Landscape structure and plant age effects on terpenoid emissions from *Pinus ponderosa* and *Pteridium aquilinum*

by

Monica B. Madronich

B. Sc. Universidad Nacional Autónoma de México, 1998

M. Sc. Universidad Nacional Autónoma de México, 2003

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written by Monica Beatriz Madronich

Has been approved for the Department of Ecology and Evolutionary Biology

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Dr. Carol A. Wessman, Chair

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Dr. Alex B. Guenther

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Dr. Russell K. Monson

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Dr. William D. Bowman

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Dr. Kenneth E. Foote

Date:_____

The final copy of this thesis has been examined by the signatories, and we Find that both the content and the form meet acceptable presentation standards Of scholarly work in the above mentioned discipline.

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Landscape structure and plant age effects on terpenoid emissions from *Pinus ponderosa* and *Pteridium aquilinum*

Thesis directed by Professors Carol Wessman and Alex Guenther.

ABSTRACT

The interactions between the atmosphere and the biosphere have been studied to a great extent in the last decades; it cannot be denied that these systems are altering each other's processes. The production of terpenoid compounds by vegetation is an example of such interactions. Ecological and atmospheric scientists are interested in the production and emission of terpenoid compounds. However, they are studying them from substantially different points of view, driven largely by the scales at which they approach the process.

The aim of my dissertation was to study biogenic emissions and their effect on atmospheric chemistry using variables such as landscape structure, landscape configuration, and developmental stage; factors that have not been considered before from an atmospheric point of view. The main questions of my study were: (1) Does understory vegetation have an impact on regional emissions? (2) Does developmental stage affect terpenoid emissions? (3) Does the light environment in the forest affect the rate at which terpenoid compounds are emitted? To answer these questions, field studies were performed and terpenoid emissions were measured using branch enclosure measurements and GC-MS techniques.

The first question was assessed by using *Pteridium aquilinum*, one of the most abundant understory species. We determined that *Pteridium* was a terpenoid emitter,

but its emissions did not influence the regional atmospheric chemistry. Nevertheless, this study is a first step to consider the herbaceous layer when studying monoterpene emissions. The second question studied the difference in emissions of *Pinus ponderosa* seedlings and mature trees, finding a significant difference in the magnitude of the emissions but not in their chemical composition; emissions of seedlings were greater. In this case, this study is one of the first on the developmental stage and monoterpene emissions in a woody species. The third question, addressing the differences of emissions of sun and shaded branches of *Pinus ponderosa*, found a significant difference between the branches' emissions. The results of the different questions addressed in this work show that it is necessary to incorporate landscape component to have a better understanding of the production and effects of BVOC's compounds in the atmosphere.

I dedicate this work to my husband Sasha. Thank you for all your encouragement, love, and patience. I love you.

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Chapter 1 INTRODUCTION

This dissertation examines at the interface of atmospheric chemistry and plant and ecological sciences. It recognizes the role of vegetation and its structure on the landscape as potentially significant contributors to atmospheric chemical processes. In this introductory chapter, I review biogenic volatile organic compounds (BVOC), important and influential compounds in the atmosphere, and the biotic factors which influence the type and magnitude of their emissions.

Terpenoid compounds

Plants emit many compounds to the atmosphere including BVOC. Terpenes, an important chemical group of BVOCs, are valuable compounds for some organisms. They function as herbivore repellents, interfering with the herbivore life cycles, or attracting their predators (Fehsenfeld, Calvert 1992, Kesselmeier and Staudt 1999). They also serve as a warning mechanism between neighboring plants (Arimura, Kost 2005/5/15, Theis and Lerdau 2003, Adler and Karban 1994, Shiojiri and Karban 2006, Heil and Karban 2010). However, in addition to their roles in plant-insect interactions, terpenes (Figure 1) are also important in atmospheric chemistry (Hewitt 1999, Hewitt and Street 1992, Atkinson and Arey 2003, Monks, Granier 2009) due to their high fluxes and high aerosol and ozone yields (Fares, Goldstein 2010, Sharkey, Wiberley 2008, Loreto and Fares 2007, Monson and Holland 2001). They also have high reactivity caused by the presence of the double bonds in the molecules that lead to short lifetimes of those compounds in the atmosphere (Karl, Harren 2005, Guenther 2002, Harley, Fridd-Stroud 1998).

Factors that affect terpenoid emissions

Biology

The emissions of terpenes to the atmosphere are regulated by the biochemistry, genetics, and physiology of the plants. Not all plants emit terpenes and not all terpene emitters release the same compounds (Kesselmeier and Staudt 1999, Loreto, Bagnoli 2009, Lerdau and Gray 2003). There are two main pathways for terpene formation (Jux, Gleixner 2001, Vickers, Gershenzon 2009): the methylerythritol phosphate pathway (MEP pathway) and the mevalonic acid pathway (MVA pathway). Monoterpenes (C_{10}) and diterpenes (C_{20}) are made in the plastids by the MPE pathway, and sesquiterpenes are produced in the cytosol by the MVA pathway (Sharkey, Wiberley 2008, Ashour, Wink 2010, Dubey, Bhalla 2003). Once synthesized, terpenes can be stored in specialized structures such as trichomes, epidermal cells, secretory ducts, cavities and cells; or they can be released to the atmosphere, mostly by diffusion through cellular compartments. The capability of the plant to store or release the compound is regulated by genetic traits (Aros, Gonzalez 2012, Wu, Schalk 2006). Due the genetic variability, the amount of terpenes stored in a plant can vary from 1 - 3% up to 15 - 20% of dry mass (Penuelas and Llusia 2001, Llusia, Penuelas 2008, Peñuelas and Llusià 2001, Harley, Monson 1999). The amount plant biomass and the rate at which terpenoid compounds are produced are known to be controlled by the genetics and the developmental stage of the plant (Quintero and Bowers 2011). Terpene production is energetically costly for plants; terpenoids precursors are produced from glucose metabolism(Sell 2003). Therefore plants have to allocate their resources to grow or to produce terpenes (Shiojiri and Karban 2006, Gray, Lerdau 2003, Kim, Kim 2005).

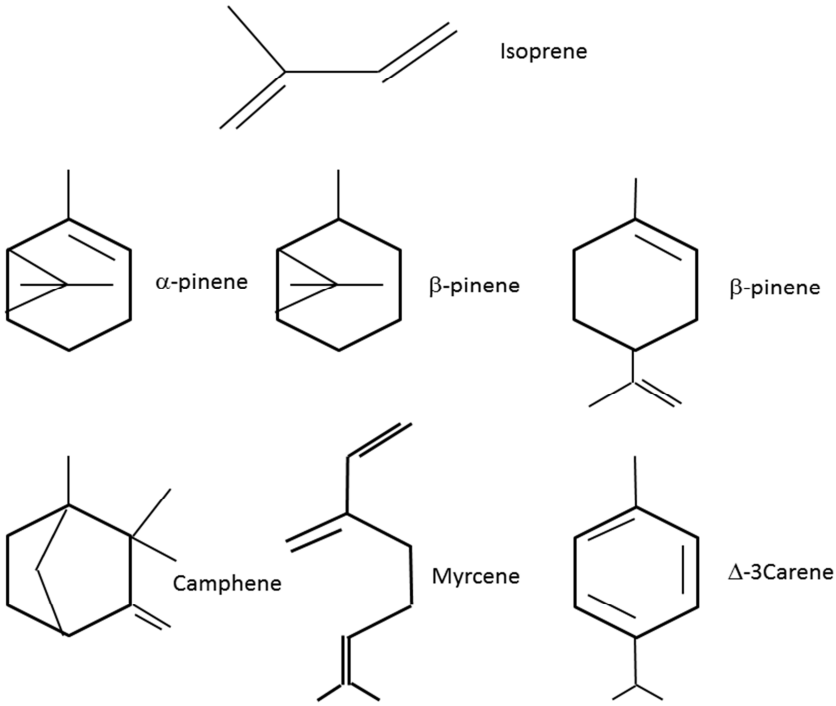

Figure 1.1 Chemical structure of isoprene and the major monoterpenes found in this study. Terpenes are defined as compounds with molecular structures containing carbon backbones made up of isoprene (2-methyl-1,3-butadiene) units (Seinfeld, J. H. and Pandis, S. 1998; Loreto, Bagnoli and Fineschi 2009).

Environmental factors and species composition

Environmental factors like photosynthetically active radiation (PAR) and temperature play an important role in terpenoid compounds emissions (Gray, Lerdau 2003, Guenther, Monson 1991, Guenther, Zimmerman 1993, Penuelas, Llusia 2005, Rinne, Guenther 2002). Isoprene and sequiterpenes emissions are triggered by increases in light intensity and leaf temperature, while monoterpene emissions increase with temperature but not always with light levels. Chemical composition of biogenic emissions is, to some extent, controlled by the species composition patterns of the vegetation (Sell 2003, Greenberg, Guenther 2004, Helmig, Vierling 1999, Lerdau,

Litvak 1997, Guenther, Greenberg 1996, Harrewijn, van Oosten 2001, Loreto, Barta 2006). If species abundances in the landscape change, the amounts and composition of the BVOC will also vary (Greenberg, Guenther 2004, Helmig, Vierling 1999, Latta 2004). Consequently, the widespread change in land cover that we see today may have a significant influence on atmospheric processes; for example, it has been pointed out that the proliferation of forest plantations with major isoprenoid emitters like Poplar and Eucalyptus can change the regional emissions budget (Gasche, Papen 2002, Guenther, Karl 2006, Peñuelas, Filella 2009, Guenther and Hills 1998).

Plants require nutrients, water, and solar radiation to survive. The excess or deficit of those factors as well as herbivore attacks will trigger stress responses by the plants. Terpenoid compounds are known to be a measure of the plant's stress (Vickers, Gershenzon 2009, Velikova, Fares 2008, Vitale, Salvatori 2008). Therefore any variation in these abiotic factors will vary the amount of terpenoids emitted to the atmosphere.

Landscape influence

The distribution of vegetation in the landscape controls the distribution and chemical composition of terpenes. As mentioned above, terpenoid emissions will depend on the biology of the plant. Therefore, if species abundances in the landscape change, the amounts and composition of terpenoid compounds will also vary.

Environmental factors are also controlled by landscape structure, specifically solar radiation and temperature. Solar radiation is attenuated with canopy depth (Stroud, Makar 2005) and temperature is intimately linked to incident radiation. Therefore,

temperature and light will differ in different parts of the forest (Jarvis, Stauch 2004), affecting the rates at which monoterpenes emissions are produced.

Implication of terpenoid emissions on atmospheric chemistry

BVOC's are a large component of the troposphere; globally, the most dominant BVOC species is isoprene, followed closely by terpenes (Table 1.1). Together, global isoprene and terpene emissions are higher than fossil fuel emissions, and of a similar magnitude to natural and anthropogenic emissions of methane.

Species	Estimated annual global emissions (TgC)
Anthropogenic[1]	**174**
Vegetation[2]	**990**
A. Terpenes	**130**
B. Isoprene	**600**
C. Other reactive BVOC	**260**

Table 1.1 Global emissions of volatile organic compounds. 1 IPCC,2005, 2Guenther et al.1995, Guenther et al. 2006

The influence of BVOC on atmospheric processes relies on their interaction with anthropogenic emissions. They contribute to the production of tropospheric ozone and secondary organic aerosols (SOA).

Ozone chemistry

Tropospheric ozone (O_3) is recognized for altering the air quality and its damaging effects on human health and agriculture (Fares, Goldstein 2010, Finlayson-Pitts and Pitts 1997). Ozone is formed when nitrogen dioxide (NO_2) is photo-dissociated by sunlight and the liberated oxygen atom adds to molecular oxygen:

$NO_2 + O_2 +$ Sunlight $\rightarrow$ $NO + O(^1D)$ (1)

$O + O_2 \rightarrow O_3$ (2)

In a reverse process, NO reacts rapidly with O_3 and destroys it continuously, even in the dark, to make NO_2 and O_2.

$NO + O_3 \rightarrow NO_2 + O_2$ (3)

BVOC's affect the balance of ozone chemistry by competing for NO by creating products (RO_2) that react with NO producing NO_2 which reduces the sink for O_3.

BVOC + OH $\rightarrow$ Organic peroxy radicals (RO_2)

$RO_2 + NO \rightarrow RO- + NO_2$

$NO_2 + O2 +$ Sunlight $\rightarrow O_3 + NO$

In summary in presence of anthropogenic emissions (NOx), terpenes contribute to ozone formation. Terpenes can also directly react with O_3 and in regions with low NO levels they can result in net ozone destruction.

Secondary organic aerosol

Ozone is not the only product of terpenes being generated in the atmosphere. It is known that oxidation of terpenes and its subsequent condensation produces aerosols (Fuzzi, Andreae 2006, Seinfeld, J. H. and Pandis, S. 1998). Hallquist et al. (Hallquist, Wenger 2009) estimated that 58% of the aerosol yield is secondary organic aerosols

produced by BVOC's. Aerosols affect the Earth system in different ways; they affect the amount of solar radiation that ecosystems receive by scattering or absorbing the energy (Monks, Granier 2009, Hallquist, Wenger 2009). Due to its implication in the energy budget, aerosols play a central role in climate (Jacobson, Hansson 2000, Goldstein and Galbally 2007). Aerosols also have an effect on human health, primarily related to the respiratory system (Lippmann 1993).

Canopy and understory emissions

The emissions measurement data that form the basis for current atmospheric models come mostly from trees in forested areas in the Northern Hemisphere (Guenther, Karl 2006, Geron, Owen 2006/3, Karl, Guenther 2003, Geron, Harley 2001/7, Geron, Rasmussen 2000), or cultivated areas where emissions can potentially affect the regional chemistry (Karl, Harren 2005, Bai, Baker 2006/9). Little or no attention has been paid to understory or smaller woody species despite their significant presence and role in many forest ecosystems (Gilliam and Roberts 2003). For example, some ferns have been shown to be significant isoprene emitters. Geron et al. found that the isoprene emission rate of *Cyclosorus parasiticus* was 284 μgC $g_{DW}^{-1}h^{-1}$ (Geron, Owen 2006/3), which is larger than emissions from *Quercus robur* that ranged from 43 to 73 μgC g_{DW}^{-1} h^{-1}(Perez-Rial, Penuelas 2009b); members of the genus *Quercus* are known to be high terpenoid emitters. Ferns are commonly found in almost every ecosystem; the fact that they reproduce by spores and have the ability to disperse long distances has made the ferns one of the more prolific living plants in the world (Barrington 1993).

Dissertation outline

The aim of my dissertation was to study the influence of variables such as landscape structure, landscape configuration, and developmental stage on biogenic emissions, factors that have not been considered before from an atmospheric point of view. I studied terpenoid emissions from three different perspectives. In chapter two, I studied the emissions from *Pteridium aquilinum*, an understory species. Research and modeling of BVOC emissions has typically neglected understory vegetation in ecosystems. In chapter three, I determined that the developmental stage affects terpenoid emissions, by studying seedlings and mature *Pinus ponderosa* trees. There are few studies considering tree age and terpenoid emissions. Lastly, in chapter 4 I studied the effects of light environment in branches of Ponderosa pine; this study is a first step to try to incorporate landscape structure as a factor to improve atmospheric models.

Chapter 2 MONOTERPENE EMISSIONS FROM AN UNDERSTORY SPECIES *Pteridium aquilinum*

Abstract

Monoterpene emissions from the dominant understory species *Pteridium aquilinum* (Bracken fern) in a mixed temperate forest were measured in the field during the summers of 2006, 2007 and 2008. Bracken fern emitted monoterpenes at different rates in the understory and in open areas. Understory plants emitted monoterpene levels ranging from 0.002 to 13 μgC g dw^{-1} h^{-1}, with an average emission of 1.6 ± 0.4 μgC g dw^{-1} h^{-1}. Open area plants emitted monoterpene levels ranging from 0.0 05 to 2.21 μgC g dw^{-1} h^{-1} with an average emission of 0.4 ± 0.07 μgC g dw^{-1} h^{-1}.. During the summer of 2008 greenhouse studies were performed to complement the field studies. Only 3% of the greenhouse Bracken fern plants emitted substantial amounts of monoterpenes. The average emission, 0.15 μgC g dw^{-1} h^{-1}± 0.9 m μgC g dw^{-1} h^{-1}, was much lower than that observed in the field. The factors controlling monoterpene emissions are not clear, but this study provides evidence of the potential importance of understory vegetation to ecosystem total hydrocarbon emissions and emphasizes the need for longer-term field studies.

Introduction

Studies of biogenic volatile organic compounds (BVOCs) from vegetation have focused on emissions of tree species that dominate the landscapes of some terrestrial biomes.

Understory vegetation has been neglected by these studies and its contribution to regional BVOC emissions is unknown.

Within the BVOC's, monoterpenes (MT - $C_{10}H_{16}$) stand out as a class due to their various ecological and atmospheric roles (Loreto, Bagnoli 2009, Fuentes, Lerdau 2000). Ecologically, monoterpenes are known to deter herbivores and/or attract their predators. These compounds are also known to serve as signaling compounds to attract pollinators (Sell 2003, Brilli, Ciccioli 2009, Theis 2006). From the atmospheric point of view, monoterpenes influence atmospheric composition through their role as precursors of ozone and aerosol formation (Hewitt 1999, Finlayson-Pitts and Pitts 2000). Monoterpenes can be stored in specialized structures in the plant; the amount of terpenes stored in a plant can vary from 1 - 3% up to 15 - 20% of dry mass (Penuelas and Llusia 2001). Monoterpenes are released to the atmosphere mostly by diffusion through cellular compartments and, thus, temperature plays an important role in monoterpene emissions because of its influence on vapor pressure and diffusion processes. As temperature increases, emissions exponentially increase. However, while some monoterpene emissions depend only on temperature, other monoterpene emissions are triggered by photosynthetically active radiation (PAR) as well (Kesselmeier and Staudt 1999, Ortega, Helmig 2008). Other factors that affect monoterpene emissions and storage in plants are herbivore attacks, drought, soil composition, and atmospheric CO_2 concentration among others (Baker, Bai 2005/1, Rapparini, Baraldi 2001, Staudt, Bertin 2000, Litvak and Monson 1998a)

PAR and temperature are normally the main drivers of monoterpene emissions from vegetation; therefore the plant's physical location plays an important role in

determining emission magnitudes. The location, specifically whether a plant is in the understory or in an open area, will determine the temperature and light conditions that may promote monoterpene emissions. *Pteridium aquilinum* (Bracken fern) is a useful example of a common understory species. This organism is one of the most widespread plants in the northern hemisphere (Moran 2004), and can also be found in some southern hemisphere regions. Bracken fern is an herbaceous perennial that grows in both open and understory environments, usually in deciduous forests (Gilliam and Roberts 2003, Atkinson 1989, Roberts and Gilliam 1995, Royo and Carson 2006). Studies of the chemical composition of Bracken fern fronds have shown that the leaves contain terpenoid compounds and, therefore, are a potential source of atmospheric hydrocarbons (Jones, Firn 1991). Despite its broad distribution, bracken fern's potential emissions and their unknown impact on atmospheric chemistry have been neglected by atmospheric chemists. The broad range of light environments in which Bracken fern will grow suggests that monoterpene emissions, if present, will be variable. If environmental variables like PAR and temperature elicit the emission of monoterpenes, it is expected that open areas will produce more emissions than understory areas.

The objectives of this work were to determine (1) if *Pteridium aquilinum* emits monoterpenes, (2) factors, including landscape configuration, that trigger any detected emissions, and (3) if detected, are emissions of this understory species substantial enough to influence atmospheric chemistry.

Methods

Study sites

The study took place at the University of Michigan Biological Station (UMBS, 45° 32' 59.9" N, 84° 39' 36" W, 238m elevation) and the National Center for Atmospheric Research (NCAR) greenhouse in Boulder, Colorado (40° 2' 6" N, 105° 14' 35" W, 1625m elevation). The study was divided into three phases; the first two phases were field measurements completed at the UMBS during the summers of 2006 and 2007 and the last phase was a greenhouse study completed at NCAR during the summer of 2008.

Sampling and analytical techniques

The emissions of terpenes from the Bracken fern foliage were determined using a dynamic plant enclosure system (Ortega and Helmig 2008) as shown in Figure 2.1. This system consists of a 20.5 L teflon bag made by Welch fluorocarbon (Dover, New Hampshire). The bag was supported by a plastic frame which prevented damage to the fronds. Since rhizomes are found in deeper soil, the frame base was buried at a depth of 10 cm 15 days before beginning the measurements in order to prevent rhizome emissions from affecting the measurements. Leaf litter and/or other vegetation were removed from the base of each plant to avoid any non-fern terpenoid emissions from litter. Although bare ground has little or no emissions (Schade and Goldstein 2001), potential contributions from soil were minimized by placing a teflon sheet between the enclosure base and the frame. The enclosure was put in place 2-3 hours prior the first measurement, and was connected to two pumps operated by 12 V marine batteries. Ambient air was pumped into the enclosure at a rate of 3 L/min giving a mean residence time of 6.83 minutes. Ozone was removed from the sweep air using a glass-fiber filter

impregnated with sodium thiosulfate ($Na_2S_2O_3$) (Helmig 1997, Pollmann, Ortega 2005), and ambient VOCs were removed using a charcoal trap. Monoterpene emissions were collected on Volatile Collector Traps (VCT, ARS Inc., Gainesville, FL) which are glass tubes filled with Super Q adsorbent (Alltech). Air was pulled from the enclosure through the VCT at a rate of 200 ml/min for 3 hours giving a sample volume of 36 L. After each measurement, fronds were cut, and leaf area and dry weight were measured to express the emissions by dry weight.

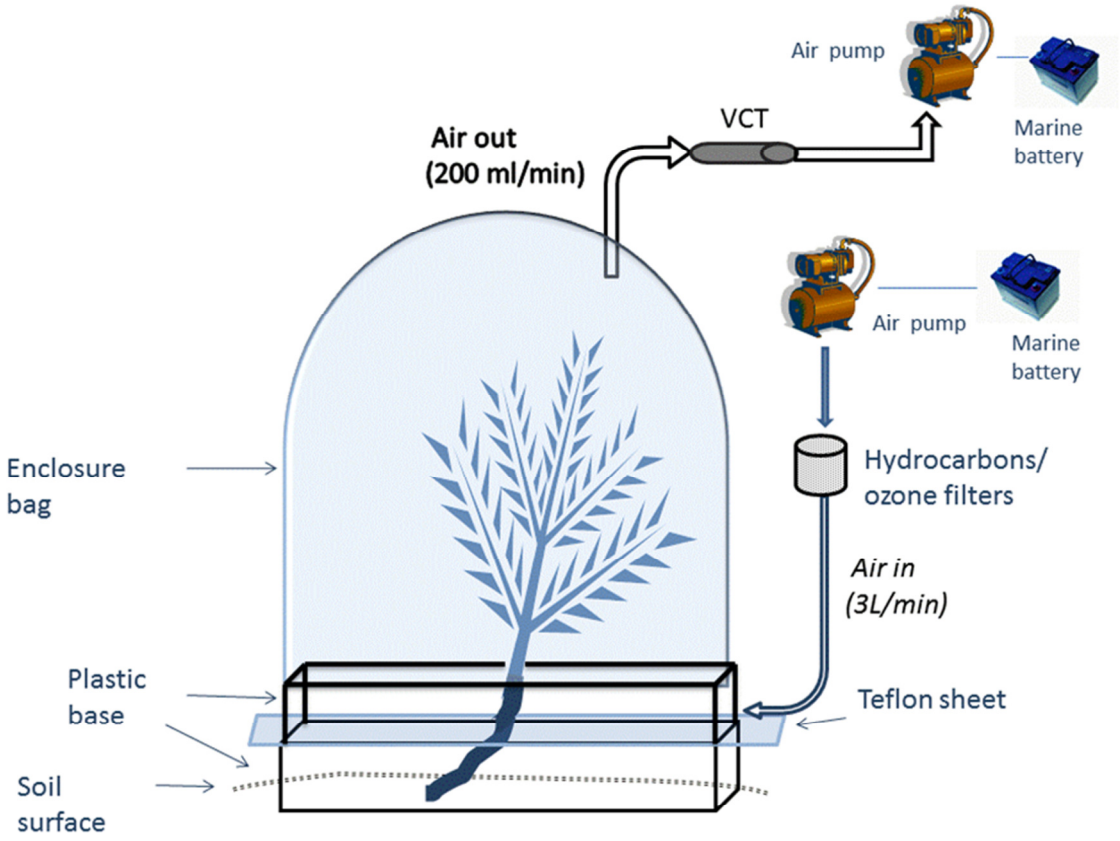

Figure 2.1 Dynamic bag enclosure. This portable system was used to obtain Bracken fern emissions. Bracken emissions were adsorbed into the VCT and posteriorly desorbed and analyzed with a GC-FID system.

PAR measurements were made using sensors (LI-COR Quantum Sensors) placed outside the enclosure. Leaf temperature was measured with a probe that was placed inside the bag (LCD External Temperature Probe, L-TMB-M002, Onset Computer Corporation, Bourne, MA). Ambient temperature was measured with a second sensor (HOBO U12, Temp/RH Probe, Onset Computer Corporation, Bourne, MA). PAR and temperature sensors were connected to the HOBO U-12 data logger and measurements were taken every minute during the measurement period.

Monoterpenes were extracted from the VCT using the technique and same parameters described by Matsunaga et al. (Matsunaga, Guenther 2009) using 2ml of dichloromethane (DCM) and 3ml of hexanes (Hexanes, Sigma-Aldrich). Details on the efficiency of the VCT technique have been published by Matsunaga et al. (2009). The conditions and procedures of the desorption were similar as the ones used by Matsunaga. The extractions were placed into 5ml glass vials and stored in a freezer (-5 centigrades) prior the chemical analysis. A internal standard, (10 µl, 20 ppm) of Dodecane (Sigma-Aldrich) was added to the extraction. A 3 µl aliquot of the extract was injected into a gas chromatograph (SRI Model 310, SRI instruments, Menlo Park, CA). The injection port was built into the system and it was kept at 200˚C. The gas chromatograph was coupled to a flame ionization detector and was equipped with a low polarity column (Restek, MXT®-5, 5% diphenyl and 95% dimethyl polisiloxane, 30m, 0.53mm ID, stainless steel, Restek Corporation, Bellephonte, PA). Helium was used as a carrier (10 cm/min). Data were recorded and analyzed using the Peak Simple Chromatography Data System software from SRI. Compound identification was performed by comparing the retention times of the field samples with the retention

times of several monoterpene standards as well as n-alkanes (including β-pinene, α–pinene,limonene and C6-C12 alkanes). Isoprene was not measured because its retention time was within the injection peak of the solvents (DCM and Hexane) and could not be separated.

Study phases

The first phase (UMBS, August 2006) focused on whether Bracken fern emitted monoterpenes. We measured 4 plants in an open area during two consecutive days, 2 plants/day. The measurements were completed through the day over a course of 3 hours for a total period of 9-12 hours; 3-4 samples total.

The second phase objectives (UMBS, summer 2007) were to examine factors triggering monoterpene emissions from Bracken fern and to determine if the landscape configuration, i.e. open areas and understory, influenced terpenoid emissions. The distribution of Bracken fern in open and understory areas at UMBS provided an opportunity to investigate plants exposed to very different light and temperature environments. Four 10mX10m plots were established, two plots were located in open areas and two in understory areas. Fifteen plants within each plot were randomly selected for measurements; not all the plants were measured. Monoterpene emission rates were measured from a total of 40 *P. aquilinum* plants. Two plants were measured the same time each day; one in the open and one in the understory area. Emissions were measured through the day over a course of 3 hours for a total period of 9 hours. The goal was to have 3 measurements/plant/day, but some days samples were lost due to power failure. The results of this phase were used to design the study's third phase.

The third phase (summer 2008) examined factors that elicited the emissions observed in the field. To reduce variability associated with genetic factors, 150 rhizomes of Bracken fern were collected at the UMBS field site and transported to NCAR's greenhouse. Bracken reproduces asexually via rhizomes to produce clones (KLEKOWSKI 2003). Once the plants matured, monoterpene emissions were measured under controlled PAR and temperature (25-28˚C and 500-750 μmol/m^2*s). These measurements were made using the same techniques and sensors used in the field.

Data analysis

The data were analyzed using SAS software 9.2 (SAS Institute, Cary, NC). Regression analysis and analysis of variance (ANOVA) used a 95% confidence interval.

Results

2006 Campaign

Survey field measurements

The survey phase in 2006 included 16 measurements from 4 plants (Fig. 2.2). Average total monoterpene emissions were 2.48 ±0.31 μgC•g_{dw}^{-1}•h^{-1}, and the minimum and maximum values were 0.41 μgC•g_{dw}^{-1}•h^{-1} and 3.93 μgC•g_{dw}^{-1}•h^{-1}, respectively. The emissions were associated with an average PAR of 1332 μmol/m^2*s and temperature of 34.1 ^oC.

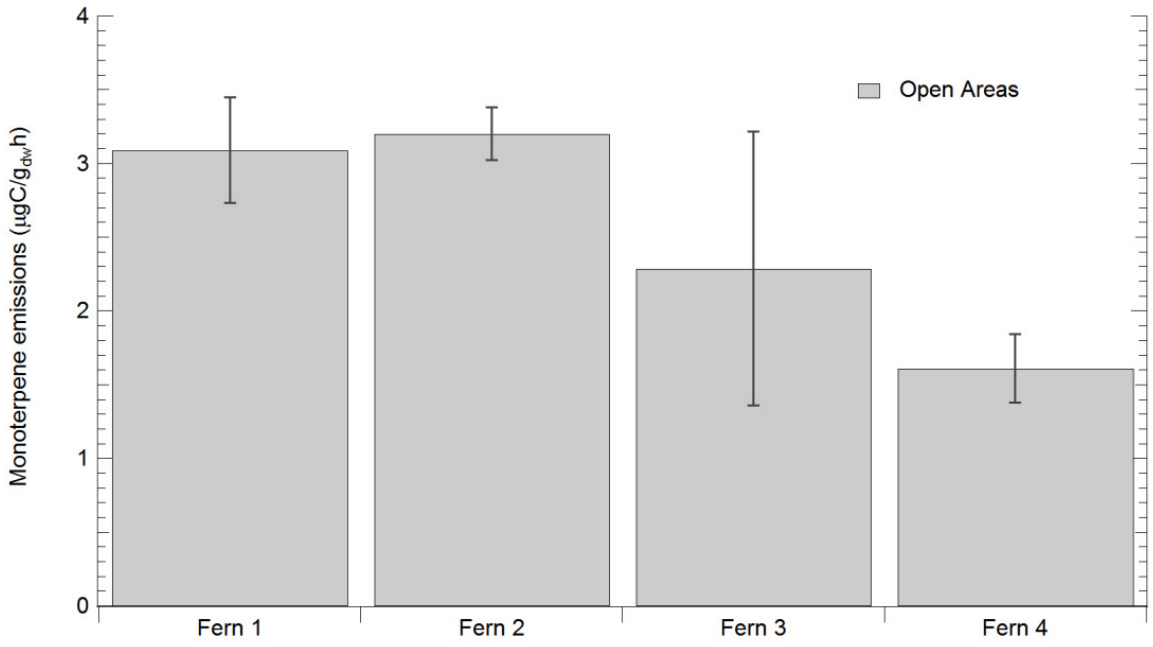

Figure 2.2 Average monoterpene emissions of Pteridium aquilinum plants during Summer 2006. All plants were located in open areas. Whisker length=Standard error of the mean.

2007 Campaign

Monoterpene variability

The 2007 campaign (early June-late August) measurements were made at irregular intervals during that time period. Bracken fronds were just expanding when the first measurements were taken and were senescing during the final measurements. Plants measured on the same day were in the same developmental stage. To facilitate understanding of emission magnitudes during the field campaign, we considered 3 periods: Early Summer (June), Mid-Summer (July), Late Summer (August). Figure 2.3 and Table 2.1 show the fern emissions during those periods as well as the averaged PAR

and temperature. The magnitude of the emissions decreased over the summer.

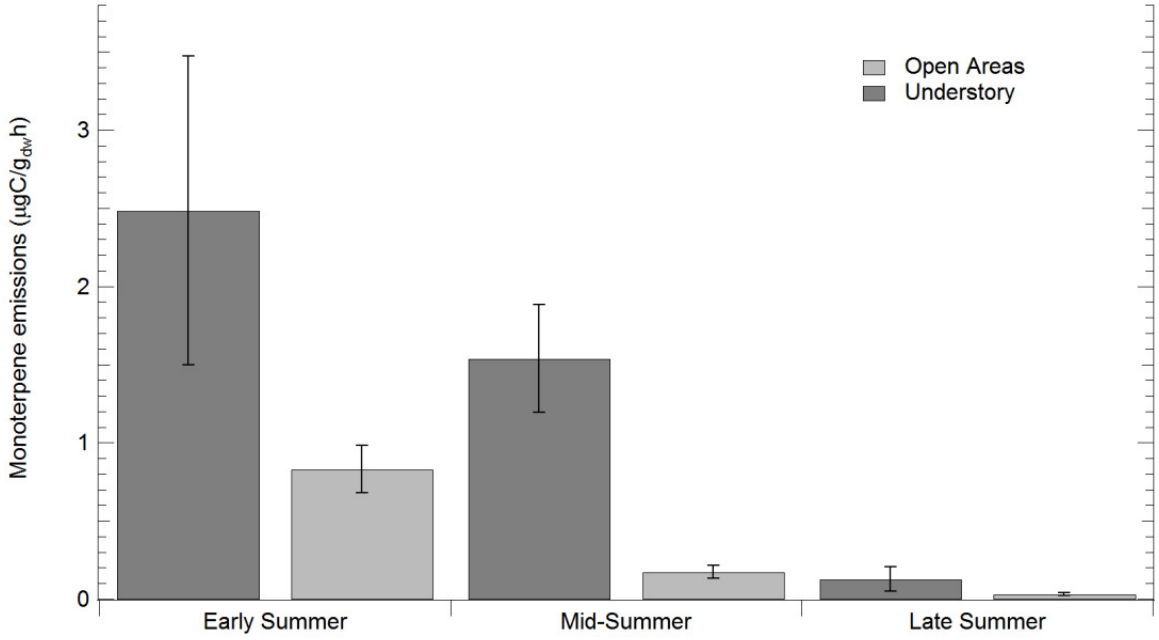

Figure 2.3. Comparison of average monoterpene emissions of *Pteridium aquilinum* plants in open and understory areas during early, mid and late summer periods. Summer 2007. Whisker length=Standard error of the mean.

Table-2.1. Average values of monoterpene emissions ($\mu gCg_{dw}^{-1}h^{-1}$), PAR ($\mu mol\ m^{-2}s^{-1}$), and temperature (^oC) during Summer 2007. N=number of samples. ±=Standard error of the mean.

	Understory			**Open areas**		
	Early Summer	Mid-Summer	Late Summer	Early Summer	Mid-Summer	Late Summer
Emissions	2.48±1	1.54±0.3	0.13±0.1	0.83±0.15	0.175±0.043	0.03±0.01
PAR,	957.8±99.0	668.5±137.2	504.0±182.2	1450.8±183.4	815.85±106.6	1335.2±147.8
TEMP	26.08±2.1	23.69±1	23.5±4.34	32.94±3.61	29.34±1.83	37.7±2.43
N	17	20	10	18	19	12

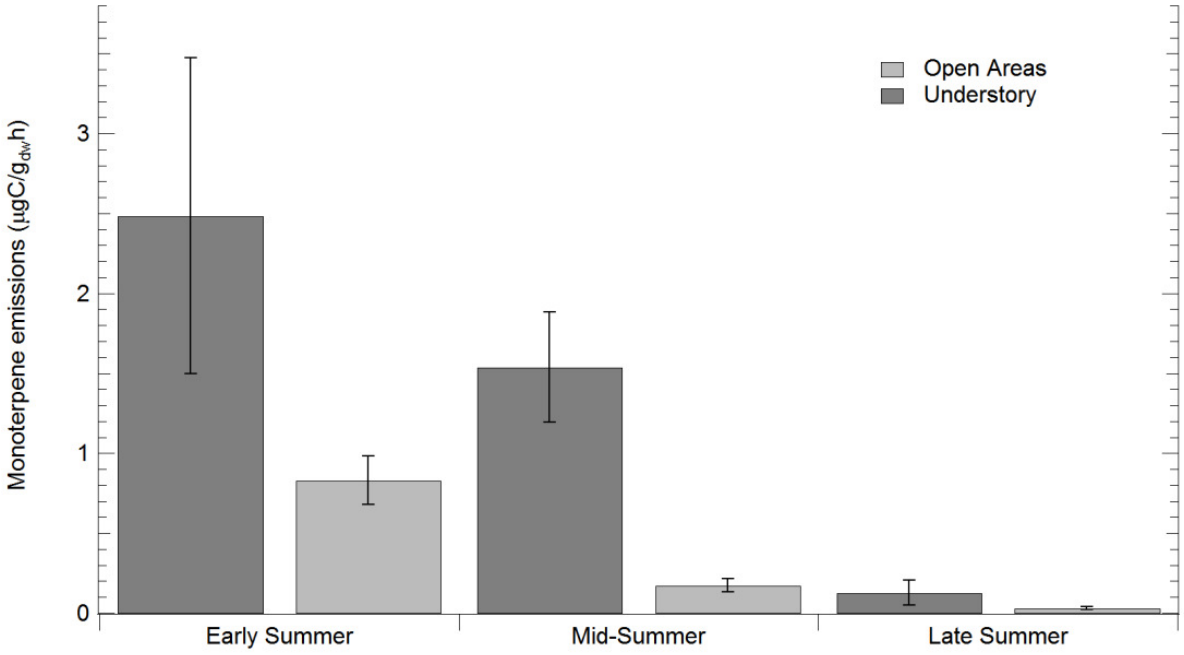

Figure 2.4. Comparison of average monoterpene emissions of *Pteridium aquilinum* plants in open and understory areas during early, mid and late summer periods. Summer 2007. Whisker length=Standard error of the mean.

Terpenoid emissions and landscape structure

To determine if there was a significant difference between the emissions of the plants located in the understory and those located in open areas, a one-way ANOVA was performed with an α=0.05($F_{1, 96}$= 8.97 p= 0.0035; Fig. 2.4). Results showed a significant difference between the emissions of the different sites. Average total monoterpene emissions in the understory were 1.6 ±0.4 µgC•g_{dw}^{-1}•h^{-1} and 0 .4 ±0.07 µgC•g_{dw}^{-1}•h^{-1} in the open areas.

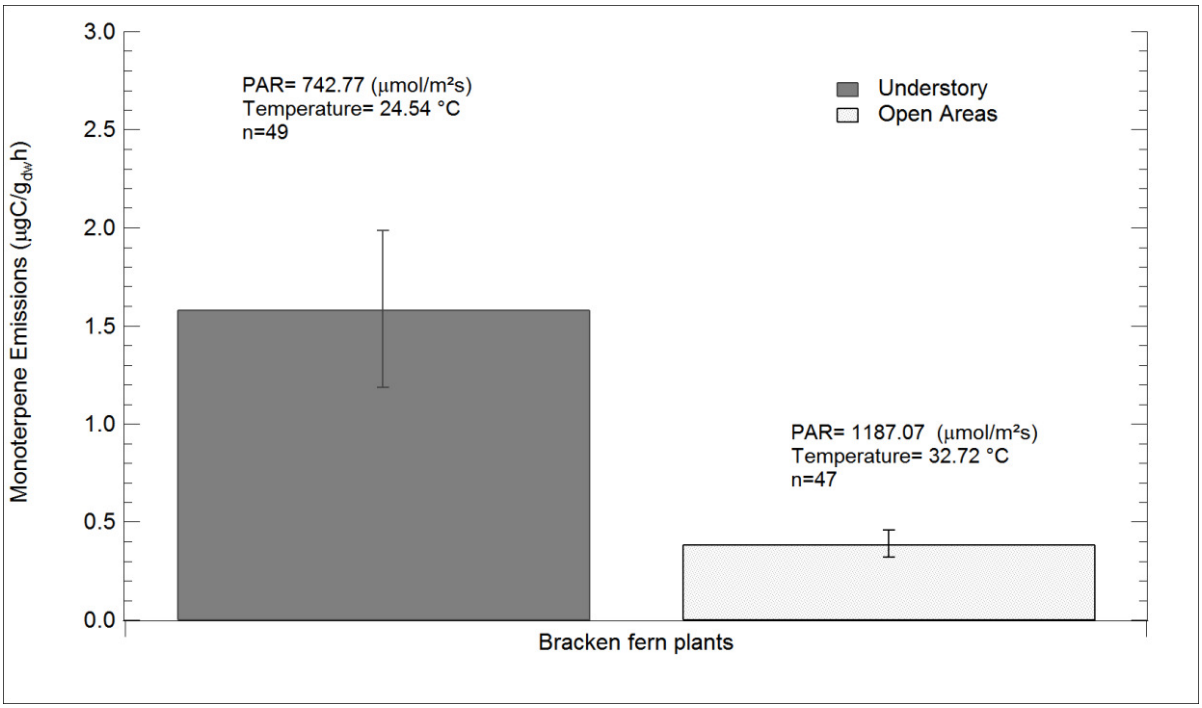

Figure2.5. Comparison of average monoterpene emissions of *Pteridium aquilinum* plants in open and understory areas during Summer 2007. Whisker length=Standard error of the mean.

Factors triggering the emissions: PAR or Temperature?

The results of this study showed emission variations across the summer and a difference between plants in open areas and in the understory. In studies of other plants species, this variation has often been explained by the effects of PAR, temperature or both. To determine which variables influenced Bracken emissions, regression analyses were performed separately on open and understory areas (Table 2.2). Results show lack of correlation with PAR (Fig. 2.5) or temperature (Fig. 2.6) in both areas; temperature and PAR explain only a fraction of the variability in emissions.

Table 2.2 Linear regression results of PAR and temperature on Bracken fern monoterpene emissions.

Area	Measurement	d.f.	F	P $_{\alpha=0.05}$	R^2
Open	Temperature, ^oC	46	1.81	0.177	0.040
	PAR, $\mu molm^{-2}s^{-1}$	46	6.53	0.014	0.126
Understory	Temperature, ^oC	43	1.33	0.255	0.030
	PAR, $\mu molm^{-2}s^{-1}$	43	0.77	0.388	0.018

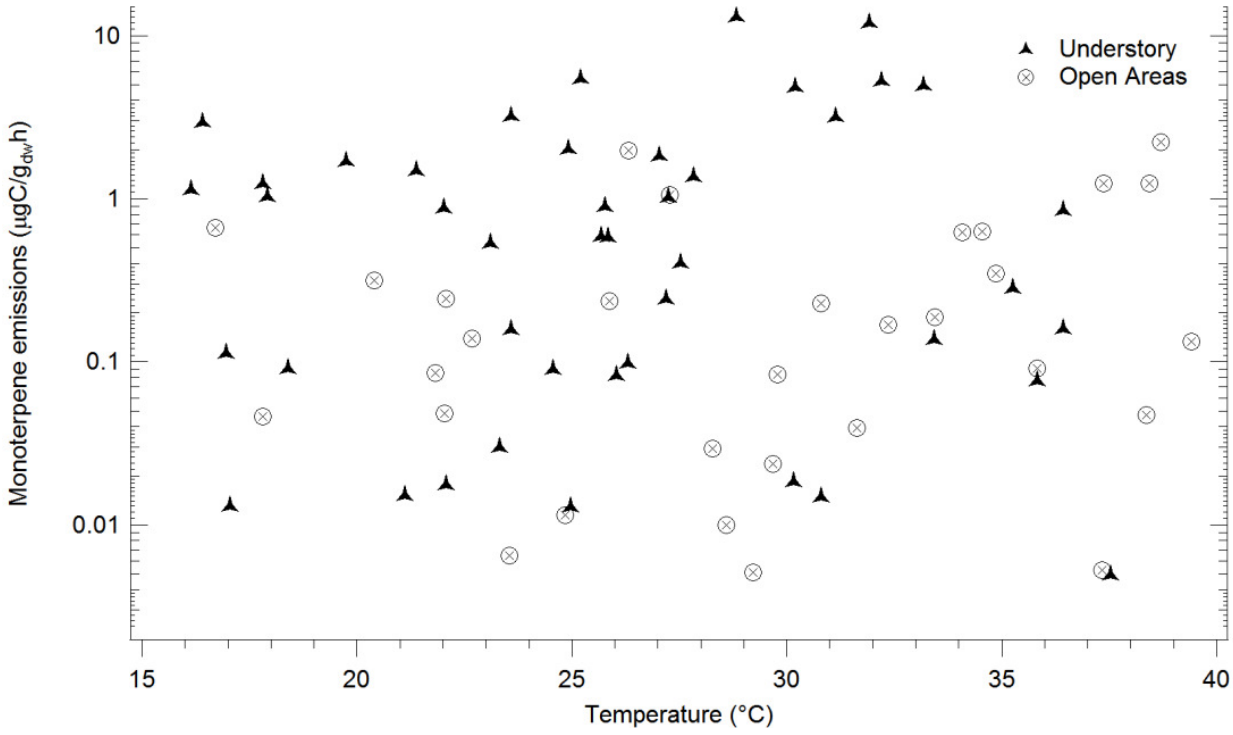

Figure 2.6. Linear regression analysis of Bracken fern emissions and temperature from Summer 2007. Open Areas: d.f.=46, F=6.53, α=0.05= 0.01, R2= 0.126. Understory Areas: d.f.=43, F=0.77, α=0.05= 0.383, R2= 0.18

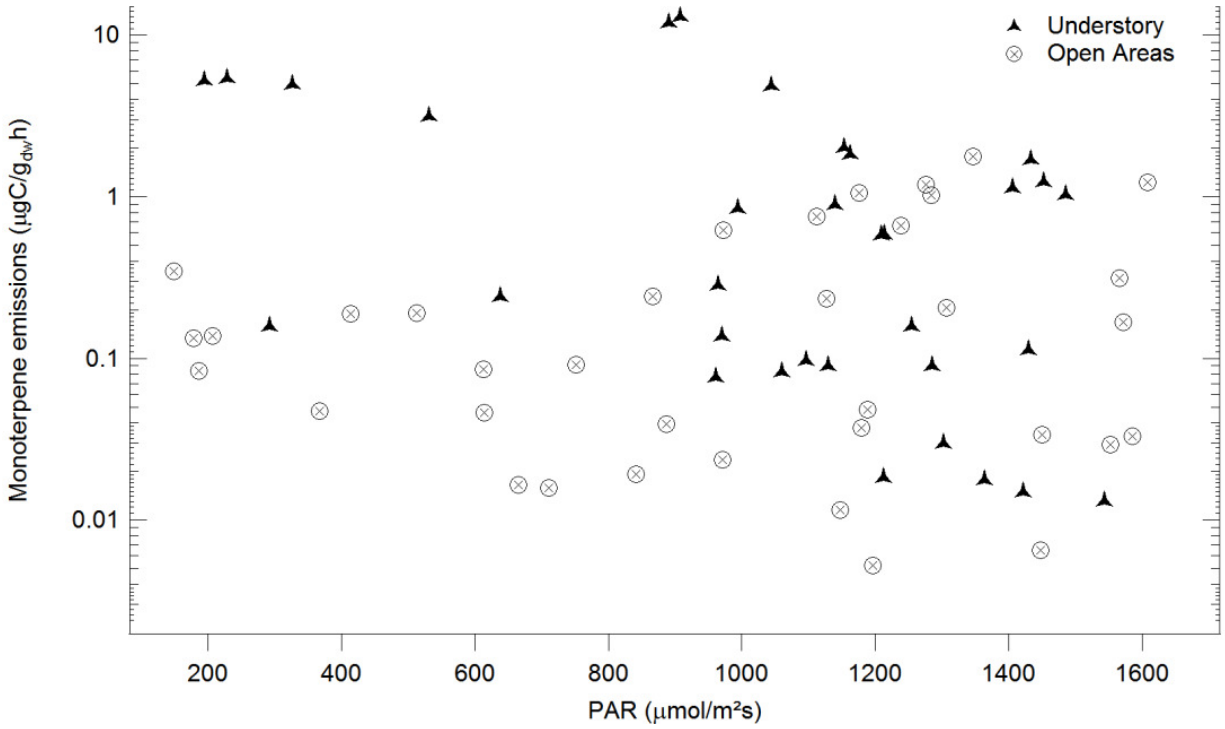

Figure 2.7. Linear regression analysis of Bracken fern emissions and PAR from Summer 2007. Open Areas: d.f.=46, F=1.88, α=0.05= 0.177, R^2= 0.040. Understory Areas: d.f.=43, F=1.33, α=0.05= 0.255, R^2= 0.030

Climate conditions

During the summer of 2007, according to the National Climatic Data Center (NCDC), the UMBS area experienced a severe drought. The average precipitation for the last 20 years was 2.29±0.117 mm*day^{-1} and during that summer was 1.43±0.117 mm*day^{-1} (NCDC). The average values or rainfall precipitation for the last 20 years and for 2007 can be seen in Figure 2.7. Due to the dry summer, the ferns began to senesce in mid-August, an event that was, according to local records, earlier than is typical.

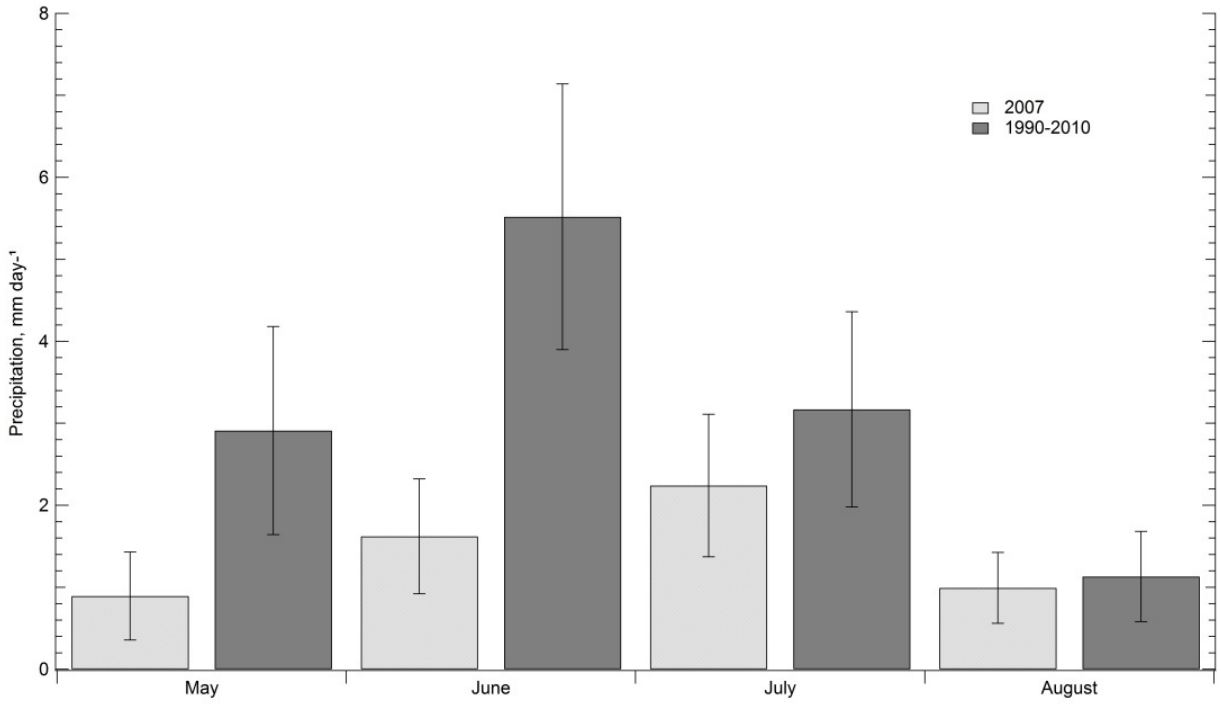

Figure 2.8 Rainfall precipitation in the UMBS area from May to August. The bars represent the monthly rainfall average during the year of 2007 and the years 1990-2010. The error bars represent the standard error of the mean. Data source: National Climatic Data Center. Whisker length=Standard error of the mean.

Change in biomass across summer 2007

The study took place during the summer 2007, beginning in the month of May. During early summer, Bracken fern plants are just expanding. In the middle of summer the plants are fully expanded and at the end of the summer, they are senescing (Fig 2.8).

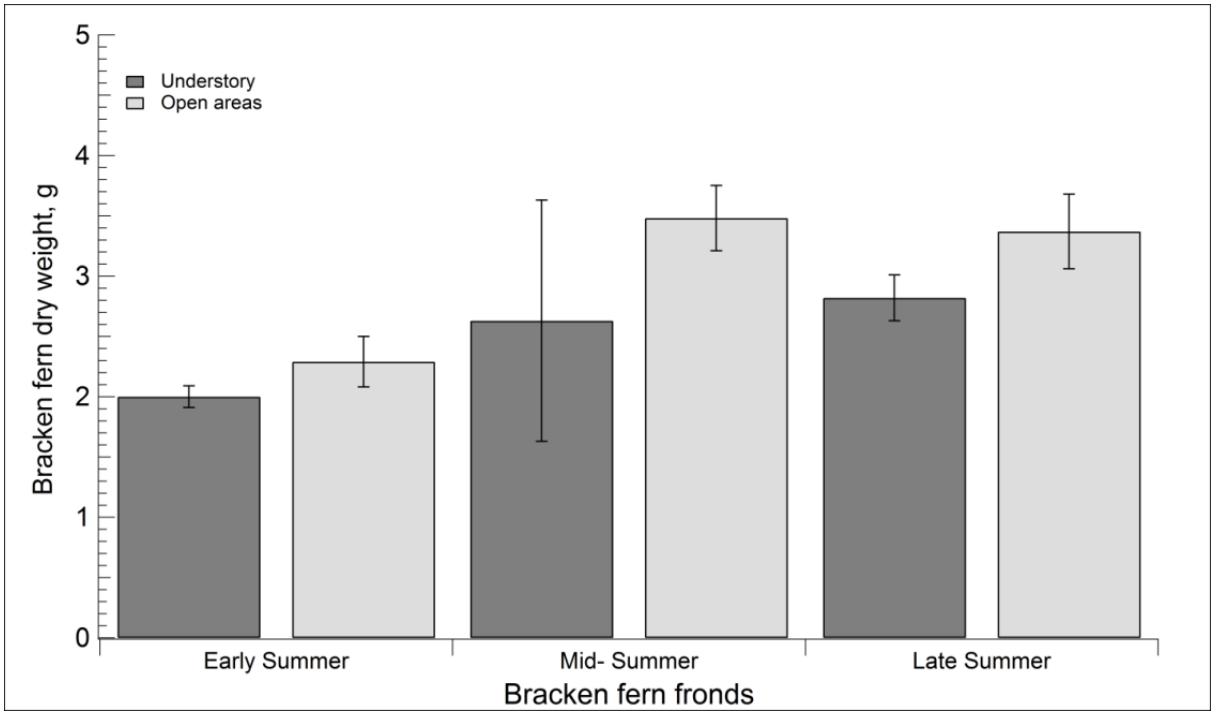

Figure 2.9. Average Bracken fern fronds biomass during the summer of 2007. Whisker length=Standard error of the mean.

2008 Campaign

Greenhouse measurements

The greenhouse-cultivated Bracken fern rhizomes developed into 100 full fronds of which just 3 were found to be terpenoid emitters. The average emission of total monoterpenes for those 3 plants was 0.15 $\mu gC \bullet g_{dw}^{-1} \bullet h^{-1} \pm 0.9 \mu gC \bullet g_{dw}^{-1} \bullet h^{-1}$ and was associated with a PAR level of 750 $\mu mol/m^2 * s$ and a temperature of 27.8 ^{o}C.

Impact of Pteridium aquilinum emissions in a Michigan Forest

The contribution of Bracken fern to total monoterpene emissions in the UMBS forest landscape was estimated using the Model of Emissions of Gases and Aerosols from Nature (MEGAN, v2) (Guenther, Karl 2006) to extrapolate enclosure measurements to the landscape scale. The average observed Bracken fern total monoterpene emission factor for the 2006 and 2007 field campaigns results in an emission of 0.11 $\mu g \bullet m^{-2} \bullet h^{-1}$ when extrapolated to the landscape scale using a the measured LAI of 0.054 $m^2 \bullet m^{-2}$, representative of a Northern Michigan forest. The total monoterpene emission rate associated with Bracken was less than 1% of the total emission estimated for the forest canopy (154 $\mu g \bullet m^{-2} \bullet h^{-1}$). It can be concluded that there is no significant contribution by Bracken fern to the total monoterpene emissions in this Michigan forest.

Discussion

Pteridium aquilinum is capable of producing and emitting monoterpenes. The factors that control these emissions are complex and were not resolved by this study. Statistical analysis showed no significant relationship between emissions and PAR or temperature. The emission variations may be attributed to other factors including air humidity, soil nutrient content, plant-insect interactions, stress or other factors not measured in this study (Hewitt and Street 1992).

During the 2007 campaign, emissions from understory plants were significantly higher than those in open areas. Initially we were expecting higher emissions in open areas.

However, those plants were exposed to harsher sun and temperature conditions. It is possible that plants in open areas allocated more resources to produce biomass in order to increase fitness, and thus had fewer resources to allocate to secondary compounds.

Emissions can be affected by the plant life cycle. Monoterpene emissions are affected by the allocation of resources between growth and production of secondary metabolites. It is known that the chemistry of Bracken fronds changes with time (Alonso-Amelot, Oliveros 2001). Emissions were measured in late summer during 2006 and throughout the summer of 2007. Different stages of development may result in different terpenoid concentrations which contribute to the large variability of the emissions.

Bracken fern biology may explain the lack of success in measuring emissions after transplanting rhizomes from the site to the greenhouse. *Pteridium aquilinum* is a plant that allocates the majority of its biomass to the rhizomes (Whitehead and Digby 1997). It is possible that the plants growing in the greenhouse were allocating the majority of their nutrient resources to their rhizomes and not to the production of secondary metabolites such as monoterpenes, resulting in a lack of measureable emissions.

Another conclusion from this work is that long measurement periods are necessary for characterizing emission factors. Long-term monitoring is needed to understand how and when emissions are produced. Since emissions from Bracken fern were detected on some days but not others, a survey conducted on a single day could conclude that Bracken does not emit any monoterpenes or could overestimate emissions. Measurements of BVOC emission factors have typically been made during short-duration field campaigns. This approach can fail to identify some significant emitters, and it is necessary to lengthen the duration of field campaigns or survey the same plants

during different periods of their life cycle in order to produce more representative emission estimates.

Results also showed that Bracken emissions did not make a significant contribution to total monoterpene emissions from the forest ecosystem investigated. However, understory terpenoid emitters could make an important contribution in other ecosystems.

Pteridium aquilinum is not the only understory species that has been neglected; additional herbaceous species are potentially significant contributors of terpenes and other BVOCs to the atmosphere. It is necessary to have a deeper understanding of potential emissions from understory vegetation to the atmosphere in order to determine if such species could be significant contributors to atmospheric chemical processes.

CHAPTER 3 TERPENOID EMISSIONS FROM PONDEROSA PINE SEEDLINGS AND MATURE TREES. DOES AGE MATTER?

Abstract

Terpenoid emissions from vegetation have been studied intensively in the field and in the laboratory. In the field, the focus has been to quantify emission rates from the dominant species in the landscape under ambient conditions. In the laboratory, studies are done primarily on seedlings or young trees, under controlled conditions. It is known that terpenoid emissions vary with plant age . However, the studies on this topic have been done predominantly on non-woody plants with the focus on plant-animal interactions. The present work is the result of a field study where the differences in terpenoid emissions from seedlings and mature trees of *Pinus ponderosa* were quantified. The study took place at the Manitou Experimental Forest, during the summer of 2009 (May-August). Emissions from 26 seedlings and 30 mature trees were measured through the measurement period. Results show that there is a significant difference in the magnitude of the emissions of seedlings and mature trees ($p<0.05$). Total monoterpene emissions from seedlings averaged 4.24 ± 0.52 μgC $g_{DW}^{-1}h^{-1}$ (mean ± se), while mature trees averaged 1.92 ± 0.16 μgC $g_{DW}^{-1}h^{-1}$ (mean ± se). This work is one the first studies that have measured and compared the emissions from different plant age in a woody plant *in situ*. Results suggest that the rates at which monoterpene compounds are emitted to the atmosphere are affected by the stage of development of the plant.

Introduction

Plants produce many chemical compounds and some of them are emitted into the atmosphere. Many of these compounds are commonly known as biogenic volatile

organic compounds (BVOC). The terpenoids are a BVOC chemical group that stands out because of their functionality and diversity among plants (Loreto, Bagnoli 2009, Lerdau and Gray 2003, Sell 2003). Terpenoids are major players in the interactions between the biosphere and the atmosphere (Monks, Granier 2009, Monson and Holland 2001, Pollmann, Ortega 2005, Kurpius and Goldstein 2003, Lerdau, Guenther 1997). When emitted to the atmosphere they form aerosols and play a key role in tropospheric ozone chemistry (Atkinson and Arey 2003, Loreto and Fares 2007, Fuentes, Lerdau 2000). In the biosphere, terpenes are known to be defensive compounds, pollinator attractants, photo-protective and anti-oxidative agents (Theis and Lerdau 2003, Harrewijn, van Oosten 2001, Chen, Kolb 2002).

Biochemically, the production and storage of terpenoid compounds are energetically costly for the plants; the "building blocks" for terpenoid production are shared with other processes in the plant (Ashour, Wink 2010, Dubey, Bhalla 2003, Sell 2003, Gershenzon 1994, Nagegowda 2010). Terpene production have as a precursors the isopentenyl diphosphate (IPP) and the dimethylallyl diphosphate (DMAPP), these isomers are produced in the mevalonate pathway (MVA). IPP and DMAPP are form using pyruvate and glyceraldehyde 3-phosphate important intermediates in several metabolic processes such as glycolysis and photosynthesis(Ashour, Wink 2010). The fact that such important intermediates are shared between metabolic pathways makes it difficult for the plant. The allocation of those compounds could determine the plants growth and/or defense capacity(Sharkey, Wiberley 2008, Sharkey and Yeh 2001, Gräwert, Groll 2011).

The growth differentiation balance hypothesis (GDBH) states that the allocation of plant resources between growth and synthesis and differentiation (changes induced in

maturing cells due to chemicals, e.g. production of pheromones) are negatively correlated, growth being the dominant factor if resources are available (Lerdau, Litvak 1994, Stamp 2004, Lorio Jr. 1986). Therefore, one would expect that typically plants would allocate more resources to grow than to produce defensive compounds. However, it has been shown that plants could maintain a constant level of defensive compounds if they are exposed to constant attack by herbivores (Hamilton, Zangerl 2001, Koricheva, Larsson 1998).

Terpene production has been studied widely by different scientific disciplines. In general, ecological studies have been focused on quantifying and identifying the different terpenes located inside and emitted by plants, and the interaction of those compounds with other species (Arimura, Kost 2005/5/15, Harley, Monson 1999, Harrewijn, van Oosten 2001, Loreto, Barta 2006, Langenheim 1994). These studies have found that the composition and amount of terpenoids produced by a plant will be determined by genetics, developmental stage, environmental factors and seasonality (Llusia, Penuelas 2008, Brilli, Ciccioli 2009, Hare 2010, Gaylord, Kolb 2006, Llusia, Penuelas 2006). Ecological and or biochemical studies generally use herbaceous plants as a study system, the reason behind it is that these plants possess fast growth rate and easy to manipulate. Atmospheric studies on the other hand, study terpenoid emissions from plants that that occupy large areas of land (Geron, Harley 2001, Guenther, Zimmerman 1996). Usually these plants are woody species that because of their size and distribution are capable of modifying the regional atmospheric chemistry(Geron, Guenther 1994). Atmospheric studies are focused more on the magnitudes of the emissions and the environmental variables that affect them (Karl, Guenther 2003, Fares, Oksanen 2010, Fuentes, Wang 2007, Pressley, Lamb 2004, Guenther 1997).

In general studies of terpenoid emissions in woody species are done in the laboratory on young trees and in the field mature trees are measured. The main question of this work is: Is there a difference between mature trees and seedlings terpenoid emissions? If the GDBH is correct we could expect expect that seedlings would possess a lower terpenoid emission rate than the mature trees. However, this study took place in the field, seedlings are more vulnerable to be attacked, thus they need to produce more defensive compounds.

The goal of this study is to investigate if there is a difference between emissions of seedlings and mature trees of *Pinus ponderosa*. The majority of research on terpene emissions and their relationship with plant age has been done under controlled conditions or with non-woody plant species with an ecological perspective. The results of this study are not only providing information on the difference between mature trees and saplings emissions, but it also investigated the magnitude of terpenoid emission rates from seedlings in ambient conditions.

Methods

Study site

This study took place at the Manitou experimental forest (MEF, Lat. 39°6'0" N, Long. 105°5'30" W, 2300 m elevation) in the summer of 2009 (late May-Late August). The study site is representative of the ponderosa pine montane zone which extends from southern Wyoming to northern New Mexico (Huckaby, Kaufmann 2003). The vegetation is dominated by *Pinus ponderosa* (ponderosa pine) in the overstory and bunchgrasses in the understory, including primarily *Muhlenbergia montana (*mountain muhly) and *Festuca arizonica* (Arizona fescue; Gary,1985). The climate is characteristic

of the eastern slopes in Colorado, dry with an average annual precipitation of 36.9 cm (April to August)(U.S. Department of Agriculture, Forest Service)(U.S. Department of Agriculture, Forest Service)(U.S. Department of Agriculture, Forest Service)(U.S. Department of Agriculture, Forest Service).

Study system Pinus ponderosa

Ponderosa pine is a perennial evergreen widely distributed across the western United States, ranging from northern Mexico to southern Canada (Huckaby, Kaufmann 2003). Due to its large distribution and importance to the timber industry, ponderosa pine has been studied extensively (Pearson 1936, Litvak and Monson 1998b, Grulke and Retzlaff 2001). The regeneration of ponderosa pine can be affected by several factors such as light environment, drought, seed dispersions, among others (Savage, Brown 1996). *P. ponderosa* is known to produce and emit a great variety of terpenoid compounds. It has been shown that the concentration of those compounds is distributed differently within the tissues of and it also varies with the location of those tissues within the tree crown. (Latta, Linhart 2003, Linhart, Mooney 2001, Kim 2001, Latta, Linhart 2000, Lerdau, Dilts 1994).

Experimental design

For this experiment, five 10mX10m plots were established. Within each plot, 5-7 mature trees and 5-7 seedlings were randomly selected. Terpenoid emissions from a total of 30 mature trees and 26 saplings were measured. Trees were measured almost every week throughout the summer on the same day at different hours. Mature trees

were part of a second experiment investigating the differences between sun and shade branches (Chapter 4) and they were measured 4-6 times through the day; seedlings were measured 2-3 times.

Sampling techniques

Figure 3.1 Ponderosa pine seedling inside of a dynamic bag enclosure.

The emissions of terpenes from ponderosa pine foliage were determined using a dynamic plant enclosure system (Ortega and Helmig 2008) . This system consists of a 10 L teflon bag made by Welch fluorocarbon (Dover, New Hampshire). The bag were placed on the branch and on the seedling 12-16 hours before beginning the measurements to allow equilibration and avoid emission enhancement by possible disturbance caused by handling the tree.

The enclosure was connected to two pumps; ambient air was pumped into the enclosure at a rate of 4-5 L/min giving a mean residence time of 2 minutes. Ozone was removed from the sweep air using a glass-fiber filter impregnated with sodium thiosulfate ($Na_2S_2O_3$) (Helmig 1997, Pollmann, Ortega 2005), and ambient VOCs were removed using a charcoal trap. Monoterpene emissions were collected on stainless steel cartridges filled with adsorbent. The two-stage cartridges were either custom-filled in-house with a mixture of ~150mg of Tenax TA (60/80 mesh, Buchem BV, Apeldoorn, The

Netherlands) and ~170 mg Carbotrap (20/40 mesh, Sigma-Aldrich Co. LLC, St. Louis, MO, USA), or purchased pre-filled with ~350 mg of Tenax GR (35/60 mesh) and Carbograph 5TD (40/60 mesh; Markes International, Llantrisant, RCT, UK). Air was pulled from the enclosure through the cartridge at a rate of 200 ml/min for half an hour giving a sample volume of 7.5 L. After the sampling was finished, the branch and seedling were cut and dry weight of needles and branch were measured.

Temperature was measured with a probe that was placed inside the bag (LCD External Temperature Probe, L-TMB-M002, Onset Computer Corporation, Bourne, MA). Ambient temperature and humidity were measured with a second sensor (HOBO U12, Temp/RH Probe, Onset Computer Corporation, Bourne, MA). Temperature sensors were connected to the HOBO U-12 data logger and measurements were taken every minute during the measurement period.

Other parameters that were measured were: 1) tree age, 2) tree height, 3) diameter at breast height (DBH), 4) dry weight of needles and bark, and 5) number of trees and seedlings within 5m radius of each tree/seedling. Seedling age was estimated by counting the number of whorls in the tree. Mature tree age was estimated with tree cores. The average age was 55±7.3 years (mean ± se) for mature trees and 5.2±0.37 years (mean ± se) for the seedlings.

Analytical techniques

Quantitative analysis

Terpenes were desorbed from the cartridges using thermal desorption and injected into a gas chromatograph (SRI Model 310, SRI instruments, Menlo Park, CA). The gas chromatograph was coupled to a flame ionization detector equipped with a low polarity column (Restek, MXT®-5, 5% diphenyl and 95% dimethyl polisiloxane, 30m, 0.53mm ID, stainless steel, Restek Corporation, Bellephonte, PA). Helium was used as a carrier gas (10 cm/min). Data were recorded and analyzed using the Peak Simple Chromatography Data System software from SRI. Compound identification was performed by comparing the retention times of the field samples with the retention times of standards (isoprene, β-pinene, camphene and C6-C12 alkanes).

Qualitative analysis

A subset of the sampling cartridges were analyzed on a gas chromatograph (GC) coupled to a mass-selective detector (MSD), to identify the compounds present in the samples. These cartridges were thermally desorbed using an autosampler (Series 2, model ULTRATD, Markes International, Llantrisant, RCT, UK) and analytes were then cryo-focused onto a thermal desorber (model Unity Markes International, Llantrisant, RCT, UK) operated in splitless mode. The samples were subsequently injected into a GC (model 7890A, Agilent Technologies, Inc., Santa Clara, CA, USA). Flow path temperatures from the autosampler to the GC were maintained at ~ 175°C. GC oven was equipped with a flame ionization detector (FID) and a MSD (5975C inert MSD, Agilent Technologies, Inc., Santa Clara, CA, USA), and had an HP-5MS column (30 m x 0.25 mm, 0.25 μm, Agilent Technologies, Inc., Santa Clara, CA, USA), using nitrogen as a

carrier gas. GC oven heating program used in the analysis of the MFE samples was as follows: Start temperature: 35°C, Hold time, 1 min; Ramp 1, 6°C/min→80°C (no hold time), Ramp 2, 3°C/min→155°C (no hold time), Ramp 3, 10°C/min→190°C (no hold time), Ramp 4, 25°C/min→260°C, final hold time 5.2 min (total run time, 45 min). Compounds were identified using authentic standards and the NIST MS search 2.0 program (National Institute of Standards and Technology library, Gaithersburg, MD) comparing the spectra with those of the NIST library (National Institute of Standards and Technology library, Gaithersburg, MD).

Data analysis

The results were analyzed using SAS software 9.2 (SAS Institute, Cary, NC). Due to the inequality of sample size between the saplings and the mature trees, an analysis of equality of variances was performed in order to select the proper analysis for the data. A t-test with a Satterthwaite approximation was performed to determine if there was a significant difference between the emissions of seedlings and mature trees. The analyses were done using 95% confidence intervals.

Data normalization

During the field campaign seedlings and mature trees experienced different temperature conditions, affecting the emission rates of terpenoids compounds. A temperature normalization model proposed by Guenther (Guenther, Monson 1991, Guenther, Zimmerman 1993) was applied to the data to better understand the behavior of the emissions. All the statistical analyses were performed on the normalized data. The following model was used (Guenther, Zimmerman 1993):

$$M = M_s \times e^{(\beta(T-T_s)}$$

Where M_s is the emission rate at a standard temperature (T_s) and β is an empirical coefficient found through a nonlinear fit. The range of values for the β coefficient found in the literature range from 0.04 to 0.15 K^{-1}. The recommended value for monoterpenes used in the atmospheric models suggested by Guenther is 0.09 K^{-1}. For this study, the β coefficients were estimated using the data from the field study.

Results

Temperature dependence

The temperature dependence of monoterpene emissions is shown in Figure 3.2. Both seedlings and mature trees show a clear increasing trend with temperature, with some evidence of upward curvature consistent with the exponential model proposed by Guenther et al (Guenther, Zimmerman 1993).

When considered at the same temperature, emissions from seedlings are more than twice as large as those from mature trees. The beta coefficient for total monoterpenes from mature trees was 0.07 K^{-1} and for the seedlings was 0.06 K^{-1}. From the linear plot (Figure 3.2) it may be difficult to see which curve has a higher beta coefficient, but on a log-linear plot (Figure 3.3) the shallower slope (and therefore smaller beta) for the seedlings is evident. An analysis of covariance corroborated that the slopes of seedlings and mature trees are significantly different ($F=210.5$, $p<0.05$).

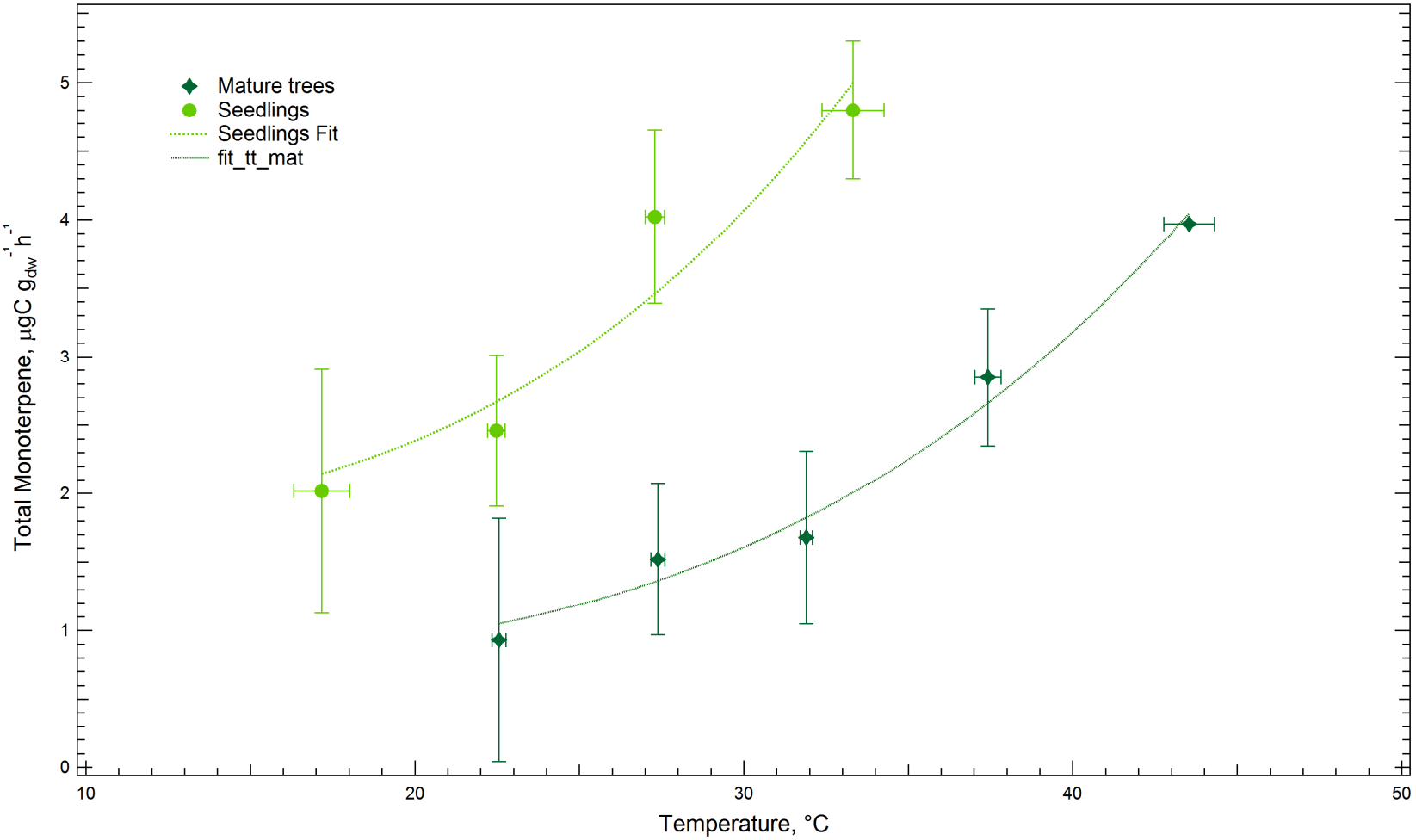

Figure 3.2 Response of total monoterpene emissions of *Pinus ponderosa* to temperature. Seedlings (circles) and mature trees (diamonds). Whiskers= standard error of the mean.

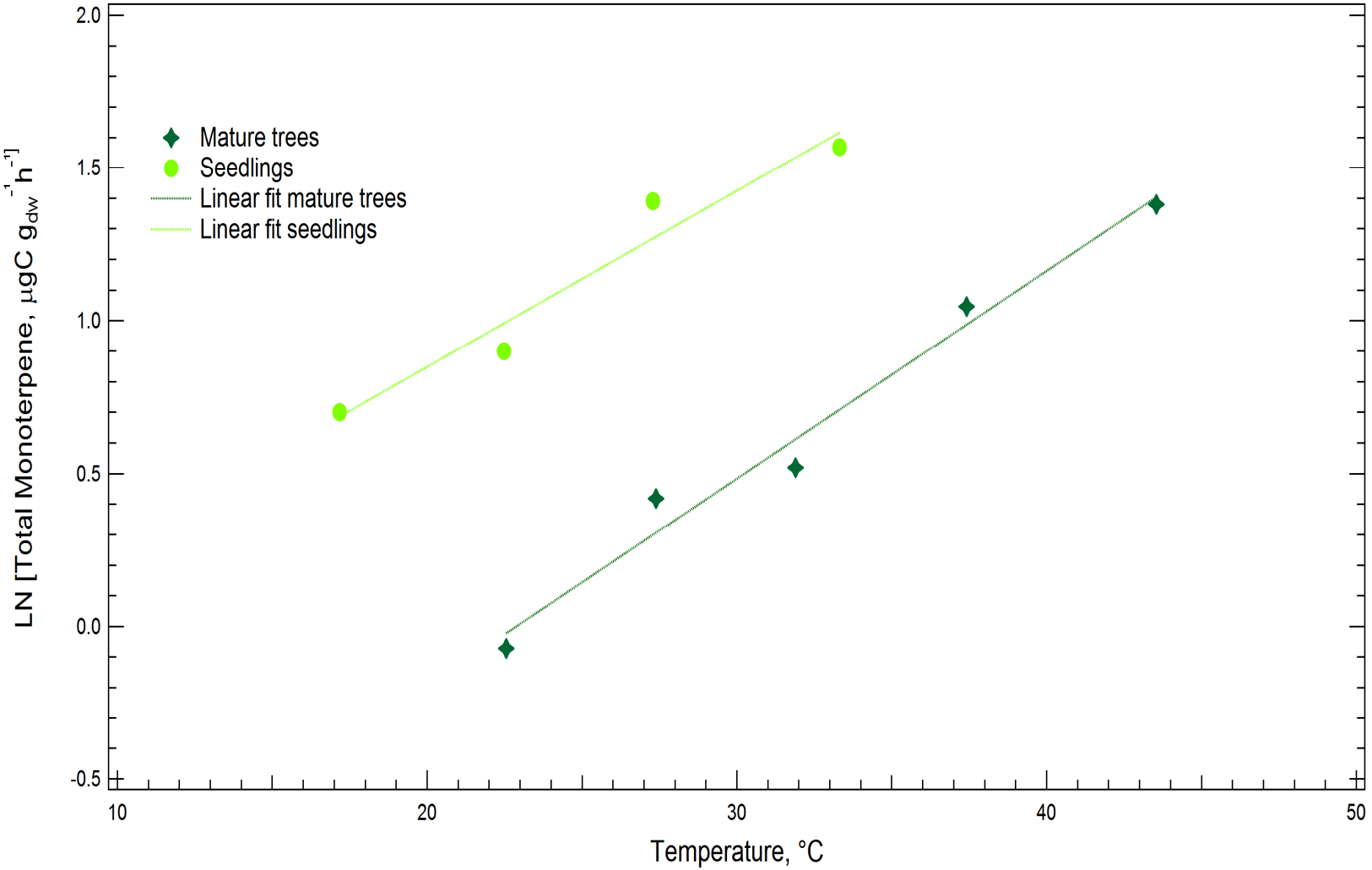

Figure 3.3 Response of total monoterpene emissions of *Pinus ponderosa* to temperature. Seedlings (circles) and mature trees (diamonds).

The β coefficients for the individual monoterpenes were calculated as well (Table 3.1). It can be seen that while the beta values of mature trees are closer to the values cited in the literature, the seedlings presented smaller values.

Compound	β mat, K^{-1}	β seed, K^{-1}
α-pinene	0.07	0.01
β-pinene	0.07	0.03
Δ-carene	0.08	0.02
Camphene	0.07	0.05
Myrcene	0.08	na
Limonene	0.04	0.03

Table 3.11 Beta factors for the different monoterpenes found in the measurements. For calculation purposes the beta value found for myrcene in the mature trees was applied to the seedlings.

Emissions composition

Chemical analysis showed that ponderosa pine seedlings and mature trees present the same terpene composition in the emissions. The major terpenoids in the emissions were 2-methyl-3-buten-2-ol (MBO), and six monoterpenes: α-pinene, β-pinene and Δ-carene, camphene, limonene and myrcene (Fig 3.4). These monoterpenes constitute the "total monoterpene emissions" referred to throughout the analyses.

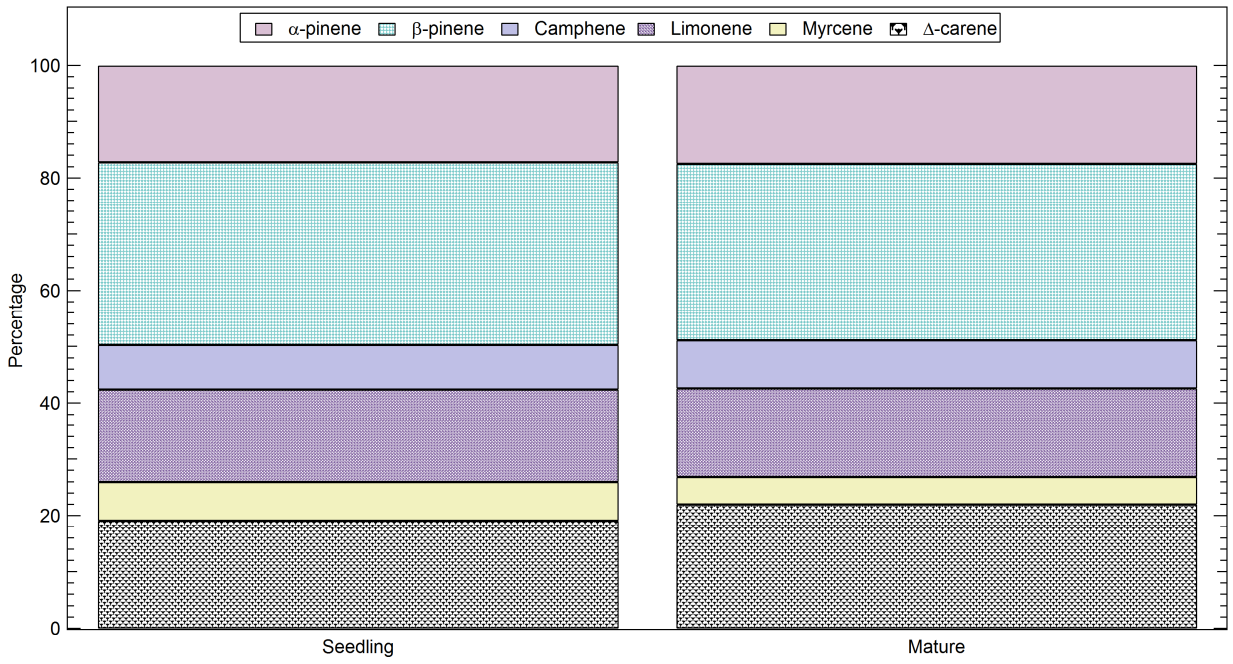

Figure 3.4 Percentages of major terpenoids found in the emissions of Pinus ponderosa from seedlings ar mature trees branches. Summer 2009

Differences between seedlings and mature trees

MBO (2-methyl-3-buten- 2-ol)

Emissions of MBO from seedlings across the measurement period averaged 22.4 ± 1.9 µgC $g_{DW}^{-1}h^{-1}$ (mean ± se). The mature trees emitted an average of 13.7 ± 1.1 µg$C g_{DW}^{-1}h^{-1}$ (mean ± se). Differences between emission rates from the mature trees and seedlings were analyzed using a Satterthwaite test. The analysis showed that there is not a significant difference between the seedlings and mature trees emissions ($t_{1,\ 69.193}$= -1.86, p > 0.05), of MBO (Fig 3.5-A).

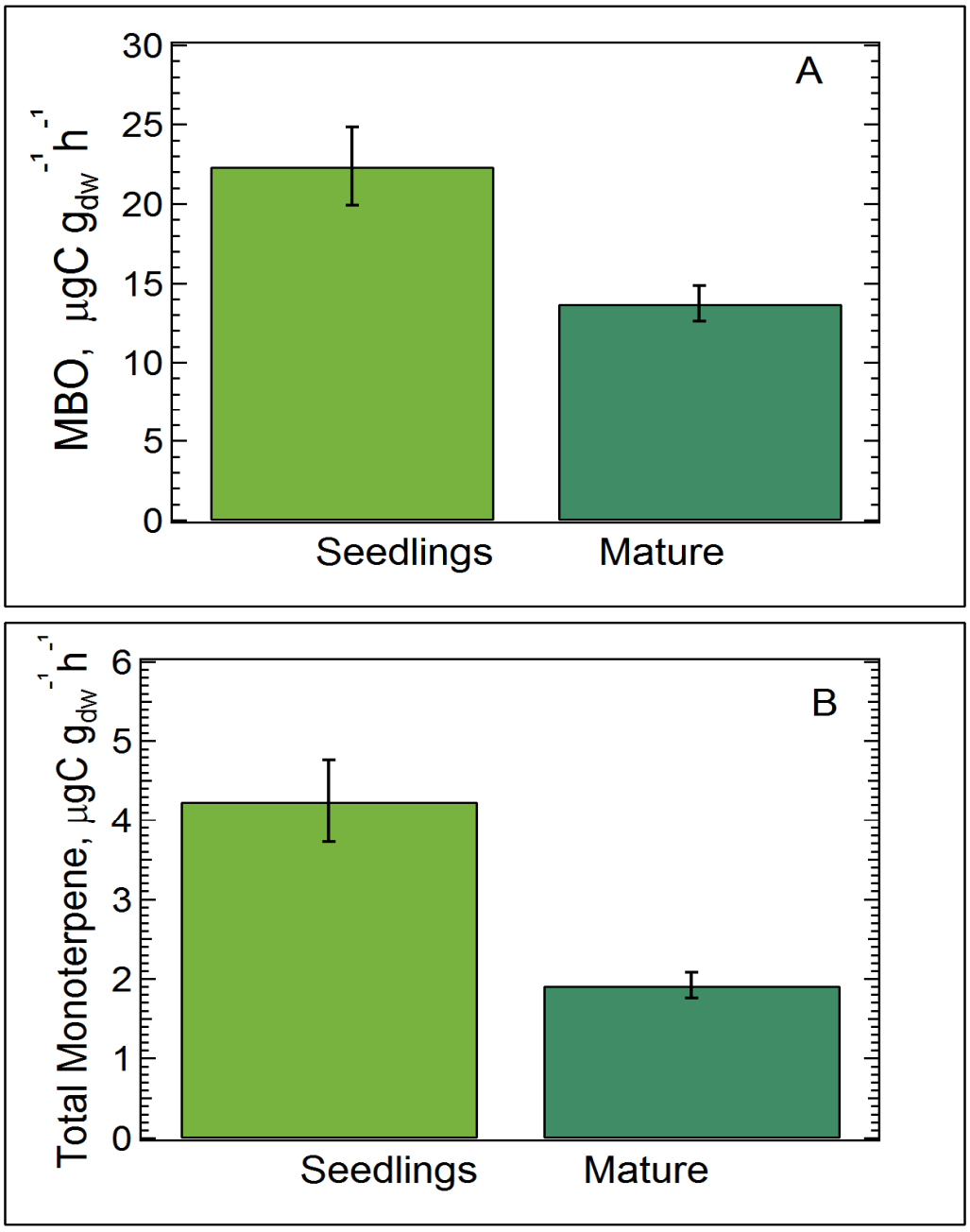

Figure 3.5 Differences between (A)MBO and (B) total monoterpene emissions of seedlings and mature trees of *P. ponderosa*. Whiskers=Standard error of the mean. Results showed that MBO do not show a statistically significant difference between seedlings and mature trees ($t_{1,\ 69.193}$= -1.86, p < 0.05), Total monoterpenes did show a statistically significant difference ($t_{1,\ 71.3}$= -3.6, p =0.0006) .

Monoterpenes

Emissions of total monoterpenes from mature trees across the summer averaged 1.92 ± 0.16 µgC $g_{DW}^{-1}h^{-1}$ (mean ± se), while the seedlings emitted an average of 4.2 ± 0.52 µgC $g_{DW}^{-1}h^{-1}$ (mean ± se). The t-test showed that there is a significant difference between the total monoterpene emissions of seedlings and mature trees emissions ($t_{1,\ 71.3}$= -3.6, p =0.0006) (Fig. 3.5-B)

Compound	**Seedlings** (µgC $g_{DW}^{-1}h^{-1}$)	**Mature trees** (µgC $g_{DW}^{-1}h^{-1}$)	**P value**
α-pinene	0.90 ± 0.18	0.39 ± 0.03	0.01*
Camphene	0.41 ± .10	0.19 ± 0.02	0.057
β-pinene	1.74 ± 0.25	0.70 ± 0.11	0.0005*
Myrcene	0.36 ± 0.05	0.11 ± 0.01	0.0001*
Δ-Carene	1.01 ± 0.17	0.49 ± 0.04	0.004*
Limonene	0.86 ± 0.14	0.34 ± 0.08	0.002

Table 2 Average emissions of seedlings and mature trees (mean ± Standard error) for the different monoterpenes found in the measurements. With the exception of camphene, all the terpenes showed significant difference between mature trees and seedlings. Statistical analysis: Satterthwaite test. Mature trees, n=169. Seedling, n=70. Significant difference is marked by an asterisk.

Differences between seedlings and mature tree emissions of the distinct monoterpenes compounds were determined by applying a Satterhwaite test as well. Results show that with the exception of camphene, there was a significant difference among all the monoterpenes. Results are shown in table 3.2 and figures 3.6 and 3.7.

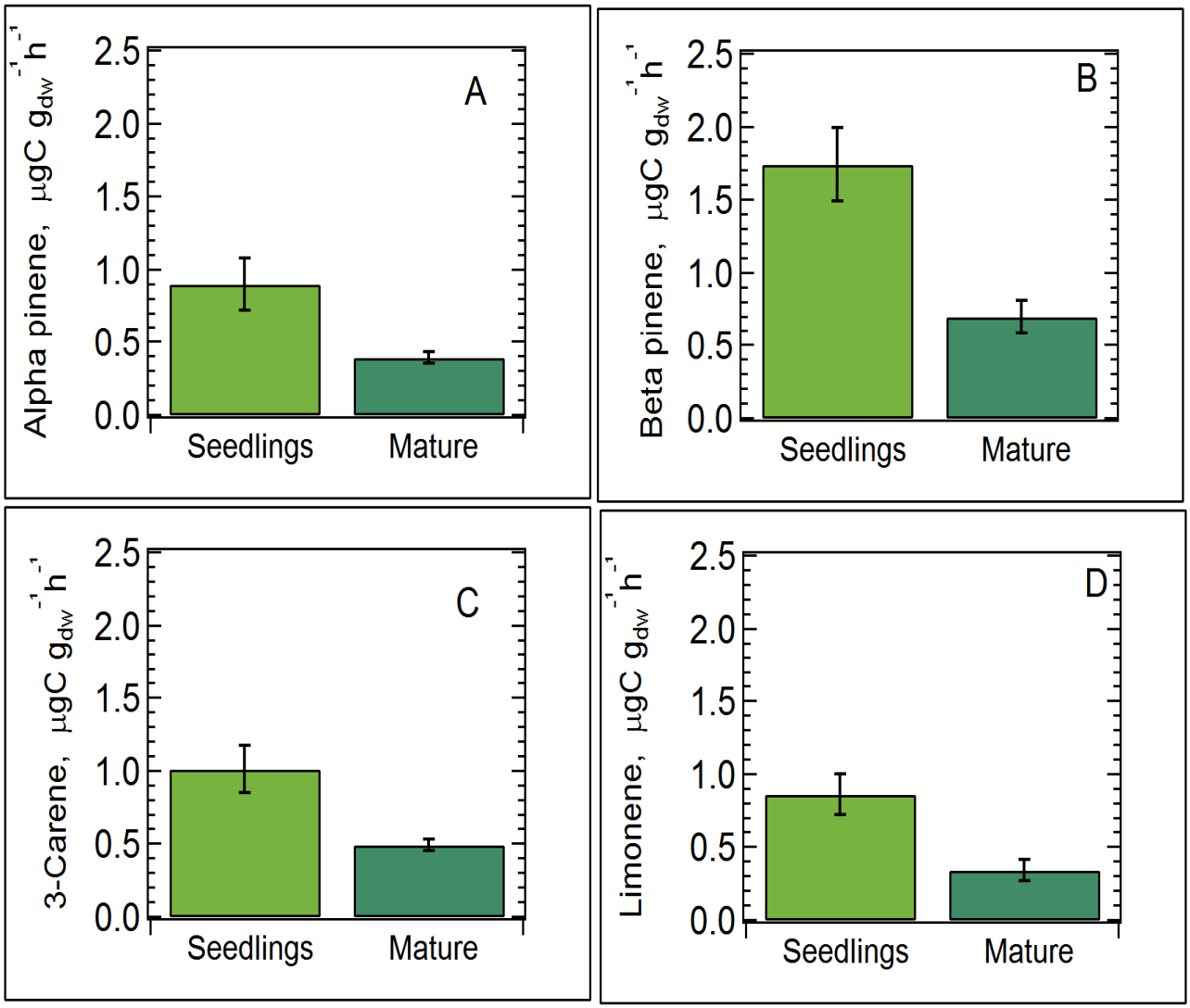

Figure 3.6 Differences between emissions of seedlings and mature trees of *P. ponderosa*. The difference between the emissions was determined with a Satterhwaite test. A) α-pinene (t= -2.66, p =0.01). B) β-pinene (t= -3.64, p =0.0005) . C) Δ-carene (t= -2.93, p =0.004). D) Limonene (t_1= -2.56, p =0.0028). Whisker length =Standard error or the mean.

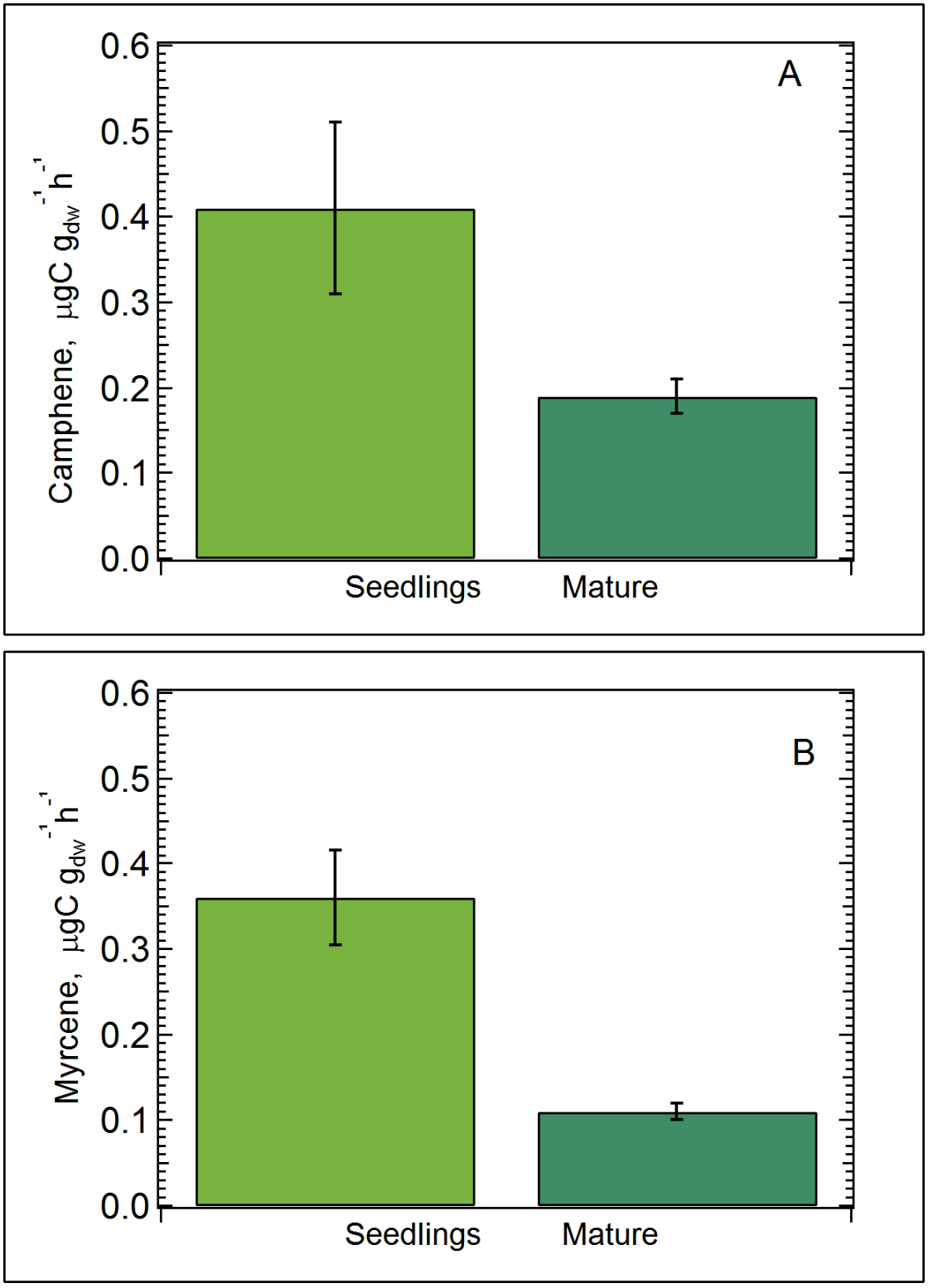

Figure 3.7 Differences between emissions of seedlings and mature trees of *P. ponderosa*. The difference between the emissions was determined with a Satterhwaite test. A) Camphene (t= -1.97, p =0.057). B) Myrcene (t= -4.34, p =0.0001). Whisker length =Standard error or the mean.

Discussion

The results of this study suggest that the rates at which monoterpene compounds that are emitted to the atmosphere are affected by tree age. This study has shown that the seedlings emit more terpenoid compounds than the mature trees. Tthere is a significant difference between the emissions of mature trees and seedlings. While there have been several studies on plant developmental stage and volatile terpenoid compounds, most of the research have been done on herbaceous species (Shiojiri and Karban 2006, Quintero and Bowers 2011, Hare 2010). From those studies we know that terpenoid compounds vary with developmental stage; young plants are more prone to produce terpenoid compounds to protect themselves from herbivore attack (Coley, Bryant 1985, Pakeman and Marrs 1994). Studies on woody species have shown that terpene production decrease with age, but these studies have measured the chemical composition of the trees oils or resins, not terpenoid emissions (Litvak and Monson 1998a, Latta, Linhart 2000, Smith and Shortle 2001, Latta and Linhart 1997, Street, Owen 1997). The work by Street et al. (Street, Owen 1997) is the only study found in the literature acknowledging the effects of tree age and terpenoid emissions. However, this study didn't found any significant difference between the mature tree and the seedling. It has to be mentioned that the sample size of this study was too small (one seedling and one mature tree).

The results of my work showed that the difference between seedlings and mature trees is significant. However, the ontogenic stage may not be completely responsible for that difference. During the measurement period, *Chionaspis pinifoliae* (pine needle scale) was observed in some trees and seedlings, approximately 25-30% of the area was affected. Therefore, the emissions could have been a response to the presence of those

insects. While the specific plants that were measured were not infected, there were some instances in which the neighboring plants were attacked by the pine scale. It is also known that plants that are attacked emit more terpenes and neighboring can be induced to produce terpenes (Adler and Karban 1994, Heil and Karban 2010, Karban and Adler 1996, Karban 2001). It may be the case that the difference in the emissions was a response of the pine scale infestation. However, it can be argued that both mature trees and seedling were exposed to the same signaling compounds, and plant development is solely causing the difference of the emissions between seedlings and mature trees.

The ability of *Pinus ponderosa* to produce and store terpenoid compounds has been shown in previous studies (Linhart, Mooney 2001, Latta, Linhart 2000). It has also been shown that ponderosa pine emits terpenoid compounds (Lerdau, Dilts 1994, Gray, Goldstein 2006). The results of this study corroborates that the major compounds emitted by P. *ponderosa* are α-pinene, β-pinene and Δ-carene, camphene, limonene and myrcene. While, the chemical composition of the emissions found in this study coincides with previous studies, the β coefficient values do not. Lerdau et al.(Lerdau, Dilts 1994), measured monoterpene emissions from mature trees of ponderosa pine and found β values ranging between 0.08 K^{-1} to 0.170 K^{-1}, closer to the value suggested by Guenther et al. (Guenther, Zimmerman 1993) . It is possible that the difference between coefficients is due to biological variability, a major challenge when characterizing terpenoid emissions (Kesselmeier and Staudt 1999, Penuelas and Llusia 2001) . However, the mentioned studies calculated the factors using small numbers of trees, and measuring the emissions of those trees measured repeatedly under controlled conditions. The results of this study are the product of measuring 30 mature trees and 26 seedlings under ambient conditions, and therefore may incorporate biological

variation more effectively. It is difficult to assess if the β coefficients change with age, as there is just one study by Street et al. (Street, Owen 1997), that has compared mature conifer trees and seedlings in ambient conditions. That study showed that seedlings emitted less than the mature trees, but the sample size was also small. They just compare 2 mature trees to 2 branches.

The complicated nature of the measurements and the biological variability associated with the emissions of terpenoid compounds calls for larger data sets and a combination of physical and biological measurements. While this study possesses the strength of a larger data set, and complete analytical measurements, it lacks a comprehensive set of ancillary biological measurements. Photosynthetic rate and stomatal conductance were not measured; these parameters are known to affect the emission rate of terpenoid compounds. (Vickers, Gershenzon 2009, Tingey, Manning May 1980, Niinemets, Hauff 2002, Fares, Loreto 2008). Further research is needed to trace the physiological origins of the significant differences observed in this study. Nonetheless, given the large sample size and the temporally extensive measurement design, these differences are very likely a function of plant developmental stage and reinforce the need for additional research elucidating important biological controls on biosphere-atmosphere interactions.

Chapter 4 DIFFERENCES BETWEEN SUN AND SHADED BRANCHES IN A PONDEROSA PINE FOREST: IMPLICATIONS ON ATMOSPHERIC MODELING.

Abstract

Temperature and solar radiation have been identified as factors that affect the rate at which plants produce and emit terpenoid compounds. In turn, light and temperature are affected by the spatial distribution of the vegetation in the landscape. While atmospheric models consider variations in temperature and solar radiation in the landscape, they assume that the emission rates will be the same for sun and shaded vegetation, and the emissions will depend solely on the amount of radiation and temperature that the plants receive. Biologically, it is known that sun and shade vegetation present different physiological characteristics. This biological difference may be the key for a more accurate emissions inventory. This chapter evaluated the emissions of terpenoid compounds from sun and shaded branches of *Pinus ponderosa* measured in the field. Using dynamic a plant enclosure and GC-MS techniques, I showed that sun and shaded branches have different terpenoid emissions rates. I also found that the major components in the emissions were 2-methyl-3-buten-2-ol (MBO), α-pinene, β-pinene and Δ-carene, camphene, limonene and myrcene. MBO emissions of the sun branches were significantly different than shaded branches. Sun branches averaged MBO emissions of 10.2 ± 0.9 μgC $g_{DW}^{-1}h^{-1}$ (mean ± se), while shade branches emitted an average of 7.3 ±0.7 μgC $g_{DW}^{-1}h^{-1}$ (mean ± se). Total monoterpenes emissions presented a significant difference as well; sun branches averaged 1.8 ±0.2 μgC $g_{DW}^{-1}h^{-1}$ (mean ± se), and shaded branches emitted an average of 1.4 ±0.1 μgC $g_{DW}^{-1}h^{-1}$ (mean ±

se). The results of this work showed that light environments affect in significant way the emissions of terpenoid compounds to the atmosphere.

Introduction

The contribution of biogenic volatile carbon (BVOC) to atmospheric chemistry and ecological processes has been studied widely in the last decades, yet there are still many uncertainties in the measurements and consequently the models quantifying those processes are uncertain as well. For example, it is known that some plants species are more prone to emit BVOC, but when those species are studied by different research groups the BVOC emissions found in those studies differ quantitatively and sometimes qualitatively. Within those BVOC there is a group that stands out because they constitute a major percentage of the BVOC emissions and they play major roles in ecological and atmospheric processes: Terpenes (Kesselmeier and Staudt 1999, Lerdau, Guenther 1997, Peñuelas and Michael 2010). The oxidation of terpenes contributes to ozone and aerosol formation, affecting the chemistry of the atmosphere (Atkinson and Arey 2003, Monks, Granier 2009).

Plants produce terpenes for a diverse array of reasons: terpenes are known to help in plant reproduction and plant defense. Terpene production, as means to mitigate stress, increases when plants are subjected to environmental stressors such as high temperature and high light levels (Lerdau and Gray 2003, Monson, Lerdau 1995, Penuelas and Llusia 2003). Thus, an important multidisciplinary challenge is to represent accurately the emissions of the terpenes, including their geographic distributions and temporal variations, and the complex factors that control emission rate.

Terpene production is known to be triggered by light and temperature, which has affected the assumptions made by atmospheric scientists when modeling the chemistry of those compounds in the atmosphere (Stroud, Makar 2005, Geron, Guenther 1994, Pressley, Lamb 2004/6). Most of those assumptions have been based on the physics of the environment in a specific time and spatial scale. In the particular case of terpenoid emissions and their light dependence, atmospheric scientists use radiative transfer models to describe how the instantaneous photosynthetic active radiation (PAR is attenuated through the canopy, to estimate emission levels(Guenther, Karl 2006, Stroud, Makar 2005, Grote 2007, Pearcy, Muraoka 2005). However, they assume a constant physiology in leaves throughout the canopy and, thus an equal capacity to emit terpenoid compounds. (Fehsenfeld, Calvert 1992, Guenther, Karl 2006, Guenther, Hewitt 1995, Simpson, Guenther 1995). They do not consider the fact that leaves are that grow in direct sunlight (sun leaves/branches) or shaded conditions (shade leaves/branches) will possess variable photosynthetic efficiency and likely, terpene emissions (Pearcy, Muraoka 2005, Boardman 1977, Valladares and Pearcy 2002).

Physically the models assumption for calculating terpenes emissions where they estimate that vegetation that grows in the sun will emit more terpenes makes sense; the amount of light that the plants receive will determine the magnitude of the emissions. However, biologically the relationship is less clear cut. Light and temperature environments will determine the photosynthetic activity of the plant, the amount and type of chlorophyll, and the distribution of chloroplast in the leaves (Lichtenthaler, AÄ 2007, Blackburn 1998). It is also known that the plants need ATP synthesized in the chloroplasts to produce terpenes (Monson, Jaeger 1992, Rosenstiel, Ebbets 2004). If the amount of ATP determines the emission rate of the terpenoid compounds, then sun

and shaded leaves should differ significantly in their production. Consequently, light distribution is not enough to model terpenoid emissions; plant biology should be considered as well.

Landscape structure which influences the light environment of vegetation might have a significant influence on regional terpene emissions. In particular, modern landscapes are subject to considerable land-cover and land-use changes that result in increased patchiness and edges; these dynamics are neglected in atmospheric modeling or regional emissions. If the landscape is changing, is it possible that the emissions are changing as well? Is it possible that we are overestimating/underestimating terpenoid emissions in the models?

This study is an initial effort to evaluate the effect of the landscape on terpenoid emissions and determine if this parameter could improve current atmospheric models. In order to accomplish that this study used field measurements of sun and shaded branches of Pinus ponderosa (ponderosa pine) and compare them to fluxes measurements of the study site and to model predictions. The first objective was to determine if emission rates of terpenoid compounds varied within the different light environments. If light did indeed affect the emissions of terpenoid emissions it was expected to find different emissions from the branches in the different light environments. The second objective was to evaluate if the results found in the field could have an effect at a landscape level with a sensitivity study. This sensitivity study showed how well we can simulate the light environments within a forest canopy. A sensitivity analysis was performed using the MEGAN model to address this objective. Different scenarios were carried out using emission factors and light and temperature response factors based on the field studies. The results of the model helped us to

recognize the need to include and study in more detail landscape structure to improve current atmospheric models

Methods

Study site

This study took place at the Manitou Experimental Forest (MEF, Lat. 39°6'0" N, Long. 105°5'30" W, 2300 m elevation) in the summer of 2009 (Late May-Late August). The study site is representative of the ponderosa pine montane zone which extends from southern Wyoming to northern New Mexico. The vegetation is dominated by *Pinus ponderosa* (ponderosa pine) in the overstory and bunchgrasses in the understory, including primarily *Muhlenbergia montana (*mountain muhly) and *Festuca arizonica* (Arizona fescue, (Gary 1985). The climate is characteristic of the eastern slopes in Colorado, dry with an average annual precipitation of 36.9 cm (April to August,(U.S. Department of Agriculture, Forest Service)(U.S. Department of Agriculture, Forest Service) U.S. Department of Agriculture, Forest Service). This site has been managed by the USDA Forest Service and the stands are thinned on a regular basis. The even distribution of the trees is ideal for the experiment, which will provide a better estimation of the emissions from a managed forest area, which in the future are most likely to be more abundant than non-disturbed areas.

Study system Pinus ponderosa

Ponderosa pine is a perennial evergreen widely distributed across the western United States, ranging from northern Mexico to southern Canada (Huckaby, Kaufmann 2003). Ponderosa pine is found at elevations from sea level to about 2700 meters in regions

with arid condition and summer rainfall. Due to its large distribution and importance to the timber industry ponderosa pine has been studied extensively (Pearson 1936, Litvak and Monson 1998b, Grulke and Retzlaff 2001). *P. ponderosa* is known to produce and emit a great variety of terpenoid compounds (Latta, Linhart 2003, Linhart, Mooney 2001, Kim 2001, Latta, Linhart 2000, Lerdau, Dilts 1994).

Experimental design

Five 10mX10m plots were established in the MEF in early summer 2009. Five to seven trees were selected for sampling within each plot. Terpenoid emissions from a total of 30 trees were measured through the summer; from each tree, one sun and one shaded branch were measured simultaneously, for three times interval: morning (8-11), noon (11-14) and afternoon (14 up). The objective of this procedure was to have measurements of different branches with different PAR and temperature profiles, which would allow us to assess any differences in emissions of sun and shaded branches.

A branch was labeled a "sun branch" when the branch was completely exposed to sunlight during the entire day; a "shaded branch", was a branch that had other branches above and did not receive direct sunlight for most of the day. Other parameters that were used to select the branches were: 1) branches coming from the same stem, and 2) occurring at the same height (~1-1.5m).

Sampling techniques

The emissions of terpenes from ponderosa pine foliage were determined using a dynamic plant enclosure system (Ortega and Helmig 2008) . This system consists of a 10 L teflon bag made by Welch fluorocarbon (Dover, New Hampshire). The bag were

placed on the branches 12-16 hours before beginning the measurements to allow equilibration and avoid emission enhancement by possible disturbance caused by handling the tree.

The enclosures were connected to two pumps; ambient air was pumped into the enclosure at a rate of 4-5 L/min giving a mean residence time of 2 minutes. Ozone was removed from the sweep air using a glass-fiber filter impregnated with sodium thiosulfate ($Na_2S_2O_3$) (Helmig 1997, Pollmann, Ortega 2005), and ambient VOCs were removed using a charcoal trap. Monoterpene emissions were collected on stainless steel cartridges filled with adsorbent. The two-stage cartridges were either custom-filled in-house with a mixture of ~150mg of Tenax TA (60/80 mesh, Buchem BV, Apeldoorn, The Netherlands) and ~170 mg Carbotrap (20/40 mesh, Sigma-Aldrich Co. LLC, St. Louis, MO, USA), or purchased pre-filled with ~350 mg of Tenax GR (35/60 mesh) and Carbograph 5TD (40/60 mesh; Markes International, Llantrisant, RCT, UK). Air was pulled from the enclosure through the cartridge at a rate of 200 ml/min for half an hour giving a sample volume of 7.5 L. After the sampling was finished, the branch and seedling were cut and dry weight of needles and branch were measured.

PAR measurements were made using sensors (LI-COR Quantum Sensors) placed outside the bag. Temperature was measured with a probe that was placed inside the bag (LCD External Temperature Probe, L-TMB-M002, Onset Computer Corporation, Bourne, MA). Ambient temperature and humidity were measured with a second sensor (HOBO U12, Temp/RH Probe, Onset Computer Corporation, Bourne, MA). PAR and temperature sensors were connected to the HOBO U-12 data logger and measurements were taken every minute during the measurement period.

Other parameters that were measured were: 1) tree age, 2) tree height, 3) diameter at breast height (DBH), 4) dry weight of needles and bark, and 5) number of trees and seedlings within 5m radius of each tree/seedling. Mature tree age was estimated with tree cores. The average age was 55±7.3 years (mean ± se).

Analytical techniques

Quantitative analysis

Terpenes were desorbed from the cartridges using thermal desorption and injected into a gas chromatograph (SRI Model 310, SRI instruments, Menlo Park, CA). The gas chromatograph was coupled to a flame ionization detector equipped with a low polarity column (Restek, MXT®-5, 5% diphenyl and 95% dimethyl polisiloxane, 30m, 0.53mm ID, stainless steel, Restek Corporation, Bellephonte, PA). Helium was used as a carrier gas (10 cm/min). Data were recorded and analyzed using the Peak Simple Chromatography Data System software from SRI. Compound identification was performed by comparing the retention times of the field samples with the retention times of standards (isoprene, β-pinene, camphene and C6-C12 alkanes).

Qualitative analysis

A subset of the sampling cartridges were analyzed on a gas chromatograph (GC) coupled to a mass-selective detector (MSD), to identify the compounds present in the samples. These cartridges were thermally desorbed using an autosampler (Series 2, model ULTRATD, Markes International, Llantrisant, RCT, UK) and analytes were then cryo-focused onto a thermal desorber (model Unity Markes International, Llantrisant, RCT, UK) operated in splitless mode. The samples were subsequently injected into a

GC (model 7890A, Agilent Technologies, Inc., Santa Clara, CA, USA). Flow path temperatures from the autosampler to the GC were maintained at ~ 175°C. GC oven was equipped with a flame ionization detector (FID) and a MSD (5975C inert MSD, Agilent Technologies, Inc., Santa Clara, CA, USA), and had an HP-5MS column (30 m x 0.25 mm, 0.25 μm, Agilent Technologies, Inc., Santa Clara, CA, USA), using nitrogen as a carrier gas. GC oven heating program used in the analysis of the MFE samples was as follows: Start temperature: 35°C, Hold time, 1 min; Ramp 1, 6°C/min→80°C (no hold time), Ramp 2, 3°C/min→155°C (no hold time), Ramp 3, 10°C/min→190°C (no hold time), Ramp 4, 25°C/min→260°C, final hold time 5.2 min (total run time, 45 min). Compounds were identified using authentic standards and the NIST MS search 2.0 program (National Institute of Standards and Technology library, Gaithersburg, MD) comparing the spectra with those of the NIST library (National Institute of Standards and Technology library, Gaithersburg, MD).

Data analysis

Differences between emissions of sun and shade branches were determined using a one way analysis of variance (ANOVA) at 95% confidence interval. Two separate ANOVAs were used to analyze the difference of the emissions during the day and across the summer time. Regression analyses were performed to calculate the coefficients used for the data normalization. The statistical analyses reported in this paper were generated using SAS software 9.2 (SAS Institute, Cary, NC).

Data normalization

Sun and shaded branches experience different environmental conditions, and as stated in Chapter 3, it is necessary to apply a model to normalize the emissions. Models of temperature and light dependence as proposed by Guenther (Guenther, Monson 1991, Guenther, Zimmerman 1993) were applied to all the data. Statistical analyses, were performed on the on the normalized data.

For MBO, which behaves similarly to isoprene in its temperature and light dependence, the following equation was applied:

$$E = E_S \times C_L \times C_T$$

where E_S is the standardized emission rate at standard temperature and PAR (30^{o}C and 1000 μmolm^{-2}s^{-1}) and C_L and C_T are functions of temperature and PAR respectively. C_L is defined by:

$$C_{L=\frac{\alpha C_{L1} L}{\sqrt{1+\alpha^2 L^2}}}$$

where α_{shaded}=0 .0052 and α_{sun}=.0008 , C_{L1}=1.066, and L=environmental PAR. C_T is defined by:

$$C_T \frac{e^{\frac{C_{T1}\times(T-T_S)}{R\times T_S\times T}}}{1+e^{\frac{C_{T2}\times(T-T_M)}{R\times T_S\times T}}}$$

where R= 0.0083 KJmol^{-1}K^{-1}, C_{T1}=95 KJ, C_{T2}= 230 KJ and T_M=314 K.

Monoterpene emissions were normalized with a model proposed by Guenther et al.,(Guenther, Zimmerman 1993):

$$M = M_s \times e^{(\beta(T-T_s)}$$

where M_s is the emission rate a standard temperature (T_s) and β is an empirical coefficient.

The α coefficients for MBO were calculated using a nonlinear best-fit procedure; normally this coefficient is calculated using light and temperature curves, where one can fix one parameter and measure the other. In this case the data were highly variable because the PAR and temperature conditions were not fixed. In order to account for that variability, the data was assembled into bins and the coefficients were calculated from the nonlinear regression of those bins (Figure 4.1).

As we can see in figure 1, MBO emissions from shaded branches have slightly higher emissions at low PAR (150-300) than the emissions from sun branches; as PAR increases monoterpene emissions decrease. While the emissions from sun branches are steadily increasing as PAR increases.

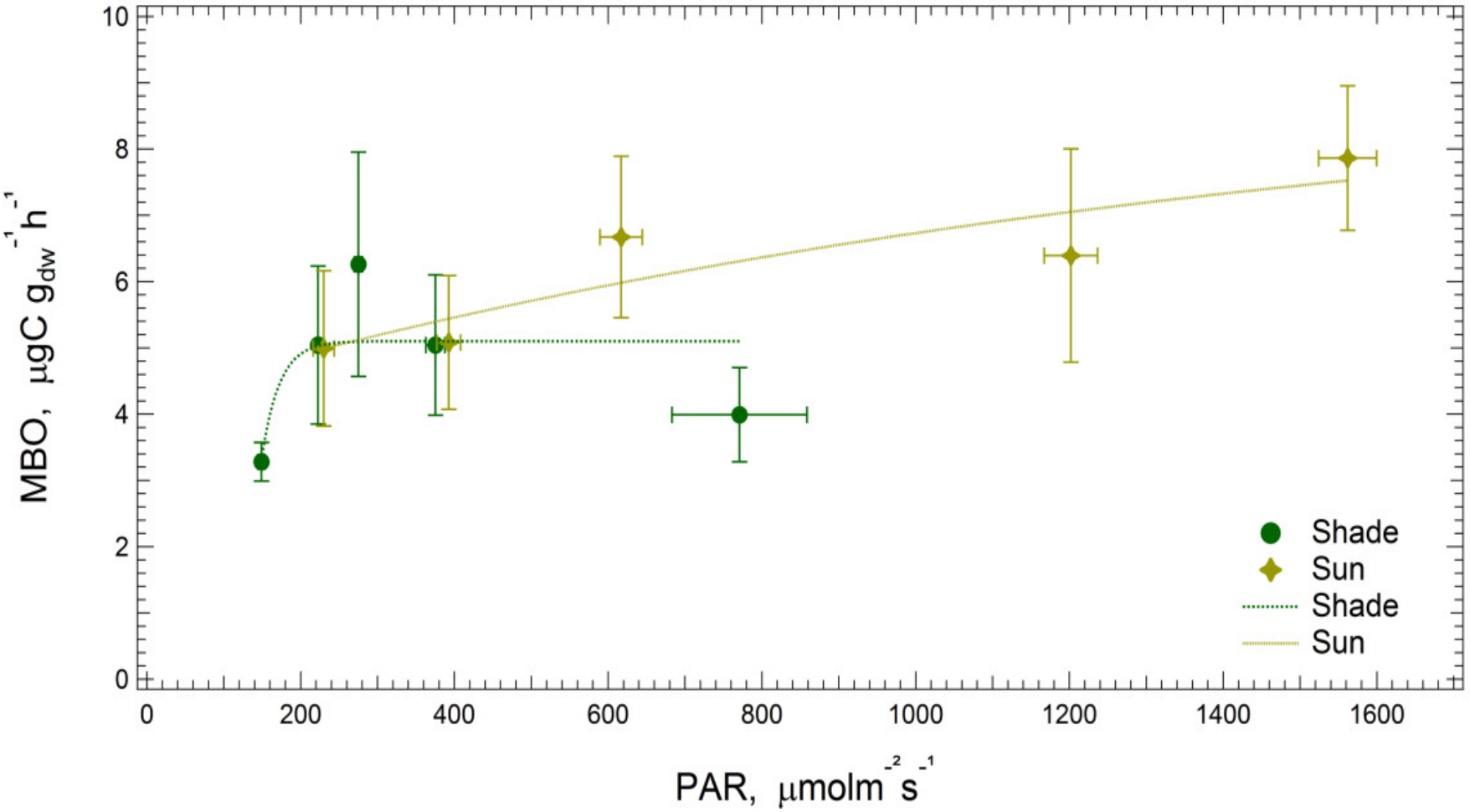

Figure 4.1 Response of MBO emission rate of Ponderosa pine sun branches (diamond) and shaded branches (circles) to PAR. Whiskers=Standard error of the mean.

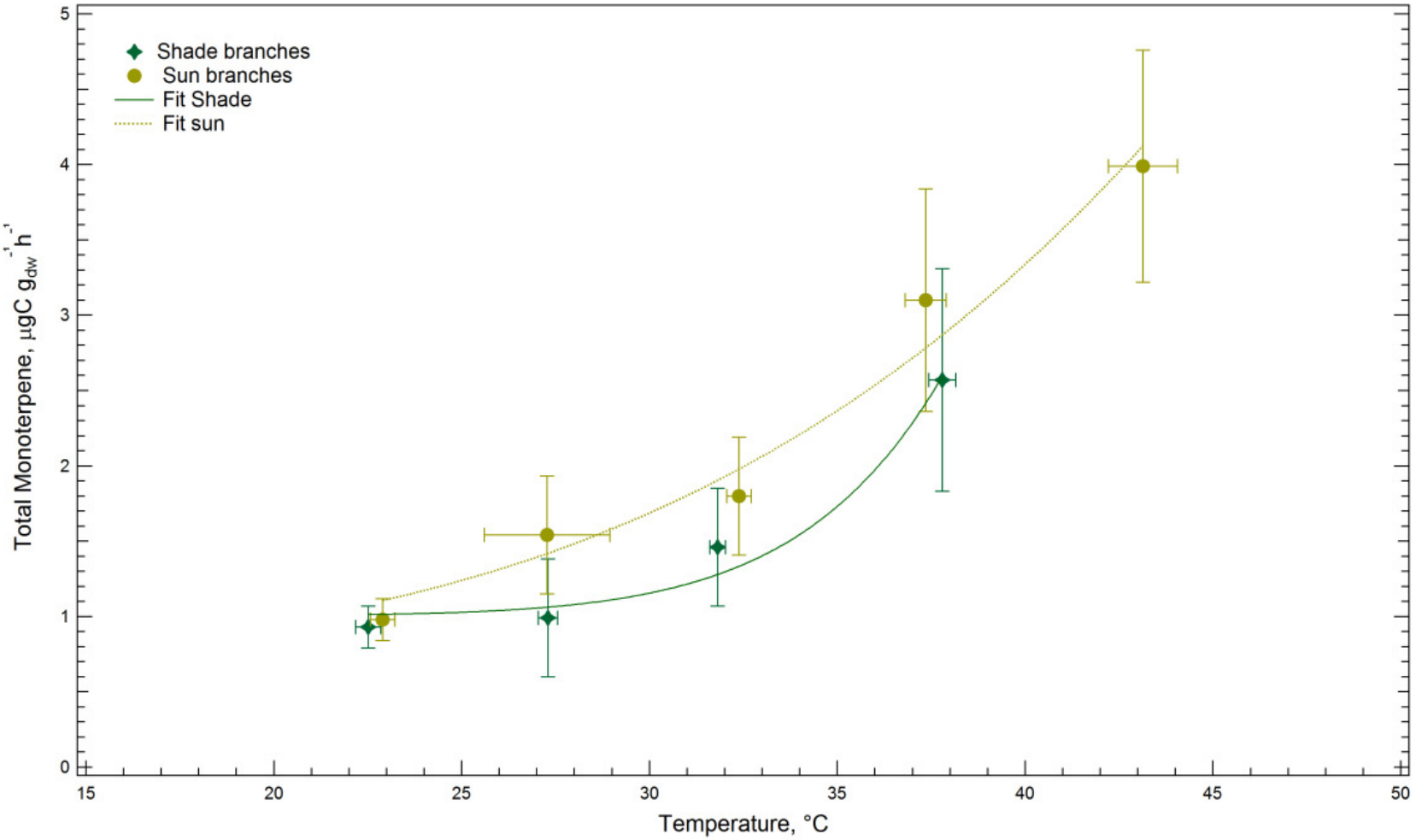

Figure 4.2 Response of total monoterpene emissions of Pinus ponderosa to temperature. Sun branches (circles) and shaded branches (diamonds). Whiskers= standard error of the mean.

Results

Monoterpene emissions temperature dependence

The coefficient β was estimated for the total (Figure 4.2) and individual monoterpenes (Table 4.1) found in the emissions using a nonlinear regression.

Compound	β sun, K^{-1}	β shade, K^{-1}
α-pinene	0.06	.04
β-pinene	0.03	0.03
Δ-carene	0.08	0.02
Camphene	0.04	0.009
Myrcene	0.08	0.03
Limonene	0.04	0.07

Table 4.1 Beta factors for the different monoterpenes found in the measurements.

Chemical composition

The chemical analysis showed ponderosa pine emits several monoterpenes and MBO. The major components of the monoterpene emissions were α-pinene, β-pinene and Δ-carene, camphene, limonene and myrcene, these 6 compounds are referred to as the "total monoterpene emissions" throughout the analysis. Figure 4.3 shows that the

relative abundances of these terpenes are similar for the shade and sun branches, with the shaded branches having somewhat higher camphene / β-pinene ratio.

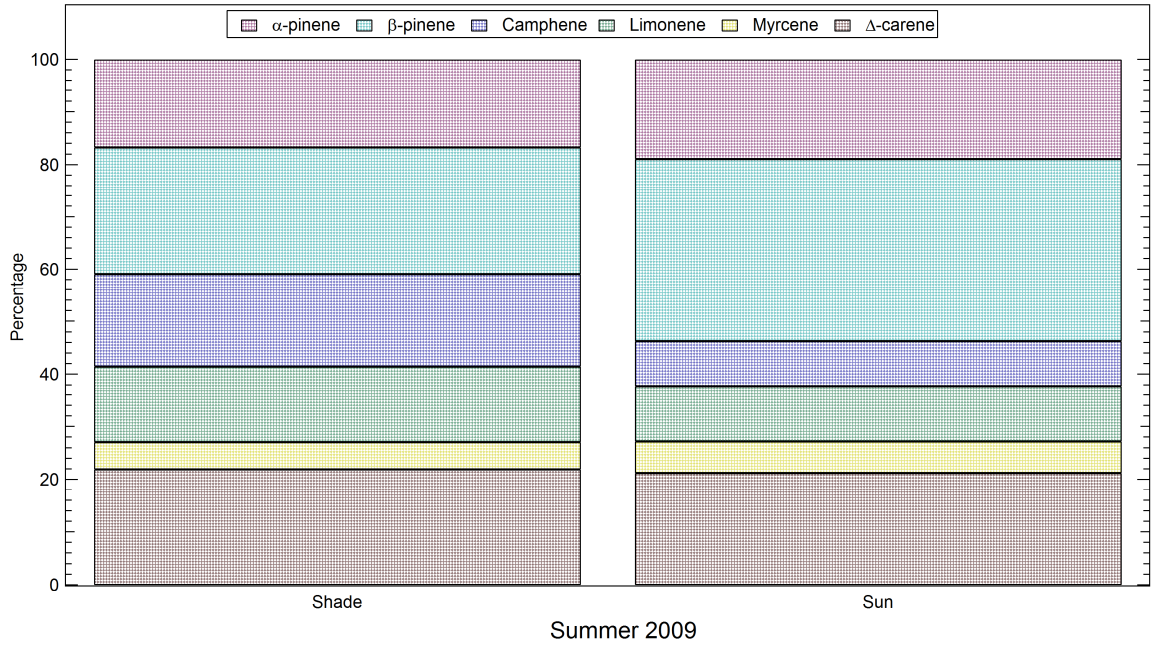

Figure 4.3 Percentages of major terpenoids found in the emissions of Pinus ponderosa from sun and shaded branches. Summer 2009

Differences between sun and shade branches

MBO

MBO emissions from sun branches across the measurement period averaged 10.23 ±0.88 μgC $g_{DW}^{-1}h^{-1}$. The shade branches emitted an average of 7.27 ±0.69 μgC $g_{DW}^{-1}h^{-1}$. The ANOVA analysis showed that there is a significant difference between the branch emissions ($p < 0.01$), for MBO (Fig 4.4).

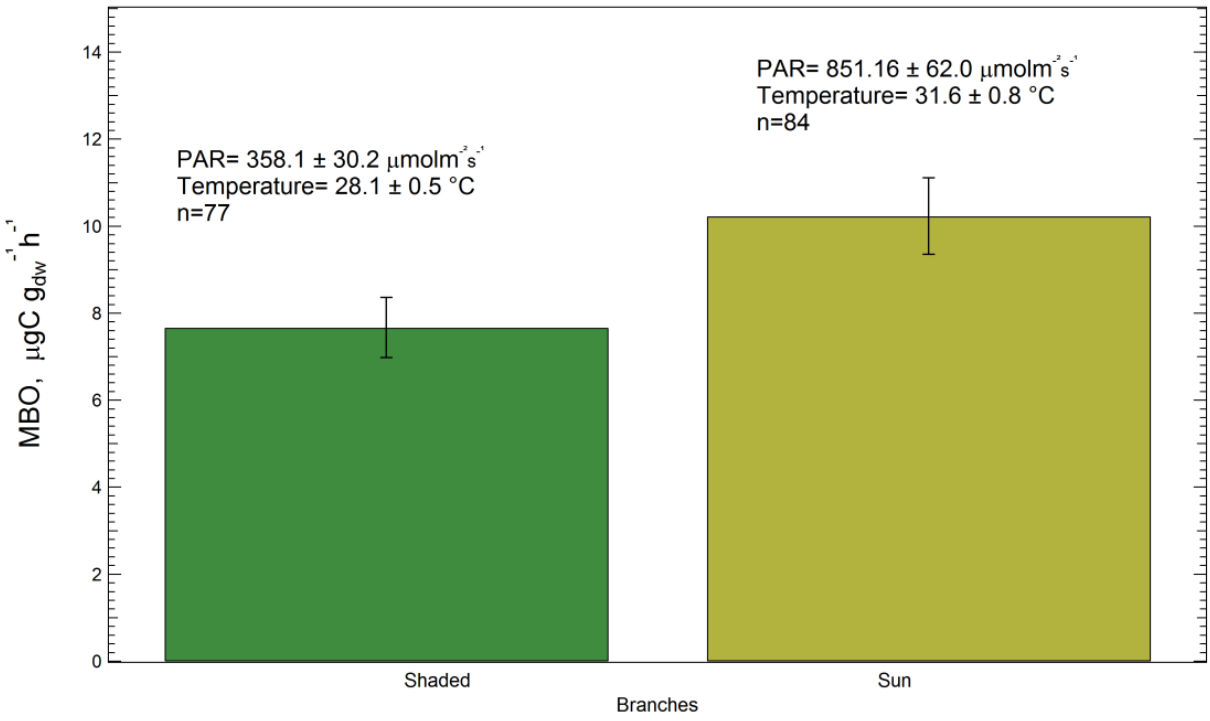

Figure 4.4. Comparisons of average MBO emissions of *Pinus ponderosa* sun and shaded branches during Summer 2009. Whisker length=standard error of the mean.

Monoterpenes

Emissions of total monoterpenes from sun branches across the summer averaged 1.85 ± 0.17 µgC $g_{DW}^{-1}h^{-1}$, while the shade branches emitted an average of 1.4 ±0.14 µgC $g_{DW}^{-1}h^{-1}$. The analysis of variance showed that there is a significant difference between the branch emissions ($p < 0.01$), and total monoterpenes (Fig 4.5).

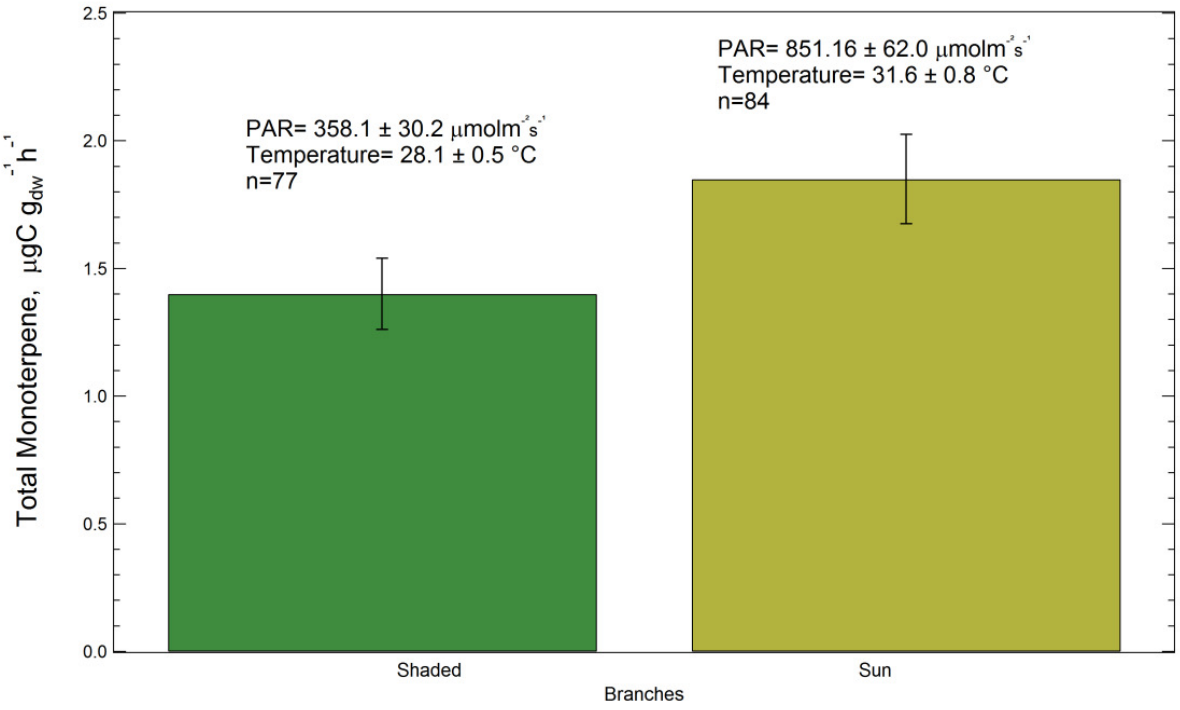

Figure 4.5 Comparisons of average total monoterpene emissions of *Pinus ponderosa* sun and shaded branches during Summer 2009. Whisker length=standard error of the mean.

The 6 six major monoterpenes were also analyzed with an ANOVA to determine if there was a difference in the emissions between sun and shaded branches. β-pinene was the only monoterpene that showed a significant difference ($p < 0.01$, Fig 4.6).

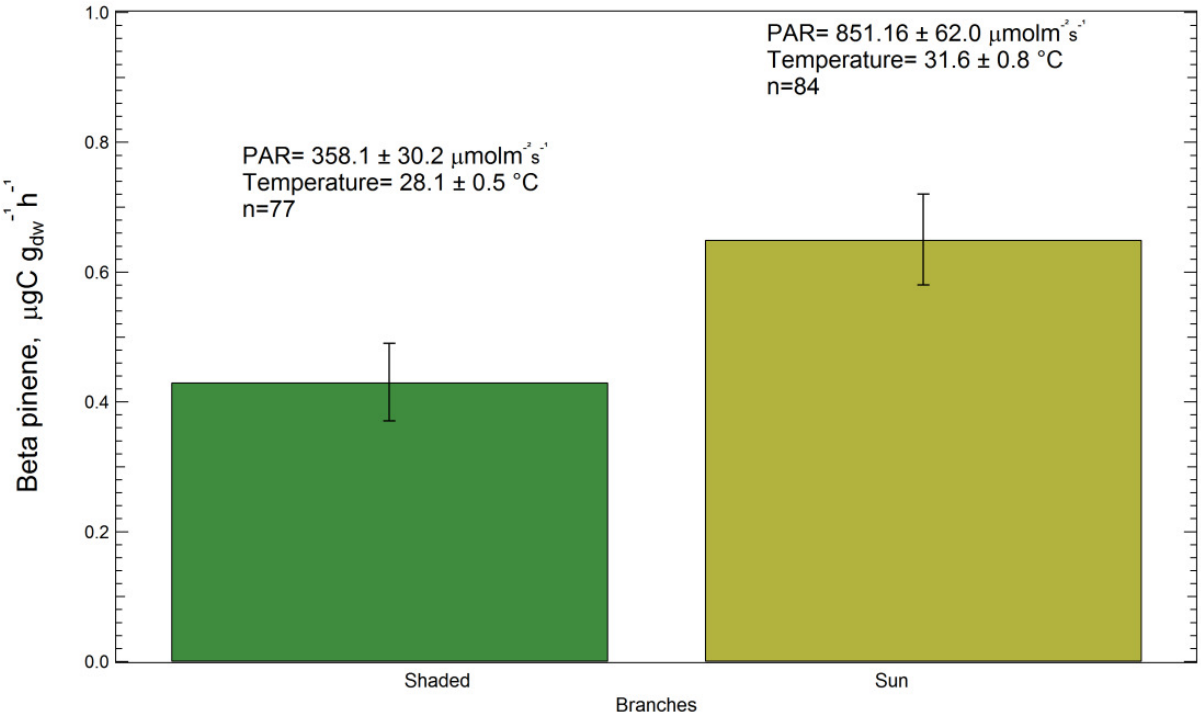

Figure 4.6 Comparisons of average β-pinene emissions of Pinus *ponderosa* sun and shaded branches during Summer 2009. Whisker length=standard error of the mean.

Daytime variability

MBO

PAR and temperature are environmental factors that change during the day, affecting the emission rate at which the terpenoid compounds are emitted. The daytime variability across the measurement period is shown in Figure 4.7. Because measurements were not taken at the exact time every day, the measurements were grouped in to: morning (8 AM-11 AM), Noon (11 AM-2 PM) and afternoon (2 PM and later). Emissions were larger in the morning period and decreased during the day for both sun and shaded branches (Figure 4.7). There was not a significant difference between the emissions of sun and shaded branches among the different times of the day ($p>0.05$).

It was expected that emissions would be larger at noon, because of the dependence of MBO with PAR and temperature, and it is precisely because of this dependence that the results are surprising. We can see that the highest PAR average was measured during the morning period for both sun and shade branches. If MBO emissions are triggered by PAR and temperature, it was expected that at the highest PAR we would measure the highest emissions, and in the case of this study that was the "morning" period.

Monoterpenes

Total monoterpenes emissions showed the same behavior as MBO (Fig. 4.8) in the shaded branches. There was a decrease on the emissions during the day, while the sun branches had a decrease at noon time and increased again during the afternoon. No significant difference was found between the emissions of sun and shaded branches across the day ($p>0.05$). While monoterpenes are more temperature dependent, we can see that the highest temperature was also achieved during the morning period.

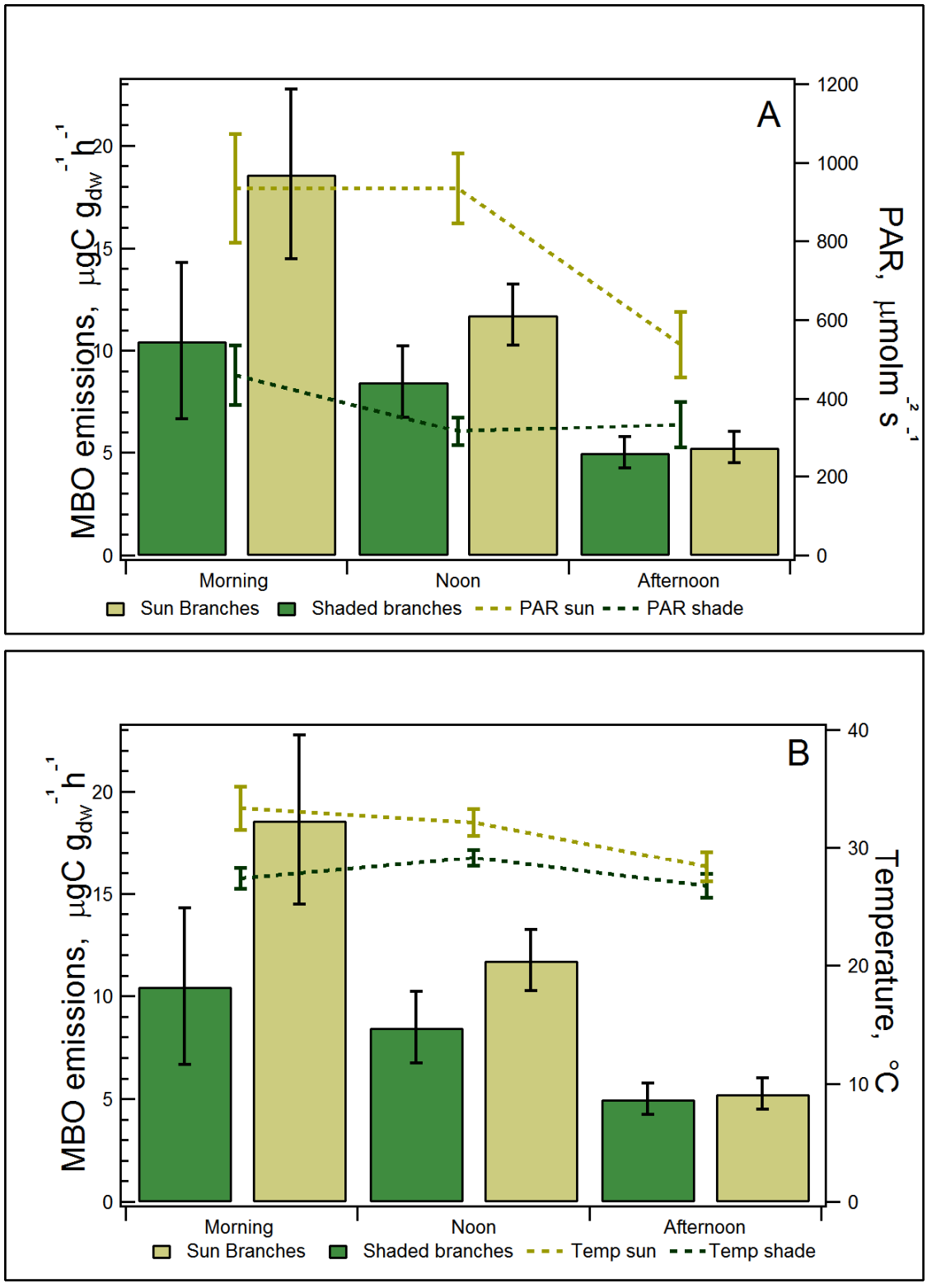

Figure 4.7 Graphs A and B show the comparisons of average MBO emissions of *Pinus ponderosa* sun and shaded branches during the day across the summer of 2009. Graph A shows the PAR average values and graph B shows the average temperature values. Whisker length=standard error of the mean.

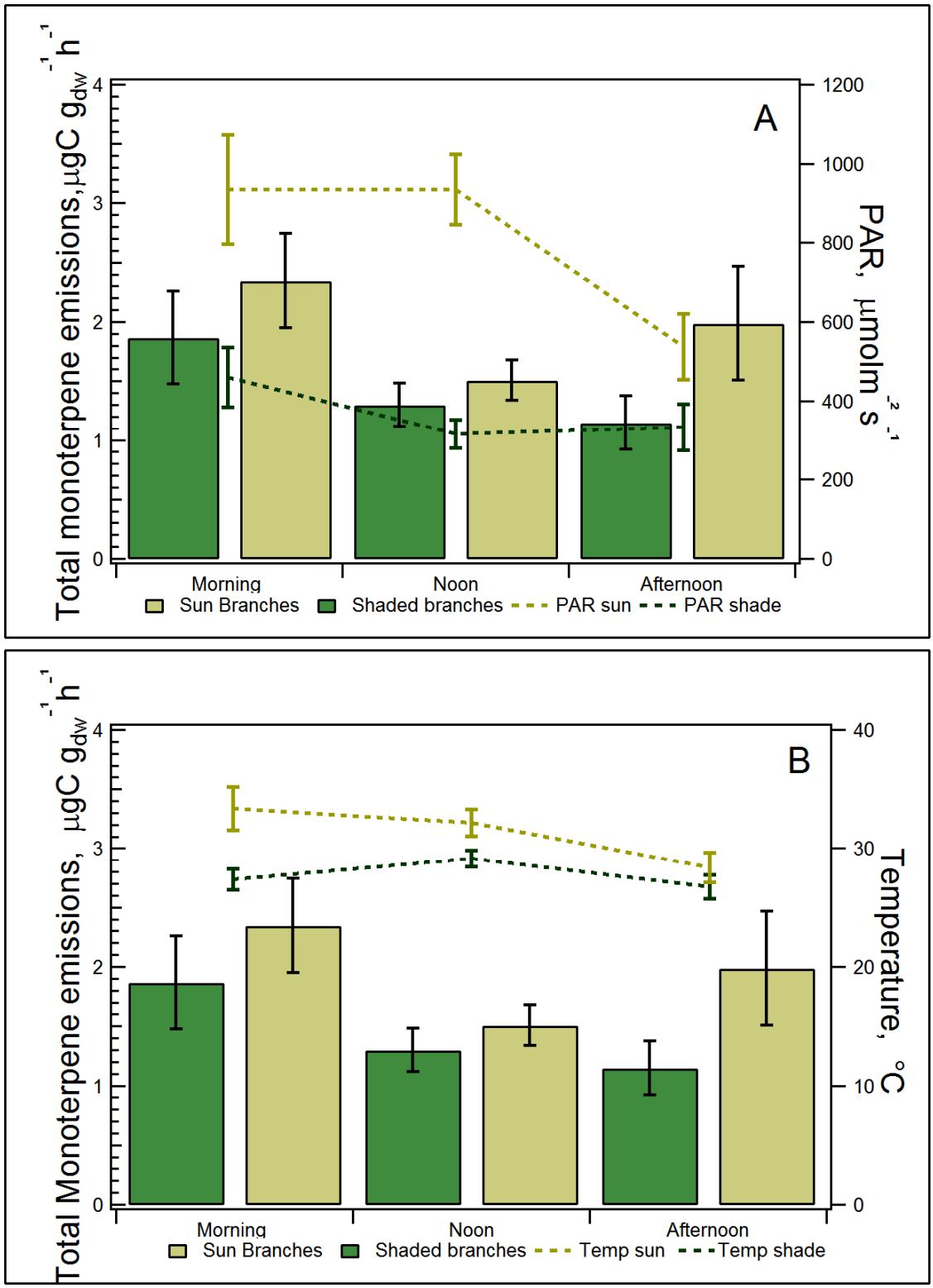

Figure 4.8 Graphs A and B show the comparisons of average of total monoterpene emissions of *Pinus ponderosa* from sun and shaded branches during the day across the summer of 2009. Graph A shows the PAR average values and graph B shows the average temperature values. Whisker length=standard error of the mean.

Summer variability

MBO

MBO emissions were measured from late May to late August. It is important to remember that solar angle (maximum solar elevation above the horizon) and weather are variables that change through the year; the time at which the emissions where measured have a very distinct solar angle and weather patterns. MBO emissions changed over the measurement period, but only at the end of the summer there was a significant difference between emissions from sun and shaded branches ($p < 0.05$, Fig.4.9).

ANOVAs were run to find if there were differences in emissions across the summer for sun and shade branches independently. No significant difference in sun branch emissions was evident between the early and mid-summer periods, or mid-summer and later summer. There was a significant difference between early and late summer ($p<0.05$). Emissions from shade branches showed significant differences between the late summer period ($p<0.05$) and the early and mid-summer.

Monoterpenes

Monoterpene emissions analysis showed a significant difference between sun and shaded branches in the late period of the summer ($p<0.05$, Fig. 4.10). A comparison of emissions of sun branches across the summer showed no significant difference between the three periods of the summer ($p>0.05$). The same result was found for the shaded branches ($p>0.05$).

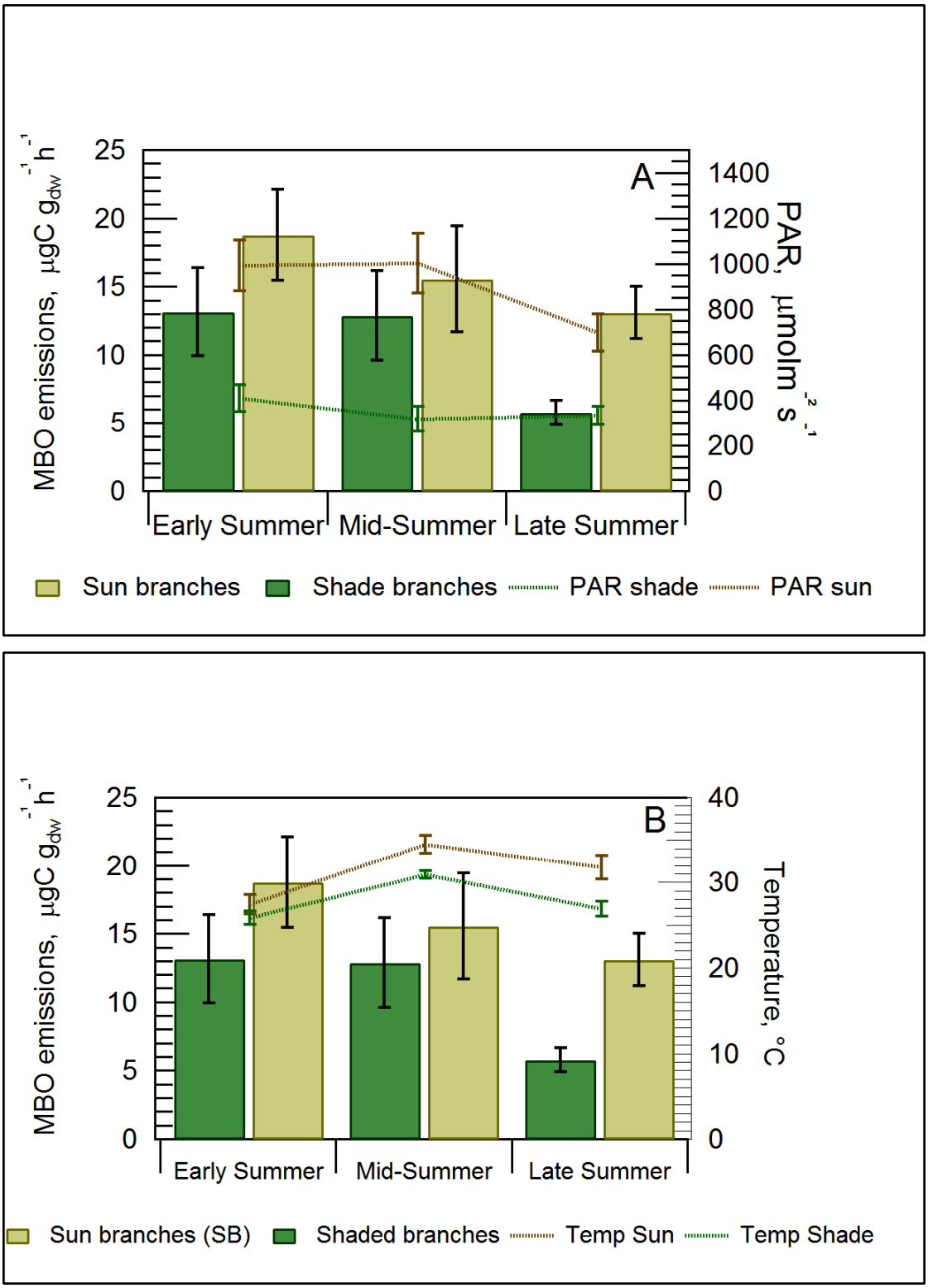

Figure 4.9 Graphs A and B show the comparisons of average of total monoterpene emissions of *Pinus ponderosa* from sun and shaded branches across the summer of 2009. Graph A shows the PAR average values and graph B shows the average temperature values. Whisker length=standard error of the mean.

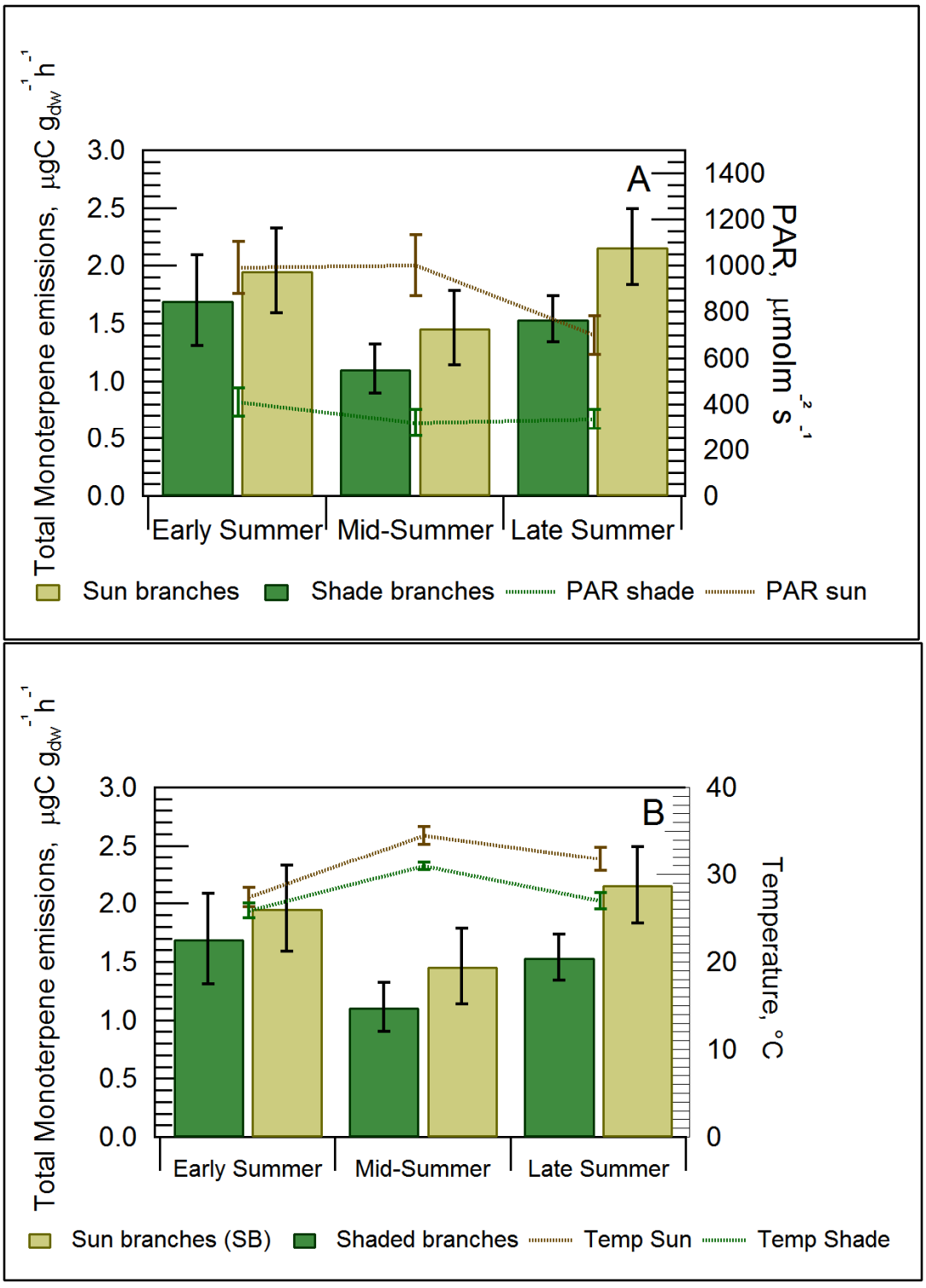

Figure 4.10 Graphs A and B show the comparisons of average of total monoterpene emissions of *Pinus ponderosa* from sun and shaded branches during the day across the summer of 2009. Graph A shows the PAR average values and graph B shows the average temperature values. Whisker length=standard error of the mean.

Summer variability per day time

MBO

Daytime and summer time analysis showed that there was variability from the environmental factors across the summer and during the day. It was of interest to see how the MBO emissions varied throughout the day during the different summer periods (Fig. 4.11). Emissions from sun and shaded branches varied significantly in early summer during the afternoon ($p<0.05$) and in late summer in the morning and at noon ($p<0.01$).

Monoterpenes

Monoterpene emissions analysis showed that emissions of sun and shaded branches were significantly different in late summer, but only in the morning ($p<0.05$, Fig . 12). Monoterpenes emissions are known to be triggered by temperature, and we can see that the temperatures during the summer times and the periods of the day did not vary significantly, until late summer in the morning ($p<0.01$, Fig 4.12).

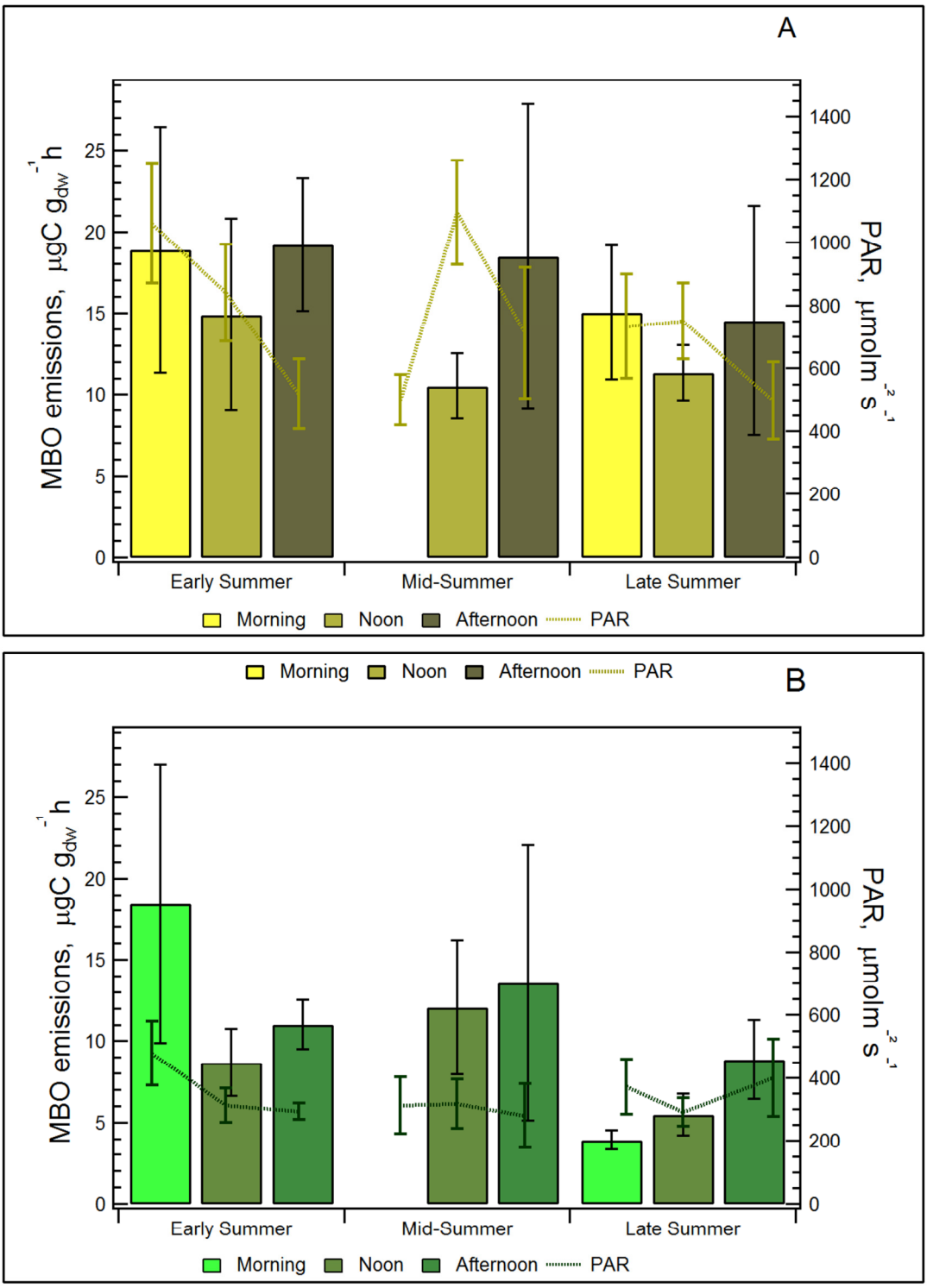

Figure 4.11 Daily averages of MBO emissions of Pinus ponderosa from sun (A) and shaded branches (B) . Lines are showing the photosynthetically active radiation variation across the summer of 2009. Whisker length=standard error of the mean. Note: There were not morning measurements available for the Mid-Summer period.

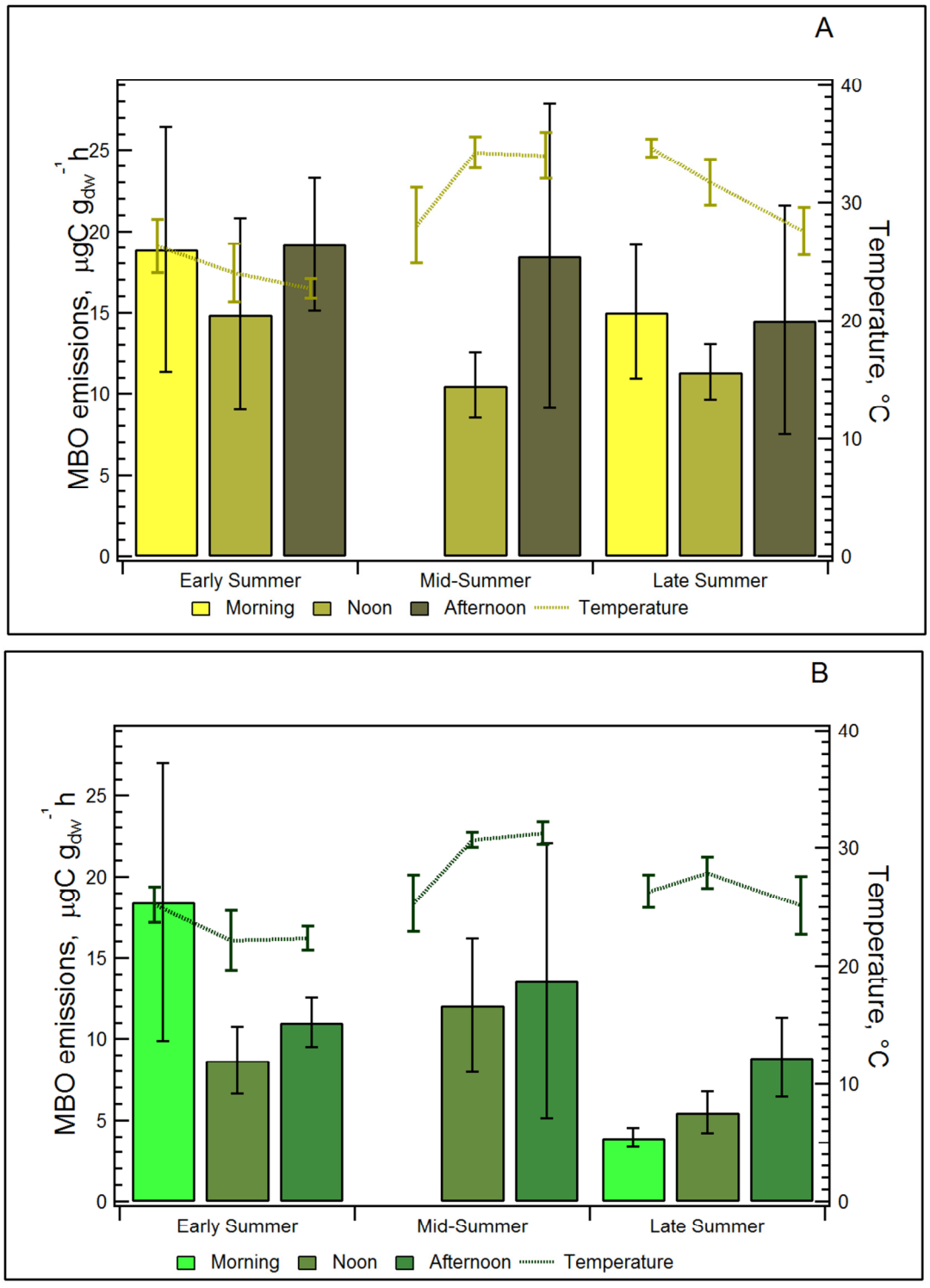

Figure 4.12 Daily averages of MBO emissions of *Pinus ponderosa* from sun (A) and shaded branches (B) . Lines are showing the temperature variation across the summer of 2009. Whisker length=standard error of the mean. Note: There were not morning measurements available for the Mid-Summer period.

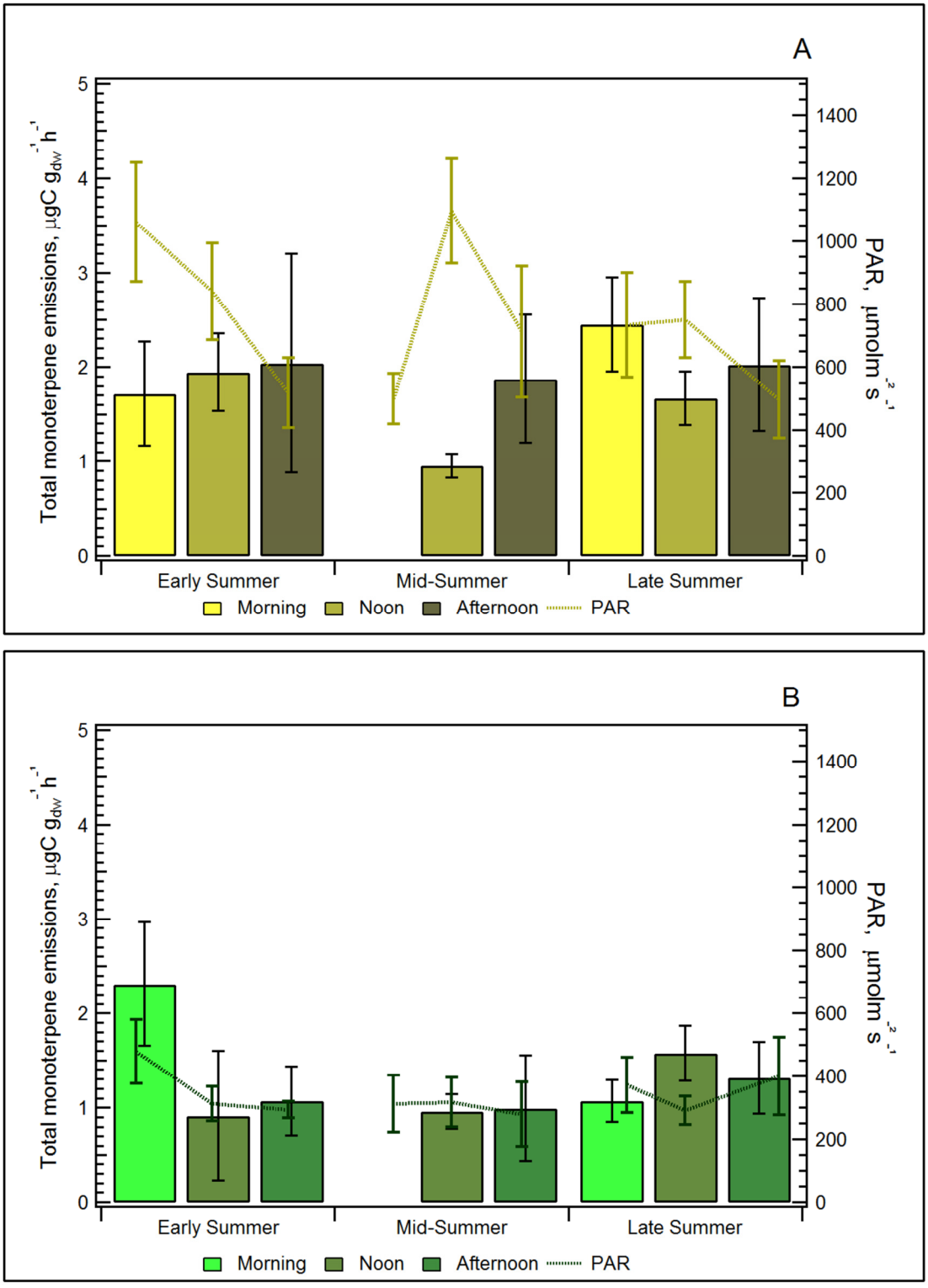

Figure 4.13 Daily averages of total terpenoid emissions of Pinus ponderosa from sun (A) and shaded branches (B) . Lines are showing the photosynthetically active radiation variation across the summer of 2009. Whisker length=standard error of the mean. Note: There were not morning measurements available for the Mid-Summer period.

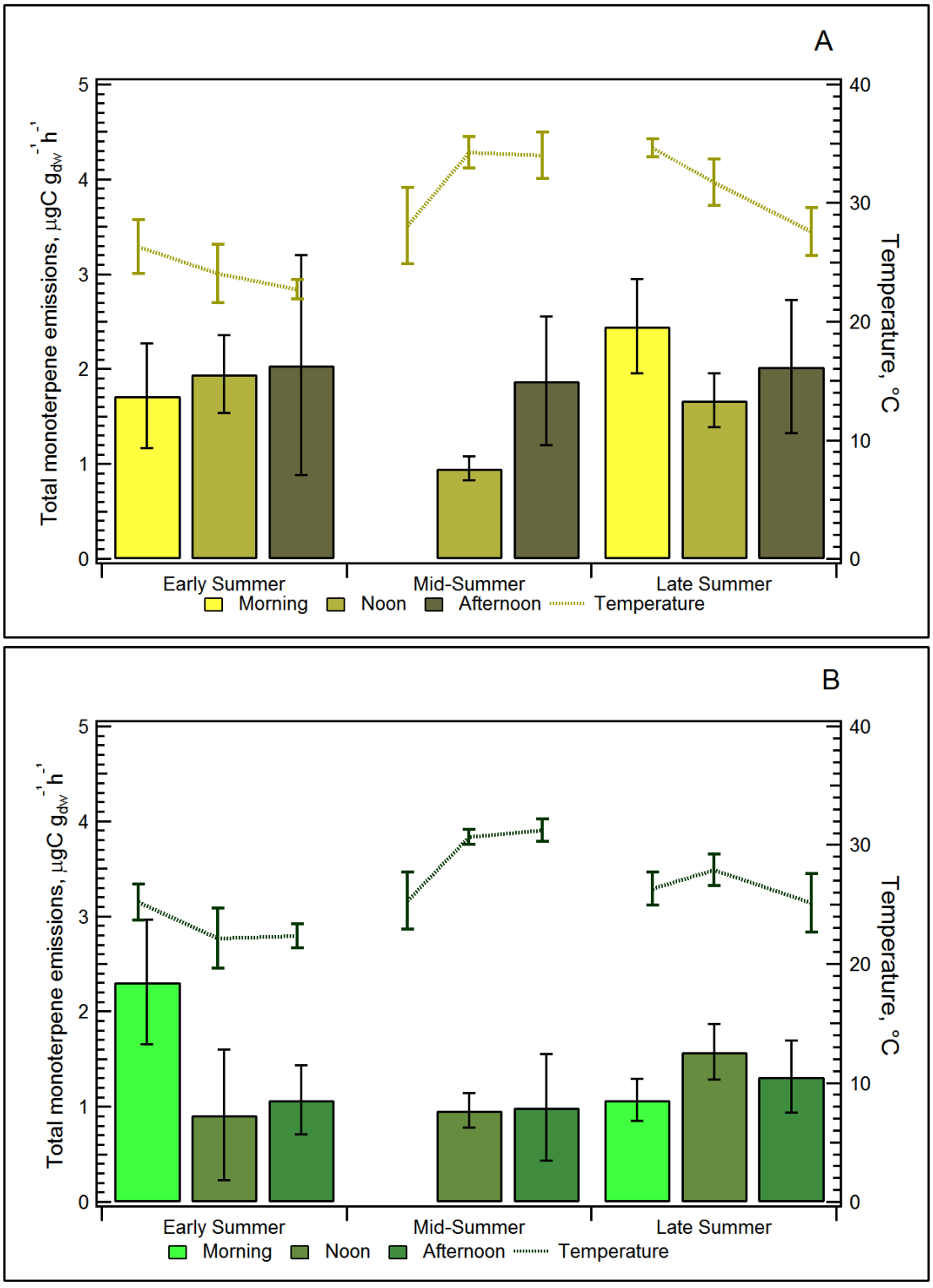

Figure 4.14 Daily averages of total terpenoid emissions of Pinus ponderosa from sun (A) and shaded branches (B) . Lines are showing the temperature variation across the summer of 2009. Whisker length=standard error of the mean. Note: There were not morning measurements available for the Mid-Summer period.

Sensitivity analysis

The results of this study were incorporated into the model of emissions of gases and aerosols from nature (MEGAN) (Guenther, Karl 2006) to estimate the magnitude of the emissions at a landscape scale. We used MEGAN to calculate the emissions rates of monoterpenes and MBO, using the model standard meteorological conditions and land cover data (MEGANst). We also calculated the emission rates using the meteorological factors and land cover data measured at the field site (MEGANf). We compared both calculations monoterpene and MBO measurements made with a proton transfer mass spectrometer (PTRMS) at the MEF by Dr. Thomas Karl (personal communication). The results of this comparison can be seen in table 4.2.

Source	MBO $\mu g\ m^{-2}s^{-1}$	Monoterpenes $\mu g\ m^{-2}s^{-1}$
MEGANst	1058	372
MEGANf	590	278
PTRMS	~6000	392

Table 4.2 Comparison of average emission rates. *MEGAN standard conditions, ** MEGAN field conditions.

It can be seen that the emission rates of MBO do not agree at all. The variability can be explained due to the light and temperature conditions above and below the canopy. If those conditions differ the emission factors may differ, as have been shown in this study. Monoterpenes emission rates did not vary as much as MBO emission rates. In this case we made the assumption that the emission of monoterpenes was completely

light independent. We can see that the emissions rate values differ from the PTRMS and MEGANst differ by 5%. While MEGANf and the PTRMS measurements differ by 29%.

Discussion

This work showed that light environment affects the emission rate of terpenoid compounds in adult ponderosa pine, with a significant difference in emissions between sun and shaded branches. Forest light environment and canopy structure have been the focus of several ecological studies since the last century (Pearcy, Muraoka 2005, Pearson 1940, Myre and Camiré 1996). It is known that sun and shade plants are significantly different in their photosynthetic rate, leaf morphology and biochemistry (Boardman 1977), and thus, as demonstrated here, terpene emissions will vary with position in the canopy. Given that information, it is surprising to discover that there are few field studies on terpenoid emissions that have taken into consideration the light environment in their measurements (Harley, Guenther 1996, Harley, Guenther 1997, Sharkey, Singsaas 1996). Most studies of terpenoid production or emissions that have been done in terms of light and temperature variation are laboratory or greenhouse studies where the conditions were controlled (Lichtenthaler, AÄ 2007, Monson and Fall 1989, Loreto and Sharkey 1990, Fang, Monson 1996, Bertin and Staudt 1996). The results of these studies have been the input of current atmospheric models. Given biological variation in the field due to environmental heterogeneity (Loreto, Bagnoli 2009, Penuelas and Llusia 2001, Wang, Owen 2007) and, particularly, in plant growing in open areas and shaded environments, these controlled studies may insufficiently represent important biological drivers of emissions.

The differences between the emissions of sun and shaded branches found in this study were expected. It is well known that the light environment where the plant develops will determine the plant's morphology and physiology (Boardman 1977, Givnish 1988, Öquist, Anderson 1992, Bond, Farnsworth 1999) . The physiology of *Pinus ponderosa* has been studied for several decades now, and it is known that light environment affects the growth patterns in ponderosa trees (Pearson 1936, Latta, Linhart 2003, Latta and Linhart 1997, Pearson 1940, Naumburg). It is also known that chemical compounds vary within tissues of *P. ponderosa*, and that chemical composition is dictated by biological variability (Latta, Linhart 2003, Latta, Linhart 2000, Mitton, Linhart 1977, Linhart, Mitton 1981, Linhart and Mitton 1985).

In this study, biological variability was a principal consideration in the experimental design and analysis. The facts that the branches were measured at the same time of day and both sun and shade branches stemmed from a larger, primary branch, helps to control for some of the biological variability that could have affected the results. Latta et al. (Latta, Linhart 2000) found that monoterpene composition varied considerably within the needles of *P. ponderosa* given the position of the branch (south or north facing). Another step to reduce variability, and which makes this study unique, is the large sample size and the length of the experiment. Most of the leaf/branch level studies done in the field have focused their attention on few branches and over a short period of time (Perez-Rial, Penuelas 2009a, Calfapietra, Mugnozza 2008, Grabmer, Kreuzwieser 2006). This study took place over a large part of the growing season (late May to late August) and a total of 30 trees (60 branches) were measured. The results also show temporal variability that can be explained by needle development. Unfortunately, this study did not measure variables that could account for development, such as

photosynthetic rate or stomatal conductance. However, those processes are known to correlate with the rate at which emissions are produced (Gray, Lerdau 2003, Gray, Goldstein 2006).

Incorporating biological processes is not an easy task because of the possible interactions within those processes. In this case the goal of this study was to determine if the light environment affected terpenoid emissions, which we showed. The second objective was to determine if the measurements in the field could have an impact in current atmospheric models. In this case the sensitivity analysis showed that there were differences between our results and those predicted by MEGAN, more evidently in the light dependent MBO(Harley, Fridd-Stroud 1998). This shows that there is a need to understand how the light environment "works "within the canopy. A radiate transfer model is not enough to predict how the emissions are going to be produced. This study is just a first step towards the integration of more biological parameters in to the current atmospheric models.

Chapter 5 CONCLUSIONS

The objective of my dissertation was to explore the potential roles that landscape structure (the arrangement of cover types) and landscape composition (plant type and plant age) play on terpenoid emissions and their consequent impacts on atmospheric chemistry. I measured terpenoid emissions rates of two different species, Pteridium aquilinum and Pinus ponderosa in two different ecosystems. In order to quantify the magnitude of the emissions I used a dynamic branch enclosures and GC-FID and GC-MS techniques. This methodology allowed me to understand how different components of the landscape may be affecting the production of terpenoid emissions.

One component of the landscape is the understory. In order to understand how this element of the ecosystem could be contributing to regional terpenoid I used *Pteridium aquilinum*, Bracken fern, a dominant understory species. Scientists who study terpenoid emissions and their roles in atmospheric chemistry have focused their efforts on measuring species that cover large areas of land. Then, why study an understory species?

I have different answers to that question. First, if we want to understand and model BVOC fluxes, we have to have a better understanding of the emissions of the whole ecosystem. Studies of trees and large shrub species' emissions commonly neglect lower strata in the forest, often a small but significant component of the biomass. Moreover, disturbances such as windthrow and fire result in early seral stages dominated by understory herbaceous and small woody species. Emission inventories do not consider dynamic changes in landscapes. While this study found that *P. aquilinum* did not have a large effect on the regional atmospheric chemistry, I cannot emphasize enough the fact

that the higher emissions measured are comparable to those of woody species (1.6 ±0.4 $\mu gC \bullet g_{dw}^{-1} \bullet h^{-1}$). If we consider a landscape that is dominated by bracken fern, which happens in a number of regions where bracken fern is an invasive species e.g. United Kingdom, the emissions will affect the regional chemistry. I think atmospheric scientists have neglected the understory vegetation because they think their emissions are negligible.

One more conclusion drawn from studying *P. aquilinum,* and in general with my whole dissertation, is that long measurement periods are necessary for characterizing emission factors. Long-term monitoring is needed to understand how and when emissions are produced. Because of biological variability, emissions can be detected on some days but not others; a survey conducted on a single day could conclude that a plant does not emit any monoterpenes or could overestimate emissions.

Another component of the landscape, and in his case I am talking in a temporal scale, is plant age. It is known that age affects biological processes, so are terpenoid emissions an exception to the rule? The approach that I took to study the influence of plants developmental stages on terpenoid emissions was to study 2 different ages of a plant species and compare their terpenoid emissions rate. The study system that I chose for this part of my dissertation was *Pinus ponderosa* a species that has been study extensively, from an ecological and atmospheric point of view. This species would provide me with comprehensive background information to achieve my goal. *P. ponderosa* is a known to produced and emitted terpenoid compounds. If all that information was available, I wondered why there were no studies about the relationship of terpenoid emissions and developmental stage. The answer is simple, is a woody

species. When doing ontogenetic studies, focus on plants that grow fast that way they can corroborate their results. This work is one the first studies focusing on the relation of developmental stage and terpenoid compounds on woody species. In Chapter 3, we discovered that mature trees emission rates were significantly lower than those of the seedlings. Seedlings are another component of the landscape that if they have not been ignored; they have not been studied in the field as much as they have been study in the laboratory. Is it possible that we are over/underestimating the emissions? There are many instances where a plant is classified as an emitter using solely laboratory studies. If the results those studies are used in current atmospheric models and the results of my study hold true for all the species. It may be the case that current atmospheric models are overestimating terpenoid fluxes. It is important to understand the scale at which we are asking the questions. Sometimes the age of the plant can be underestimated and there are instances where the results of this study could have major implications such as land use management and reforestation. If we are trying to understand the regional chemistry we have to take in consideration the type and age of vegetation present on the landscape and in this study it can be seen that age matters.

Finally, in Chapter 4, we investigated the possibility that landscape configuration could have an effect on terpenoid emissions, and discussed the possibility of integrating it as a parameter into atmospheric models to improve their estimates. We showed that there is a significant difference between the emissions of the sun and shaded branches. Is it possible to assume that sun and shaded branches could be an analogy to a dense and open forest? Unfortunately that is not the case because as we have seen, the environment where the plant grows will determine its morphology and physiological

functions. However, what happen when a forest is disturbed? The plants will face different light and temperature regimes that will affect the rate at which terpenoid emissions are emitted to the atmosphere.

Atmospheric models do not currently deal with landscape structural change. Currently the models take into consideration light, temperature, humidity, and type of vegetation. But, what happens when the landscape change? And by change I mean the distribution of the vegetation in the area where terpenoid compounds are measured.

This study it is the first step towards understanding the implications of landscape on atmospheric chemistry. One of the premises of landscape ecology is "spatial patterns affect ecological processes"(Turner 2005). I think the theory behind landscape ecology, not only applies to ecosystems and biological processes; as it has been shown in this work landscape has the potential to affect the chemistry of the atmosphere.

LITERATURE CITED

Adler F. R., R. Karban. 1994. Defended Fortresses or Moving Targets? Another Model of Inducible Defenses Inspired by Military Metaphors. The American Naturalist **144**:pp. 813-832.

Alonso-Amelot M. E., A. Oliveros, M. P. Calcagno, and E. Arellano. 2001. Bracken adaptation mechanisms and xenobiotic chemistry. Pure and Applied Chemistry **73**:549-553.

Arimura G., C. Kost, and W. Boland. 2005/5/15. Herbivore-induced, indirect plant defences. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids **1734**:91-111.

Aros D., V. Gonzalez, R. K. Allemann, C. T. Müller, C. Rosati, and H. J. Rogers. 2012. Volatile emissions of scented Alstroemeria genotypes are dominated by terpenes, and a myrcene synthase gene is highly expressed in scented Alstroemeria flowers. Journal of experimental botany :.

Ashour M., M. Wink, and J. Gershenzon. 2010. Biochemistry of Terpenoids: Monoterpenes, Sesquiterpenes and Diterpenes. Pages 258-303 *In* Anonymous Annual Plant Reviews Volume 40: Biochemistry of Plant Secondary Metabolism, Wiley-Blackwell, .

Atkinson R., J. Arey. 2003. Gas-phase tropospheric chemistry of biogenic volatile organic compounds: a review. Atmospheric Environment **37**:S197-S219.

Atkinson T. P. 1989. Seasonal and Altitudinal Variation in Pteridium-Aquilinum (L) Kuhn - Frond and Stand Types. New Phytologist **113**:359-365.

Bai J., B. Baker, B. Liang, J. Greenberg, and A. Guenther. 2006/9. Isoprene and monoterpene emissions from an Inner Mongolia grassland. Atmospheric Environment **40**:5753-5758.

Baker B., J. Bai, C. Johnson, Z. Cai, Q. Li, Y. Wang, A. Guenther, J. Greenberg, L. Klinger, C. Geron, and R. Rasmussen. 2005/1. Wet and dry season ecosystem level fluxes of isoprene and monoterpenes from a southeast Asian secondary forest and rubber tree plantation. Atmospheric Environment **39**:381-390.

Barrington D. S. 1993. Ecological and Historical Factors in Fern Biogeography. Journal of Biogeography **20**:275-279.

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

Blackburn G. A. 1998. Quantifying chlorophylls and caroteniods at leaf and canopy scales: An evaluation of some hyperspectral approaches. Remote Sensing of Environment **66**:273-285.

Boardman N. K. 1977. Comparative Photosynthesis of Sun and Shade Plants. Annual Review of Plant Physiology **28**:355-377.

Bond B. J., B. T. Farnsworth, R. A. Coulombe, and W. E. Winner. 1999. Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. Oecologia **120**:183-192.

Brilli F., P. Ciccioli, M. Frattoni, M. Prestininzi, A. F. Spanedda, and F. Loreto. 2009. Constitutive and herbivore-induced monoterpenes emitted by Populus x euroamericana leaves are key volatiles that orient Chrysomela populi beetles. Plant Cell and Environment **32**:542-552.

Calfapietra C., G. S. Mugnozza, D. F. Karnosky, F. Loreto, and T. D. Sharkey. 2008. Isoprene emission rates under elevated CO2 and O-3 in two field-grown aspen clones differing in their sensitivity to O-3. New Phytologist **179**:55-61.

Chen Z., T. E. Kolb, and K. M. Clancy. 2002. The Role of Monoterpenes in Resistance of Douglas Fir to Western Spruce Budworm Defoliation. Journal of chemical ecology **28**:897-920.

Coley P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource Availability and Plant Antiherbivore Defense. Science **230**:895-899.

Dubey V. S., R. Bhalla, and L. Rajesh. 2003. An overview of the non-mevalonate pathway for terpenoid biosynthesis in plants. Journal of Biosciences **28**:637-646.

Fang C. W., R. K. Monson, and E. B. Cowling. 1996. Isoprene emission, photosynthesis, and growth in sweetgum (Liquidambar styraciflua) seedlings exposed to short- and long-term drying cycles. Tree physiology **16**:441-446.

Fares S., F. Loreto, E. Kleist, and J. Wildt. 2008. Stomatal uptake and stomatal deposition of ozone in isoprene and monoterpene emitting plants. Plant Biology **10**:44-54.

Fares S., A. Goldstein, and F. Loreto. 2010. Determinants of ozone fluxes and metrics for ozone risk assessment in plants. Journal of experimental botany **61**:629-633.

Fares S., E. Oksanen, M. Lannenpaa, R. Julkunen-Tiitto, and F. Loreto. 2010. Volatile emissions and phenolic compound concentrations along a vertical profile of Populus nigra leaves exposed to realistic ozone concentrations. Photosynthesis Research **104**:61-74.

Fehsenfeld F., J. Calvert, R. Fall, P. Goldan, A. B. Guenther, C. N. Hewitt, B. Lamb, L. Shaw, M. Trainer, and H. Westberg et al. 1992. Emissions of Volatile Organic Compounds From Vegetation and the Implications for Atmospheric Chemistry. Global Biogeochemical Cycles **6**:389-430.

Finlayson-Pitts B. J., J. N. Pitts. 2000. Chemistry of the upper and lower atmosphere: theory, experiments and applications. Academic Press, San Diego, Calif. ; London.

Finlayson-Pitts B. J., J. N. Pitts. 1997. Tropospheric Air Pollution: Ozone, Airborne Toxics, Polycyclic Aromatic Hydrocarbons, and Particles. Science **276**:1045-1051.

Fuentes J. D., D. Wang, D. R. Bowling, M. Potosnak, R. K. Monson, W. S. Goliff, and W. R. Stockwell. 2007. Biogenic hydrocarbon chemistry within and above a mixed deciduous forest. Journal of Atmospheric Chemistry **56**:165-185.

Fuentes J. D., M. Lerdau, R. Atkinson, D. Baldocchi, J. W. Bottenheim, P. Ciccioli, B. Lamb, C. Geron, L. Gu, A. Guenther, T. D. Sharkey, and W. Stockwell. 2000. Biogenic hydrocarbons in the atmospheric boundary layer: A review. Bulletin of the American Meteorological Society **81**:1537-1575.

Fuzzi S., M. O. Andreae, B. J. Huebert, M. Kulmala, T. C. Bond, M. Boy, S. J. Doherty, A. Guenther, M. Kanakidou, K. Kawamura, V. -. Kerminen, U. Lohmann, L. M. Russell, and U. P\oschl. 2006. Critical assessment of the current state of scientific knowledge, terminology, and research needs concerning the role of organic aerosols in the atmosphere, climate, and global change. Atmospheric Chemistry and Physics **6**:2017-2038.

Gary L. H. 1985. A summary of Research at the Manitou Experimental Forest in Colorado 1937-1983. General Technical report RM-116. U.S. Department of Agriculture, Forest Service. Rocky Mountain Research Station, Fort Collins, CO :.

Gasche R., H. Papen, and H. Rennenberg. 2002. Trace gas exchange in forest ecosystems. Kluwer Academic Publishers, Dordrecht ; Boston.

Gaylord M. L., T. E. Kolb, K. F. Wallin, and M. R. Wagner. 2006. Seasonality and lure preference of bark beetles (Curculionidae : Scolytinae) and associates in a Northern Arizona ponderosa pine forest. Environmental Entomology **35**:37-47.

Geron C., P. Harley, and A. Guenther. 2001. Isoprene emission capacity for US tree species. Atmospheric Environment **35**:3341-3352.

Geron C. D., A. B. Guenther, and T. E. Pierce. 1994. An Improved Model for Estimating Emissions of Volatile Organic-Compounds from Forests in the Eastern United-States. Journal of Geophysical Research-Atmospheres **99**:12773-12791.

Geron C., P. Harley, and A. Guenther. 2001/7. Isoprene emission capacity for US tree species. Atmospheric Environment **35**:3341-3352.

Geron C., R. Rasmussen, R. R. Arnts, and A. Guenther. 2000. A review and synthesis of monoterpene speciation from forests in the United States. Atmospheric Environment **34**:1761-1781.

Geron C., S. Owen, A. Guenther, J. Greenberg, R. Rasmussen, J. Hui Bai, Q. Li, and B. Baker. 2006/3. Volatile organic compounds from vegetation in southern Yunnan Province, China: Emission rates and some potential regional implications. Atmospheric Environment **40**:1759-1773.

Gershenzon J. 1994. Metabolic costs of terpenoid accumulation in higher plants. Journal of chemical ecology **20**:1281-1328.

Gilliam F. S., R. M. Roberts. 2003. The herbaceous layer in forest of Eastern North America. Oxford University Press, New york, New York.

Givnish T. J. 1988. Adaptation to Sun and Shade: a Whole-Plant Perspective. Functional Plant Biology **15**:63-92.

Goldstein A. H., I. E. Galbally. 2007. Known and Unexplored Organic Constituents in the Earth's Atmosphere. Environmental science & technology **41**:1514-1521.

Grabmer W., J. Kreuzwieser, A. Wisthaler, C. Cojocariu, M. Graus, H. Rennenberg, D. Steigner, R. Steinbrecher, and A. Hansel. 2006. VOC emissions from Norway spruce (Picea abies L. [Karst]) twigs in the field - Results of a dynamic enclosure study. Atmospheric Environment **40**:S128-S137.

Gräwert T., M. Groll, F. Rohdich, A. Bacher, and W. Eisenreich. 2011. Biochemistry of the non-mevalonate isoprenoid pathway. Cellular and Molecular Life Sciences **68**:3797-3814.

Gray D. W., A. H. Goldstein, and M. T. Lerdau. 2006. Thermal history regulates methylbutenol basal emission rate in Pinus ponderosa. Plant Cell and Environment **29**:1298-1308.

Gray D. W., M. T. Lerdau, and A. H. Goldstein. 2003. Influences of temperature history, water stress, and needle age on methylbutenol emissions. Ecology **84**:765-776.

Greenberg J. P., A. B. Guenther, G. Petron, C. Wiedinmyer, O. Vega, L. V. Gatti, J. Tota, and G. Fisch. 2004. Biogenic VOC emissions from forested Amazonian landscapes. Global Change Biology **10**:651-662.

Grote R. 2007. Sensitivity of volatile monoterpene emission to changes in canopy structure: a model-based exercise with a process-based emission model. New Phytologist **173**:550-561.

Grulke N. E., W. A. Retzlaff. 2001. Changes in physiological attributes of ponderosa pine from seedling to mature tree. Tree physiology **21**:275-286.

Guenther A. 2002. The contribution of reactive carbon emissions from vegetation to the carbon balance of terrestrial ecosystems. Chemosphere **49**:837-844.

Guenther A., T. Karl, P. Harley, C. Wiedinmyer, P. I. Palmer, and C. Geron. 2006. Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). Atmospheric Chemistry and Physics **6**:3181-3210.

Guenther A., J. Greenberg, P. Harley, D. Helmig, L. Klinger, L. Vierling, P. Zimmerman, and C. Geron. 1996. Leaf, branch, stand and landscape scale measurements of volatile organic compound fluxes from U.S. woodlands. Tree physiology **16**:17.

Guenther A., P. Zimmerman, L. Klinger, J. Greenberg, C. Ennis, K. Davis, W. Pollock, H. Westberg, G. Allwine, and C. Geron. 1996. Estimates of regional natural volatile organic compound fluxes from enclosure and ambient measurements. Journal of Geophysical Research-Atmospheres **101**:1345-1359.

Guenther A., C. N. Hewitt, D. Erickson, R. Fall, C. Geron, T. Graedel, P. Harley, L. Klinger, M. Lerdau, W. A. Mckay, T. Pierce, B. Scholes, R. Steinbrecher, R. Tallamraju, J. Taylor, and P. Zimmerman. 1995. A Global-Model of Natural Volatile Organic-Compound Emissions. Journal of Geophysical Research-Atmospheres **100**:8873-8892.

Guenther A. B., A. J. Hills. 1998. Eddy covariance measurement of isoprene fluxes. Journal of geophysical research **103**:13.

Guenther A. B., R. K. Monson, and R. Fall. 1991. ISOPRENE AND MONOTERPENE EMISSION RATE VARIABILITY - OBSERVATIONS WITH EUCALYPTUS AND EMISSION RATE ALGORITHM DEVELOPMENT. Journal of Geophysical Research-Atmospheres **96**:10799-10808.

Guenther A. B., P. R. Zimmerman, P. C. Harley, R. K. Monson, and R. Fall. 1993. Isoprene and Monoterpene Emission Rate Variability - Model Evaluations and Sensitivity Analyses. Journal of Geophysical Research-Atmospheres **98**:12609-12617.

Guenther A. 1997. Seasonal and Spatial Variations in Natural Volatile Organic Compound Emissions. Ecological Applications **7**:34-45.

Hallquist M., J. C. Wenger, U. Baltensperger, Y. Rudich, D. Simpson, M. Claeys, J. Dommen, N. M. Donahue, C. George, A. H. Goldstein, J. F. Hamilton, H. Herrmann, T. Hoffmann, Y. Iinuma, M. Jang, M. E. Jenkin, J. L. Jimenez, A. Kiendler-Scharr, W. Maenhaut, G. McFiggans, T. F. Mentel, A. Monod, A. S. H. Pr\'ev\^ot, J. H. Seinfeld, J. D. Surratt, R. Szmigielski, and J. Wildt. 2009. The formation, properties and impact of secondary organic aerosol: current and emerging issues. Atmospheric Chemistry and Physics **9**:5155-5236.

Hamilton J. G., A. R. Zangerl, E. H. DeLucia, and M. R. Berenbaum. 2001. The carbon-nutrient balance hypothesis: its rise and fall. Ecology Letters **4**:86-95.

Hare D. H. 2010. Ontogeny and Season Constrain the Production of Herbivore-Inducible Plant Volatiles in the Field. Journal of chemical ecology **36**:1363-1374.

Harley P., A. Guenther, and P. Zimmerman. 1997. Environmental controls over isoprene emission in deciduous oak canopies. Tree physiology **17**:705-714.

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

Harley P., V. Fridd-Stroud, J. Greenberg, A. Guenther, and P. Vasconcellos. 1998. Emission of 2-methyl-3-buten-2-ol by pines: A potentially large natural source of reactive carbon to the atmosphere. Journal of Geophysical Research-Atmospheres **103**:25479-25486.

Harley P. C., R. K. Monson, and M. T. Lerdau. 1999. Ecological and evolutionary aspects of isoprene emission from plants. Oecologia **118**:109-123.

Harley P., V. Fridd-Stroud, J. Greenberg, A. Guenther, and P. Vasconcellos. 1998. Emission of 2-methyl-3-buten-2-ol by pines: A potentially large natural source of reactive carbon to the atmosphere. J.Geophys.Res. **103**:25479-25486.

Harrewijn P., A. M. van Oosten, and P. G. M. Piron. 2001. Natural terpenoids as messengers : a multidisciplinary study of their production, biological functions, and practical applications. Kluwer Academic, Dordrecht ; Boston.

Heil M., R. Karban. 2010. Explaining evolution of plant communication by airborne signals. Trends in Ecology & Evolution **25**:137-144.

Helmig D. 1997. Ozone removal techniques in the sampling of atmospheric volatile organic trace gases. Atmospheric Environment **31**:3635-3651.

Helmig D., L. Vierling, C. Geron, P. Zimmerman, L. F. Klinger, and A. Guenther. 1999. Biogenic volatile organic compound emissions (BVOCs). II. Landscape flux potentials from three continental sites in the U.S. Chemosphere **38**:2189-2204.

Hewitt C. N. 1999. Reactive hydrocarbons in the atmosphere. Academic Press, San Diego.

Hewitt C. N., R. A. Street. 1992. A qualitative assessment of the emission of non-methane hydrocarbon compounds from the biosphere to the atmosphere in the U.K.: Present knowledge and uncertainties. Atmospheric Environment.Part A.General Topics **26**:3069-3077.

Huckaby L. S., M. R. Kaufmann, P. J. Fornwalt, J. M. Stoker, and C. Dennis. 2003. Identification and ecology of old ponderosa pine trees in the Colorado Front Range. Gen. Tech. Rep. RMRS-GTR-110. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 47 p.. :.

Jacobson M. C., H. -. Hansson, K. J. Noone, and R. J. Charlson. 2000. Organic atmospheric aerosols: Review and state of the science. Reviews of Geophysics **38**:267-294.

Jarvis A. J., V. J. Stauch, K. Schulz, and P. C. Young. 2004. The seasonal temperature dependency of photosynthesis and respiration in two deciduous forests. Global Change Biology **10**:939-950.

Jones C. G., R. D. Firn, and S. B. Malcolm. 1991. On the Evolution of Plant Secondary Chemical Diversity [and Discussion]. Philosophical Transactions: Biological Sciences **333**:273-280.

Jux A., G. Gleixner, and W. Boland. 2001. Classification of Terpenoids according to the Methylerythritolphosphate or the Mevalonate Pathway with Natural 12C/13C Isotope Ratios: Dynamic Allocation of Resources in Induced Plants. Angewandte Chemie **40**:2091.

Karban R. 2001. Communication between sagebrush and wil tobacco in the field. Biochemical Systematics and Ecology **29**:995-1005.

Karban R., F. R. Adler. 1996. Induced resistance to herbivores and the information content of early season attack. Oecologia **107**:379-385.

Karl T., A. Guenther, C. Spirig, A. Hansel, and R. Fall. 2003. Seasonal variation of biogenic VOC emissions above a mixed hardwood forest in northern Michigan. Geophysical Research Letters **30**:2186.

Karl T., F. Harren, C. Warneke, J. de Gouw, C. Grayless, and R. Fall. 2005. Senescing grass crops as regional sources of reactive volatile organic compounds. Journal of Geophysical Research-Atmospheres **110**:D15302.

Kesselmeier J., M. Staudt. 1999. Biogenic Volatile Organic Compounds (VOC): An Overview on Emission, Physiology and Ecology. Journal of Atmospheric Chemistry **33**:23.

Kim J. C. 2001. Factors controlling natural VOC emissions in a southeastern US pine forest. Atmospheric Environment **35**:3279-3292.

Kim J. C., K. J. Kim, D. S. Kim, and J. S. Han. 2005. Seasonal variations of monoterpene emissions from coniferous trees of different ages in Korea. Chemosphere **59**:1685-1696.

KLEKOWSKI E. J. 2003. Plant clonality, mutation, diplontic selection and mutational meltdown. Biological Journal of the Linnean Society **79**:61-67.

Koricheva J., S. Larsson, E. Haukioja, and M. Keinänen. 1998. Regulation of Woody Plant Secondary Metabolism by Resource Availability: Hypothesis Testing by Means of Meta-Analysis. Oikos **83**:pp. 212-226.

Kurpius M. R., A. H. Goldstein. 2003. Gas-phase chemistry dominates O-3 loss to a forest, implying a source of aerosols and hydroxyl radicals to the atmosphere. Geophysical Research Letters **30**:1371.

Langenheim J. H. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles. Journal of chemical ecology **20**:1223-1280.

Latta R. G. 2004. Relating processes to patterns of genetic variation across landscapes. Forest Ecology and Management **197**:91-102.

Latta R. G., Y. B. Linhart. 1997. Path analysis of natural selection on plant chemistry: The xylem resin of ponderosa pine. Oecologia **109**:251-258.

Latta R. G., Y. B. Linhart, M. A. Snyder, and L. Lundquist. 2003. Patterns of variation and correlation in the monoterpene composition of xylem oleoresin within populations of ponderosa pine. Biochemical systematics and ecology **31**:451-465.

Latta R. G., Y. B. Linhart, L. Lundquist, and M. A. Snyder. 2000. Patterns of monoterpene variation within individual trees in ponderosa pine. Journal of chemical ecology **26**:1341-1357.

Lerdau M., D. Gray. 2003. Ecology and evolution of light-dependent and light-independent phytogenic volatile organic carbon. New Phytologist **157**:199-211.

Lerdau M., A. Guenther, and R. Monson. 1997. Plant production and emission of volatile organic compounds. Bioscience **47**:373-383.

Lerdau M., M. Litvak, P. Palmer, and R. Monson. 1997. Controls over monoterpene emissions from boreal forest conifers. Tree physiology **17**:563-569.

Lerdau M., S. B. Dilts, H. Westberg, B. K. Lamb, and E. J. Allwine. 1994. Monoterpene emission from Ponderosa Pine. Journal of Geophysical Research-Atmospheres **99**:16609-16615.

Lerdau M., A. Guenther, and R. Monson. 1997. Plant Production and Emission of Volatile Organic Compounds. Bioscience **47**:pp. 373-383.

Lerdau M., M. Litvak, and R. Monson. 1994. Plant chemical defense: monoterpenes and the growth-differentiation balance hypothesis. Trends in Ecology & Evolution **9**:58-61.

Lichtenthaler H. K., A. AÄ , M. V. Marek, J. Kalina, and O. Urban. 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiology and Biochemistry **45**:577-588.

Linhart Y. B., K. A. Mooney, M. A. Snyder, and N. Swoboda-Colberg. 2001. Phloem chemistry: Effects of genotype and environment and implications for nutritional ecology. International journal of plant sciences **162**:1009-1016.

Linhart Y. B., J. B. Mitton, K. B. Sturgeon, and M. L. Davis. 1981. Genetic variation in space and time in a population of ponderosa pine. Heredity **46**:407-426.

Linhart Y. B., J. B. Mitton. 1985. Relationships Among Reproduction, Growth Rates, and Protein Heterozygosity in Ponderosa Pine. American Journal of Botany **72**:pp. 181-184.

Lippmann M. 1993. Health effects of tropospheric ozone: review of recent research findings and their implications to ambient air quality standards. J Expo Anal Environ Epidemiol **3**:103-129.

Litvak M. E., R. K. Monson. 1998a. Patterns of induced and constitutive monoterpene production in conifer needles in relation to insect herbivory. Oecologia **114**:531-540.

Litvak M. E., R. K. Monson. 1998b. Patterns of Induced and Constitutive Monoterpene Production in Conifer Needles in Relation to Insect Herbivory. Oecologia **114**:pp. 531-540.

Llusia J., J. Penuelas, G. A. Alessio, and M. Estiarte. 2008. Contrasting species-specific, compound-specific, seasonal, and interannual responses of foliar isoprenoid emissions to experimental drought in a mediterranean shrubland. International journal of plant sciences **169**:637-645.

Llusia J., J. Penuelas, G. A. Alessio, and M. Estiarte. 2006. Seasonal contrasting changes of foliar concentrations of terpenes and other volatile organic compound in four domiant species of a Mediterranean shrubland submitted to a field experimental drought and warming. Physiologia Plantarum **127**:632-649.

Loreto F., T. D. Sharkey. 1990. A Gas-Exchange Study of Photosynthesis and Isoprene Emission in Quercus-Rubra L. Planta **182**:523-531.

Loreto F., S. Fares. 2007. Is ozone flux inside leaves only a damage indicator? Clues from volatile isoprenoid studies. Plant Physiology **143**:1096-1100.

Loreto F., F. Bagnoli, and S. Fineschi. 2009. One species, many terpenes: matching chemical and biological diversity. Trends in plant science **14**:416-420.

Loreto F., C. Barta, F. Brilli, and I. Nogues. 2006. On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant Cell and Environment **29**:1820-1828.

Lorio Jr. P. L. 1986. Growth-differentiation balance: A basis for understanding southern pine beetle-tree interactions. Forest Ecology and Management **14**:259-273.

Matsunaga S., A. Guenther, J. Greenberg, M. Potosnak, M. Rapiez, T. Hiura, S. Kato, S. Nishida, P. Harley, and J. J. Karchesy. 2009. Leaf level emission measurement of sesquiterpenes and oxygenated sesquiterpenes from desert shrubs and temperate forest trees using a liquid extraction technique. Geochemical Journal **43**:179.

Mitton J. B., Y. B. Linhart, J. L. Hamrick, and J. S. Beckman. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado front range. TAG Theoretical and Applied Genetics **51**:5-13.

Monks P. S., C. Granier, S. Fuzzi, A. Stohl, M. L. Williams, H. Akimoto, M. Amann, A. Baklanov, U. Baltensperger, I. Bey, N. Blake, R. S. Blake, K. Carslaw, O. R. Cooper, F. Dentener, D. Fowler, E. Fragkou, G. J. Frost, S. Generoso, P. Ginoux, V. Grewe, A. Guenther, H. C. Hansson, S. Henne, J. Hjorth, A. Hofzumahaus, H. Huntrieser, I. S. A. Isaksen, M. E. Jenkin, J. Kaiser, M. Kanakidou, Z. Klimont, M. Kulmala, P. Laj, M. G. Lawrence, J. D. Lee, C. Liousse, M. Maione, G. McFiggans, A. Metzger, A. Mieville, N. Moussiopoulos, J. J. Orlando, C. D. O'Dowd, P. I. Palmer, D. D. Parrish, A. Petzold, U. Platt, U. Pöschl, A. S. H. Prévôt, C. E. Reeves, S. Reimann, Y. Rudich, K. Sellegri, R. Steinbrecher, D. Simpson, H. ten Brink, J. Theloke, G. R. van der Werf, R. Vautard, V. Vestreng, C. Vlachokostas, and R. von Glasow. 2009. Atmospheric composition change – global and regional air quality. Atmospheric Environment **43**:5268-5350.

Monson R. K., E. A. Holland. 2001. Biospheric trace gas fluxes and their control over tropospheric chemistry. Annual Review of Ecology and Systematics **32**:547-+.

Monson R. K., M. T. Lerdau, T. D. Sharkey, D. S. Schimel, and R. Fall. 1995. BIOLOGICAL ASPECTS OF CONSTRUCTING VOLATILE ORGANIC-COMPOUND EMISSION INVENTORIES. Atmospheric Environment **29**:2989-3002.

Monson R. K., C. H. Jaeger, W. W. Adams, E. M. Driggers, G. M. Silver, and R. Fall. 1992. RELATIONSHIPS AMONG ISOPRENE EMISSION RATE, PHOTOSYNTHESIS, AND ISOPRENE SYNTHASE ACTIVITY AS INFLUENCED BY TEMPERATURE. Plant Physiology **98**:1175-1180.

Monson R. K., R. Fall. 1989. Isoprene Emission from Aspen Leaves. Influence of Environment and Relation to Photosynthesis and Photorespiration. Plant Physiology **90**:pp. 267-274.

Moran C. R. 2004. A natural history of ferns. Timber Press, Inc., Portland, Oregon.

Myre R., C. Camiré. 1996. The effect of crown position and date of sampling on biomass, nutrient concentrations and contents of needles and shoots in European larch. Trees - Structure and Function **10**:339-350.

Nagegowda D. A. 2010. Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation. FEBS letters **584**:2965-2973.

Naumburg . Relationships between Pinus ponderosa forest structure, light characteristics, and understory graminoid species presence and abundance. Forest Ecology and Management **124**:205.

Niinemets U., K. Hauff, N. Bertin, J. D. Tenhunen, R. Steinbrecher, and G. Seufert. 2002. Monoterpene emissions in relation to foliar photosynthetic and structural variables in Mediterranean evergreen Quercus species. New Phytologist **153**:243-256.

Öquist G., J. M. Anderson, S. McCaffery, and W. S. Chow. 1992. Mechanistic differences in photoinhibition of sun and shade plants. Planta **188**:422-431.

Ortega J., D. Helmig. 2008. Approaches for quantifying reactive and low-volatility biogenic organic compound emissions by vegetation enclosure techniques – Part A. Chemosphere **72**:343-364.

Ortega J., D. Helmig, R. W. Daly, D. M. Tanner, A. B. Guenther, and J. D. Herrick. 2008. Approaches for quantifying reactive and low-volatility biogenic organic compound emissions by vegetation enclosure techniques – Part B: Applications. Chemosphere **72**:365-380.

Pakeman R. J., R. H. Marrs. 1994. The Effects of Control on the Biomass, Carbohydrate Content and Bud Reserves of Bracken (Pteridium-Aquilinum (L.) Kuhn), and an Evaluation of a Bracken Growth-Model. Annals of Applied Biology **124**:479-493.

Pearcy R. W., H. Muraoka, and F. Valladares. 2005. Crown architecture in sun and shade environments: assessing function and trade-offs with a three-dimensional simulation model. New Phytologist **166**:791-800.

Pearson G. A. 1940. Shade effects in ponderosa pine. Journal of Forestry **38**:778-780.

Pearson G. A. 1936. Some Observations on the Reaction of Pine Seedlings to Shade. Ecology **17**:pp. 270-276.

Penuelas J., J. Llusia. 2003. BVOCs: plant defense against climate warming? Trends in plant science **8**:105-109.

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

Penuelas J., J. Llusia, D. Asensio, and S. Munne-Bosch. 2005. Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. Plant Cell and Environment **28**:278-286.

Peñuelas J., J. Llusià. 2001. The Complexity of Factors Driving Volatile Organic Compound Emissions by Plants. Biologia Plantarum **44**:481-487.

Peñuelas J., I. Filella, R. Seco, and J. Llusià. 2009. Increase in isoprene and monoterpene emissions after re-watering of droughted *Quercus ilex* seedlings. Biologia Plantarum **53**:351-354.

Peñuelas J., S. Michael. 2010. BVOCs and global change. Trends in plant science **15**:133-133-144.

Perez-Rial D., J. Penuelas, P. Lopez-Mahia, and J. Llusia. 2009a. Terpenoid emissions from Quercus robur. A case study of Galicia (NW Spain). Journal of Environmental Monitoring **11**:1268-1275.

Perez-Rial D., J. Penuelas, P. Lopez-Mahia, and J. Llusia. 2009b. Terpenoid emissions from Quercus robur. A case study of Galicia (NW Spain). - Journal of Environmental Monitoring :1268-1275.

Pollmann J., J. Ortega, and D. Helmig. 2005. Analysis of atmospheric sesquiterpenes: Sampling losses and mitigation of ozone interferences. Environmental Science and Technology **39**:9620-9629.

Pressley S., B. Lamb, H. Westberg, A. Guenther, J. Chen, and E. Allwine. 2004. Monoterpene emissions from a Pacific Northwest Old-Growth Forest and impact on regional biogenic VOC emission estimates. Atmospheric Environment **38**:3089-3098.

Pressley S., B. Lamb, H. Westberg, A. Guenther, J. Chen, and E. Allwine. 2004/6. Monoterpene emissions from a Pacific Northwest Old-Growth Forest and impact on regional biogenic VOC emission estimates. Atmospheric Environment **38**:3089-3098.

Quintero C., M. Bowers. 2011. Plant Induced Defenses Depend More on Plant Age than Previous History of Damage: Implications for Plant-Herbivore Interactions. Journal of chemical ecology **37**:992-1001.

Rapparini F., R. Baraldi, and O. Facini. 2001. Seasonal variation of monoterpene emission from Malus domestica and Prunus avium. Phytochemistry **57**:681-687.

Rinne H. J. I., A. B. Guenther, J. P. Greenberg, and P. C. Harley. 2002. Isoprene and monoterpene fluxes measured above Amazonian rainforest and their dependence on light and temperature. Atmospheric Environment **36**:2421.

Roberts M. R., F. S. Gilliam. 1995. Disturbance effects on herbaceous layer vegetation and soil nutrients in Populus forests of northern lower Michigan. Journal of Vegetation Science **6**:903-912.

Rosenstiel T. N., A. L. Ebbets, W. C. Khatri, R. Fall, and R. K. Monson. 2004. Induction of poplar leaf nitrate reductase: A test of extrachloroplastic control of isoprene emission rate. Plant Biology **6**:12-21.

Royo A. A., W. P. Carson. 2006. On the formation of dense understory layers in forests worldwide: consequences and implications for forest dynamics, biodiversity, and succession. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 36:1345-1362.

Savage M., P. M. Brown, and J. Feddema. 1996. The role of climate in a pine forest regeneration pulse in the southwestern United States. Ecoscience. Sainte-Foy [ECOSCIENCE] 3:310-318.

Schade G. W., A. H. Goldstein. 2001. Fluxes of oxygenated volatile organic compounds from a ponderosa pine plantation. Journal of Geophysical Research-Atmospheres **106**:3111-3123.

Seinfeld, J. H. and Pandis, S. 1998. Atmospheric chemistry and physics: from air pollution to climate change. Wiley, New York.

Sell C. 2003. A fragrant introduction to terpenoid chemistry. Royal Society of Chemistry, Cambridge, UK.

Sharkey T. D., S. S. Yeh. 2001. Isoprene emission from plants. Annual Review of Plant Physiology and Plant Molecular Biology **52**:407-436.

Sharkey T. D., A. E. Wiberley, and A. R. Donohue. 2008. Isoprene Emission from Plants: Why and How. Annals of Botany **101**:5-18.

Sharkey T. D., E. L. Singsaas, P. J. Vanderveer, and C. Geron. 1996. Field measurements of isoprene emission from trees in response to temperature and light. Tree physiology **16**:649-654.

Shiojiri K., R. Karban. 2006. Plant age, communication, and resistance to herbivores: young sagebrush plants are better emitters and receivers. Oecologia **149**:214-220.

Simpson D., A. Guenther, C. N. Hewitt, and R. Steinbrecher. 1995. Biogenic Emissions in Europe .1. Estimates and Uncertainties. Journal of Geophysical Research-Atmospheres **100**:22875-22890.

Smith K. T., W. C. Shortle. 2001. Conservation of element concentration in xylem sap of red spruce. Trees-Structure and Function **15**:148-153.

Stamp N. 2004. Can the growth?differentiation balance hypothesis be tested rigorously? Oikos **107**:439-448.

Staudt M., N. Bertin, B. Frenzel, and G. Seufert. 2000. Seasonal variation in amount and composition of monoterpenes emitted by young Pinus pinea trees - Implications for emission modeling. Journal of Atmospheric Chemistry **35**:77-99.

Street R. A., S. Owen, S. C. Duckham, C. Boissard, and C. N. Hewitt. 1997. Effect of habitat and age on variations in volatile organic compound (VOC) emissions from Quercus ilex and Pinus pinea. Atmospheric Environment **31, Supplement 1**:89-100.

Stroud C., P. Makar, T. Karl, A. Guenther, C. Geron, A. Turnipseed, E. Nemitz, B. Baker, M. Potosnak, and J. D. Fuentes. 2005. Role of canopy-scale photochemistry in modifying biogenic-atmosphere exchange of reactive terpene species: Results from the CELTIC field study. Journal of Geophysical Research-Atmospheres **110**:D17303.

Theis N. 2006. Fragrance of canada thistle (Cirsium arvense) attracts both floral herbivores and pollinators. Journal of chemical ecology **32**:917-927.

Theis N., M. Lerdau. 2003. The evolution of function in plant secondary metabolites. International journal of plant sciences **164**:S93-S102.

Tingey D. T., M. Manning, L. C. Grothaus, and W. F. Burns. May 1980. Influence of Light and Temperature on Monoterpene Emission Rates from Slash Pine. Plant Physiology **65**:797-801.

Turner M. G. 2005. Landscape ecology: What is the state of the science? Annual Review of Ecology Evolution and Systematics **36**:319-344.

U.S. Department of Agriculture, Forest Service. Manitou Experimental Forest - Site Description **2012**:.

Valladares F., R. W. Pearcy. 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. Plant, Cell & Environment **25**:749-759.

Velikova V., S. Fares, and F. Loreto. 2008. Isoprene and nitric oxide reduce damages in leaves exposed to oxidative stress. Plant Cell and Environment **31**:1882-1894.

Vickers C. E., J. Gershenzon, M. T. Lerdau, and F. Loreto. 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nature Chemical Biology **5**:283-291.

Vitale M., E. Salvatori, F. Loreto, S. Fares, and F. Manes. 2008. Physiological responses of Quercus ilex leaves to water stress and acute ozone exposure under controlled conditions. Water Air and Soil Pollution **189**:113-125.

Wang Y. F., S. M. Owen, Q. J. Li, and J. Penuelas. 2007. Monoterpene emissions from rubber trees (Hevea brasiliensis) in a changing landscape and climate: chemical speciation and environmental control. Global Change Biology **13**:2270-2282.

Whitehead S. J., J. Digby. 1997. The morphology of bracken (Pteridium aquilinum (L) Kuhn) in the North York Moors - a comparison of the mature stand and the interface with heather (Calluna vulgaris (L) Hull) .2. The rhizome. Annals of Applied Biology **131**:117-131.

Wu S., M. Schalk, A. Clark, R. B. Miles, R. Coates, and J. Chappell. 2006. Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. Nat Biotech **24**:1441-1447.