 2.71/--	*Brachipodium retusum*	**fragment of isoprene**	Ammann *et al.* (2004)
	8.72 ± 5.92/--	*Chamaerops humulis*	fragment of α-humulene	Demarcke *et al.* (2009)
	4.95 ± 0.61/--	*Ficus carica*	fragment of p-cymene	Tani *et al.* (2004)
	7.40 ±3.89/--	*Spartium junceum*	propylene	Warneke *et al.* (2003)
			fragment of 2-Methyl-3-buten-2-ol	Demarcke et al. (2010)
			fragment of 1-penten-3-ol	Demarcke et al. (2010)
			fragment of trans-3-hexen-1-ol	Demarcke et al. (2010)
			fragment of 6-Methyl-5-hepten-2-ol	Demarcke et al. (2010)
			fragment of 1-octen-3-ol	Demarcke et al. (2010)
			fragment of linalool	Demarcke et al. (2010)

m/z [amu]	[ppb] E/N = 96 Td/130 Td	plant species	possible compound	references
43	4.05 ± 1.65/--	*Brachipodium retusum*	fragment of 1 or 2-	
	1.73 ± 0.56/0.84 ± 0.56	*Ceratonia siliqua*	Propanol	Williams *et al.* (2001)
	1.34 ± 0.84/--	*Cistus albidus*	Propene	Kuster *et al.* (2004),
	1.63 ± 1.55/0.14 ± 0.01	*Cistus monspeliensis*		Karl *et al.* (2003c)
	0.84 ± 0.26/--	*Ficus carica*		Warneke *et al.* (2003)
	1.35 ± 0.67/0.63 ± 0.40	*Quercus suber*	**fragment of aceticacid**	Williams *et al.* (2001)
	0.76 ± 0.88/ 0.66 ± 0.53	*Spartium junceum*	fragment of vinyl acetate	Kuster *et al.* (2004)
			heavier acetates	Kuster *et al.* (2004)
			Cyclopropane	Warneke *et al.* (2003)
			fragment of linalool	Demarcke et al. (2010)
			non-specific alkane	
			fragments	Buhr *et al.* (2002)
			Ethyl-, Propyl-, Butyl-	
			and Hexyl-acetate	Buhr *et al.* (2002)
51	--/2.09 ± 1.09	*Brachipodium retusum*	-	n.f.
	0.08 ± 0.07/2.57 ± 1.82	*Ceratonia siliqua*		
	--/ 1.68 ± 1.84	*Cistus monspeliensis*		
	0.08 ± 0.02/5.17 ± 3.9	*Coronilla valentina*		
	--/1.47 ± 0.70	*Ficus carica*		
	0.07 ± 0.007/ 2.16 ±	*Olea europea*		
	1.65	*Quercus suber*		
	--/ 0.58 ± 0.1	*Spartium junceum*		
	--/3.46 ± 3.82			
57	0.94 ± 0.71/--	*Brachipodium retusum*	trans-2-butene	Warneke *et al.* (2003)
	0.59 ± 0.17/--	*Ceratonia siliqua*	butanol	Karl *et al.* (2003)
			methyl tertiary butyl	
			ether (MTBE)	Warneke *et al.* (2003),
				Karl *et al.* (2003)
			Methylcyclohexane	Warneke *et al.* (2003)
			octane	Warneke *et al.* (2003)
			decane	Warneke *et al.* (2003)
			butenes	Karl *et al.* (2003)
			butanol	Karl *et al.* (2003),
				Holzinger *et al.* (2000)
			non-specific alkane	Buhr *et al.* (2002)
			fragments	
61	3.67 ± 1.75/7.87 ± 3.14	*Brachipodium retususm*	**Aceticacid**	Holzinger *et al.* (2000),
	1.87 ± 0.41/4.07 ± 0.86	*Ceratonia siliqua*		Karl *et al.* (2004),
	0.75 ± 0.52/2.05 ± 1.67	*Cistus albidus*		Williams *et al.* (2001),
	--/0.52 ± 0.2	*Cistus monspeliensis*	methyl formate	Williams *et al.* (2001)
	--/1.48 ± 0.78	*Ficus carica*	glycoladehyde	Karl *et al.* (2004b)
	1.59 ± 0.62/2.29 ± 1.58	*Quercus suber,*	(photochemical	De Gouw *et al.* (2003)
	0.95 ± 0.74/0.99 ± 1.3	*Spartium junceum*	produced)	
	1.43 ± 0.54/3.07 ± 1.91	*Olea europea*	fragment of	
	1.73 ± 0.12/3.22 ± 0.24	*Prunus persica,*	Peroxyacetic acid	De Gouw *et al.* (2003)
	0.44 ± 0.18/1.02 ± 0.71	*Quercus afares*	fragment of 1-or 2-	
	1.13 ± 1.27/3.24 ± 3.37	*Rosmarinus officinalis*	propanol	Buhr *et al.* (2002)
			Ethyl-, Propyl-, Butyl-	
			and Hexyl-acetate	Buhr *et al.* (2002)
73	--/0.48 ± 0.36	*Brachypodium retusum*	methyl ethyl ketone	De Gouw *et al.* (1999)
	--/0.1 ± 0.02	*Prunus persica*		Isidorov *et al.* (1985)
	not done/0.2 ± 0.16	*Quercus coccifera*		Kirstine *et al.* (1998)
			methylglyoxal	Holzinger *et al.* (2007)
75	0.61 ± 0.56/1.39 ± 0.71	*Brachipodium retusum*	Hydroxyacetone	Williams *et al.* (2001),
	7.01 ± 3.81/11.51 ±	*Cistus albidus,*		Williamns *et al.* (2000)
	11.17	*Cistus monspeliensis*		
	10.94 ± 5.93/ 7.65 ±			
	4.11			

m/z [amu]	[ppb] E/N = 96 Td/130 Td	plant species	possible compound	references
79	0.06 ± 0.07/2.91 ± 1.52 0.02 ± 0.02/1.18 ± 0.42	*Brachipodium retusum* *Ceratonia siliqua*	Benzene	Kuster *et al.* (2004), Kato *et al*. (2004), De Gouw *et al.* (2003), Warneke *et al.* (2001), Ammann *et al.* (2004), De Gouw *et al*. (2006)
			fragment of ethylbenzene, iso- and n-propylbenzene	De Gouw *et al.* (2003)
93	1.52 ± 1.20/1.94 ± 1.35 1.08 ± 0.81/1.39 ± 1.37 0.47 ± 0.52/ 2.82 ± 3.24 1.37 ± 0.39/-- 1.35 ± 0.23/2.24 ± 2.21	*Buxus sempervirens* *Chamaerops humilis* *Pinus halepensis* *Quercus suber* *Rosmarinus officinalis*	Toluene	Kato *et al.* (2004), De Gouw *et al.* (2003), Warneke *et al.* (2001), Kuster *et al.* (2004), Ammann *et al.* (2004), De Gouw *et al*. (2006)
			fragment of p-cymene	Tani *et al.* (2003)
			fragment of α-pinene	Warneke *et al.* (2003)
			fragment of ß-pinene	Warneke *et al.* (2003)

n.f references not found

3.4 DISCUSSION

The objective of this study was to perform a multi-method approach for VOC emissions from Mediterranean vegetation in order to identify possible compounds that could have been overlooked due to earlier technical limitations. Due to the diversity of VOC no study will be able to detect and identify all compounds present in natural mixture. In general, the emission studies focus on a specific set of VOC species limited by technical constraints. The use of the online PTR-MS system allowed a detection of a large variety of masses that corresponded to several isoprenoids as well as oxygenated VOC species. Assignment of these masses to known species could be partly confirmed with GC-systems (online GC, GC-FID and GC-MS) making our measurements more reliable. Furthermore, the PTR-MS system permitted us to discover a certain number of masses that correspond to a manifold of possible compounds that could have been overlooked in earlier investigations performed with these plant species, as suggested in recent studies of OH chemistry performed over coniferous and tropical forest, where undetected reactive VOC were supposed (Di Carlo *et al.*, 2004; Kuhn *et al.*, 2007).

3.4.1 SCREENING

This study reflected the domination of monoterpene emitting species in the Mediterranean area, which is composed by a multitude of aromatic plants. In a few cases quantitative monoterpene emissions were observed comparable to literature, like e.g. the monoterpene emission from *Cistus albidus, Pinus halepensis, Quercus coccifera* and *Rosmarinus officinalis* as reported by Owen *et al.* (2001). But in general, there were quantitative and qualitative differences reflecting the complex background for the emission and detection of this isoprenoid group. It has been demonstrated that seasonality is one of the main factors, which can influence the amount of emission and the monoterpene-species composition (Llusia and Penuelas, 2000; Owen *et al.*, 2001; Penuelas and Llusia, 1997), but other factors like the plant species origin can not be excluded. Investigations performed with conifers demonstrated that the geographical variation and population dynamics can exert genetic variations in trees within the same species (Hanover, 1992), leading to a differentiation in monoterpene composition. In this study plant species from the south east French Mediterranean area were compared with studies performed in the Italian and Spanish Mediterranean area, Portugal and California's Central Valley supposing different genetics due to evolutionary variation. Another possible source of variability can be expected by measurements beyond standard conditions which should be normalized by mathematical algorithms such as the G93 (Guenther *et al.*, 1995). Such normalization was not performed in all studies and can not be

applied for all compounds (see Table 3.3.1.1 and 3.3.1.2). Without the normalization to standard light intensity and temperature a quantitative comparison has to be discussed carefully. Some authors realized only a temperature normalization of the data in species that have monoterpene storage organs as *Pinus halepensis, Cistus monspeliensis* and *Rosmarinus officinalis*. Such a temperature dependency have been already demonstrated for *Rosmarinus officinalis* and *Cistus monspeliensis* finding considerable emissions in the darkness (Owen *et al.*, 2002). But for example in case of *Pinus halepensis* it has been demonstrated that monoterpene species like ß-trans-ocimene and linalool were emitted being light dependent, whereas α-pinene and myrcene emissions were only temperature dependent (Simon *et al.*, 2005).

Total monoterpene emissions were also characterized qualitatively. In several studies only the main monoterpene species were accounted, whereas other investigations took into account also the compounds that were emitted in traces, finding a variety of monoterpene species measured or detected. The observed monoterpene emission pattern coincided rarely among the literature. Alpha-pinene, ß-pinene, myrcene, sabinene, limonene, camphene, α-phellandrene, eucalyptol, Z-ocimene and E-ocimene were the main emitted compounds among the species investigated. However, we have to accept that the species composition may be influenced or even induced by external factors too. Strong emissions of ocimene have already been observed for example in case of *Pinus pinea* (Staudt *et al.*, 2000; Staudt *et al.*, 1997), probably associated to an induction on the emission due to a high temperature exposure (Staudt and Bertin, 1998; Staudt *et al.*, 2003).

High isoprene emitting species (*Brachypodium retusum, Buxus semprevirens, Chamaerops humilis, Ficus carica* and *Spartium junceum*) were found, recording emissions in the range found for several known isoprene emitters already reported by Kesselmeier and Staudt (1999). Similar high isoprene emissions have been already reported for *Buxus sempervirens* from the same area, measured at the end of summer (September) (Owen *et al.*, 2001). On the other hand much lower, than those recorded here, or none emissions have been found for *Ficus carica, Spartium junceum, Chamaerops humilis* and *Brachypodium retusum* in earlier studies (Benjamin *et al.*, 1996; Owen *et al.*, 2001; Pio *et al.*, 1993). The quantitative differences could be due to the different origins of the plants among different Mediterranean areas, suggesting genetic differences, but technical aspects and missing normalization of the data can not be ignored. In some cases no direct emission was reported, but assigned, based on genus average

as in the case of isoprene emission from *Ficus carica* and *Chamaerops humilis* reported by Benjamin *et al.* (1996).

Since years there has been a challenge to detect sesquiterpene emissions, qualitatively and quantitatively. Due to their high reactivity and relatively low vapour pressure, these compounds can be either missed or overestimated (due to disturbances) (Duhl *et al.*, 2008). But special analytical adaptations can facilitate their quantification (Helmig *et al.*, 2004; Merfort, 2002; Tholl *et al.*, 2006). Results obtained in this study reflect the difficulties depending on the VOC detection method, observing higher sensitivity by offline gas chromatographic methods than by online PTR-MS method, which is however more unspecific. Previous studies have confirmed the difficulty of PTR-MS for sesquiterpenes measurements, reporting high fragmentation patterns of sesquiterpenes (Demarcke *et al.*, 2009; Tani *et al.*, 2003).

The characterization of sesquiterpene emissions on the Mediterranean area is sparse and only a few oaks, birches and pines typical for the Mediterranean area are reported to emit sesquiterpenes (Ciccioli *et al.*, 1999; Hansen and Seufert, 1999; Llusia and Penuelas, 1998; Ormeno *et al.*, 2007b; Ormeno *et al.*, 2007d). Sesquiterpene emission is known to vary considerably between vegetation species (Duhl *et al.*, 2008) and such high variability has been found concerning the emission -rate and -pattern in case of the Mediterranean vegetation characterized here, comparing our data with reports of Ormeno *et al.* (2007a, b, c, d, 2009) and Llusia *et al.* (1998). Such variation may be due to differences in analysis protocols and sampling techniques. But other factors like differences of temperature, light and time of the year may also contribute. For example highest emissions of *Rosmarinus officinalis* were measured by Ormeno *et al.* (2007d) in June, whereas lower emission rates were found in March or January (Ormeno et al. 2007b, c, 2009), demonstrating a clear seasonal trend. This was not the case in other plant species like *Cistus albidus* and *Pinus halepensis*.

Regarding the limitation for a general view, it is important to highlight that in this study important species from the Mediterranean area like *Brachipodium retusum, Buxus sempervirens, Ceratonia siliqua, Chamaerops humilis, Ficus carica, Olea europea, Prunus persica, Quercus suber* and *Spartium junceum* were characterized to emit sesquiterpenes, though finding in some cases only trace emissions. Furthermore, VOC emissions by the Mediterranean species *Coronilla valentina* and *Quercus afares*, an oak original from Algeria

and Tunisia, were characterized for the first time. *Coronilla valentina* showed low emissions of isoprene and monoterpenes (<1 µg g^{-1} h^{-1}), whereas *Quercus afares* also exhibited low emissions of isoprene but high emissions of monoterpenes (10.9 ± 0.71 µg g^{-1} h^{-1}). The emission of both types of isoprenoids in *Quercus afares* can be explained since this tree is a stabilized species that originates from hybridization between two oak species during plant evolution: *Quercus suber* and *Quercus canariensis* (Mir *et al.,* 2006). *Quercus suber*, as mentioned before, is a known monoterpene emitter and *Quercus canariensis* is an isoprene emitter (Kesselmeier and Staudt, 1999).

Information on oxygenated VOC compounds emission from vegetation is also sparse, as shown in a recent review by Seco *et al.* (2007). The technological improvement using a PTR-MS facilitated the study of short-chain oxygenated VOCs. Such compounds can play many roles in plant physiology and ecology, but also in air chemistry (Fall, 2003). The exchange of the oxygenated VOC like methanol, acetone and methyl ethyl ketone has been detected by a variety of plant species (De Gouw *et al.*, 1999; Isidorov *et al.*, 1985; Kirstine *et al.*, 1998; Seco *et al.*, 2007). In our study emissions of these compounds from the chosen plant species have been characterized for the first time, except for *Pinus halepensis* (Filella *et al.*, 2009). Although only one report was found for these VOCs, emissions of methanol were reported in similar ranges already for crop plants (Nemecek-Marshall *et al.*, 1995) and of acetone for the Mediterranean species *Quercus ilex* (Holzinger *et al.*, 2000; Kreuzwieser *et al.*, 2002), the north European species *Pinus sylvestris* and *Picea abies* (Janson and de Serves, 2001), the middle European tree *Fagus sylvatica* (Cojocariu *et al.*, 2005) as well as for *Populus fremontil* and *Pinus ponderosa* from New Mexico (USA) (Villanueva-Fierro *et al.*, 2004). Exceptionally, emissions of acetone from *Ficus carica* measured here were curiously high (4.17 ± 1.18 µg g^{-1} h^{-1}). Interestingly, emissions of methanol and acetone of the Mediterranean species investigated in this study were significantly lower ($p<0.05$) than those detected for tropical vegetation (see chapter 2). It is important to note that the difference observed in the methanol emission is probably due to different developmental stages of the plants. The tropical plants investigated were younger than the Mediterranean plant species, although in both experiments full expanded mature leaves were measured. However, acetone emissions from the Mediterranean vegetation, the species from middle and northern Europe and from North America are not comparable to the acetone emissions of the Tropical vegetation. However, only a few plant species were used to perform this comparison and further investigation is needed in order to make any conclusions.

3.4.2 MASS SCAN OF PLANTS

In order to screen for unidentified new compounds emitted by plant species chosen for this experiment a low and a high E/N value was applied. It is well known that the ratio of the electric field to the buffer gas number density (E/N) in the drift tube reactor of a PTR-MS can affect the product ion distribution of a variety of compounds (Demarcke *et al.*, 2010; Demarcke *et al.*, 2009; Tani *et al.*, 2003). Increasing the E/N ratio results in more energetic collisions, which reduces the proportion of cluster ions such as $H_3O^+(H_2O)_n$ in the drift tube. However, at the same time, this increased average collision energy might rise the fragmentation of ions produced by the reaction between H_3O^+ and the analysed gas, which is often undesirable because of the complications it causes for the analysis.

Most of the studies performed with PTR-MS uses the standard operational settings (E/N = 130 Td; E electric field strength, N buffer gas number density, 1 Td = 10^{-17} cm² V molecule^{-1}) recommended by the manufacturer and other authors (Blake *et al.*, 2009; Hansel *et al.*, 1995), probably overlooking compounds that can be detected better with lower E/N (with lower fragmentation rates), as was demonstrated for ethanol and acetaldehyde (Boamfa *et al.*, 2004). Results of this study demonstrated that m/z 41, 43 and 57 are better detected with 96 Td, whereas detectability of m/z 51, 61, 75, 79 and 93 was increased with 130 Td. Studies for E/N characterization by the measurement of sesquiterpenes like ß-caryophyllene and monoterpenes like limonene found the lowest fragmentation rate by 80 Td (Demarcke *et al.*, 2009; Tani *et al.*, 2003).

With the help of the literature an identification of the m/z 41, 43, 51, 57, 61, 75, 79 and 93 was attempted. A variety of fragments of isoprene, sesquiterpenes, monoterpenes and alcohols usually emitted from plants are measured in m/z 41 (Ammann *et al.*, 2004; Demarcke *et al.*, 2010; Demarcke *et al.*, 2009; Tani *et al.*, 2004; Warneke *et al.*, 2003). In these cases the fragmentation rate of these compounds is higher with higher E/N (130 Td). As mentioned above, in this study the detected fragment of m/z 41 is likely a fragment of isoprene, as all the plant species where m/z 41 was found, were high isoprene emitters. The compound measured as m/z 43 could also not be attributed to a defined VOC species. 2- and 1-Propanol isomers were suggested and have already been measured from grasses (Kirstine *et al.*, 1998) and from tropical forest canopies (Williams *et al.*, 2001). An indication for significant source of propanol could be derived from the measurements of acetone, as 2-propanol can oxidise to acetone (Grosjean, 1997). But acetone emissions were detected only in two of the seven m/z

43 emitting plant species (*Brachipodium retusum* and *Ficus carica*). Furthermore, several plants (*Olea europea, Pinus halepensis, Prunus persica* and *Quercus coccifera*) emitted acetone and no signal on m/z 43 was detected in case of those plants. Several gases found in urban environments like propene, fragments of vinyl acetate and heavier acetates are detected also on m/z 43 (Karl *et al.*, 2003c; Kuster *et al.*, 2004; Warneke *et al.*, 2003). On the other hand, linalool fragments can be measured at m/z 41 and 43 but no linalool emissions were detected with GC-FID measurements by the plant species that emitted both m/z. Since all plant species that emitted m/z 43 emitted also m/z 61, as mentioned above, m/z 43 and m/z 61 are likely a fragment of acetic acid. Acetic acid has already been identified by PTR-MS over tropical forest canopies (Karl *et al.*, 2004; Williams *et al.*, 2001), and as direct emission from vegetation (*Quercus ilex)* as reported by Holzinger *et al.* (2000). But not all plant species that emitted m/z 61 emitted m/z 43, as observed in *Olea europea, Prunus persica, Quercus afares* and *Rosmarinus officinalis*. Other possible identifications were suggested for m/z 61, but none of vegetation origin, except for propanol suggested by Buhr et al (2002), that has been measured already from grasses (Kirstine *et al.*, 1998). Karl *et al.* (2004b) supposed a contribution of gycoladehyde, a photochemical product ion, Buhr *et al.* (2002) measured Ethyl-, Propyl-, Butyl- and Hexyl-acetate in m/z 61 from flavour substances used for the food industry and Williams *et al.* (2001) and De Gouw *et al.* (2003) supposed a contribution of methyl formate and peroxyacetic acid to m/z 61, respectively.

Brachipodium retusum and *Ceratonia siliqua* emitted significant quantities (0.6-0.9 ppb) of m/z 57. In measurements of urban air m/z 57 was identified as butanol, MTBE or butanes (Karl *et al.*, 2003c). But also trans-2-butene, Methylcyclohexane, octane and decane could contribute to the m/z 57 (Warneke *et al.*, 2003). Furthermore, a total of eight plant species (*Brachipodium retusum, Ceratonia siliqua, Cistus monspeliensis, Coronilla valentina, Ficus carica, Olea europea,Quercus suber, Spartium junceum)* showed emissions of m/z 51, better measured with an E/N of 130 Td. No reports were found describing this mass. Its identification remains open. The identification of m/z 75 is also not clear for the plant species *Cistus albidus* and *Cistus monspeliensis*. Hydroxyacetone was proposed by Williams *et al.* (2000, 2001), describing this compound as an oxidation product of isoprene. This could be the case for *Brachipodium retusum* that was a high isoprene emitter, but no isoprene emission was detected for *Cistus albidus* and *Cistus monspeliensis.* No information could be found regarding potential gaseous emissions in the literature for m/z 75. On the other hand, the mass 73 had two identification possibilities, MEK or methylglyoxal. As mentioned above Reports

of direct MEK emission from vegetation has been measured with GC-FID and PTR-MS (De Gouw *et al.*, 1999; Isidorov *et al.*, 1985; Kirstine *et al.*, 1998). Methyl glyoxal was also identified by m/z 73 (Holzinger *et al.*, 2007). Methylglyoxal is a less possible candidate, since this compound is an oxidation product from ozonolysis of isoprene (Lee *et al.*, 2006) and ozone was removed during the measurements, in order to avoid such reactions.

Finally several plant species emitted gases at m/z 79 and 93. These two masses have been usually described by atmospheric chemists as benzene and toluene, respectively. Benzene and toluene are usually from anthropogenic origin (Ammann *et al.*, 2004; De Gouw *et al.*, 2003; Kato *et al.*, 2004; Kuster *et al.*, 2004), or the result of vegetation burning (De Gouw *et al.*, 2006). Other possible gases like fragments of ethylbenzene, iso- and n-propylbenzene have contributed to m/z 79 in measurements of urban air (De Gouw *et al.*, 2003). No vegetation sources for m/z 79 was ever reported but for m/z 93, fragments of different monoterpenes (p-cymene, α-pinene and ß-pinene) were identified at m/z 93 (Tani *et al.*, 2003; Warneke *et al.*, 2003). From the species of our study that emitted gas at m/z 93 only *Quercus suber* and *Rosmarinus officinalis* emitted α- and ß-pinene (confirmed by GC).

The identification of the masses with more than one possible compound could only be partly resolved and more work is required to clarify the identity of all the observed mass signals.

CHAPTER 4: PLANT SPECIFIC VOLATILE ORGANIC COMPOUND EMISSION FACTORS FROM YOUNG AND MATURE LEAVES OF MEDITERRENEAN VEGETATION

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Plant specific volatile organic compound emission factors from young and mature leaves of Mediterranean vegetation

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Abstract

The seasonality of vegetation, i.e. the developmental stage and phenological processes, affects the emission of volatile organic compounds (VOCs). Despite the potential significance, the seasonal contributions to VOC emission quality and quantity are not well understood, and are therefore often ignored in emission simulations. We investigated the VOC emission patterns of young and mature leaves of several Mediterranean plants species in relation to their physiological and developmental changes during the growing period, and estimated standard emission factors. Foliar isoprenoid emissions as well as emissions of oxygenated VOC like methanol and acetone were measured during the spring and summer of 2008 at the CEFE-CNRS institute in Montpellier, France. A proton transfer reaction mass spectrometer (PTR-MS) was used for online measurements of VOCs. While PTR-MS is an excellent technique for fast chemical measurements, it lacks specificity, consequently compounds with the same mass cannot be distinguished. Therefore, additional offline VOC analyses of cartridge samples were made with gas chromatography (GC) coupled with a mass spectrometer and flame ionization detector. The results demonstrate that VOC emission is a developmentally regulated process and that quantitative and qualitative variability is plant species specific. Leaf ontogeny clearly influenced not only the standard emission rate, but also the VOC composition. Methanol was the major compound that contributes to the total VOC emissions in young leaves and its contribution decreased with leaf maturity. Several plant species showed also a decrease or complete subsidence for monoterpene, sesquiterpene and acetone emission capacity upon maturity, indicating a response to the higher defence demands of young emerging leaves (*demand-side control*).

1. Introduction

Terrestrial vegetation is the most important source of atmospheric volatile organic compounds (VOCs) (Guenther *et al.*, 2006) and has significant influence on the chemistry and physics of the atmosphere (Atkinson and Arey, 2003). Since the atmospheric transformation and degradation of VOCs influences the oxidative capacity of the atmosphere, the study of VOC

emissions from plants is of great interest. Furthermore, VOCs contribute to the formation and growth of secondary organic aerosols which affects cloud development and precipitation (Andreae and Crutzen, 1997). VOCs also represent a significant loss of the photosynthetically fixed carbon. This loss of carbon may be relevant for the global carbon budget of terrestrial ecosystems (Guenther, 2002; Kesselmeier *et al.*, 2002a; Llusia and Penuelas, 2000; Penuelas and Llusia, 2004). In recent years, great effort has been made to improve regional and global models which estimate source strengths for these important tropospheric constituents (Grote and Niinemets, 2008; Guenther *et al.*, 2006; Niinemets *et al.*, 2002; Zimmer *et al.*, 2000). Specifically, the effects of exogenous environmental factors such as the CO_2–level and drought have been extensively studied (Penuelas and Staudt, 2010) and incorporated into emission models (Arneth *et al.*, 2007; Grote *et al.*, 2009; Heald *et al.*, 2009). The role of endogenous factors such as plant developmental and phenological processes and their contributions to seasonal effects are less understood and therefore often ignored in emission simulations. This is particularly true for emissions of VOCs other than isoprene, the most abundant and best studied VOC species.

Young leaves undergo physiological and developmental changes during the growing period and the VOC standard emission factors (E_s, basic emission strength defined as the VOC emission rate at standard light and temperature conditions of 1000 µmol m^{-2} s^{-1} Photosynthetic Active Radiation (PAR) and 30°C) may vary accordingly. Only a few studies, limited to a few plant species, have been performed to characterize the influence of plant developmental stages on the E_s of terpenes such as isoprene, monoterpenes and sesquiterpenes (Batten *et al.*, 1995; Centritto *et al.*, 2004; Geron *et al.*, 2000; Grinspoon *et al.*, 1991; Guenther *et al.*, 1991; Hakola *et al.*, 1998; Harley *et al.*, 1994; Kuhn *et al.*, 2004b; Kuzma and Fall, 1993; Lehning *et al.*, 2001; Lerdau *et al.*, 1995; Loreto *et al.*, 2007; Mayrhofer *et al.*, 2005; McConkey *et al.*, 2000; Monson *et al.*, 1994; Wiberley *et al.*, 2005; Zhang *et al.*, 2008). Information on sesquiterpene emission from plants is rare (Duhl *et al.*, 2008). Furthermore, studies on the relationship of plant development and oxygenated VOC emissions are very sparse being limited almost only to methanol (Fukui and Doskey, 1998; Harley *et al.*, 2007; Hüve *et al.*, 2007; Macdonald and Fall, 1993a; Nemecek-Marshall *et al.*, 1995), and we are aware of only one report about developmental effects on acetone emission (Macdonald and Fall, 1993b).

Leaf ontogeny has a direct impact on VOC emission. For example, isoprene emissions have been demonstrated to be controlled on a transcriptional level by isoprene synthase (IspS) in

developing leaves, and by a combination of IspS and DMADP (Dimethyl allyl diphosphate) supply from the plastidic MEP (methyl erythritol phosphate) pathway in mature leaves (Sharkey *et al.*, 2008; Wiberley *et al.*, 2005). Isoprene emission can be reduced in young leaves due to the more dominant respiratory processes that compete with isoprene biosynthesis for pyruvate, the only cytosolic substrate in the MEP pathway (Fares *et al.*, 2008; Loreto *et al.*, 2007). As shown recently, different developmental stages may also cause emission quality changes, e.g. changes in the monoterpene composition, and other VOC diversity, possibly representing a protection mechanism against pathogenic fungi for the young tissue (Zhang *et al.*, 2008). Not only isoprenoid emission quality can change during leaf growth, but also the production rate of methanol can vary within an order of magnitude before leaves reach maturity (Harley *et al.*, 2007). Methanol production in plants is thought to be mainly related to pectin demethylation during cell wall expansion and hence to growth processes, or to maintenance of cell wall plasticity (Fall and Benson, 1996; Galbally and Kirstine, 2002). Acetone is another oxygenated compound whose production and emission has been poorly investigated. Seasonal emission changes for acetone have been observed in temperate forests (Karl *et al.*, 2003b) with higher emissions during autumn, most likely attributed to senescing and decaying biomass. On the other hand, emission of acetone from young tissue, like buds of conifers, has been reported (Macdonald and Fall, 1993b).

The aim of this study was to investigate potential differences in VOC emission patterns quantitatively and qualitatively for young and mature leaves of nine typical Mediterranean plant species at standard conditions (1000 µmol m^{-2} s^{-1} PAR and 30 °C). The Mediterranean area was chosen due to its unique diversity in VOC emitting plant species. This area is one of only five small regions worldwide sharing a distinct climate (Seufert *et al.*1997). These regions cover only 2% of the earth's land surface, but hold 20% of its plant-life, and are especially rich in odorant plants emitting a lot of BVOCs (Seufert *et al.*, 1997). Isoprenoid emissions as well as emissions of oxygenated VOCs such as methanol and acetone were measured from leaves of common Mediterranean tree and shrub species during the spring and summer of 2008 at the CEFE-CNRS institute in Montpellier, France. A proton transfer reaction mass spectrometer (PTR-MS) was used for online measurement of VOCs. While PTR-MS is an excellent technique for fast chemical measurements, it lacks specificity and compounds with the same mass can not be distinguished from one another. For this reason, parallel offline VOC analyses were made using gas chromatography (GC) coupled with a

mass spectrometer and a flame ionization detector. The combination of the PTR-MS and GC analyses enabled accurate and online identification of the VOCs emitted.

2. Materials and Methods

2.1 Plant material

A total of nine potted plant species common to the Mediterranean area were studied during the months of April to July 2008, included were deciduous and non-deciduous trees and shrubs from different distribution ranges (see table 1). Triplicates of each measured species were placed in an enclosure system at standard conditions and given at least one hour to adapt to the enclosure conditions before measurements. Measurements from branches with young leaves were made some days after bud break. Afterwards the investigated leaves were cut off for the determination of leaf area and leaf dry weight. Measurements from mature leaves of the same plant were made at least two weeks after measurements of the young leaves to avoid possible stress effects due to leaf removal. The conifer *Pinus halepensis* and the aromatic shrub *Rosmarinus officinalis* possess glandular organs producing and storing volatile compounds. It has been shown that wounding and mechanic stress can cause large bursts of VOCs from these plants which in turn can lead to large overestimations in emission rates. In order to avoid such an artifact, an adaptation period in the enclosure system of at least 12 hours was chosen for these species.

2.2 Gas exchange measurements

A dynamic enclosure system was used to determine exchange rates of VOCs at the leaf level, for a description see (Staudt *et al.*, 2004). The enclosure was constantly flushed with air at a flow of 650 ml min^{-1} resulting in an air exchange of the enclosure system on average every 14 s. The air was passed through a clean air generator (AIRMOPURE, Chromatotec, France) that purified and dried the ambient air. The air was then re-humidified by passing a variable portion of the air stream through a water bubbler. Homogenous mixing of the air in the enclosure was accomplished by a Teflon fan. One or several terminal leaves of an individual plant were placed horizontal to the light to ensure homogenous light repartition on the adaxial surface of the leaves. For most of the plants, some leaves needed to be removed to enable placement in the enclosure, this was done at least one week before any measurements. Chamber and plants were illuminated with white light (OSRAM 1000 W) filtered by a 5-cm water bath. At the end of each measurement the studied leaves were harvested and leaf area and dry weight were determined.

All tubing was made of Teflon and maintained at a constant temperature of 45°C, which was always higher than the enclosure temperature. Measurements were performed under controlled conditions. Temperature, PAR and relative humidity were kept constant at 30 ± 1°C, 1055 ± 36 µmol m^{-2} s^{-1} and 48 ± 13 %, respectively. These micrometeorological parameters were monitored during the experiment and data were collected by a 21X datalogger (Campbell Scientific Ltd., Shepsherd, UK). Physiological parameters (photosynthesis and transpiration) were measured by analyzing a constant portion of the inlet and outlet air, using a CO_2/H_2O infrared gas analyzer (LI-COR inc. 7000, Lincoln, Nebraska, USA).

2.3 VOC measurements

VOCs were analyzed using different analytical methods. For online detection, the PTR-MS was connected to the outlet and the inlet of the enclosure system using two meter long heated PFA tubing (1/8 inch). Gas sampling was switched at 16 min cycles by means of a magnetic valve and alternated between the inlet and outlet. PTR-MS employs chemical ionization based on proton transfer reactions from H_3O^+ reagent ions to VOCs and has been reviewed elsewhere (Blake *et al.*, 2009; Lindinger *et al.*, 1998a, c). The instrument was operated in selected ion-monitoring mode at standard operational settings (E/N = 130 Td; E electric field strength, N buffer gas number density, 1 Td = 10^{-17} cm² V molecule^{-1}) by a drift tube voltage of 600 V, measuring eleven cycles (dwell time of 1s per mass). Each of the eleven measurement cycles were interspersed with seven cycles of instrumental background measurements by passing the sample air over a heated platinum catalyst maintained at 350 °C. This measurement sequence was repeated at least thrice for each sample. The background signals were subtracted from the outlet and inlet air measurements. The standard emission rates E_s of each compound was then calculated in µg g^{-1} h^{-1} according to Equation 1 from the measured concentration difference ($\Delta c = c_{outlet} - c_{inlet}$), the enclosure flush rate Q, and the leaf dry weight (dw).

$$E_s = \Delta c(Q/dw). \qquad (1)$$

Since measurements were performed at standard conditions, emission rates in this study were considered as standard emission rates.

The main compounds (protonated masses (Molecular Weight + 1)) detected by the PTR-MS in the emissions and given as atomic mass per unit (amu) were methanol (33), acetone (59) isoprene (69), monoterpenes (137, fragment on 81) and sesquiterpenes (205). Note that

different isomers of monoterpenes or sesquiterpenes cannot be distinguished with a PTR-MS. Therefore, PTR-MS based standard emissions factors for monoterpenes and sesquiterpenes always refer to the sum of all monoterpenes and sesquiterpenes, respectively. The PTR-MS instrument was calibrated using a gas standard (Deuste Steininger GmbH, Germany) mixed with nitrogen at concentrations of ~300 ppb (± 10% accuracy). The standard gas contained most of this study's target VOCs, except for sesquiterpenes. The standard gas was diluted with synthetic air to final concentrations of 0.5 – 10 ppb. The quantification of sesquiterpenes was calculated using simple ion-molecule reaction kinetics described elsewhere (Hansel *et al.*, 1995; Wisthaler *et al.*, 2001). The detection limit of this method was estimated as being the greater of the variability levels in the difference between the outlet and the inlet concentrations in the empty enclosure (at the 95% confidence level) and was typically 1.6 ppb for methanol, 579 ppt for acetone, 493 ppt for isoprene, 201 ppt for monoterpenes and 94 ppt for sesquiterpenes.

In addition to the online analysis, cartridges were collected for gas chromatographic analysis (GC) with a flame ionization detector (FID) as well as mass spectrometric (MS) detection with a sample flow of 0.1 l min^{-1} for 10 minutes. The cartridges consisted of glass tubes containing a mixture of 200 mg of Tenax (20-35 mesh, Chrompack). The samples were analysed by a Chrompack CP9003 gas chromatograph equipped with a FID and a Chrompack TCT4002 thermo-desorber (all Varian Inc.) located at the CEFE-CNRS in Montpellier, France. Furthermore, parallel VOC samples were taken for analyses with gas chromatography coupled with Mass Spectrometry (Varian CP3800/Saturn2000 MS plus a Perkin-Elmer Turbomatrix thermo-desorber) using the same analytical column and temperature program as in the GC-FID instrument. VOCs were separated on a Chrompack Sil 8 CB low bleed capillary column (30m x 0.25 mm) using the following temperature program: 3 min at 40°C, 3°C min^{-1} to 100°C, 2.7°C min^{-1} to 140°C, 2.4°C min^{-1} to 180°C, 6°C min^{-1} to 250°C. Peaks were identified by comparing their mass spectra and retention times with those from authentic standards analysed under the same conditions. The offline GC system was calibrated using the same gas standard mixture used to calibrate the PTR-MS and with the same concentration dilutions (0.5 - 10 ppb).

In order to identify statistical differences between young and mature leaves, analyses of a two factor ANOVA with replication were performed, followed by Turkey's post hoc test.

3. Results

3.1. Combination of different techniques

The combination of the PTR-MS and GC techniques enabled us to combine the identification of a certain number of VOC species with a low time resolution by the GC to the fast online screening for the masses of different volatile organics and tentative identification by the PTR-MS. With this combination of techniques, we were able to investigate the emission variability of a larger group of plant species with highly diversified VOC spectra. Standard emission factors were generated based on mean VOC emission rates as observed with three plant replicates per species measured between 9:00 and 18:00 h. PTR-MS based quantifications of identifiable VOC (masses) were in reasonable agreement with GC-FID data showing mean differences of 10 and 14% for monoterpenes and isoprene, respectively. Overall, the correlation between PTR-MS and GC emission data were better for monoterpenes than for isoprene (r^2 of 0.90 versus 0.78, data not shown). In cases where the emission rate was close to the detection limit, PTR-MS data could not always be confirmed by GC data as observed for the monoterpene measurements on *Prunus persica*, on young leaves of *Buxus sempervirens* and on mature leaves of *Coronilla valentina* and *Cistus albidus*.

3.2. Total VOC release and relative abundance of VOC classes

The total VOC released by the investigated Mediterranean plants varied from 2.5 to 32 and 3.8 to 56.1 µg g^{-1} h^{-1} by young and mature leaves, respectively (Table 2). Results of the two factor ANOVA showed, in general, significant differences in the VOC emissions from young and mature leaves ($p<0.01$). Percent contribution of each VOC species to the total amount of VOC between young and mature leaves was also compared by ANOVA (see table 3). In this case, the overall ANOVA showed significant differences with a probability less than 0.05-0.001 for each VOC.

The overall VOC composition for the studied plant species is shown in Figure 1. In young as well as in mature leaves of *Cistus albidus*, *Coronilla valentina* and *Prunus persica*, methanol was the main VOC emitted on a leaf dry weight basis with a contribution of 51 % and 88 % (*Cistus albidus)*, 87 % and 85 % (*Coronilla valentina*), 91 % and 81 % (*Prunus persica*) to the total amount of VOC for young and mature leaves, respectively. Methanol was also the dominant emission from young leaves of *Ceratonia siliqua*, *Pinus halepensis* and *Rosmarinus officinalis*. The methanol contribution is usually higher in young leaves with significant

differences between young and mature leaves in *Ceratonia siliqua, Cistus albidus, Pinus halepensis, Prunus persica* and *Spartium junceum* (see table 3).
The observed absolute decrease in monoterpene E_s upon maturity in *Buxus sempervirens, Cistus albidus, Coronilla valentina, Olea europea* and *Spartium junceum* is also reflected in a decreased relative contribution to the total amount of VOC in the case of *Coronilla valentina* and *Spartium junceum*, but not in the case of *Buxus sempervirens, Olea europea* and *Cistus albidus*, where the contribution of monoterpenes did not change. On the other hand a significant increase in the relative monoterpene contribution to the total VOC emission was detected in *Prunus persica*, mainly because of the decreased methanol contribution upon maturity in this plant species (see table 3). The relative contribution of monoterpenes to the total VOC emission of *Ceratonia siliqua* and *Pinus halepensis* increased in close accordance with the increase of the monoterpene E_s. In the case of *Rosmarinus officinalis* the E_s as well as the % contribution of monoterpenes to the total amount of VOC did not vary.
The VOC emission was dominated by isoprene in the species *Buxus sempervirens* and *Spartium junceum*, accompanied by a significant increase ($P<0.05$) in the isoprene contribution upon maturity.

The contribution of acetone and sesquiterpenes to the total amount of VOC emissions was less than 10 %, except for young leaves of *Cistus albidus* that emitted sesquiterpenes which accounted for 27 % of the total volatile organic compounds emitted, decreasing its contribution significantly ($p<0.05$) upon maturity. Only *Prunus persica* showed significant differences in the acetone contribution to the total VOC emissions.

3.3. Emission of methanol

In a comparison of young and mature leaves methanol emissions are of special interest as the synthesis of this compound is regarded to be related with growth processes (Harley *et al.*, 2007).

Methanol emissions from young leaves were found to be significantly higher than emissions from mature leaves in almost all plant species studied (Table 2). Only *Cistus albidus* did not show any significant differences between young and mature leaves. It should be noted that all the young leaves, or shoots, that were studied already exhibited photosynthetic net carbon assimilation except for *Ceratonia siliqua* activities, though they were still in the growing phase as indicated by the increased methanol emissions. In some plant species, photosynthesis

in the young leaves was of equal intensity as that of mature tissue, or even higher, as in the case of *Cistus albidus*.

3.4. Emissions of acetone

Low emissions (<1 µg g^{-1} h^{-1}) of acetone were detected in mature leaves of *Coronilla valentina, Olea europea, Pinus halepensis* and *Prunus persica.* Young leaves of the last two mentioned species and *Cistus albidus* were also acetone emitters. Acetone emissions were significantly lower at maturity for *Prunus persica* and *Pinus halepensis* and disappeared completely upon maturity in *Cistus albidus*. The dependence of acetone emissions on leaf development was highly variable among species (table 2).

3.5. Emission of isoprene

Of the nine investigated plant species only *Spartium junceum and Buxus sempervirens* were identified as strong isoprene emitters, the latter exhibiting a significantly ($P< 0.01$) higher isoprene E_s for mature leaves. *Olea europea, Pinus halepensis,* and *Prunus persica* turned out to be low isoprene emitters with weak insignificant differences between young and mature leaves, whereas *Cistus albidus* and *Rosmarinus officinalis* did not show any isoprene emission above the detection limit. Finally, *Ceratonia siliqua* and *Coronilla valentina* emitted low amounts of isoprene exclusively from mature leaves.

3.6. Emission of monoterpenes

All studied plant species emitted monoterpenes, but in different amounts. *Ceratonia siliqua, Olea europea, Pinus halepensis* and *Rosmarinus officinalis* were classified as strong monoterpene emitters (> 1 µg g^{-1} h^{-1}) with E_s in mature leaves ranging from 1.21 to 7.94 µg g^{-1} h^{-1} while *Buxus sempervirens, Cistus albidus* and *Coronilla valentina* were found to emit only low rates of monoterpenes from mature leaves (< 1 µg g^{-1} h^{-1}). In the case of *Buxus sempervirens, Cistus albidus, Coronilla valentina, Olea europea,* and *Spartium junceum,* we observed significantly higher emission rates in young leaves than in mature leaves, whereas it was the opposite for *Ceratonia siliqua* and *Pinus halepensis.*

An increase of 96 and 29 % was detected in the monoterpene E_s of *Ceratonia siliqua* and *Pinus halepensis*, respectively, upon maturity, coinciding with an increase of assimilation in the case of *Ceratonia siliqua*. On the other hand, monoterpene E_s from *Buxus sempervirens, Cistus albidus, Coronilla valentina* and *Olea europea* in mature leaves were significantly ($P< 0.01$) lower compared to young leaves, showing a decrease in the E_s of 57-79 % upon

maturity. *Spartium junceum* no longer emitted monoterpenes upon maturity. The reduction in monoterpene emission potential was accompanied by a decrease in assimilation for *Coronilla valentina* and *Spartium junceum*, but an increase for *Buxus sempervirens* and *Olea europea*. Monoterpene E_s and assimilation rates for *Prunus persica* leaves, which showed only trace emissions, did not significantly change during leaf development. Similarly, monoterpene emissions from *Rosmarinus officinalis* did not show noticeable differences between young and mature leaves.

Monoterpene emission not only changed in quantity but also in quality. *Ceratonia siliqua*, for example, showed substantial monoterpene emissions at maturity (Table 2), whereas young leaves exhibited very low emissions of monoterpenes with α-pinene being the only compound identified (see table 4). Mature leaves, showed high standard emission factors for *E*-ocimene and *Z*-ocimene and traces of myrcene, whereas α-pinene was missing.
Similarly, *Pinus halepensis* showed additionally released monoterpene species for young leaves (table 4). The highest single emission was for *E*-ocimene in both young and mature leaves of *Pinus halepensis*, contributing 63 ± 7.6% and 92 ± 4.6 % of the total amount of monoterpenes emitted by young and mature leaves, respectively. The difference in the % contribution of *E*-ocimene to the total amount of monoterpene in young and mature leaves was significant ($p<0.05$). Young leaves emitted linalool as the second highest emitted compound (22 ± 11.9% of the total monoterpene emission from young leaves), followed by low emissions of α-pinene, myrcene and 3-carene (<0.1 $\mu g\ g^{-1}\ h^{-1}$), while traces of terpinene-4-ol (<0.01 $\mu g\ g^{-1}\ h^{-1}$) were released only from mature leaves. The high linalool emission was not followed upon maturity (see table 4). Myrcene and *Z*-ocimene were also emitted by young and mature leaves of *Pinus halepensis,* but in similar proportion and without significant difference (see table 4). The monoterpene speciation did not differ very much in young and mature leaves of *Olea europea*, emissions mainly of *E*-ocimene (90%) followed by Z-ocimene (10%) were observed. As mentioned above, monoterpene emissions of PTR-MS data in *Prunus persica*, young leaves of *Buxus sempervirens* and mature leaves of *Coronilla valentina* and *Cistus albidus* were not confirmed with GC data, maybe due to interferences of other VOC in the masses classified as monoterpene (m/z 81 and 137) on the PTR-MS. For example, interferences from sesquiterpene fragments like ß-caryophyllene and α-humulene in m/z 81 and 137 have been already reported contributing for 7.5 and 6 % in each m/z respectively in the case of ß-caryophyllene (Demarcke *et al.*, 2009). Also low interferences in

m/z 59 due to fragments of linalool in monoterpene emitting species could not be excluded, contributing for less than 6 % to the emission (Demarcke *et al.*, 2010).

3.7. Emission of Sesquiterpenes

Standardized emission rates for sesquiterpenes were detected which ranged from 0.03 to 3.33 µg g^{-1} h^{-1}. Young leaves of *Cistus albidus, Coronilla valentina* and *Olea europea* emitted higher levels of sesquiterpenes compared to mature leaves. Highest values were found for the aromatic shrub *Cistus albidus* (see table 2). Only trace levels of sesquiterpenes (<0.1 µg g-1 h-1) were detected in young leaves of *Pinus halepensis* and mature leaves of *Buxus sempervirens* and *Ceratonia siliquia* with no significant difference between young and mature leaves. Emission rates of sesquiterpenes were in most cases low (<1 µg g^{-1} h^{-1}), except for young leaves of *Cistus albidus*.

Cistus albidus also showed a significant difference in the sesquiterpene composition emitted by young and mature leaves (see table 4). Around half of the sesquiterpene emission from young leaves and 83 % from mature leaves of *Cistus albidus* was composed of (-) trans-caryophyllene, δ-cadinene, ß-bourboubene, *E*-ß-farnesene and ß-cubebene. The difference between young and mature leaves is mainly due to the significantly increased contribution of ß-cubebene in mature leaves (from 8.8 ± 0.1 to 20.2 ± 6 %) ($p<0.05$) (See table 4). Also the appearance and disappearance of sesquiterpene species with maturity influenced the % distribution of sesquiterpenes. For example, sesquiterpene species like α-zingiberene and germacrene D were emitted only by young leaves, whereas α-cubebene, α- and ß-copaene were emitted only from mature leaves (see table 4). On the other hand, the sesquiterpene emission pattern of young and mature leaves of *Olea europea* differed also qualitatively (see table 4). Both young and mature leaves emitted (-) trans-caryophyllene and germacrene-D, but only young leaves of *Olea europea* emitted a notable quantity of α-farnesene (30.6 ± 2.4% of the total sesquiterpene emission). The difference in the % contribution of (-) trans-caryophyllene to the total amount of sesquiterpenes emitted between young and mature leaves was significant ($p<0.05$) (see table 4).

4. Discussion

This study provides clear evidence that the developmental phase has an impact on VOC emissions. Development influences not only the standard emission rate, but also the VOC composition, with methanol being the major compound contributing to the total VOC emissions from young leaves of most of the plant species investigated. The contribution of methanol is maintained or decreases with maturity. Only in *Cistus albidus* was the relative contribution of methanol higher upon maturity, due to higher contribution of monoterpenes, sesquiterpenes and acetone in the young leaves. Isoprene contribution is much less affected by development, whereas monoterpene contribution to total VOC emissions may be enhanced or reduced with maturity, depending on the plant species. This distinct behaviour observed for monoterpenes within plant species and the variability in their composition during development, as observed in this study, may reflect the functional diversity of these compounds at different developmental stages within the plant kingdom. This is also consistent with the significant variation observed in the composition of sesquiterpenes despite their low contribution to total VOC emissions. Furthermore, the emission of acetone, which is very low when compared to the total VOC emissions, was also quite variable amongst the plant species considered in this study.

The production of methanol was found to be clearly linked to the growth period of the plant in most of the plant species investigated, with an average decrease of 25-90% in the methanol E_s in case of mature leaves. This supports the hypothesis that methanol production is related to cell wall growth and expansion, and that the demethylation of pectin during cell wall formation in the growing young leaves is a dominant contributer to methanol emission (Galbally and Kirstine, 2002; Harley *et al.*, 2007). The decrease in methanol E_s upon maturity was observed in almost all the tree species investigated except for *Cistus albidus*, even though significant methanol emissions were observed in both young and mature leaves of this species. Mature leaves of all investigated species continued emitting significant levels of methanol probably due to the continued primary cell wall elongation (Galbally and Kirstine, 2002). Other processes like root growth (Folkers *et al.*, 2008), protein repair, or DNA demethylation which are described as contributing to the methanol emission in a minor way could also account for this emission (Finnegan *et al.*, 1998; Mudgett and Clarke, 1993).

Development can also play a significant role in the variability of standard emission rates of monoterpenes and sesquiterpenes. A significant decrease, or complete subsidence, in the monoterpene E_s was observed in *Buxus sempervirens*, *Coronilla valentina, Olea europea, Spartium junceum* and *Cistus albidus,* and for the sesquiterpene E_s in *Cistus albidus, Olea*

europea and *Coronilla valentina*. Also, the isoprenoid species composition can change as is demonstrated in the case of *Pinus halepensis, Ceratonia siliqua* for monterpenes, or *Cistus albidus* and *Olea europea* for sesquiterpenes. In previous studies, an enhanced production of monoterpenes in the early developmental stages of the leaves has been reported, e.g. in the case of peppermint monoterpene production in the days 12 to 20 with a peak on day 15 prior to full leave expansion (Gershenzon *et al.*, 2000). A decrease of monoterpene emission after leaf expansion has been reported for tea-leafed willow (*Salix phylicifolia*), aspen (*Populus tremula*) (Hakola *et al.*, 1998) and the tropical tree species *Hymenaea courbaril* (Kuhn *et al.*, 2004b). Sesquiterpene E_s similar to that measured in young leaves during this study for *Cistus albidus*, but higher for *Pinus halepensis* and *Rosmarinus officinalis* have been reported during leaf growth periods in spring by Ormeño et al. (2007b), whereas much lower emissions have been detected for *Cistus albidus* in summer and autumn (Llusia and Penuelas, 2000), suggesting a link between leaf development and seasonal induction of sesquiterpenes, but no developmental studies have been performed on sesquiterpene emitting species previously. Since monoterpenes and sesquiterpenes are known to be protective against pathogens due to their antimicrobial or antifungal activity, or due to their toxic, repellent and deterrent properties (Croft *et al.*, 1993; De Moraes *et al.*, 2001; Shiojiri *et al.*, 2006), the decrease or loss of monoterpene and/or sesquiterpene emission potential in several studied species upon maturity might be the consequence of a response to higher defence demands of young emerging leaves (*demand-side control*). This theory of "*demand-side control*" was discussed by Kuhn *et al.* (2004b) who argued that young leaves have a stronger requirement for defence than mature leaves, as they are more attractive for herbivores and plant pathogens due to the fact that expanding leaves have higher nutritional quality than mature leaves. Experiments performed by Shiojiri and Karban (2006) support this theory, demonstrating that young plants released higher amounts of monoterpenes and sesquiterpenes above influence of herbivores in order to induce a systemic resistance among young branches.

In our study, monoterpene and/or sesquiterpene emission potential decreased or completely disappeared during maturation for several plant species. For example in the case of *Buxus sempervirens* and *Coronilla valentina*, a decrease in monoterpene emission potential, and in the case of *Coronilla valentina* a complete loss of sesquiterpene emission potential was observed. Note also that the decrease, or loss, in both plant species was accompanied by an increase in isoprene emission. Such behaviour in the isoprenoid emissions during leaf development has also been reported by Hakola *et al.* (1998) and Kuhn *et al*. (2004b). Kuhn *et al.* (2004b), postulated based on the "*supply-side control theory*" (Coley and Barone, 1996),

that the low Dimethylallyl Diphosphate (DMAPP) availability in young leaves can lead to an ontogenetic delay of isoprene production and emission (Rosenstiel *et al.*, 2002), which does, however, not affect the production of monoterpenes and sesquiterpenes. Thus, DMAPP supply seems not to be a limiting factor in young leaves for monoterpene and sesquiterpene production. The low K_m of the enzymes involved in monoterpene sesquiterpene production (Cane, 1999; Müller, 2003; Nieuwenhuizen *et al.*, 2009; Rajaonarivony *et al.*, 1992) allows a high monoterpene and sesquiterpene production rate even at low DMAPP availability. In contrast, Isoprene Snythase (IspS) has a high Km, thus high substrate (DMAPP) availability is needed to form isoprene. On the other hand, it has been shown that the isoprene production in young leaves is mainly regulated at the level of *IspS* transcription, whereas in mature leaves, the regulation of emission is shared between the *IspS* and DMAPP supply from the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway (Sharkey *et al.*, 2008; Wiberley *et al.*, 2005). However, *Olea europea* did not follow this behavioural pattern, only the monoterpene and sesquiterpene emission potential upon maturity decreased and the isoprene emission potential was maintained. Furthermore, the monoterpenes E_s rose in the tree species *Pinus halepensis* and *Ceratonia siliqua* upon maturity. This increase is mostly due to an increment in ocimene emissions. Strong seasonal increases in ocimene emissions have already been observed in *Pinus pinea* (Staudt *et al.*, 2000; Staudt *et al.*, 1997) and are probably associated with an induction on the emission due to exposure to high temperatures (Staudt and Bertin, 1998; Staudt *et al.*, 2003). *Pinus halepensis* is a terpene storing species that is known to emit monoterpenes with a variety of terpene composition (Llusia and Penuelas, 2000; Owen *et al.*, 2001; Simon *et al.*, 2006; Simon *et al.*, 2005). Llusia and Penuelas (2000) found a seasonal trend in the composition variation of the emitted monoterpenes, but not in the E_s. However, in the case of *Ceratonia siliqua*, where a monoterpene enhancement with maturity was observed, a seasonal trend could not be excluded as reported by Owen *et al.* (2001), where *Ceratonia siliqua* plants emitted monoterpenes mostly in fall and only low emissions were found in late spring. Even though there was no apparent net carbon gain in the young leaves of *Ceratonia siliqua* small quantities of monoterpenes were detected suggesting that monoterpenes can be produced in chloroplasts lacking photosynthetic autonomy. Results obtained for *Rosmarinus officinalis* and *Prunus persica*, showing no influence of development, were in close accordance with earlier reports for spring and summer emissions (Benjamin and Winer, 1998; Ormeño *et al.*, 2007; Owen *et al.*, 2002; Seufert *et al.*, 1997).

No uniform developmental trend was found for acetone emissions. Emissions of this oxygenated compound depended on the plant species, with a random distribution seen for

young and mature leaves. The metabolic pathways for acetone formation in plants are not yet clearly understood, making it difficult to draw conclusions for the observed behaviour. One of the possible metabolic pathways described for acetone is the cyanogenic pathway, activated to deter herbivores. This pathway induces the production of hydrogen cyanide (HCN) and, as a by-product, acetone (Fall, 2003). The magnitude of cyanogen metabolism varies greatly between different plant species. Several thousand plant species synthesize cyanogenic glycosides (Vetter, 2000). Acetone emission could be inferred from the cyanogenic pathway in *Olea europea*, *Coronilla valentina* and *Prunus persica*. Accumulation of the cyanogenic glycoside prunasin is widespread in the families Rosaceae and Oleaceae. Significant levels of this compound have been reported for several *Prunus* species like *P. lyonii* and *P. serotina* (Xu *et al.*, 1986; Yemm and Poulton, 1986). On the other hand, the cyanogenic glycoside linamarin has been found, among others, in the Fabaceae family, to which *Coronilla valentina* belongs (Vetter, 2000). The decrease or loss in the acetone emission capacity by *Prunus persica* and *Cistus albidus*, respectively, could be explained with the above mentioned "*demand-side control theory*", which explains the enhancement of acetone emission by the higher requirements of young leaves for defence. But other possible acetone production pathways cannot be excluded, such as the production of acetone from acetotacetate that has been supposed for conifer buds by Macdonald and Fall (1993b) and is also reported for soil bacteria (Fall, 2003).

Our results emphasise that the emission of volatile organic compounds is a developmentally regulated process and quantitative and qualitative variability is plant species specific. They highlight the necessity of compiling plant specific emission inventories for the development of VOC predictive regional and global models, which should represent both environmental changes as well as seasonality and developmental aspects.

Acknowledgments

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Table 1: Name, functional type, family, and regions of prevalence of the 9 investigated Mediterranean plant species

Species, trivial name	Functional type	Family	Range of distribution
Buxus sempervirens, European box	2	Buxaceae	Western and southern Europe, northwest Africa, southwest Asia
Ceratonia siliqua, Carob tree	3	Fabaceae	Mediterranean region
Cistus albidus, Rock rose	2	Cistaceae	South west Europe to north Africa, Mediterranean region
Coronilla valentina, Honey coronilla	2	Fabaceae	Mediterranean region
Olea europea, Olive tree	3	Oleaceae	Coastal areas of the eastern Mediterranean region, Lebanon, Syria and the maritime parts of Asia Minor and northern Iran at the south end of the Caspian Sea
Pinus halepensis, Aleppo pine	3	Pinaceae	Mediterranean region
Prunus persica, Peach tree	4	Rosaceae	China, Iran, Mediterranean region
Rosmarinus officinalis, Rosemary	1	Lamiaceae	Mediterranean region
Spartium junceum, Spanish broom	2	Fabaceae	Mediterranean region

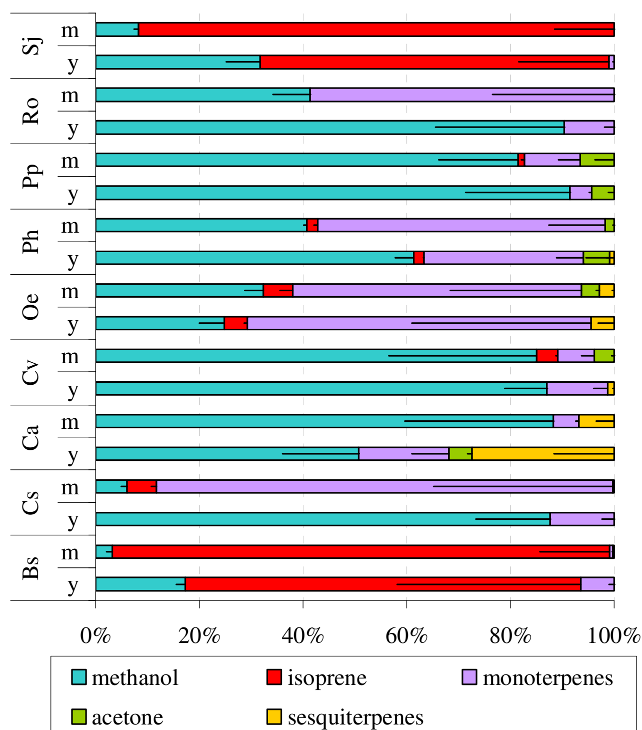
Functional type: 1 evergreen herb, 2 evergreen shrub, 3 evergreen tree, 4 deciduous tree

Table 2 Emission quantities of VOC (methanol (MetOH), isoprene (Isp), monoterpene (Mtp), acetone (Ac) and sesquiterpenes (Sqt)) E_s in µg g^{-1} h^{-1} and CO_2 Assimilation rates (A) in µmol m^{-2} s^{-1} from young (y) and mature (m) leaves of 9 Mediterranean species. Values are means ± standard deviation (SD) of n replicates measured with PTR-MS with the exception of some sesquiterpene measurements, which were below the detection limit of the PTR-MS. In such cases the GC-FID data are shown.

Plant species	y/m	n	A	MetOH	Ac	Isp	Mtp	Sqt
Buxus	Y	3	4.17 ± 0.90 ***	1.68 ± 0.3 ***	<d.l.	5.06 ± 4.07 ***	0.42 ± 0.10 ***	< d.l.
sempervirens	M	3	7.97 ± 0.82	1.04 ± 0.63	< d.l.	21.46 ± 5.21	0.13 ± 0.06	0.07 ± 0.05
Ceratonia	Y	3	-0.17 ± 0.41 ***	3.51 ± 1.00 ***	<d.l.	<d.l. ***	0.33 ± 0.11 ***	< d.l.
siliqua	M	3	3.34 ± 0.66	0.79 ± 0.23	< d.l.	0.52 ± 0.17	7.94 ± 5.41	0.03 ± 0.02
Cistus albidus	Y	3	18.04 ± 1.62 **	6.17 ± 3.11	0.53 ± 0.09 ***	<d.l.	1.44 ± 1.04 ***	3.33 ± 1.41 *
	M	3	12.46 ± 5.99	8.20 ± 4.61	< d.l.	< d.l.	0.30 ± 0.07	0.63 ± 0.32
Coronilla	Y	3	19.06 ± 1.46 **	29.35 ± 4.76 ***	<d.l. ***	<d.l. ***	2.70 ± 1.05 ***	0.42 ± 0.07 *
valentina	M	3	14.99 ± 4.26	13.48 ± 7.84	0.61 ± 0.07	0.43 ± 0.08	0.75 ± 0.45	< d.l.
Olea europea	Y	3	9.20 ± 2.89 ***	1.56 ± 0.53 **	<d.l. ***	0.19 ± 0.05	2.84 ± 2.44 ***	0.28 ± 0.19 *
	M	3	13.76 ± 1.76	1.03 ± 0.20	0.11 ± 0.02	0.12 ± 0.09	1.21 ± 0.95	0.09 ± 0.01
Pinus	Y	3	8.64 ± 3.04	5.70 ± 0.58 ***	0.47 ± 0.42 **	0.12 ± 0.01	1.95 ± 0.57 *	0.08 ± 0.13
halepensis	M	3	5.01 ± 1.21	2.95 ± 0.09	0.09± 0.01	0.10 ± 0.07	2.75 ± 0.94	< d.l.
Prunus persica	Y	3	9.93 ± 0.39	14.23 ± 5.4 **	0.66 ± 0.16 **	<d.l.	0.45 ± 0.10	< d.l.
	M	3	10.92 ± 2.27	4.01 ± 1.30	0.32 ± 0.18	0.04 ± 0.03	0.36 ± 0.25	< d.l.
Rosmarinus	Y	2	24.90 ± 8.53	21.3 ± 10.1 ***	< d.l.	<d.l.	1.55 ± 0.51	<d.l.
officinalis	M	3	14.77 ± 5.88	2.03 ± 0.61	< d.l.	< d.l.	1.97 ± 1.36	<d.l.
Spartium	Y	3	10.10 ± 1.50 ***	22.77 ± 8.1 ***	<d.l.	33.0 ± 14.79	0.44 ± 0.15 ***	<d.l.
junceum	M	3	7.53 ± 1.45	3.74 ± 0.72	< d.l.	28.28 ± 6.13	< d.l.	< d.l.

<d.l. = under detection limit

Differences in VOC E_s and A between young and mature leaves were tested with ANOVA (Tukey's post hoc test). P-value < 0.1 F ratio is significant (*), when P-value < 0.05 F ratio is quite significant (**) and when P-value < 0.01 F ratio is very significant (***)



plant sp.	MetOH	Isp	Mtp	Sqt	Ac
Bs	n.s.	*	n.s.	n.s.	-
Cs	**	n.s.	**	n.s.	-
Ca	*	-	n.s.	***	n.s.
Cv	n.s.	n.s.	**	n.s.	n.s.
Oe	n.s.	n.s.	n.s.	n.s.	n.s.
Ph	*	n.s.	**	n.s.	n.s.
Pp	**	***	**	-	**
Ro	n.s.	-	n.s.	-	-
Sj	*	*	*	-	-

Figure 1

Comparison of total VOC composition in % emitted from young (y) and mature (m) leaves of 9 Mediterranean plant species (sp.): (Bs) *Buxus sempervirens*, (Cs) *Ceratonia siliqua*, (Ca) *Cistus albidus*, (Cg) *Coronilla valentina,* (Oe) *Olea europea*, (Ph) *Pinus halepensis*, (Pp) *Prunus persica*, (Ro) *Rosmarinus officinalis*, (Sj) *Spartium junceum.* Standard error bars are given.

Table 3

Statistical significant differences of VOC % composition between VOC emitted from young and mature leaves was tested with ANOVA (Tukey's post hoc test). Given are the p-values indicating levels of significance at 5% (0.05, *), 1% (0.01, **) and 0.1% (0.001, ***). Not significant (n.s.).

Table 4

Statistical significant differences on monoterpene or sesquiterpene % composition between VOC emitted from young and mature leaves was tested with ANOVA (Tukey's post hoc test). Given are the p-values indicating level of significance at 5% (0.05, *) and not significant (n.s.) differences.

	Monoterpene sp.	young (y) leaves [%]	mature (m) leaves [%]	difference y/m
Ceratonia siliqua	α –pinene	100	0	-
	myrcene	0	1 ± 0.8	-
	Z-ocimene	0	19 ± 1.6	-
	E-ocimene	0	80 ± 2.4	-
Pinus halepensis	*E*-ocimene	63 ± 7.6	92 ± 4.6	*
	Z-ocimene	1 ± 0.9	2 ± 3	n.s.
	linalool	22 ± 11.9	0	-
	myrcene	5 ± 3.7	5 ± 2	n.s.
	3-carene	3 ± 2	1 ± 0.3	n.s.
	α -pinene	6 ± 5	0	-
	terpinene-4-ol	0	0.6 ± 0.3	n.s.
Olea europea	*E*-ß-ocimene	90	90	n.s.
	Z-ß-ocimene	10	10	n.s.

	Sesquiterpene sp.	young (y) leaves [%]	mature (m) leaves [%]	difference y/m
Cistus albidus	α –cubebene	0	14.2 ± 7	-
	ß-cubebene	8.8 ± 0.1	20.2 ± 6.0	*
	(-)trans-caryophyllene	26.5 ± 3.3	34.8 ± 7.8	n.s.
	α-zingiberene	31.0 ± 8.7	0	n.s.
	α-copaene	0	8.8 ± 2.2	n.s.
	ß-copaene	0	3.3 ± 1.8	n.s.
	delta-cadinene	1.7 ± 0.5	8.1 ± 5.9	n.s.
	ß-bourboubene	2.3 ± 0.7	8.1 ± 1.5	n.s.
	e-ß-farnesene	12.4 ± 0.7	11.8 ± 5.9	n.s.
	germacrene D	17.0 ± 10.3	0	n.s.
Olea europea	(-)trans-caryophyllene	35.4 ± 8	80.7 ± 17.2	*
	germacrene	34 ± 15.5	19.2 ± 17.1	n.s.
	α-farnesene	30.6 ± 2.4	0	-

CHAPTER 5: SHORT-TERM AND LONG-TERM FLOODING EFFECTS ON PHYSIOLOGY AND VOC EMISSIONS UNDER ROOT ANOXIA

5.1. INTRODUCTION

In the Amazon basin, a land area as big as Germany (300.000 km²) is subjected to a continuous flooding pulse; it is flooded for 210 days a^{-1} on an average (Junk, 1989). This floodplain area covered with forest is characterized by several types of inundation areas (see figure 5.1.1). The most representative in central Amazonia are the periodically flooded forest of várzea and igapó. Várzea regions are influenced by nutrient-rich and sediment-carrying whitewater, whereas igapó regions are flooded with clear water that appears dark blue to black, containing low quantities of nutrient and sediments and a lower pH (Prance, 1979; Soili, 1954).

Figure 5.1.1 Distribution and characterization of major neotropical wetlands (Junk, 1997)

To survive the flooding period, vegetation has developed several morphological, anatomical and physiological strategies to mitigate the produced stress due to root anoxia (Parolin, 2009; Parolin *et al.*, 2004). In many species, the reduction of root activity by root anoxia leads to a water deficit in the crown and leaf shedding down-regulating their metabolism (Waldhoff and Furch, 2002), whereas other better adapted species maintain their leaves for several months even below water. Some of the morphological and anatomical strategies to provide oxygen in the roots during the stress period are: the formation of lenticels, adventitious roots, wood rings or aerenchyma (see figure 5.1.2 a)-b)) (Parolin *et al.*, 2004). Despite these strategies, the CO_2 uptake and Chlorophyll content can be affected by the stress produced during flooding. Under waterlogged conditions, most species reduce the assimilation rate in the aerial leaves, but in

some specific cases an increase was reported (Parolin, 2000a, 2001a; Parolin *et al.*, 2004). On the other hand the chlorophyll content in leaves of flooded trees is lower than in non flooded trees (Waldhoff and Furch., 1998), whereas in submerged leaves, chlorophyll content is little affected (2002; Waldhoff and Furch., 1998).

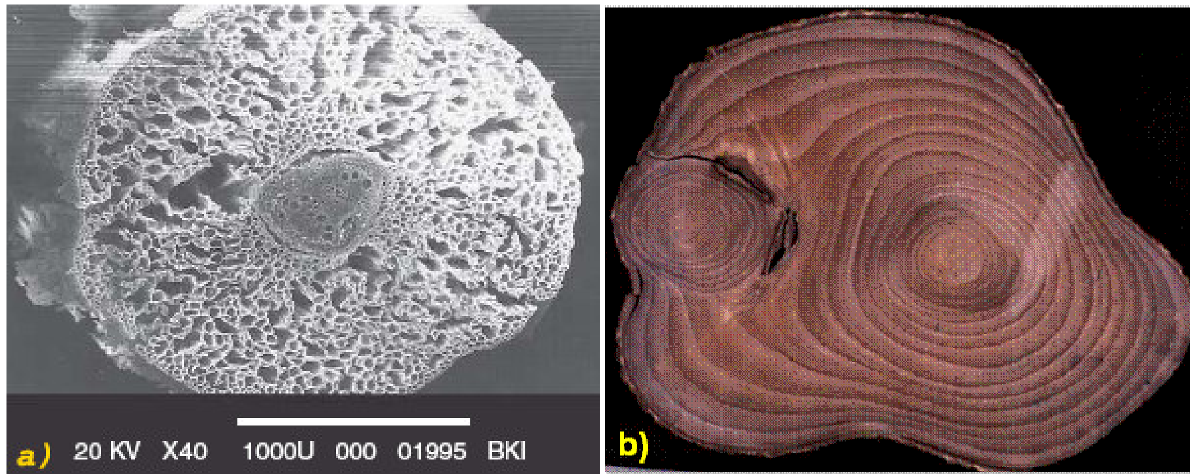


Figure 5.1.2 Anatomical adaptation from plants to the flooding pulse. a) *Cecropia latiloba*: cross section of young adventitious root with aerenchyma (pictures by Danielle Waldhoff) b) annual growth rings (Worbes, 1988)

The fermentation of sucrose in the roots to comply with the energy demand under anoxia is a physiological strategy to adapt to waterlogging. The resulting toxic metabolite - ethanol - is transported through the transpiration stream to the leaves. From there it can either be directly emitted into the atmosphere or can be converted to acetaldehyde and/or acetate, still volatile enough to be partly released (Kreuzwieser *et al.*, 1999b; Rottenberger *et al.*, 2008). This release may contribute to the abundance of such oxygenated compounds in the atmosphere. Thus, root anoxia, caused by flooding, may contribute to significant changes of volatile organic compound composition in the Amazonian atmosphere. This is of special importance, as short-chain oxygenated compounds may play an important role in atmospheric chemistry (Karl *et al.*, 2003a; Kirstine *et al.*, 1998; Lamanna and Goldstein, 1999; Schade and Goldstein, 2001, 2002; Seco *et al.*, 2007). However, not only oxygenated VOCs (oVOCs) are emitted from vegetation of floodplain areas. Stress induced by flooding might also have effects on isoprenoid emissions.

During this study, plants of two different environments, várzea and igapó, were subjected to short-term and long-term flooding periods. The effects on assimilation, transpiration, stomatal conductance and VOC emissions were investigated. Plant species typical from the floodplain

environment várzea (*Pseudobombax munguba, Pouteria glomerata* and *Hura crepitans*) and igapó (*Garcinia macrophylla*) were studied. Additionally, *Hevea spruceana* and *Vatairea guianensis* from várzea and igapó respectively were analyzed in order to compare both floodplain environments.

The recent investigations, we present in this work were complemented by an evaluation of a previous and unpublished isoprenoid data set gained within two doctoral theses performed by a close cooperation between Dr. Betina Kleiss and Dr. Steffanie Rottenberger, who carried out experiments at the University of Oldenburg, Germany in 2000. In the Oldenburg study short-term flooding studies on *Laetia corymbulosa* and *Salix martiana* of VOC emissions were measured with the PTR-MS technique. Results of the emission of oVOC and about the corresponding physiology, assimilation and transpiration, are already published in Rottenberger *et al.*, 2008, whereas results on the emission of isoprenoids will be presented here for the first time.

5.2 MATERIAL AND METHODS

5.2.1 PLANT MATERIAL

Typical widely distributed plant species from the floodplain areas of the central Amazon basin were chosen for this study. The tree species *Vatairea guianensis* Aubl. (Fabaceae), *Hevea spruceana* (Benth.) Müll.Arg. (Euphorbiaceae) and *Garcinia macrophylla* (Mart.) Planch. & Triana (Clusiaceae) are of commercial importance and occur in both floodplain forest types (Ferreira, 1997; Parolin, 2000b; Wittmann *et al.*, 2006; Worbes, 1986). *Hevea spruceana* and *Garcina macrophylla* are common also in terra firme forest but not *Vatairea guianensis* (Wittmann *et al.*, 2006). For our experiment, *Vatairea guianensis* and *Hevea spruceana* were collected from várzea and igapó respectively and *Garcinia macrophylla* from igapó. In addition, species like the pioneer tree *Pseudobombax munguba* (Mart. & Zucc.) Dugand (Bombacaceae) and two species commonly used in the wood industry - *Hura crepitans* L. (Euphorbiaceae) and *Pouteria glomerata* (Miq.) Radlk. (Sapotaceae) - were also collected for our experiments as they are widely distributed in the várzea environment. The six species differ in their phenology being *P.munguba* a stem-succulent tree, *V. guianensis* a deciduous tree, *H. spruceana* and *H. crepitans* a brevi-deciduous tree and *G. macrophylla* and *P. glomerata* an evergreen tree (Schöngart *et al.*, 2002). *V. guianensis* is a non pioneer species (Ziburski, 1991), dominating late successional stages in the low várzea and old-growth forests in the igapó (Wittmann *et al.*, 2006). The wood is used and commercialized mainly for house

construction (Duke and Vasquez Martinez, 1994). *H. spruceana* is a middle high tree typical from the floodplain areas, it is very common on the Rio Negro riversides, but can be often found also in the Solimões River, belonging to the low várzea (Wittmann *et al.*, 2006; Ziburski, 1991). *G. macrophylla* is native from the Amazonian lowlands, where it can grow up to 9 m as an understory tree. Its fruit is not much esteemed but widely eaten and sold in native markets (Campbell, 1983). *Hura crepitans* is recognized by the many dark pointed spines and a smooth brown bark. These spines have caused it to be called "Monkey no-climb". The caustic milky sap from this tree is used by fishermen to poison fish and its wood is of great importance in the wood industry. *H. crepitans* is one of the most important trees in the high várzea forest (Wittmann *et al.*, 2006) but it is also well distributed in terra firme forest (Worbes *et al.*, 1992). *P. glomerata* is a late-successional tree of great importance in the wood industry (De Simone *et al.*, 2003b). The formation of sunken stomata and the suberization of old and young root zones are some of the strategies of this tree to survive watterlogging conditions (De Simone *et al.*, 2003b; Parolin *et al.*, 2004). *P. munguba* is distributed from the middle of the Solimões river to the Amazonas river. This pioneer tree is a light depending plant and is the most important and representavie tree of the low and high várzea forest (Wittmann *et al.*, 2006).

In addition of screening flooding effects on isoprenoid emission of *Laetia corymublosa* Spruce ex. Benth. (Flacourtiaceae) and *Salix martiana* Leyb. (formally called *S. humboldtiana* var. *martiana* (Leyb.) Anders) (Salicaceae) raw data provided by Dr. Betina Kleiss and Dr. Stefanie Rottenberger were included in this study. *L. corymbulosa* is a non-pioneer tree, being one of the most abundant species from the low várzea areas and reaching a stem height of about 25 m (Parolin, 2002; Wittmann *et al.*, 2006). *S. martiana* is a rapid-growing and light-demanding pioneer species occurring mainly in the low elevation sites of the várzea forest, with stem heights reaching about 10-12 m (De Simone *et al.*, 2003b; Parolin, 2000b). Both tree species are evergreen and well adapted to flooding conditions (De Simone *et al.*, 2003a; Schöngart *et al.*, 2002). One of the strategies to mitigate the effects of flooding and found in *L. corymbulosa* as well as in *S. martiana*, is the suberization of the root exodermis. This strategy is restricted to evergreen species. The formation of adventitious roots by *S. martiana* provide an additional adaptation pattern to tolerate high sedimentation rates on the sand banks (De Simone *et al.*, 2003a).

All species are naturally subjected to a similar water regime. Leaves or eventually part of the crown of *V. guianensis, H. spruceana, G. macrophylla, P. glomerata* and *L. corymbulosa* are usually not affected by rising water levels and remain in contact with the atmosphere throughout the whole inundation period. In contrast, *S. martiana* can be subjected to total submergence over a period of several month during the high water peak, with leaves being well developed during the aquatic phase (Parolin, 2005; Worbes *et al.*, 1992). *P. munguba* is the only stem succulent species loosing all its leaves during the flooding period and flushing its leaves later than normal deciduous trees.

Saplings of *Garcinia macrophylla, Vatairea guianensis* and *Hevea spruceana* were collected at the river side of the Tarumã Mirim River, an affluent of the Negro River. The forest on the river sides of the Tarumã Mirim River is considered igapó forest (see figure 5.2.1.1). The várzea saplings of *Vatiarea guianensis, Hura crepitans, Pouteria glomerata* and *Pseudobombax munguba* were collected on the Ilha da Marchanteria, an island situated in the white water Solimões River (see figure 5.2.1.1). *Hevea spruceana* from várzea saplings were collected at the Costa do Catalão (figure 5.2.1.1). The plants were potted with the earth collected on their original emplacement and at least after one month adaptation were measured. The plants were kept in the vegetation house of the INPA under ambient conditions before the measurements and were irrigated every day.

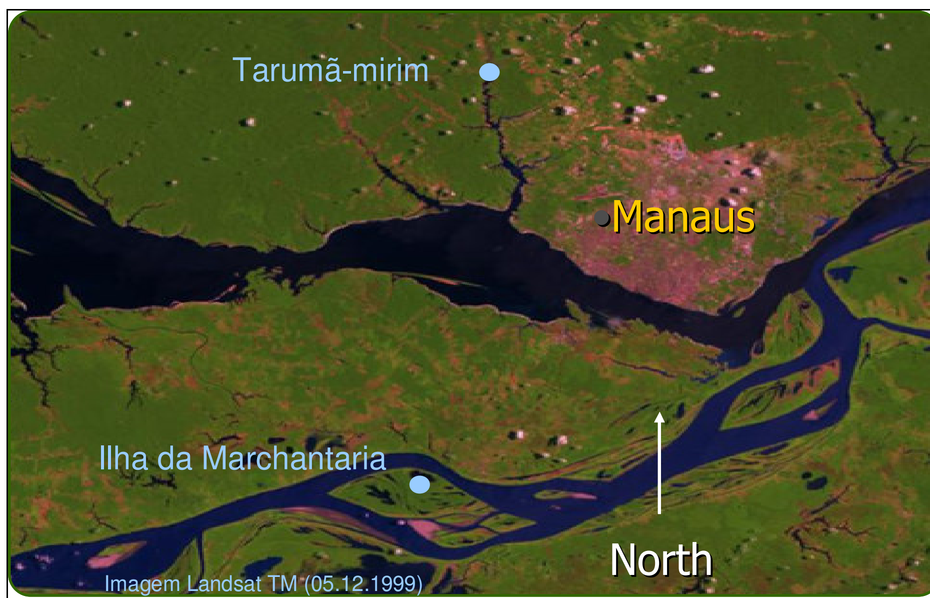


Figure 5.2.1.1 Satellite image of the Ilha da Marchantaria, Costa do Catalão and the Tarumã-mirim River

5.2.2 GAS EXCHANGE MEASUREMENTS WITH ENCLOSED PLANTS

5.2.2.1. ENCLOSURES

An enclosure system developed at the Max Planck Institute of Chemistry in Mainz, Germany, for the measurements of trace gas exchange (Dindorf *et al.*, 2006; Kesselmeier *et al.*, 1997; Kesselmeier *et al.*, 1993; Kesselmeier *et al.*, 1996; Kuhn *et al.*, 2000; Kuhn *et al.*, 2002b; Schäfer *et al.*, 1992) was set up in the surroundings of the Max Planck station at the Instituto Nacional da Pesquisa da Amazônia (INPA), Manaus, Brazil All experiments were performed outside the building in a natural environment. The open enclosure system consisted of two 9 l Teflon (Norton, 50 mm thickness, Saint-Gobain Performance Plastics, Germany) enclosures with an incorporated Teflon (Xylan 1514-946 white; Whitford GmbH, Germany) coated fan. Depending on the plant size a small (9 l) or a big enclosure (100 l) was chosen. For the long term flooding experiment a small enclosure was used and for the short term flooding experiment a big enclosure (for details in the experimental procedure see chapter 5.2.4). The air was pumped with three Teflon membrane pumps (Vacuubrand, Germany) through an ozone scrubber (filter of copper nets covered with MnO_2, Ansyco, Germany) in order to avoid oxidant interferences in the VOC measurements and followed by a Teflon filter (Zefluor Teflon filters, 2 µm pore size, Gelman Science, USA) to remove small particles and a flow meter (EL-Flow, 50 l min^{-1}, Bronkhorst Hi-Tec, Germany) for flow regulations of each enclosure. The flow was controlled by a needle valve situated in front of the flow meter. The air flow in the enclosure was adjusted at 6-10 l min^{-1} for the small enclosure and at 20 l min^{-1} for the large ones. Air was dispersed through a ring of Teflon tubes with holes placed at the bottom of the enclosure and the fan at the top of the enclosures mixed the air into the opposite flow direction to assure a well air mixture in the enclosure. The chosen flow allowed an air exchange of the total enclosure volume every 0.9-1.5 minutes and 5 minutes, respectively. A whole plant or a branch of a tree was placed in the enclosure. Air samples were taken to measure VOCs by cartridge sampling or by continuous flow for physiological and micrometeorological measurements. All Teflon tubes connecting the enclosure with instruments were heated to a temperature above ambient temperature (~45°C) in order to avoid condensation of volatiles (Larsen *et al.*, 1997). The mixing ratio F was calculated according to formula (1) taking into account the measured concentration difference between the reference (empty) and the sample (with the plant) enclosure ($\Delta c = c_{sample} - c_{reference}$), the enclosure flush rate Q, and the leaf dry weight (dw).

$$F = \Delta c(Q/dw). \qquad (1)$$

Light intensities were monitored with a LI-190SZ Quantum Sensor (LI-COR, Lincoln, Nebraska, USA) placed next to the enclosure. Leaf and enclosure temperatures were measured with Teflon covered thermocouples (0.005",Chromel-Constantan, Omega, UK) and were recorded with a datalogger CR23X (Campbell Scientific Inc., UK) as one minute averages. Relative humidity and temperature from each enclosure was monitored by the use of two combined temperature/humidity sensors (Model Rotronics MP-100A, Walz, Germany).

5.2.2.2. ASSIMILATION/TRANSPIRATION MEASUREMENTS

Physiological parameters (photosynthesis and transpiration) were measured with a CO_2/H_2O infrared gas analyzer (LI-COR inc. 7000, Lincoln, Nebraska, USA). This equipment was operated in differential mode receiving the absolute reference concentration signal measured by a second CO_2/H_2O infrared gas analyzer (LI-COR inc. 7000, Lincoln, Nebraska, USA). Nitrogen (N_2 5.0, Messer Griesheim, Germany) was used as reference gas for the absolute CO_2 and H_2O in the reference enclosure. The gas flow to the instrument was supplied by a custom made pump unit (membrane pump, 12 Volt; KNF-NEUBERGER) and regulated by a rotameter (Omega, USA) placed in front of the analyzer inlet adjusting the flow to 0.5 l min^{-1}. Calibration of the analyzer was accomplished prior to the experiments by use of a calibration gas standard for the calibration of CO_2 (512 ± 2 ppm CO_2 in synthetic air, LI-COR, Lincoln, Nebraska, USA) and a dew point generator for the calibration of water vapor (Li 610; LI-COR, Lincoln, Nebraska, USA). At the end of each experiment the calibration of the analyzer was checked and the signal response was corrected for sensitivity and zero drifts as a function of time. Furthermore, the signal response of the instrument was corrected for temperature effects and with regard to the offset of specified and measured reference concentrations. Stomatal conductance was calculated according to Pearcy, Schulze & Zimmermann (1989).

5.2.3 VOC DETERMINATION

VOCs were analyzed with an online and an offline method. For the online detection a Proton Transfer Reaction – Mass Spectrometer (PTR-MS) was connected to the enclosure with a 1/8 inch tube. The PTR-MS as the name indicates is a mass spectrometric method with chemical ionisation, based on proton transfer reactions from H_3O^+ reagent ions to VOC species and has been thoroughly reviewed elsewhere (Lindinger *et al.*, 1998a, c). The PTR-MS was maintained in an air conditioned room during all experiments. Sample and reference enclosure and the background signal were probed alternately. The instrument was operated in selected

ion-monitoring mode at standard operation conditions (E/N = 130 Td; E electric field strength, N buffer gas number density, 1 Td = 10^{-17} cm² V molecule^{-1}) measuring eleven cycles of the sample/reference enclosures and seven cycles the background signal in 16 minutes. A total of 27 masses were selected (protonated masses given in atomic mass units [amu]: 21, 29, 31, 32, 33, 39, 42, 45, 47, 55, 59, 61, 69, 71, 73, 75, 81, 83, 87, 93, 95, 107, 121, 137, 139, 151, and 205) and detected with a dwell time of 1s. The measurement of one complete cycle took 30 seconds. The considerable background signals, caused probably by desorption of impurities inside the sampling system and the drift tube (Steinbacher *et al.*, 2004), was determined by applying VOC free air to the instrument that was generated by a catalytic converter (Zero-air generator, Parker Co., USA). This offset was subtracted from the sample and reference signal and the emission rate was calculated according to (1). Emission rate in µg g^{-1} h^{-1}were standardized using a mathematical algorithm developed by Guenther *et al.* in 1993, 1995 and 1997 (in following referred to as G93). This algorithm was developed to predict isoprene emissions, but it has been demonstrated that G93 can very well predict monoterpene emissions (Ciccioli *et al.*, 1997; Kuhn *et al.*, 2002b, 2004a). G93 describes the emission of volatile organics as a function of a basic emission strength (i.e. a standard emission factor or basal emission rate) combined with environmental parameters like ambient light and temperature conditions. Standard emission factors (Es) that describes the basal VOC emission at standard conditions (PAR 1000 µmol m^{-2} s^{-1} and 30°C temperature) were calculated according to (2)

$$Es = \frac{F}{C_L \cdot C_T} \tag{2}$$

$$C_L = \frac{\alpha \cdot C_{L1} \cdot L}{\sqrt{1+\alpha^2 \cdot L}} \tag{3}$$

$$C_T = \frac{\exp\left(\frac{C_{T1}\cdot(T-T_s)}{R\cdot T_s \cdot T}\right)}{C_{T3} + \exp\left(\frac{(C_{T2}\cdot(T-T_M)}{R\cdot T_s\cdot T}\right)} \tag{4}$$

where α is the empirical coefficient 0.0027, C_{L1} the empirical coefficient 1.066, C_L the light dependent term of the G93 function, C_T the temperature dependent term of the G93 function, C_{T1} the empirical coefficient 95000 J mol^{-1}, C_{T2} the empirical coefficient 230000 J mol^{-1}, C_{T3} the empirical coefficient 0.961, L the photosynthetic active radiation (PAR) in µmol m^{-2} s^{-1}, R the universal gas constant 8.314 J K^{-1} mol^{-1}, E_s the standard emission factor in µg g^{-1} h^{-1}, T

the leaf temperature in °K, T_M the empirical coefficient 314 °K, T_S the leaf temperature at standard condition 303 °K and F the VOC emission rate in µg g^{-1} h^{-1}.

The main emissions detected by PTR-MS were methanol (33 amu), acetaldehyde (45 amu), ethanol (47 amu), acetone (59 amu), isoprene (69 amu) and monoterpenes (137 amu, fragment on 81 amu). It is important to note that different types of monoterpenes cannot be distinguished separately with PTR-MS. Therefore, standard emissions factors calculated for monoterpenes with the PTR-MS in this study always refer to the sum of all monoterpenes. The PTR-MS instrument was calibrated using a gas standard (Apel Riemer, USA or Deuste Steininger GmbH, Germany) containing most of the given target VOC, except ethanol, in nitrogen (± 5 % accuracy for Apel Riemer and ± 10% accuracy for Deuste standard) and diluted with synthetic air to concentrations of 0.5 – 10 ppb. The volume mixing ratio of ethanol was calculated using simple ion-molecule reaction kinetics, where the count rate of RH^+ product ions, $i(RH^+)$, can be calculated from

$$i(RH^+) = i(H_3O^+)_0(1-e^{-k[R]t}) \sim i(H_3O^+)_0k[R]t \qquad (5)$$

$i(H_3O^+)$ is the count rate of H_3O^+ ions, k is the proton transfer reaction rate coefficient, [R] is the number of density of trace molecules in the reactor and t is the transit time of H_3O^+. A detailed description of this calculation method was explained elsewhere (Hansel *et al.*, 1995; Wisthaler *et al.*, 2001). The detection limit of this method was estimated as the greater of the variability levels in background signal (at the 95% confidence level) and was typically 1.153 ppb for methanol, 291 ppt for acetone, 445 ppt for isoprene and 574 ppt for monoterpenes.

Gas Chromatography with Flame Ionisation Detection (GC-FID) (Model AutoSystem XL, Perkin Elmer, Germany) complemented the PTR-MS measurements by offline determination and quantification of VOC. Chromatographical methods are still the most effective method for the gas analyses capable to identify and quantify a high variety of individual volatile compounds. Silicosteel coated cartridges (¼" OD, 89 mm length, RESTEK, USA) filled with the absorbers Cabrograph 1 and 5 were used to collect air from the sample and reference enclosure. Sampling was performed with an automated sampler developed at the MPI for Chemistry in Mainz, Germany (Kuhn *et al.*, 2005) at a flow rate of 100 ml min^{-1} over forty minutes resulting in a sampling volume of 4 liters air. After sampling the cartridges were kept in under air conditioning before transport to the Max-Planck Institute for Chemistry in Mainz, Germany, for GC analysis. Samples were desorbed from the cartridge by heating for 10

minutes at 260°C with a Thermodesorber (Model ATD400, Perkin and Elmer, USA) and introduced in the GC gas flow. Helium 6.0 was chosen as carrier gas. In order to enhance the chromatographic resolution the sample was preconcentrated at -30°C in a cold trap filled with 20 mg Carbograph 5 (cryofocusing). Afterwards the pre concentrated gas mixture was desorbed from the cold trap by heating it rapidly to 280 °C for 2-3 seconds and separated on a GC-column (model HP-1, 100 m length, 0.25 mm ID, 100% dimethylpolysiloxane ($(CH_3)_3$-Si-[O-Si-$(CH_3)_2$-]$_n$-O-Si-$(CH_3)_3$). The temperature program used for analysis ranged between -10 and 220°C (-10 to 40°C at 20°C min^{-1}, 40 to 145°C at 1.5°C min^{-1}, and 145 to 220°C at 30°C min^{-1}). Chromatographic separation of one sample was performed within 90 min. Peak detection was achieved by utilisation of a flame ionisation detector (FID). GC-FID calibration was performed with a gaseous standard mixture containing isoprene and several n-alkanes (n-pentane, n-hexane, n-heptane, n-octane, n-nonane, and n-decane) in high purity nitrogen (calibration gas standard, Apel-Riemer, USA) (for details see Dindorf (2006), Kuhn *et al.* (2002b)). In addition to isoprene 10 different monoterpene compounds were evaluated from these analyses: camphene, Δ3-carene, p-cymene, limonene, myrcene, α-pinene, β-pinene, sabinene, α-terpinene, and γ-terpinene. The detection limit of the GC-FID was calculated again as the greater of the variability levels blank cartridges (at the 95% confidence level) was 141 ppt for isoprene and 319 ppt for monoterpenes.

5.2.4 EXPERIMENTAL PROCEDURE

A short term and a long term inundation experiment were carried out at the surroundings of the Max Plank quarts at the INPA, Manaus, Brazil. The roots of typical plants of the floodplain areas were inundated and reactions on the physiology and VOC emissions were monitored. For the short term experiment the sapling's root of *Vatairea guianensis* and *Hevea spruceana* from igapó and várzea were inundated during three to four days. The selection of this inundation period is based on results observed by other studies in several tree species tested to VOC emission during inundation, in which 24 hours of inundation were enough to see significant enhancement on VOC emissions (Kreuzwieser *et al.*, 1999a; Rottenberger *et al.*, 2008). Two replicates of each species were measured. This experiment took place from the beginning of March to the beginning of May 2007. Measurements started after a preceeding adaptation period. Plants were enclosed in the evening (around 20:00 pm) and data of the first night (~10 hours) were not taken into account. Although such adaptation time is variable according to previous reports (from 20 minutes to 48 h) (Dindorf *et al.*, 2006; Hayward *et al.*, 2004; Llusia *et al.*, 2008; Rottenberger *et al.*, 2008), we considered and

validated a ~10 hours of adaptation to the enclosure environment as sufficient. The next complete day was measured under non flooded conditions. On the second day at 12:00 pm the plant's root system was flooded by inundation in a water filled bucket. The plant was maintained flooded for three to four days. VOC emissions as well as the physiology of the plant were monitored online with the PTR-MS and gas analysers, respectively, during the whole experiment. Additionally, some cartridges were collected during the non flooded and flooded state of the plant. A potential decrease in the O_2 water concentration was monitored by measuring the O_2 concentration (Profiline Dissolved Oxygen Meter Oxi197, WTW, Weilheim, Germany) every flooded day at noon.

For the long term experiment the species *Hevea spruceana* and *Garcinia macrophylla* growing at the igapó and the várzea species *Hura crepitans*, *Pouteria glomerata* and *Pseudobombax munguba* were selected. The experiment took placed during the end of July to middle of August and middle of October to beginning of November 2006. Three saplings per plant species were measured in the long term flooding experiment. Enclosure started around 20:00 pm and after one night adaptation VOC emissions were measured until 14:00 of the next day when the plant's root system was inundated. After 2 months and three weeks of inundation the plants were enclosed again following the same protocol as describe above. VOC measurements were performed until 18:00 of this final day. This inundation period was regarded as "long term" in order to distinguish this experiment from the short term (3-4 days) inundation experiment. Although, a 3 months period of inundation may not be a long period of inundation for these plants, if we take into account that the investigated plants can be subjected to longer inundation periods in the nature (up to 210 days $year^{-1}$) (Junk, 1989).

CO_2 exchange and transpiration data were recorded as 1 minute average. VOC Standard emission factors were calculated according to Guenther *et al.* (1995) taking into account light intensity and temperature. Statistical differences between flooded and not flooded conditions were performed by one-way ANOVA and proven by Tukey test. Differences were considered significant at a probability level of $P<0.01$ (*), very significant at $P<0.001$ (**) and highly significant at $P<0.0001$ (***). A number of two replicates were used by each treatment. Although the power of ANOVA analysis increases with the number of replicates, reliability of this analysis of variance remains high even when a low number of replicates is used (Rosner, 2006).

5.3. RESULTS

5.3.1 ENVIRONMENTAL CONDITIONS

5.3.1.1 MICROMETEOROLOGICAL CONDITIONS DURING THE SHORT TERM FLOODING EXPERIMENT

Micrometeorological parameters, like photosynthetic active radiation (PAR), leaf temperature, relative humidity and CO_2 absolute concentration in the enclosure system, were monitored before and after flooding during the three to four days inundation period. Experiments were performed under semi-controlled conditions at the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil. The slight variation of micrometeorological parameters during this experiment did not affect the physiology of the investigated plants (see table 5.3.1.1). In order to avoid physiological differences light intensities were maintained constant at 498.4 ± 1.6 µmol m^{-2} s^{-1} by artificial radiation (see Materials and Methods). Control of relative humidity (RH) and temperature (T_{leaf} and $T_{enclosure}$) was not possible under the experimental conditions. Nevertheless, temperature and relative humidity variation was not very high during the experiment, reaching values of to 29.5 ± 3.4 °C for $T_{leaf,}$ 29.2 ± 3.1 °C for $T_{enclosure}$ and 85 ± 29 % for RH. Almost no difference between $T_{enclosure}$ and T_{leaf} was detected, probably due to the efficient mixing of the air in the enclosure, which allows the leaf temperature range around ambient temperature (Cook and Dixon, 1964). Carbon dioxide concentrations varied between 414 ± 21 ppm.

Table 5.3.1.1 Micrometeorological parameters during the short term flooding experiments performed with *Vatairea guianensis* and *Hevea spruceana*. Given is the mean of 5 min average values of the three to four days of experiment for two replicates of each tree species (n = 25.92 * 10^4 – 34.56 * 10^4) at maximal photon flux ± standard deviation (SD). Photosynthetic active radiation (PAR) in µmol m^{-2} s^{-1}, leaf temperature (T_{leaf}) and enclosure temperature ($T_{enclosure}$) in °C, relative humidity (RH) in % and absolute concentration of CO_2 in ppm. Species of várzea (v) and igapó (i) environments (En) were investigated.

Plant Species	En	PAR [µmol m^{-2} s^{-1}]	T_{leaf} [°C]	$T_{enclosure}$ [°C]	RH [%]	CO_2 [ppm]
Vatairea guianensis	V	499 ± 5	33 ± 0.8	32.3 ± 0.6	77 ± 5	410 ± 7
	I	498 ± 4	32 ± 0.5	31.6 ± 0.8	81 ± 4	408 ± 9
Hevea spruceana	V	499 ± 1	32 ± 0.1	31.4 ± 0.1	78 ± 1.4	401 ± 6
	I	499 ± 1	32.7 ± 0.3	32.1 ± 0.3	75 ± 3	404 ± 2

During another short term flooding experiment carried out at the University of Oldenburg, Germany, light conditions were maintained constant during the experiment at 185 ± 36 and 216 ± 39 µmol m^{-2} s^{-1} for *L. corymbulosa* and *S.martiana* respectively, which is marginally

lower than those recorded for the Manaus experiment. On the other hand, leaf temperature was very similar to the Manaus experiments varying between 32.2 ± 3.3 and 32.7 ± 3.3 °C for *L. corymbulosa* and *S.martiana* respectively. For details of the micrometeorology and physiology of this experiment see Rottenberger *et al.* (2008).

5.3.1.2 MICROMETEOROLOGICAL CONDITIONS DURING THE LONG TERM FLOODING EXPERIMENT

No control of the micrometeorology was possible during the long term flooding experiment. Table 5.3.1.2 gives the photosynthetic active radiation, leaf temperature, relative humidity and absolute CO_2 concentration before and after a long flooding period (2 months and 3 weeks). Light intensity values before and after the flooding were above the photosynthetic light saturation point (see figure 2.3.3) and above the VOC light saturation point for the isoprenoids emitters *G. macrophylla* and *H. spruceana* (see figure 2.3.4.3 and 2.3.4.4). PAR was therefore not a limiting factor for photosynthesis and VOC emissions. On the other hand, slight differences in leaf temperature could affect in some cases the physiology of the plant (see table 5.3.1.2). Leaf temperature in this experiment was either at, or 2-6 °C above the temperature optimum for tropical trees (25-30 °C, (Larcher, 2003)). Leaf temperatures of *G. macrophylla* and *H. spruceana* were lower in flooded conditions than before inundation, in both cases approaching the temperature optimum described for tropical trees by Larcher 2003, whereas leaf temperatures of *H.crepitans*, *P. glomerata* and *P.munguba* did not differ very much (Δt_{leaf} = 0.4 - 0.8 °C). Thus critical temperature effects on physiology and VOC emissions could be excluded. It is noteworthy to mention that again like in the short term flooding experiment enclosure temperature was similar to leaf temperature. Plants were maintained under natural conditions of CO_2 supply, with ambient CO_2 concentrations of 342-382 ppm. Ambient relative humidity was lower in non flooded conditions, except for the case of *P. glomerata*. Usually in the nature, lower relative humidity together with optimal temperature, light intensity and CO_2 concentration would lead to a decrease of stomatal conductance and thus transpiration and CO_2 exchange. However, in our experiments the opposite effect was observed, with lower relative humidity, stomatal conductance was higher implying that in this experiment the humidity only slightly influences the physiological parameters. Only the transpiration of *H. crepitans* and *G. macrophylla* was found to be affected by the difference on relative humidity between not flooded and flooded condition (see figure 5.3.3.2.1 b).

Table 5.3.1.2 Micrometeorological parameters during the long term flooding experiment performed with *Garcinia macrophylla*, *Hevea spruceana, Hura crepitans, Pouteria glomerat and Pseudobombax munguba*. Given are means for one non flooded (nf) day (n = $3.42 * 10^4 – 4.68 * 10^4$) and a flooded day (f) of three replicates after two months and three weeks of inundation by maximal photon flux (n = $12.87 * 10^4$) ± SD. Photosynthetic active radiation (PAR) is given in µmol m^{-2} s^{-1}, leaf temperature (T_{leaf}) and enclosure temperature ($T_{enclosure}$) in °C, relative humidity (RH) in % and absolute concentration of CO_2 in ppm. Várzea (v), Igapó (i).

Plant species	En	nf/f	PAR [µmol m^{-2} s^{-1}]	T_{leaf} [°C]	$T_{enclosure}$ [°C]	RH [%]	CO_2 [ppm]
Garcinia macrophylla	i	nf	1263 ± 431.6	36.3 ± 2.6	34.9 ± 1.8	36.7 ± 12.7	348 ± 7.4
		f	614 ± 33.6	32.2 ± 2.1	31.1 ± 2.1	76.5 ± 9.0	377 ± 19.5
Hevea spruceana	i	nf	1331 ± 411.4	35.3 ± 2.5	35.4 ± 1.8	33.5 ± 14.8	349 ± 10.3
		f	603 ± 25.0	28.6 ± 2.2	27.9 ± 2.3	85.5 ± 10.7	383 ± 17.8
Hura crepitans	v	nf	828 ± 339.8	32.4 ± 1.2	33.5 ± 1.2	49.0 ± 10.1	342 ± 8.5
		f	620 ± 40.5	32.0 ± 2.1	32.0 ± 2.4	70.0 ± 11.9	368 ± 19.9
Pouteria glomerata	v	nf	573.1 ± 7.7	32.0 ± 1.4	31.7 ± 1.7	65.4 ± 14.5	349 ± 25.0
		f	636.2 ± 77.5	34.8 ± 3.1	34.9 ± 3.1	55.4 ± 14.5	357 ± 15.0
Pseudobombax munguba	v	nf	1183 ± 433.4	33.0 ± 2.1	32.9 ± 2.1	45.6 ± 17.0	344 ± 10.0
		f	624 ± 49.7	32.6 ± 1.9	33.2 ± 2.2	64.8 ± 12.1	369 ± 15.6

5.3.1.3 SOIL CONDITIONS

Due to low solubility of O_2 in water and its consumption by respiring roots and microorganisms the soil of inundated plants turns to hypoxic conditions (Visser et al., 2003). In this study, the oxygen concentrations in the water measured at surface level reached values between 4.3 to 7.2 mg l^{-1} corresponding to O_2 conditions occurring naturally in the Amazon water (Furch and Junk, 1997). No difference between long term and short term inundation was found.

Table 5.3.1.3 Dissolved oxygen in the flooding water in mg l^{-1} and in % and water temperature in °C during the short and long term flooding experiments.

Plant species	En		exp	O_2 concentration [mg l^{-1}]	[%]	H_2O Temperature [°C]
Hevea spruceana	v	tree1	short	4.30	53.28	25.90
		tree2	short	5.37	68.05	27.13
Hevea spruceana	i	tree1	short	6.66	85.73	27.78
		tree2	short	6.14	81.60	28.60
Vatairea guianensis	v	tree1	short	6.12	74.88	26.74
		tree2	short	5.99	76.70	29.00
Vatairea guianensis	i	tree1	short	5.99	69.36	26.52
		tree2	short	6.66	77.60	26.20

Plant species	En		exp	O_2 concentration		H_2O Temperature
				[mg l^{-1}]	[%]	[°C]
Garcinia macrophylla	i	tree1	long	6.43	83.00	29.20
		tree2	long	6.77	87.60	28.80
		tree3	long	7.20	90.30	25.30
Hevea spruceana	i	tree1	long	6.53	81.40	25.30
		tree2	long	6.51	80.20	26.00
		tree3	long	6.47	79.00	25.50
Hura crepitans	v	tree1	long	7.2	90.0	25.9
		tree2	long	6.27	87	26.6
		tree3	long	5.75	78.5	25.7
Pouteria glomerata	v	tree1	long	7.1	87.0	25.3
		tree2	long	7.05	86.7	25.4
		tree3	long	7.01	84.0	25.2
Pseudobombax munguba	v	tree1	long	7.05	84.5	28.9
		tree2	long	6.07	83.9	28.6
		tree3	long	6.09	82.8	28.8

5.3.2 MORPHOLOGICAL ADAPTATION

In oxygen depleted soil, plants have evolved a wide range of characteristic responses that appear to reduce the impact of the stress. For example plants develop morphological features that are induced by the flooding event, like the adventitious roots and lenticels that were found during our investigation (see figure 5.3.2.1). Adventitious roots were observed in the oxygenated layer at the surface of the floodwater table, as well as lenticels at the stem above the water table, both features improve the plant's oxygen status by facilitating the entry of oxygen into the root and the stem. As expected, these morphological formations were observed only during the long term flooding period, but not during the short term flooding experiment. The pioneer tree *Pseudobombax munguba* showed the longest and most developed adventitious roots as well as well developed lenticels. None of the flooded tree species had signs of senescence except for *Pouteria glomerata* (see 5.3.2.2), but new leaves were still observed on the apical region after the long period of inundation.

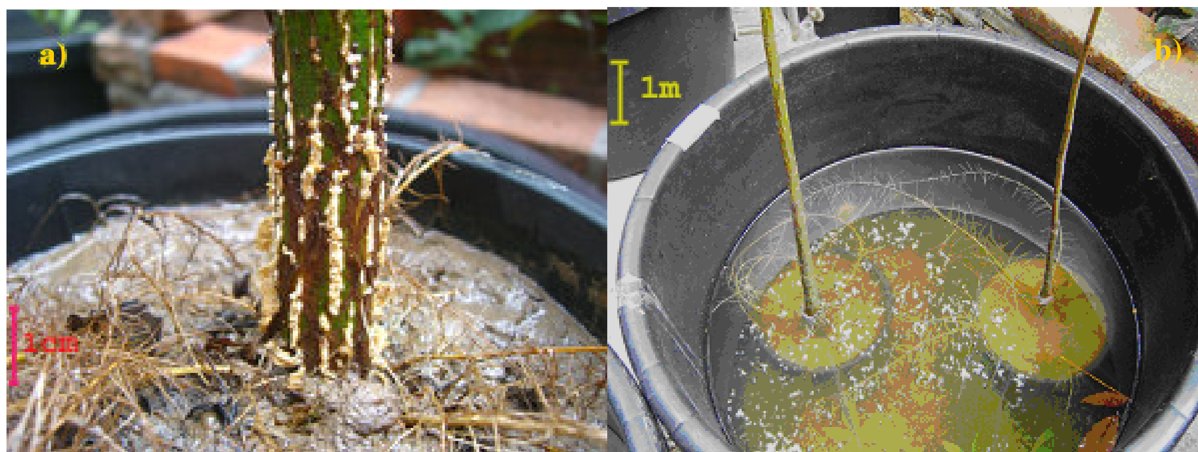


Figure 5.3.2.1 lenticels (a) and adventitious roots (b) observed by *Pseudobombax munguba* after 2 months and 3 weeks inundation.

Figure 5.3.2.2 *Pouteria glomerata* mature leaves after 2 months and 3 weeks inundation

5.3.3 PHYSIOLOGICAL ADAPTATION AND VOC EMISSION RESPONSES TO WATERLOGGING CONDITIONS

5.3.3.1 PHYSIOLOGY AND VOC EMISSIONS BY SHORT-TERM FLOODING

The physiological response to flooding was very variable among the different plant species studied and also among the várzea and igapó environments (see figure 5.3.3.1.1 a)-j)). Rates of assimilation and transpiration, stomatal conductance and internal concentration of CO_2 were found to be significantly higher ($P<0.0001$) after only one day of flooding in case of the igapó species *Hevea spruceana* and *Vatairea guianensis*. This increase of the assimilation and stomatal conductance was further enhanced in case of *H. spruceana* during the following flooding days. Physiological parameters of *H. spruceana* from várzea increased significantly ($P<0.001$-$P<0.0001$) in the first days of inundation but decreased again in the fourth day of waterlogging, whereas *V. guianensis* from várzea showed a significant reduction in its physiological parameters from the first day of inundation ($P<0.0001$). The common várzea species *Laetia corymbulosa* reduced its assimilation and stomatal conductance from the third

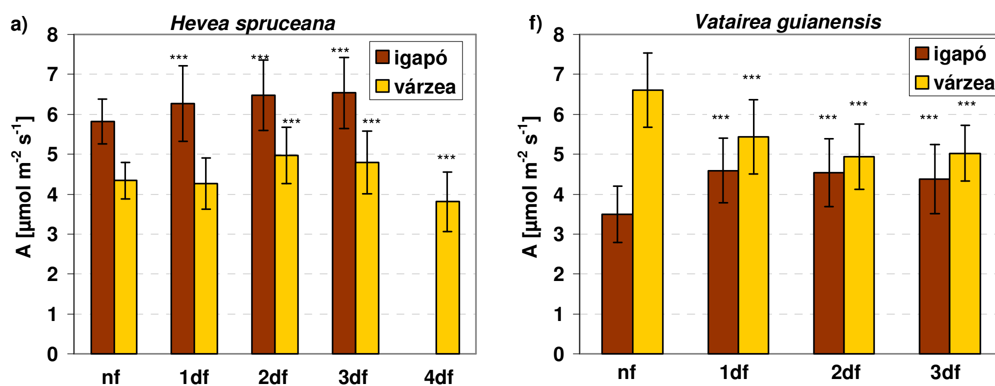
day and its transpiration from the fifth day of waterlogging significantly ($P<0.0001$) (figure 5.3.3.1.4 a)-d)). On the other hand physiology of the várzea pioneer tree *Salix martiana*, that is normally completely flooded under natural conditions, was not affected by the root inundation (figure 5.3.3.1.4 a)-d)). Assimilation variations were conditioned by changes in stomatal conductance which - since the micrometeorological parameters were kept constant - might vary because of the stress caused by inundation. Studies performed by Crawford and Braendle in 1996, in which they studied the inundation of the root of tomato plants for 24-48 h, found that the inundation caused an increase of abscisic acid and thus a decrease on stomatal conductance. In this study we could certify that this is not always the case and that physiological responses are species specific and environment specific; we found that *H. spruceana* and *V. guianensis* from igapó tended to increase physiological parameters during short term flooding, whereas *H. spruceana* and *V. guianensis* form várzea showed no response or decreased their physiological parameters.

Isoprenoid emissions can be affected by short term flooding periods in different manners depending on the tree species and the origin. For example, the change in isoprenoid emission was greater for the tree species *H. spruceana* than by *V. guianensis* (see figure 5.3.3.1.1 e) and j)). Plants from the igapó and várzea environments reacted in different ways, e.g. *H. spruceana* from igapó showed an increase of monoterpene standard emission factors (E_s) whereas the várzea species showed a decrease (see figure 5.3.3.1.1 e)). *V. guianensis* from várzea showed a decline in isoprene emissions, whereas almost no effects on igapó species were observed (see figure 5.3.3.1.1 j)). Cartridges collected before and after 4h, 24h and 72h inundation were analyzed with the GC-FID system in order to detect the monoterpene composition emitted by *H. spruceana* from igapó and várzea respectively. There was no significant difference between the monoterpene composition emitted by *H. spruceana* of igapó and várzea and inundation did also not affect this composition. The main emitted monoterpenes was alpha-pinene (51-80% of the total monoterpene emission) followed by limonene (12-26 % of the total monoterpene emission) and sabinene, ß-pinene, camphene, myrcene and p-cymene (0-6.5 %, 0-4%, 2-11% 0.4-2 %, 1-8 % respectively of the total monoterpene emission). Also traces of alpha-phellandrene 3-carene and gamma-terpinene were detected (<1% of the total monoterpene emission).

Isoprene E_s by *L. corymbulosa* from várzea were also not affected by flooding whereas *S. martiana* showed higher isoprene Es in the last days of inundation (see figure 5.3.3.1.2 d)). A

clear correlation of assimilation with isoprenoid emission was only observed by *H. spruceana* from igapó (see figure 5.3.3.1.3). A decoupling of photosynthesis from isoprenoid emissions may be the result of stress due to waterlogging.

In the event of total and near-total oxygen deficiency (anoxia), respiration switches to anaerobic dissimilation. In the absence of terminal oxidation, the toxic metabolites ethanol and/or acetaldehyde are produced in the roots and transported through the transpiration stream to the leaves and emitted in the atmosphere (Parolin *et al.*, 2004; Rottenberger *et al.*, 2008; 2004). Emission of ethanol and/or acetaldehyde was observed during the short term inundation periods (3 days) in the tree species *Vatairea guianensis* (see figure 5.3.3.1.4 and 5.3.3.1.5). Differences in the emission of oxygenated VOC were detected between species from the várzea and igapó regions. Igapó species seem to adapt more quickly to the stress produced by flooding, emitting only small amounts of ethanol on the first day after the inundation and some rests on the second day. On the other hand várzea species emitted acetaldehyde and higher quantities of ethanol from the first day of inundation. But várzea species were also well adapted to flooded conditions; the emission of both acetaldehyde and ethanol decreased within the three days of measurement. The same effect could be observed for ethanol and acetaldehyde emissions from *L. corymbulosa* and *S. martiana* (Rottenberger *et al.*, 2008). Adaptation to flooding of *H. spruceana* seems to be very effective, since no ethanol and acetaldehyde emission was detected.



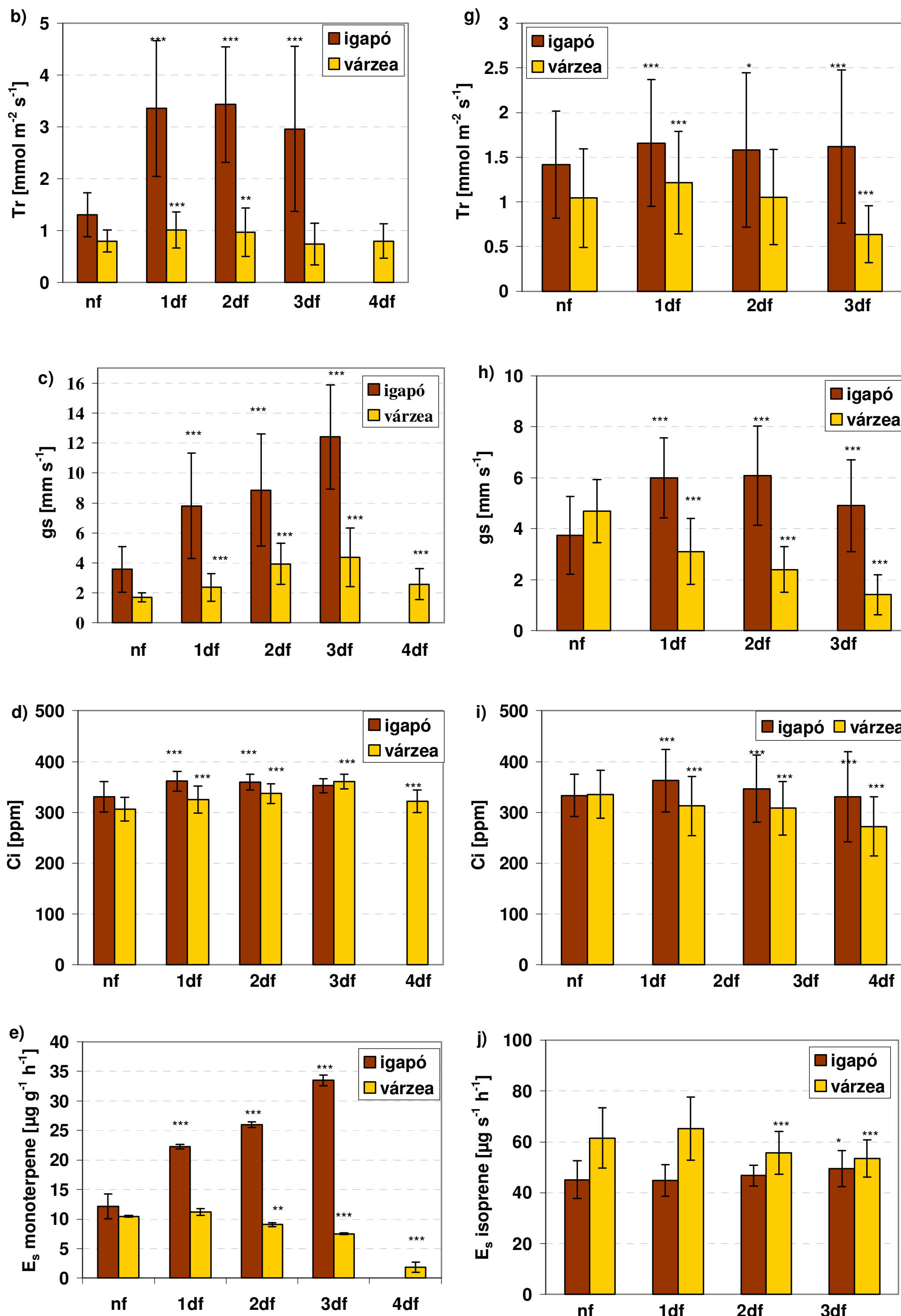


Figure 5.3.3.1.1 a)-j) Means of assimilation (A) in µmol m^{-2} s^{-1}, transpiration (Tr) in mmol m^{-2} s^{-1}, stomatal conductance (gs) in mm s^{-1} and leaf internal CO_2 concentration (Ci) under maximal photon flux density (see Material and Methods) ± standard deviation (SD). Standard emission factors (Es) of monoterpene by *Hevea spruceana* and isoprene by *Vatairea guianensis* from várzea and igapó respectively in µg g^{-1} h^{-1} ± estimated error calculated from the standard error of the regression line. Differences between non flooded (nf) and 1, 2, 3 and 4 days flooded (df) respectively were tested with ANOVA. When P-value < 0.01 F ratio is significant (*), when P-value < 0.001 F ratio is very significant (**) and when P-value < 0.0001 F ratio is highly significant (***)

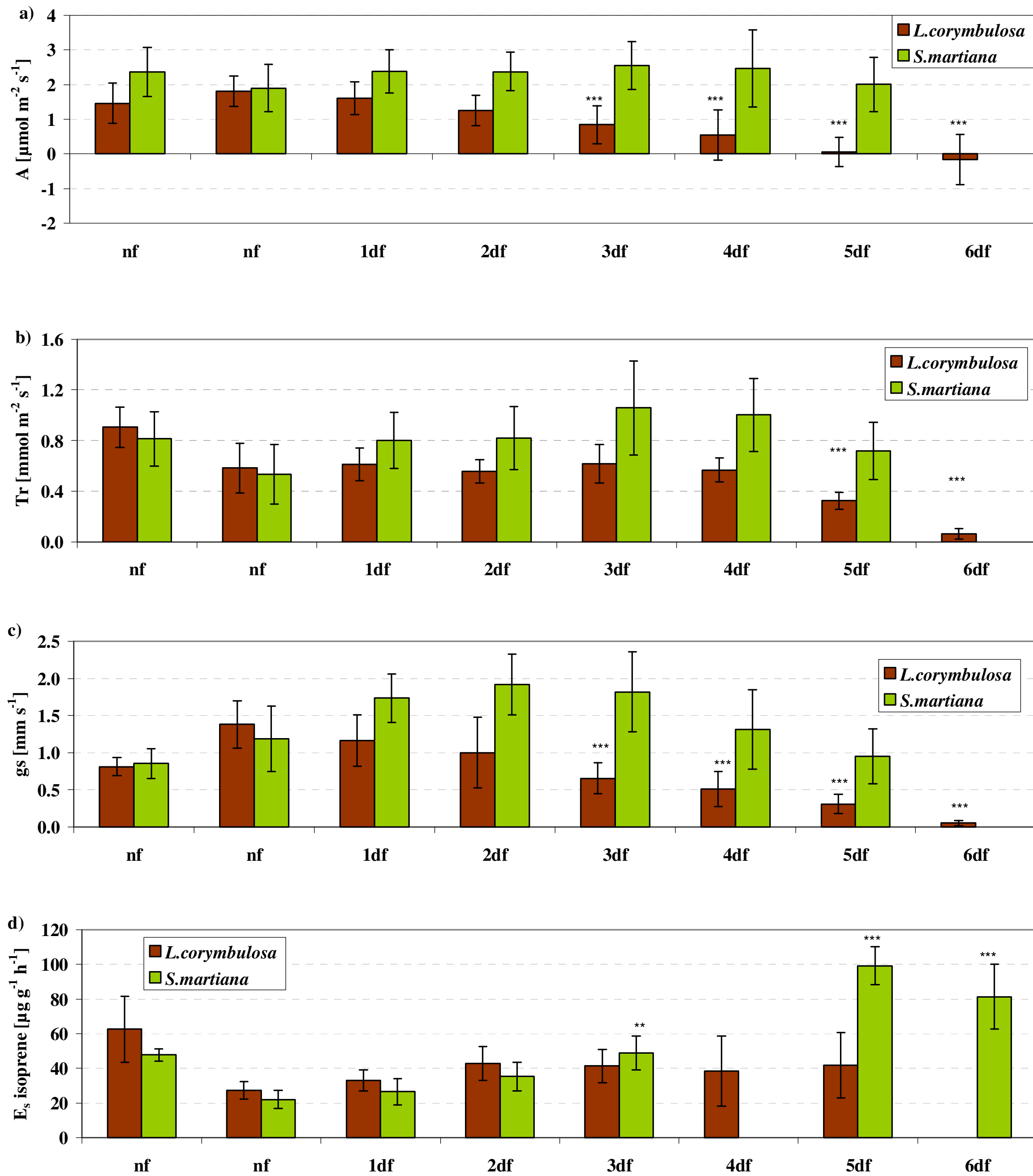


Figure 5.3.3.1.2 a)-d) Day time means of assimilation (A) in µmol m^{-2} s^{-1}, transpiration (Tr) in mmol m^{-2} s^{-1} and stomatal conductance (gs) in mm s^{-1} ± SD. Standard emission factors (Es) of isoprene in µg g^{-1} h^{-1} ± estimated error calculated from the standard error of the regression line. Differences between non flooded (nf) and 1, 2, 3 and 4 days flooded (df) respectively were tested with ANOVA. When P-value < 0.01 F ratio is significant (*), when P-value < 0.001 F ratio is very significant (**) and when P-value < 0.0001 F ratio is highly significant (***)

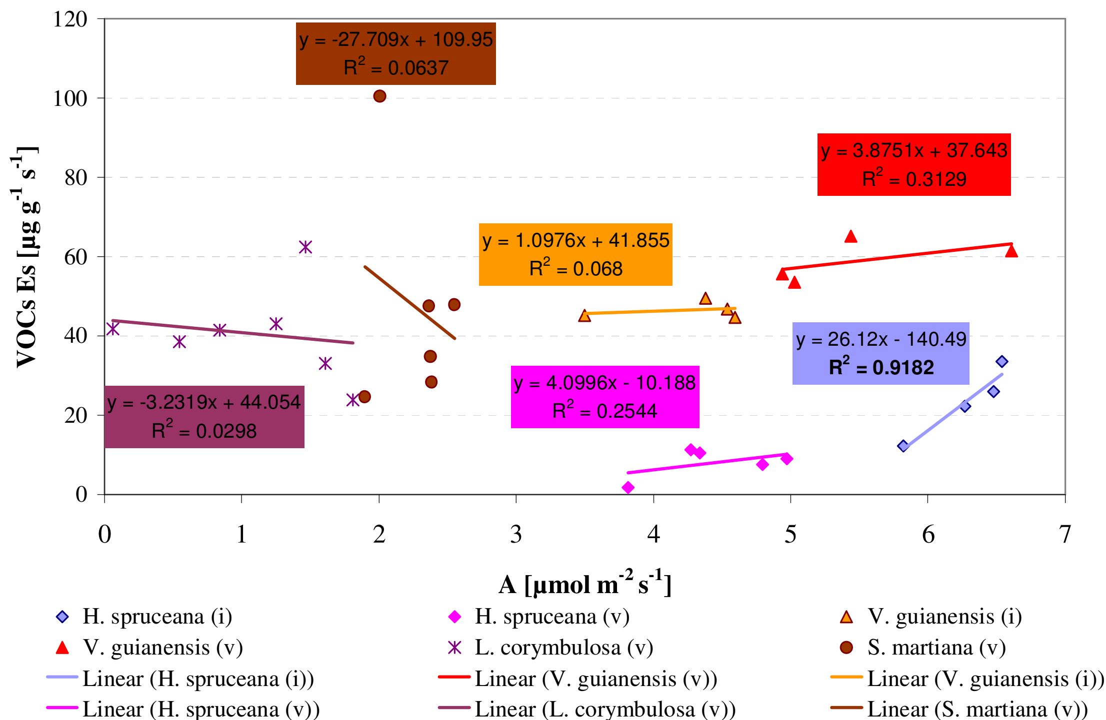


Figure 5.3.3.1.3 Correlation of Assimilation [µmol m^{-2} s^{-1}] with isoprene Es for *V. guianensis* and with monoterpene Es for *H. spruceana* from igapó and várzea, respectively, given in [µg g^{-1} h^{-1}]

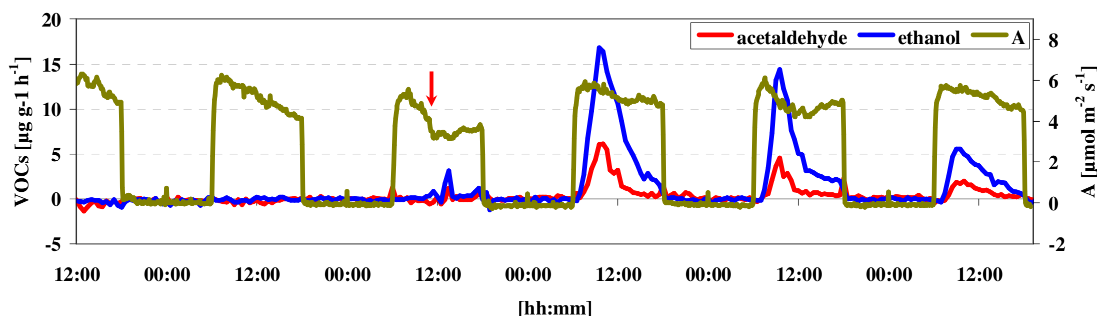


Figure 5.3.3.1.4 Assimilation (A) in µmol m^{-2} s^{-1}, ethanol and acetaldehyde emissions in µg g^{-1} h^{-1} during the short term flooding experiment by *Vatairea guianensis* from várzea. The red arrow represents the moment of inundation.

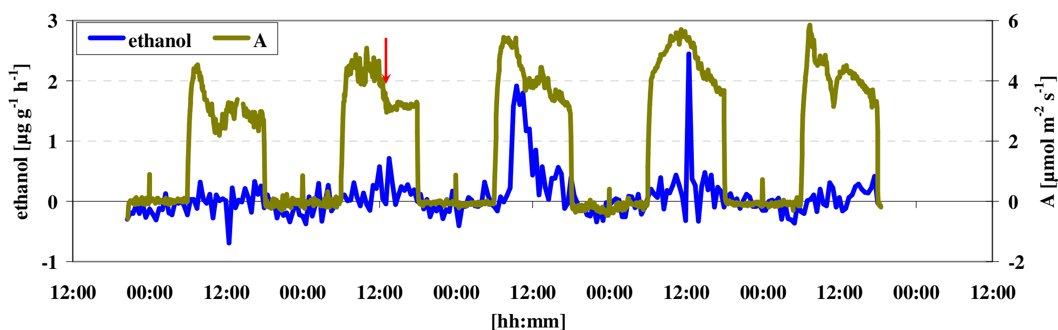
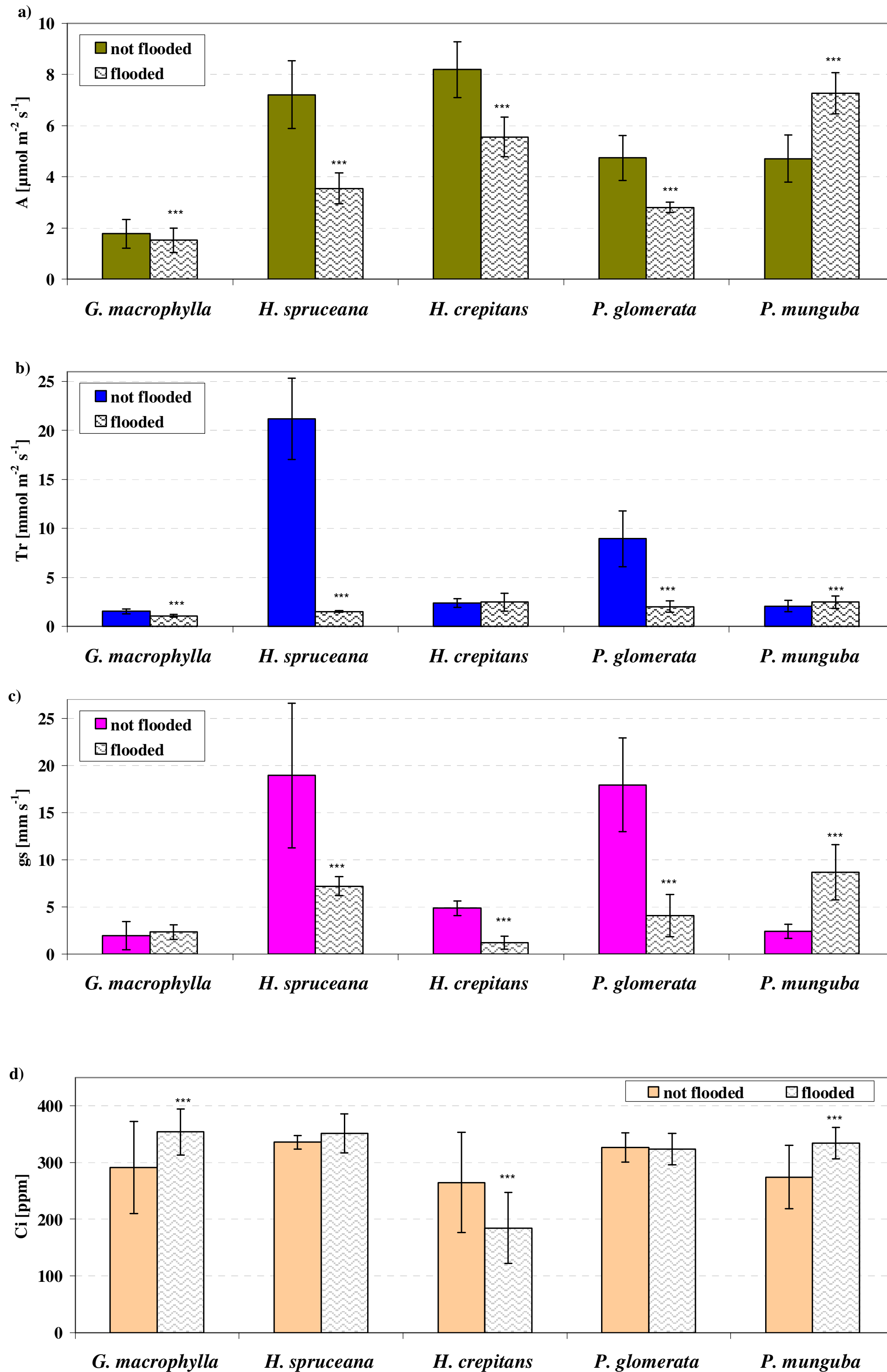


Figure 5.3.3.1.4 Assimilation (A) in µmol m^{-2} s^{-1} and ethanol emissions in µg g^{-1} h^{-1} during the short term flooding experiment by *Vatairea guianensis* from igapó. The red arrow represents the moment of inundation.

5.3.3.2 PHYSIOLOGY AND VOC EMISSIONS AFTER LONG-TERM FLOODING

Plant species that are typical of the floodplain areas which are flooded for several months in the year were chosen for the investigation on the effects of long term flooding. The VOC emissions of potted plants was measured first under non flooding conditions, the plants were then inundated and finally, after almost three months, VOC emissions were measured again. Physiological variables were monitored during the experiment. Assimilation decreased significantly ($P<0.0001$) probably due to waterlogging in all species investigated except for *P. munguba* which showed higher photosynthetic rates after the long term flooding period. Photosynthesis correlated well with stomatal conductance in all species apart from *G. macrophylla* in which stomatal conductance did not suffer significant changes due to the inundation, whereas the increase of internal CO_2 concentrations (Ci) suggests a non stomatal inhibition of photosynthesis. Also, a non stomatal inhibition of photosynthesis can be assumed in the case of *H. spruceana* and *P. glomerata,* where a decrease of photosynthesis (and thus stomatal conductance) occurs, but no drop in Ci was observed. According to Bradford *et al.* (1983) stomatal behaviour and photosynthetic capacity are independently altered by root flooding stress. This stress could affect stomatal conductance indirectly by diminishing photosynthetic capacity and intercellular CO_2 concentrations (Ci) could then remain constant or even increase under ambient conditions as observed in the three plant species mentioned above. On the other hand a decrease of assimilation was accompanied with a decrease on stomatal conductance and Ci by *Hura crepitans* showing in this case a stomatal inhibition of photosynthesis. In contrast to the other investigated plants to long term flooding conditions, *P. munguba* seems to be better adapted to waterlogging, since stomatal conductance, Ci and consequently assimilation are higher in flooding conditions.

Transpiration is dependent on the physical conditions affecting evaporation as well as on the stomatal regulation. Transpiration was found also to follow stomatal conductance in the investigated plants with exception of *H. crepitans* and *G. macrophylla*, suggesting in these cases an influence of the low relative humidity recorded during the non flooded period that affect transpiration by enhancing the leaf-to-air vapour pressure deficit.


a)
A [µmol m-2 s-1]
not flooded
flooded
10
8
6
4
2
0
***
G. macrophylla
H. spruceana
H. crepitans
P. glomerata
P. munguba
b)
Tr [mmol m-2 s-1]
25
20
15
10
5
0
c)
gs [mm s-1]
d)
Ci [ppm]
400
300
200
100

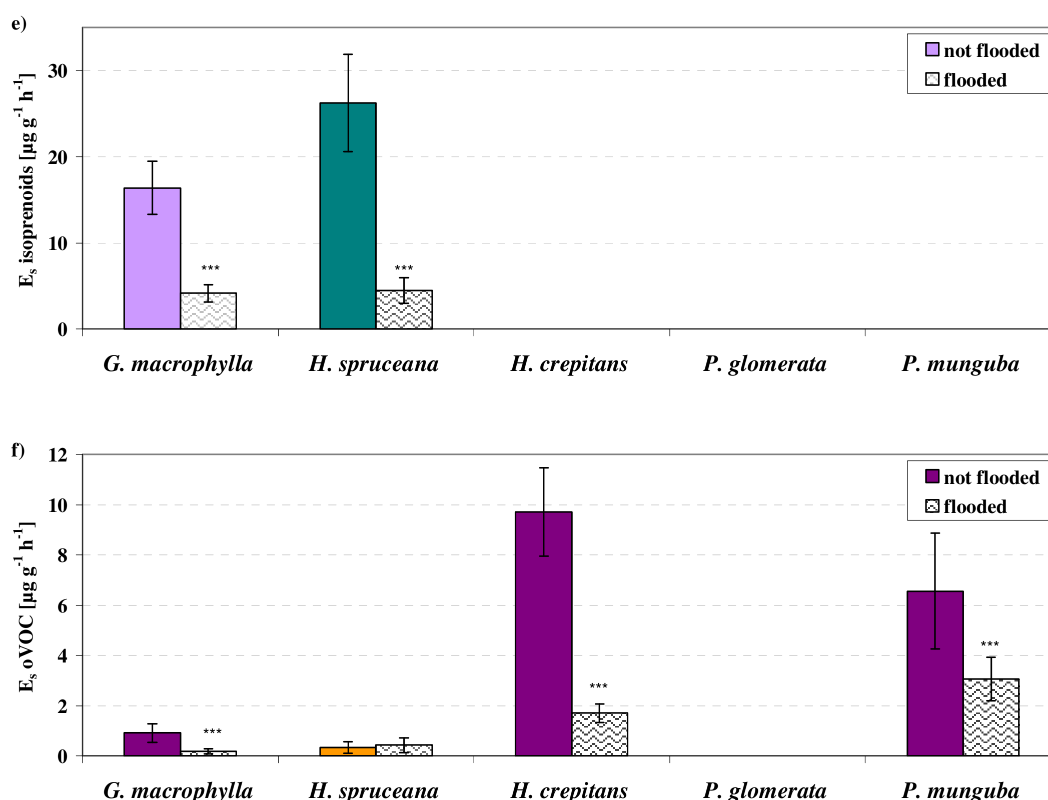


Figure 5.3.3.2.1 a)-f) Means of assimilation (A) in µmol m^{-2} s^{-1}, transpiration (Tr) in mmol m^{-2} s^{-1}, stomatal conductance (gs) in mm s^{-1} and internal CO_2 concentration (Ci) under maximal photon flux density (see Material and Methods) ± standard deviation (SD). Standard emission factors (Es) for isoprene in case of *Garcinia macrophylla* from igapó, for monoterpene in case of *Hevea spruceana* from igapó, for methanol in case of *Garcinia macrophylla, Hura crepitans* and *Pseudobombax munguba* and for acetone in case of *Hevea spruceana* are given in µg g^{-1} h^{-1} ± estimated error calculated from the standard error of the regression line. Differences between not flooded and flooded conditions were tested with ANOVA. When P-value < 0.01 F ratio is significant (*), when P-value < 0.001 F ratio is very significant (**) and when P-value < 0.0001 F ratio is highly significant (***)

Isoprenoid emission was clearly affected by a long term waterlogging. Standard emission factors of isoprene and monoterpenes measured for the isoprene emitter *G. macrophylla* and the monoterpene emitter *H. spruceana* respectively dropped by ~75% and ~83% (see figure 5.3.3.2.1 e). In contrast photosynthesis decreased only by 16.3 % and a 50.8 % respectively (see figure 5.3.3.2.1 a). There is a tendency of down-regulation of the physiological parameters in most of the plants species investigated after a flooding period of around 3 months, except for *P. munguba*. The rises in the physiological parameters showed by species of igapó environments, i.e. by *H. spruceana*, during short term flooding seems not to be maintained in the long term flooding.

Methanol emissions by *G. macrophylla, H. crepitans* and *P. munguba*, showed a decrease of the standard emission factors after the long term flooding. There are a few potential reasons for this. First it is known that methanol is controlled by stomatal conductance (Fall and Benson, 1996; Hüve *et al.*, 2007; Macdonald and Fall, 1993a; Nemecek-Marshall *et al.*, 1995), but a decrease on stomatal conductance was observed in *H. crepitans* only. Since methanol emission is produced by the pectin formation during cell wall expansion, the age of the leaves could also affect the standard emission factor of methanol. As the flooded plants were around three months older they were probably growing at a lower growth rate, and thus a lower methanol Es would be reasonable. In addition to methanol release a low acetone emission was observed for *H. spruceana* but no flooding effects could be detected.

5.4 DISCUSSION

Emission of VOCs by several Amazonian tree species from two ecosystems of the floodplain areas, várzea (nutrient rich soil and water of neutral pH) and igapó (nutrient poor soil and water of acid pH), was investigated after short term and long term inundation. Experiments were performed under semi-controlled conditions. All VOC emission studies were accompanied by measurements of primary physiological processes, i.e. CO_2 and water exchange.

5.4.1 SHORT-TERM FLOODING

Under semi-controlled conditions, the physiology and the VOC emission of young plants of *Hevea spruceana* and *Vatairea guianensis* from várzea as well as from igapó environments showed different adaptation strategies to short term flooding. Under non stress conditions we can observe that *Hevea spruceana* has developed strategies to survive in nutrient poor environments (igapó) better than in nutrient rich environments (várzea) in the terrestrial phase, whereas for *Vataitera guianensis* the opposite effect was observed.

It seems that inundation had a higher impact on physiology and VOC emission in case of *Hevea spruceana* growing in igapó than in várzea ecosystems. During the inundation days A, Tr, gs, Ci and monoterpene emissions of *Hevea spruceana* from igapó increased responding to new anoxia conditions and low nutrient availability. Plants in várzea ecosystems might be naturally better adapted to resist flooding conditions. Obviuosly, *Hevea spruceana* plants from várzea better regulate physiological processes, such as assimilation and transpiration, during the short term inundation, supporting speculating that the observed variation of physiology (oscillating above and below the physiology value of the non flooded day) is due to a circadian rhythm of the plant. VOCs emission is also affected by inundation showing a slight reduction of emission from the second day of inundation. On the other hand, *Vatairea guianensis*, a species that under non flooded conditions is better adapted to várzea ecosystem (see figure 5.3.3.1.1 f), exhibited drastic changes in physiology and isoprene emission in both ecosystem, suggesting that these plants have a low response to regulate and adapt their metabolism to the new flooding condition. The changes detected by *Vatairea guianensis* from igapó were the same as observed by *Hevea spruceana* from the same environment, which considerably increased its physiology and VOC emission during the inundation period, whereas *Vatairea guianensis* from várzea reduced its physiology and VOC emission from the first day of inundation on. Another species that seems to be bad adapted to waterlogging

conditions is *Laetia corymbulosa*, showing a massive reduction of assimilation during the inundation period. In contrast, physiology of the pioneer well adapted to inundation tree *Salix martiana* was not affected. These data for both tree species are in close agreement with earlier reports by Rottenberger *et al.* (2008).
In general, a reduction of CO_2-uptake in aerial leaves of inundated plants have been reported (Parolin *et al.*, 2004), but single measurements – in contrast to average values of the complete aquatic period – show that photosynthetic activity during waterlogging could reach the same or even higher values than in the terrestrial phase (Parolin, 2000a), as also reported in this study for igapó species.

Isoprenoid emission is usually related to plant defense as a response to biotic and abiotic stress (Beauchamp *et al.*, 2005; Davison *et al.*, 2008; Ibrahim *et al.*, 2008; Plaza *et al.*, 2005; Sharkey *et al.*, 2008). Such a response can be related to photosynthesis (Delwiche and Sharkey, 1993; Ferrieri *et al.*, 2005; Kuhn *et al.*, 2002b; Schnitzler *et al.*, 2004), but not under stress situations (Funk *et al.*, 2004). Under stress situations like flooding a higher carbon loss by VOC emission in relation to photosynthesis can be expected (Kesselmeier *et al.*, 2002a), due to other compounds than isoprenoids (Holzinger *et al.*, 2000; Rottenberger *et al.*, 2008). Such behavior has been confirmed in this study for *Hevea spruceana* and *Vatairea guianensis* from igapó and *Salix martiana* from várzea, finding in these cases an enhancement of the monoterpene, isoprene and isoprene release, respectively, but also of assimilation in case of the igapó species. In contrast, a decrease or maintenance of VOC emission is observed for *Hevea spruceana* and *Vatairea guianensis* from igapó and *Laetia corymbulosa* from várzea, suggesting a decrease of this secondary metabolism in order to preserve the primary metabolism (carbohydrates, aminoacids, proteins and lipids).

As already published in several reports (Holzinger *et al.*, 2000; Kennedy *et al.*, 1992; Kreuzwieser *et al.*, 1999b; Macdonald and Kimmerer, 1993; MacDonald *et al.*, 1989; Rottenberger *et al.*, 2008; Schlüter *et al.*, 1993; Visser *et al.*, 2003), leaves of plants subjected to waterlogging conditions can emit ethanol and acetaldehyde as a response to root anoxia. The ethanol produced in the roots due to alcoholic fermentation under anoxia is transported via the transpiration stream through the xylem to the leaves, where it can be directly emitted to the atmosphere or oxidized to acetaldehyde and/or acetate, both of them also partly escaping to the atmosphere. Acetate can be further converted to acetyl-CoA by acetyl-CoA

synthetase and then enter again the metabolism pathways such as the TCA cycle or lipid synthesis (Macdonald and Kimmerer, 1993).

The accumulation of ethanol in the plant can be toxic (Kennedy *et al.*, 1992), especially in the restricted ambient created for the experiment, where the water was not changed during the whole experiment. The absence of morphological injury during the short-term waterlogging and of ethanol and acetaldehyde emission from *Hevea spruceana* plants from both environments, suggests this as an strategy of the plant in order to economize energy returning this anaerobically produced oxygenated VOCs to metabolism. *Vatairea guianensis* from both environments seems to be worst adapted to flooding conditions, since, although no apparent morphological injury was observed, emissions of oxygenated VOCs were detected from the first day of inundation decreasing continuously with the time. However, *Vatairea guianensis* from igapó could cope better with the anoxia, emitting only ethanol in the first and second day of inundation and in lower quantities as emitted from *Vatairea guianensis* from várzea. This varzea species emitted additionally acetaldehyde until the third day of inundation.

5.4.2 LONG TERM FLOODING

Under natural conditions in the floodplains adventitious roots and hypertrophy of lenticels have been observed in juvenile and adult individuals for several species (Parolin, 2001b). The long term flooding experiment also showed the potential capacity to produce morphological adaptations forming adventitious roots and hypertrophy of lenticels, which might have a function in the improvement of internal oxygen status by facilitating the entry of oxygen into the root and the stem.

In general, assimilation rates were lower under waterlogging conditions after long term inundation, confirming the results already reported by Parolin et al. (2000a). The pioneer tree *Pseudobombax munguba* exhibited a higher assimilation rate under inundation as compared to non flooding conditions. Such a behavior could also be observed for the pioneer tree *Cecropia latiloba*, with a mean CO_2-uptake not significantly differing between the non inundated and the inundated period, though in special cases an increased assimilation can be observed (Parolin, 2000a). Stomatal conductance did not always follow assimilation, as observed for *Garcinia macrophylla*. Since the internal concentration of CO_2 of this species was enhanced, a non stomatal suppression of photosynthesis may be suggested.

This study reported for the first time the effects of long-term flooding on emissions of isoprenoids and oxygenated VOC such as methanol and acetone. Much lower isoprene and monoterpenes emission after the long term inundation of *Garcinia macrophylla* and *Hevea spruceana* was observed in close accordance to primary physiology. But the reduction of isoprenoid emissions was much higher (75 and 83%, respectively) than that of assimilation (14 and 51%, respectively). Reduction of methanol emission was also observed. This latter tendency might be the result of growth reduction due to the stress conditions, as already reported by Parolin and Ferreira (1998) but also due to the developmental stage of the plant (Hüve *et al.*, 2007). In contrast, waterlogging did not exert any influence on the low acetone emissions (< 1µg g^{-1} h^{-1}) detected in case of *Hevea spruceana*, for example.

The lack of ethanol and acetaldehyde emission under long term flooding conditions is of high interest for biosphere atmosphere exchange under natural conditions. We may assume that this metabolic strategy is only relevant for the first days of adaptation to oxygen lack in the roots. By long term flooding periods the alcoholic fermentation seems to lose its role in gaining energy. The accumulation of organic acids and aerobic respiration, dependent on oxygen transport, begin to play the dominant roles at this stage. The respiration process is fed by a high consumption of reserve materials under low oxygen partial pressure (Schlüter *et al.*, 1993).

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CHAPTER 6: CONCLUSIONS AND PERSPECTIVES

In conclusion, this study provides a categorization of 16 common plant species from the Mediterranean area, 22 from the tropical area as known as VOC emittent species and two known as non emitters. For the terpenoid emission pattern, there was a clear difference between tropical and Mediterranean vegetation. Among the investigated tropical vegetation, isoprene emission dominated in comparison with monoterpene emission. Seven tropical plant species were isoprene emitting species, whereas only four species emitted monoterpenes. In contrast, monoterpene emission predominated in the Mediterranean vegetation, finding nine plants that emitted this VOC. Also five were classified as high isoprene emitters and seven as low isoprene emitters. Isoprene standard emission factors were usually higher than monoterpene emission factors, with mean values by the high emitting plants of 25.8 $\mu g\ g^{-1}\ h^{-1}$ and 17.7 $\mu g\ g^{-1}\ h^{-1}$ for isoprene and monoterpenes, respectively. Furthermore, another very important terpenoid family for the atmospheric chemistry, the sesquiterpenes were detected only in Mediterranean vegetation. Although our experiments did not detect any sesquiterpene emissions from tropical vegetation, emissions of this terpenoid class has been demonstrated recently from other tropical species (Courtois *et al.*, 2009).

On the other hand, emissions of oxygenated VOCs like methanol and acetone were found to be widely distributed in plants of both ecosystems. Most of the plant species studied emitted methanol as an indicator of growth. Since the plants which were investigated, were still saplings and probably in the growth period, such a high emission of methanol was expected, although for the screening experiments always mature leaves were chosen. Interestingly, methanol and acetone emissions of the Mediterranean species investigated in this study were significantly lower than those detected for tropical vegetation. This difference on methanol emission could have been caused by differences on the developmental stage among the plants. For acetone emission reports are rare. However, it seems that acetone emissions from the Mediterranean vegetation, the species from middle and northern Europe and from North America are not comparable to the acetone emissions of the Tropical vegetation. But as only a few plant species were used to make this comparison, further investigation is needed in order to draw final conclusions.

Although in the last years complicated models have been developed in order to better simulate VOC emissions (Guenther *et al.*, 2006; Niinemets *et al.*, 2002) related to plant physiological activities such as photosynthesis or seasonal behaviour. Despite the need for better models, the simulation of VOC emissions with the algorithm of Guenther *et al.* (1993) (G93), based

only on temperature and light intensity changes, showed a good correlation. We demonstrated that isoprene, monoterpenes, methanol (without morning peak) and acetone emission rates could be well represented with G93. The use of this algorithm helped us to standardize the data in order to compare them with the literature. Despite standardization of the data, great differences between our measurements and the data of other studies have been found. A variety of reasons have been discussed during this study, such as the seasonal variation or the differences between technical systems which were used. On the other hand, many of the species described here were described for the first time as VOC emitting species, especially regarding the tropical area, that is worst characterized.

This work also demonstrates the necessity of comparing different VOC measurement systems to reach an accurate and complete view of the composition of VOC emissions from plants. The use of different techniques for the VOC emission allowed a faster and more complete view of the VOC emitted from the plants. For example PTR-MS techniques showed fast online VOC emissions covering a mass range of 32-205 m/z. On the other hand, GC-FID and GC-MS techniques are offline techniques, where analysis takes some hours, but compounds with the same mass, which cannot be differentiated with PTR-MS, are identified with GC-FID or GC-MS. These GC techniques were used to detect the individual monoterpene and sesquiterpene emissions. Interestingly, the monoterpene composition in case of Mediterranean species was in some cases quite similar to the monoterpene composition found in case of the tropical vegetation. For the tropical vegetation, α-Pinene was the main monoterpene species emitted, followed by Limonene, ß-Pinene, Sabinene, and/or p-Cymene. In the case of the Mediterranean species, the mentioned monoterpene species were also abundant, but also monoterpenes as *Z*- and *E*-ocimene were important in a variety of plant species, such as *Olea europea*. For the emission of sesquiterpenes the compound ß-caryophyllene is the most common, as already found in a variety of reports (Duhl *et al.*, 2008), even though this compound is one of the most reactive sesquiterpenes (Shu and Atkinson, 1995).

Measurements of Mediterranean vegetation could be performed at standard conditions (1000 µmol m^{-2} s^{-1} light intensity and 30°C temperature) and VOC emissions from tropical vegetation could be, as mentioned above, standardized by G93. The provision of standard emission factors by this work is of great importance, since such factors at leaf level from vegetation are essential for the development of prediction models on regional and global scale. This arduous but necessary task should be continued in order to realize more reliable

models and predictions. However, it has to be noted that the G93 parameterization is not necessarily the adequate normalization for all kinds of VOC species.

Standard emission factors are usually plant species specific, but not always constant, since they can vary by the influence of several stress factors, as drought, nutrients availability, etc. During this work less studied influences factors, such as flooding or ontogeny of the plant were investigated. We could conclude that root anoxia, a stress factor that occurs among others in the surroundings areas of the Amazon rivers when the water rises after the rain period, affect not only the emission of oxygenated compounds as ethanol and acetaldehyde, but also isoprenoid emissions. Data show that at the beginning of root anoxia due to inundation, emissions of ethanol and acetaldehyde can be detected, especially in less anoxia adapted species such as *Vatairea guianensis*. The better adapted species *Hevea spruceana* could probably directly remetabolize the formed ethanol, without emitting it. After long periods of inundation a total adaptation to flooding could be assumed in those cases where no ethanol or/and acetaldehyde emissions were detected any more. On the other hand, isoprenoid emissions can shortly increase after some days of flooding but then after a long period of waterlogging emissions tend to decrease considerably, as well as photosynthesis, transpiration and stomatal conductance.

Ontogenetic studies were performed with the well characterized Mediterranean species, providing clear evidence of the differences on standard emission factors of young and mature leaves. Standard emission factors of isoprene were lower in young leaves than in mature leaves. Contrasting, higher monoterpene and sesquiterpene emissions were found in case of several plant species, indicating a role for these VOC species as defense compounds against herbivores or pathogens during the youth period. Monoterpene and sesquiterpene species composition varied also in some cases with maturity. On the other hand, methanol emissions were, as expected, higher in young leaves than in mature leaves, corroborating the theory that methanol is the product of demethylation of pectine by cell wall elongation. These changes in emission capacity of vegetation can be accounted for in recent model estimates (Guenther *et al.*, 2006).

A better understanding is required on interactions between vegetation and atmosphere. To obtain an estimate of this interaction, it is necessary that future experiments provide a more complete carbon budget for each ecosystem. In particular, the primary objective is to identify

emissions of extra carbon emitted in form of BVOCs as several experiments have shown a certain amount of “missing carbon” that should be responsible for the unexpected high concentrations of OH radicals (Di Carlo *et al.*, 2004; Kuhn *et al.*, 2007; Lelieveld *et al.*, 2008). Secondly, long term BVOCs measurements should be performed in order to explore seasonality effects. Until now prediction models are based on data of a few plant species or punctual areas (tower measurements). The characterization of VOC emissions from representative species from the different ecosystems and areas will support our understanding of emission processes and regulation. In order to perform reliable prediction models, experiments should comprise a high representative number of plant species and the exact geographical distribution should be known, even though, the access to some areas, e. g. in the tropics, makes this task difficult. As shown in this work a variety of stress factors, as well as several biotic and abiotic factors can influence BVOCs emissions. Such factors can influence and vary standard emission rates in several orders of magnitude influencing the emission pattern of the plant. If such factors are not taken into account in prediction models, high errors can be expected, particularly on a regional scale.

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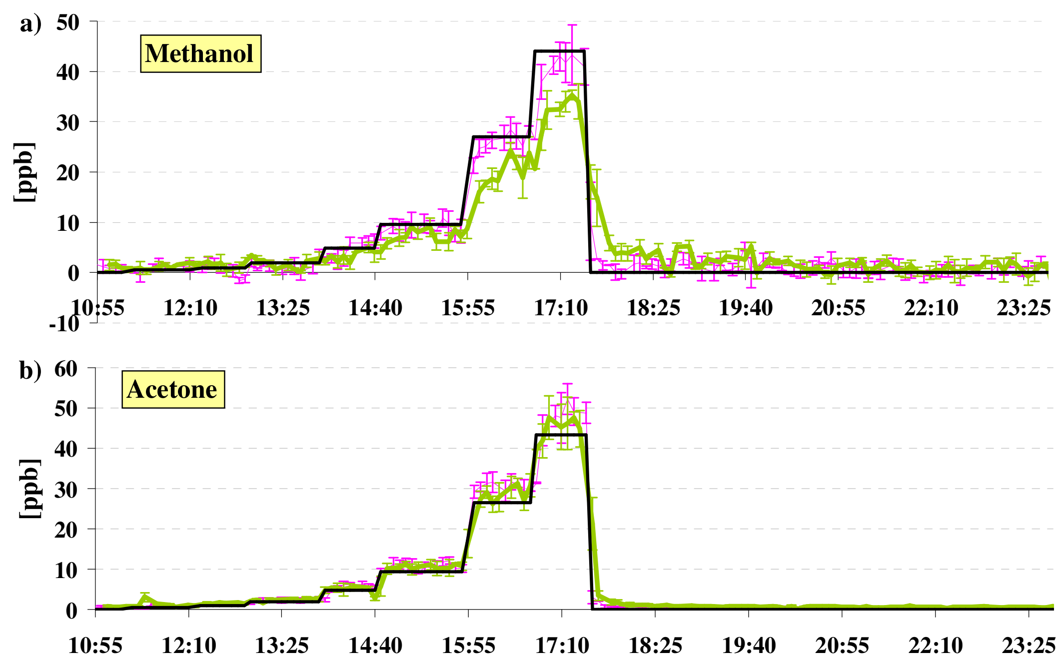
Appendix 1: Validation of GFS-3000 for VOC measurements

Considerable uncertainties are present in the estimates of biogenic emission rates of different VOCs mainly attributable to the sampling and analytical procedures used in the emission rate measurements. VOC are in general very reactive compounds that in contact with certain materials can be exerting to adsorptive processes or catalytic reactions. In order to avoid these types of reactions or absorptions inner materials are needed to performed VOC measurements. Teflon is one of the materials commonly used in enclosure systems for this task, but also silicosteel or glass are often utilized. During the experiments performed in the botanical garden of Kiel, Germany, a new enclosure system, the GFS-3000, performed by the company Walz GmbH was used for VOC measurements. This system was developed for physiology measurements but we adapted it for the VOC measurements. The material used to construct this enclosure was not completely defined, having parts of glass, but also aluminium, Teflon covered tubes and silicon tubes. For this reason the uncertainties provoked by this system were tested flushing through the empty cuvette a standard gas mixture (Deuste GmbH, Germany) containing compounds that are present in the emissions of the experimental plant species like isoprene, alpha-pinene, methanol and acetone diluted with synthetic air to different concentrations (0; ~0.5; ~1; ~2; ~5; ~10; ~25 and ~43 ppb ± 10 uncertainty). The concentrations of the tested VOCs were measured in the inflowing and outflowing air with the online technique PTR-MS. The instrument was operated in selected ion-monitoring mode at standard operational settings (E/N = 130 Td; E electric field strength, N buffer gas number density, 1 Td = 10^{-17} cm^2 V $molecule^{-1}$) by a drift tube pressure of 2.1 mbar and a drift tube voltage of 600 V, measuring a part of the inflowing and the outflowing air for seven cycles respectively (dwell time of 1s per mass; each cycle took ~16s). Each of the seven measurement cycles were interspersed with three cycles of instrumental background measurements by passing a part of the inflowing air over a heated platinum catalyst maintained at 350 °C (Parker co., USA). This measurement sequence was repeated during at least 40 minutes by each concentration from the moment that the signal was constant. The background signals were subtracted from the sample and reference air measurements.

Since diffusion and permeation processes are temperature dependent, the standard temperature used during experiments performed with plants (30°C) was set in the enclosure for this study.

One way Anova tests were carried out in order to detect possible significant differences between the inflow and outflow air. The differences found were confirmed with Tukey's post hoc test.

Figure A.1 shows the VOCs concentration (in ppb) measured before and after the enclosure in comparison with the concentration expected from the standard gas. The inflow air reflected the expected standard gas concentration in all four investigated VOCs. In the case of acetone and isoprene no significant differences were observed between the concentration of the inlet and the outlet air, whereas methanol and alpha-pinene concentrations in the outlet air was significantly lower than in the inlet air (Anova test confirmed by Tukey test: There was a significant difference by a methanol concentration of 27 and 44 ppb with $p<0.001$ and by an alpha-pinene concentration of 1,9 ppb with a $p<0.05$ and of 4.7; 9.3; 26.4 and 43.1 ppb with $p<0.001$). Differences of 25 % and 34 % in methanol and alpha pinene have been detected. A stark memory effect was observed by these two compounds that were still realised from the system during several hours after stopping the standard gas supply. On the other hand, it is important to remark that in all measured compounds the detection limit was increased by measuring with this enclosure system, to 1.5; 4.3; 1 and 2.3 ppb for acetone, methanol, isoprene and alpha-pinene, respectively.



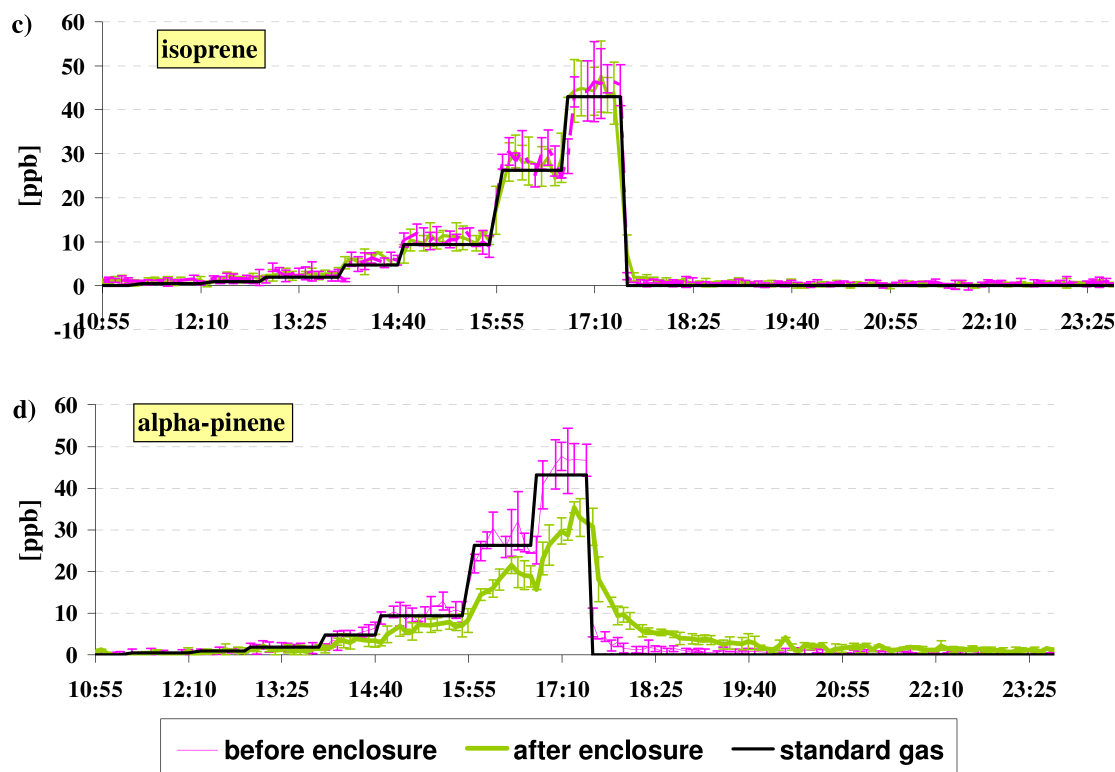


Figure A.1 a)-d) GFS-3000 surface material test. Each data point represent a mean value of five minutes (n=8) ± standard deviation.

Possible unknown compounds emitted by the system measured at the same mass of acetone, methanol, isoprene and alpha-pinene could be the cause of these high detection limits. Furthermore, as mentioned above, the zero-air signals of the PTR-MS determined empirically by the catalytic converter were relatively high, their origin is not well explained up to now and they show considerable variability. This background was measured every 5 minutes, but it still leaves significant uncertainty that might affect the measurement of low concentrations in particular (Ammann *et al.*, 2004).

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Given are the p-values indicating level of significance at 5% (0.05, *) and not significant (n.s.) differences.

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Figure 2.2.1.1 Satellite image of the Reserva Ducke Copyright © 2002-2009 authorSTREAM

Figure 2.2.1.2 Satellite image of the Ilha da Marchantaria and the Tarumã-mirim

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[2] http://www.ptrms.com/technology/technology.html

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Figure 1

Comparison of total VOC composition in % emitted from young (y) and mature (m) leaves of 9 Mediterranean plant species (sp.): (Bs) *Buxus sempervirens*, (Cs) *Ceratonia siliqua*, (Ca) *Cistus albidus*, (Cg) *Coronilla valentina,* (Oe) *Olea europea*, (Ph) *Pinus halepensis*, (Pp) *Prunus persica*, (Ro) *Rosmarinus officinalis*, (Sj) *Spartium junceum.* Standard error bars are given.

Chapter 5

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Apendix 1

Figure A.1 a)-d) GFS-3000 surface material test. Each data point represent a mean value of five minutes (n=8) ± standard deviation.

Appendix 4: List of abbreviations

AMU: Atomic Mass per Unit

BVOC: Biogenic Volatile Organic Compound

CEFE-CNRS: Centre d'Ecologie Fonctionnelle et Evolutive – Centre National de Recherche Scientifique

CNR: Consiglio Nazionale delle Ricerche

DMAPP: Dimethylallyl Pyrophosphate

$E_{s:}$ Standard Emission Factor

FPP: Farnesyldiphosphate

GC-FID: Gas Chromatograph with Flame ionization detector

GC-MS: Gas Chromatograph with Mass Spectrometer

GFS: Gas Exchange Fluorescence System

GGPP: Geranylgeranyl Diphosphate

GPP: Geranyldiphosphate

HCN: Hydrogen Cyanide

INPA: Instituto Nacional de Pesquisas da Amazônia

IPCC: The Intergovernmental Panel on Climate Change

IPP: Isopentenyl Pyrophosphate

IspS : Isoprene Synthase

LCP: Light Compensation Point

LGM: Last Glacial Maximum

LSP: Light Saturation Point

MBO: 2-Methyl-3-butene-2-ol

MeJA: Methyl Jasmonate

MEK: Methyl Ethyl Ketone

MEP: Methyl Erythritol Phosphate

MeSA: Methyl Salicylate

MPAN: Methylperoxyacetylnitrat

MPI: Max Planck Institute

NBP: Net Biome Productivity

NEP: Net Ecosystem Productivity

NMVOCs: Non-Metane VOC

ORCHIDEE: ORganizing Carbon and Hydrology In Dynamic EcosystEms

PAN: Peroxyacetyl Nitrate

PAR: Photosynthetic Active Radiation

PME: Pectin Methyl Esterases

PTR-MS: Proton Transfer Reaction-Mass Spectrometer

RH: Relative Humidity

ROS: Reactive Oxygen Species

SD: Standard Deviation

SEM: Secondary Electron Multiplayer

SLA: Specific Leaf Area

TCA: Citric Acid Cycle

VOC: Volatile Organic Compounds

WUE: Water Use Efficiency

Appendix 5: Curriculum Vitae

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RESEARCH EXPERIENCE

From February 2005 to present

From 2005 I am PhD student in the Biogeochemistry department of the Max Planck Institute for atmospheric chemistry (**MPI-CH**) under supervision of Prof. Dr. Jürgen Kesselmeier. I am working in the Plant Physiology Group, which develops methods for the assessment of biosphere atmosphere interaction. I performed Volatile Organic Compound (VOC) analysis using mass spectrometry and gas chromatography methods. I realized screening of tropical and Mediterranean vegetation, taking part in international cooperations with the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil and the Centre National de la Recherche Scientifique (CNRS) – Centre d'Ecologie Fonctionelle et Evolutive in Montpellier, France. I developed an enclosure system to analyze primary emissions of VOC, CO_2 and H_2O exchange at leaf level under controlled conditions. As a part of my job I realized logistic organization for scientific equipment's transport and experiment set up in field campaigns, which has improved my management skills. Teaching was also a duty that I performed supervising students during laboratory practical work. My communication skills in different languages (English, German, Portuguese, Italian, French and Spanish) were developed i.a. by the participation in several international conference events and the cooperation work.

In 2004 I realized my diploma thesis at the Max Planck Institute for Chemistry working on the physiological elements that controls VOC emissions in the plant.

From March 2000 to December 2001 I participated in a research project developing statistics of the occurrence of Parvovirus B19 and its antibodies in human blood. This project was performed at the Johannes Gutenberg – Universität Klinikum in Mainz, Germany. During this job I analyzed viral Nuclei acids via Polymerase Chain Reaction techniques (PCR) and Antibodies using ELISA-Tests.

ACADEMIC FORMATION

- From February 2005: PhD “Screening the exchange of Volatile organic compounds in central Amazonian Terra firme and floodplain forest” Group of plant physiology of the Biogeochemistry Department. Max Planck Institute for Chemistry. Mainz. Germany
- From February 2004 to February 2005: Diploma Thesis “The influence of the stomatal conductance on the emission of Isoprene and Monoterpenes” Group of plant physiology of the Biogeochemistry Department. Max Planck Institute for Chemistry. Mainz. Germany
- From October 1998 to February 2005 Study of Biology. Specialization on Microbiology, Immunology and Botanic. Johannes Gutenberg University of Mainz. Germany
- From June 1998 to August 1998 German course intermediate level, Inlingua, Wiesbaden, Germany
- From November 1997 to April 1998 German course basic level, Benedict Schule, Krefeld, Germany
- From September 1995 to June 1997 BETEC in information technology and communications skills, Deeside College, Chester, England
- From September 1995 to June 1997 Professional formation on international secretary skills in Academia Técnica, Santander, Spain
- From September 1994 to June 1995 C.O.U (Curso de orientación universitaria), Insitituto Sta. Clara, Santander, Spain

COURSES AND TRAINING

- Course of Introduction in the applied Statistic, Johannes Gutenberg University. Winter Semester 2007/08.
- Course of Atmospheric Chemistry, Johannes Gutenberg University. Winter Semester 2007/08.
- Course of Atmospheric Thermodynamic, Johannes Gutenberg University. Winter Semester 2007/08.
- Seminar of the project INPA/Max-Planck, Manaus, Brazil. March – May 2007: Oral Presentation: “Investigation on primary emission of VOCs from tropical vegetation from floodplain and terra firme areas”.
- Workshop for scientific publishing. Johannes Gutenberg University. November 10th 2007.
- Course of Adobe Photoshop cs2 software for windows. Systeme24 IT-Training, Mainz February 16th -17th 2006.
- Seminar: “Earth System Cycles”, Johannes Gutenberg University. Winter Semester 2005/06.
- English for scientist, Max Planck Institute for Chemistry, Mainz. Summer semester 2005 and winter semester 2005/06.
- Training Course of PTR-MS (Proton Transfer Reaction-Mass Spectrometry) at University Centre Obergurgle, Austria. February 3rd-5th 2005.
- Course of Atmospheric Chemistry methods, Max Planck Institute for Chemistry, Mainz, Germany. Summer Semester 2005.

- Course of „Modern Mass Spectrometry“, Prof. Klaus Blaum, Johannes Gutenberg University, Department of Physics, Mainz. Summer Semester 2005.
- Course of Project Management: “How to structure a PhD Project?”, Max-Planck für ausländisches und internationals Strafrecht, Freiburg. December 09th – 10th 2005.
- International Summer School organized by ISONET Marie Curie Research and Training Network and VOCBAS research network program of the European science foundation (ESF): “Biogenic Volatile Organic Compounds in the Plant-Environment Interaction” in Pieve Tesino (Trento, Italy) September 20th -24th 2004.
- Seminar of the Botanic department at the Johannes Gutenberg University of Mainz, Germany,
    - Winter Semester 2004/05, Oral Presentation: “influence of the stomatal conductance on isoprene and monoterpene emissions: Preliminary results”
    - Summer Semester 2005, Oral Presentation: “influence of the stomatal conductance on isoprene and monoterpene emissions: Final results”
    - Winter Semester 2007/08, Oral Presentation: “Investigation on primary emission of VOCs from tropical vegetation and flooding effects on VOCs emissions”

AWARDS

- European Scientific Foundation (ESF) scholarship April 2008 – July 2008 to support doctorate studies at the CNRS – CEFE in Montpellier, France.
- Outstanding mark for the diploma thesis, 2004: “Influence of stomatal conductance on the isoprene and monoterpene emissions”. Max Planck Institute for Chemistry Mainz, Germany

RELEVANT COMPUTER EXPERIENCE

- Specialized software for scientific graphing, data analysis, image processing and programming IGOR Pro 6.
- Specialized software for the utilization of Quadrupole Mass Spectrometer (Quadstar 32-bit). Aplication for PTR-MS (PTR-MS Workplace)
- Operative System MS-DOS
- Use of WEB browsers: Netscape Communicator, Internet Explorer.
- Applications of Microsoft Office (Word, Excel, Power Point, Outlook).

PUBLICATIONS

Bracho Nunez, A., Knothe N., Schebeske, G., Ciccioli P., Piedade Maria T.F., Kesselmeier J., "VOC Emissions from Amazonian tree species: Investigation of primary emission at leaf level" In preparation

Bracho Nunez, A., Welter, S., Schebeske, G., Staudt, M., Kesselmeier J., "Screening plant species for regional and global VOC budgets: a multi-method experiment to determine plant-specific emission factors" In preparation

Bracho Nunez, A., Welter, S., Staudt, M., Kesselmeier, J. "Plant specific volatile organic compound emission factors from young and mature leaves of Mediterranean vegetation", Submitted to Atmospheric Environment.

POSTER AND PRESENTATIONS IN CONGRESS

- Oral presentation at the ISONET-VOCBAS Summer School "Biogenic Volatile Organic Compounds in the Plant-Environment Interaction" at Pieve Tesino (Trento) Italy. October 20th – 24th 2004: "Increase of monoterpene emissions after artificially induced stomatal closure" **A. Bracho-Nunez**, G. Schebeske, J. Kesselmeier.

- Poster at General Assembly of the EGU (European geosciences union), April 24th -29th 2005: "Increase of monoterpene emissions after artificially induced stomatal closure" **A. Bracho-Nunez**, G. Schebeske, J. Kesselmeier.

- Oral presentation at the International Science Meeting of the ESF research networks VOCBAS and INTROP: "Biogenic Volatile Organic Compounds: Sources and fates in a changing world" October 02nd – 05th 2007: "Investigation of primary emission of VOCs from tropical floodplain and terra firme vegetation" **A. Bracho-Nunez**, N. Knothe, W. R. Costa, M.A. Liberato, G. Schebeske, P. Ciccioli, M.F.T. Piedade, J. Kesselmeier.

- Oral presentation at the International Scientific Conference of the LBA, GEOMA and PPBio: "Amazon in Perspective: Integrated Science for a Sustainable Future" November 17th -20th 2008. Manaus Amazonas Brazil: "Investigation of primary emission of VOCs from tropical floodplain and terra firme vegetation" **A. Bracho-Nunez**, N. Knothe, W. R. Costa, M.A. Liberato, G. Schebeske, P. Ciccioli, M.F.T. Piedade, J. Kesselmeier.

- Poster at General Assembly of the EGU (European geosciences union), April 20th – 24th 2009: "VOC Emissions from Amazonian tree species: Investigation of primary emission at leaf level" **A. Bracho-Nunez**, N. Knothe, W. R. Costa, M.A. Liberato, G. Schebeske, P. Ciccioli, M.F.T. Piedade, J. Kesselmeier.

- Poster at General Assembly of the EGU (European geosciences union), April 20th – 24th 2009: "Flooding effects on plant physiology and VOC emissions from Amazonian tree species from two different flooding environments: Varzea and Igapo" **A. Bracho-Nunez**, N. Knothe, W. R. Costa, M.A. Liberato, G. Schebeske, P. Ciccioli, M.F.T. Piedade, J. Kesselmeier.

- Poster at General Assembly of the EGU (European geosciences union), May 3th-7th 2010: "Plant specific volatile organic compound emission factors from young and mature leaves of Mediterranean vegetation". **A. Bracho Nunez**, S. Welter, M. Staudt, J. Kesselmeier.

- Poster at Gordon Research Conference 23th-28th May, "Revealing the role of interspecific gene flow in the diversification of isoprenoid emissions of Mediterranean oaks – a case study on *Quercus suber*, *Quercus canariensis*, and its putative hybridisation product *Quercus afares*" S. Welter, C. Mir, I. Zimmer, **A. Bracho Nunez**, J. Kesselmeier, R. Lumaret, J.-P. Schnitzler, M. Staudt.

TEACHING EXPERIENCE

Supervisor of the monthly FII practical course: "Exchange of trace gases between the atmosphere and the biosphere" February 2005, June 2005, November 2007, Max Planck Institute for Chemistry, Mainz, Germany.

PROFESIONAL EXPERIENCE

- Coordination of the administration and accountancy. Public relation Skills. Non governmental organization "Human Help Network". Mainz, Germany. May 1998 to February 2000.
- Spanish Teacher. School "Vamos", Frankfurt. May 1998 to November 1998
- Laboratory technical assistant. Johannes Gutenberg Universität Klinikum. Mainz Germany. March 2000 to November 2001