

Monoterpene emission from coniferous trees in response to elevated CO₂ concentration and climate warming

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Abstract

It was hypothesized that high CO₂ availability would increase monoterpene emission to the atmosphere. This hypothesis was based on resource allocation theory which predicts increased production of plant secondary compounds when carbon is in excess of that required for growth. Monoterpene emission rates were measured from needles of (a) Ponderosa pine grown at different CO₂ concentrations and soil nitrogen levels, and (b) Douglas fir grown at different CO₂ concentrations. Ponderosa pine grown at 700 μmol mol⁻¹ CO₂ exhibited increased photosynthetic rates and needle starch to nitrogen (N) ratios when compared to trees grown at 350 μmol mol⁻¹ CO₂. Nitrogen availability had no consistent effect on photosynthesis. Douglas fir grown at 550 μmol mol⁻¹ CO₂ exhibited increased photosynthetic rates as compared to growth at 350 μmol mol⁻¹ CO₂ in old, but not young needles, and there was no influence on the starch/N ratio. In neither species was there a significant effect of elevated growth CO₂ on needle monoterpene concentration or emission rate. The influence of climate warming and leaf area index (LAI) on monoterpene emission were also investigated. Douglas fir grown at elevated CO₂ plus a 4 °C increase in growth temperature exhibited no change in needle monoterpene concentration, despite a predicted 50% increase in emission rate. At elevated CO₂ concentration the LAI increased in Ponderosa pine, but not Douglas fir. The combination of increased LAI and climate warming are predicted to cause an 80% increase in monoterpene emissions from Ponderosa pine forests and a 50% increase in emissions from Douglas fir forests. This study demonstrates that although growth at elevated CO₂ may not affect the rate of monoterpene emission per unit biomass, the effect of elevated CO₂ on LAI, and the effect of climate warming on monoterpene biosynthesis and volatilization, could increase canopy monoterpene emission rate.

Keywords: atmospheric chemistry, carbon dioxide, climate change, nitrogen, *Pinus ponderosa*, *Pseudotsuga menziesii*

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Introduction

The emission of volatile organic compounds (VOCs) from vegetation exerts the dominant control over regional oxidative chemistry of the atmosphere in many terrestrial ecosystems (Monson *et al.* 1995; Lerdau *et al.* 1997). In the case of coniferous forests, monoterpene compounds represent the dominant biogenic VOC (Lerdau 1991). Monoterpenes are 10-carbon compounds used primarily for herbivore defence, and volatilization to the atmo-

sphere occurs from storage reservoirs in the needles and bark of conifers. Once in the atmosphere, monoterpenes react readily with oxidative species (e.g. hydroxyl radical, nitrate radical, and ozone) to form a variety of secondary products that influence atmospheric chemistry and climate change (Wuebbles *et al.* 1989). Among the products of monoterpene oxidation are CO, organic acids, organic nitrates, and reactive organic peroxy compounds (Fehsenfeld *et al.* 1992). Thus, monoterpene emissions from coniferous forests represent a primary contribution to the broader discipline of biosphere–atmosphere interactions.

Theoretical models of resource allocation suggest that

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monoterpene production and tissue concentration should be determined by (i) tradeoffs between the allocation of carbon to growth vs. the production of carbon-based defenses, and (ii) past herbivory and evolutionary pressures that influence the expression of genes responsible for monoterpene production (Lerdau *et al.* 1994b). In the case of control by carbon allocation, it has been hypothesized that carbon in excess of that required for immediate growth should be used in the production of carbon-based defensive compounds (e.g. monoterpenes) (Lerdau *et al.* 1994b). Using this hypothesis, one might predict higher rates of monoterpene production and emission from plants growing at elevated CO₂ and limited nitrogen availability.

It has generally been assumed that monoterpene emissions will increase in response to climate warming. This is based on the knowledge that monoterpene emissions are highly temperature-sensitive, exhibiting a 2–3 fold increase for each 10 °C increase in temperature (Guenther *et al.* 1991, 1993; Lerdau *et al.* 1994a, 1997). In order to sustain elevated emission rates, however, monoterpene production rate would have to increase proportionally, otherwise the tissue reservoir would become depleted over time. Given that monoterpene production is catalysed by a group of enzymes, monoterpene cyclases, and that enzyme catalysis rates tend to approximately double for each 10 °C increase in temperature, it is reasonable to predict monoterpene production rates of sufficient magnitude to support enhanced emission rates in a warmer global climate.

In the current study, we took advantage of two existing experiments in which coniferous tree species have been grown at elevated CO₂ concentrations, and in one case a warmer climate regime, to investigate how monoterpene production and emission might respond to future environmental change. Two specific hypotheses were addressed: (i) monoterpene concentrations in needle tissues and emission rates per unit of needle biomass increase when trees are grown under elevated CO₂ concentration, but low nitrogen availability (i.e. in conditions where carbon assimilation is enhanced, but overall tree growth is constrained due to low N availability), and (ii) tissue monoterpene concentration will be sufficient to support higher monoterpene emission rates in a warmer climate.

Materials and methods

Site characteristics and growth conditions

Two coniferous tree species were studied, Ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). The Ponderosa pine trees were studied as part of an ongoing experiment

in Placerville, California, USA (Ball 1990). Ponderosa pine seeds from Eldorado County, California were germinated under three CO₂ concentrations (350, 550, and 700 µmol mol⁻¹), and in the spring of 1990 were transplanted into hexagonal 3.35 m diameter open-top chambers placed on the natural soil, a xeric Haplohumult. In order to establish different treatments within the chambers, CO₂ was added to ambient air using a mass flow meter and blown into the plant canopy through a perforated plastic plenum at the chamber base. The conditions in the chambers were established as a randomized combination of the three CO₂ treatments and three levels of N fertilization using ammonium sulphate –0, 10, and 20 g m⁻² N y⁻¹. The 3 × 3 experimental design was incomplete as the 550 µmol mol⁻¹ CO₂ and 10 g m⁻² N treatment was not created to increase sample size for the remaining treatments. Trees have grown continuously in their respective treatments for the last six years and in the winter of 1995 a limited number of chambers in each treatment were enlarged to circular chambers 5 m in diameter. Subsequent examination of growth characteristics comparing the 5 m and 3.35 m chambers indicated no differences within treatments (J.T. Ball, Desert Research Institute, pers. comm.).

The Douglas fir trees were studied as part of an ongoing experiment in Corvallis, OR, USA (Tingey *et al.* 1995). Seeds of Douglas fir were germinated and grown for two years in ambient conditions at Corvallis before 14 seedlings were planted into each of the controlled-environment chambers (referred to as Terracosms) in the spring of 1993. The Terracosms are composed of equal sized above-and below-ground compartments measuring 2 m wide, 1 m deep, and 1.5 m tall. Terracost soil is a reconstructed forest soil collected in the Cascade Mountains (Typic Hapludand). The environment of the above-ground compartment of the Terracost is controlled to create four growth treatments: (i) ambient CO₂ and ambient temperature, (ii) ambient CO₂ with ambient temperature elevated by 4 °C, (iii) CO₂ elevated to 550 µmol mol⁻¹ and ambient temperature, and (iv) CO₂ elevated to 550 µmol mol⁻¹ with ambient temperature elevated by 4 °C. Two additional chambers with the above-ground compartment open to the ambient atmosphere were designated as chamberless controls. In June 1996, the trees had been growing in the Terracosms for ≈ 2.5 years.

In early June 1996, gas exchange characteristics, needle monoterpene concentrations and needle monoterpene emission samples were collected from the 1996 (current year) and 1995 (previous year) needle age classes from the following five treatments for Ponderosa pine trees: 350 µmol mol⁻¹ CO₂ with no added N (350/CN), 350 µmol mol⁻¹ CO₂ with 20 g m⁻² added N (350/HN), 700 µmol mol⁻¹ CO₂ with no added N (700/CN),

700 $\mu\text{mol mol}^{-1}$ CO_2 with 20 g m^{-2} added N (700/HN), and chamberless control trees with no added N (Ch'less). In mid- to late-September 1996, a second series of measurements in the above treatments were collected for 1995 needles. In late June 1996, gas exchange characteristics, needle monoterpene concentrations and needle monoterpene emission samples were collected from the 1996 and 1995 needle age classes from the following three treatments for Douglas fir trees: 350 $\mu\text{mol mol}^{-1}$ CO_2 with ambient temperature (350/+ 0), 550 $\mu\text{mol mol}^{-1}$ CO_2 with ambient temperature (550/+ 0), and chamberless control trees grown at ambient temperature (Ch'less). Additionally, tissue monoterpene concentrations, but no emission rates were measured for trees grown with 350 $\mu\text{mol mol}^{-1}$ CO_2 with the ambient temperature increased by 4 °C (350/+ 4), and 550 $\mu\text{mol mol}^{-1}$ CO_2 with the ambient temperature increased by 4 °C (550/+ 4). A second series of all measurements for 1996 needles of the Douglas fir trees was performed in early October of 1996.

Needle gas exchange measurements and collection of monoterpene emission samples

Gas exchange rates were measured with an open gas exchange system (MPH-1000, Campbell Scientific Inc., Logan, Utah, USA) connected to a microcomputer to calculate real-time values according to the equations of von Caemmerer & Farquhar (1981) for carbon assimilation rate (A); needle conductance (g); and intercellular CO_2 concentration (c_i). Relative humidity in the chamber was held constant at 60% and needle temperature was maintained at 30 °C (Ponderosa pine) or 25 °C (Douglas fir). The CO_2 and H_2O concentration differentials between the cuvette inlet and outlet lines were measured with an infrared gas analyser (LI-6262, LiCOR, Inc., Lincoln, NE, USA). All measurements used hydrocarbon-free source air to prevent equipment and sample contamination.

For all measurements, \approx 4–6 needles were placed into the cuvette with extreme care to avoid needle injury that would cause abnormally high needle monoterpene emission rates. If needle injury was suspected, the needles were removed and the cuvette was heated to 45 °C and flushed with hydrocarbon-free air at \approx 1 L min^{-1} for one hour, a procedure shown effective in eliminating contamination. The closed-cell foam pads that sealed the needles in the cuvette were changed after every second measurement to minimize monoterpene cross-contamination between measurements. To check for the efficacy of these procedures, measurements were occasionally made with an empty cuvette following a series of actual emissions measurements. Tests showed that these empty cuvette measurements contained no measurable monoterpene contamination. The needles used in each measure-

ment were collected for determination of (i) total needle area as determined by needle volume displacement followed by drying at 60 °C and weighing (BOREAS 1994), and (ii) needle monoterpene concentration (see below).

Following equilibration of gas exchange conditions within the cuvette and collection of gas exchange measurements, a solid-phase adsorbent sample cartridge containing 175 mg of 60/80 mesh Tenax TA (Supelco Inc., Bellefonte, PA, USA) was connected to the cuvette outlet. Using a small external pump (model 224-PCXR4, SKC Inc., Eighty Four, PA, USA), gas exiting the cuvette was pulled through the cartridge at 140–170 $\text{cm}^3 \text{min}^{-1}$ as monitored by a portable mass flow meter (model GFM17, Aalborg Instruments, Orangeburg, NY, USA) to load a total volume of 4 L on each sample cartridge. Studies in the laboratory using terpenes generated from permeation tubes showed no sample breakthrough at these loading rates or terpene interconversion. Cartridges were sealed and stored at –20 °C until analysis in the laboratory.

Needle starch and nitrogen determination

Needle starch was determined using 15 mg of dried tissue following the procedure of Fredeen *et al.* (1989). Tissue was dried with an initial 1 h exposure to 90 °C to stop respiration, followed by three days at 60 °C. Dried tissue was suspended in 1 mL dH_2O and autoclaved for 45 min to solubilize the starch. For Ponderosa pine a 100 μL sample aliquot was added to 200 μL sodium-acetate buffer (pH 4.8) and 100 μL of 15 units/mL amyloglucosidase (Boehringer Mannheim #1202 367, Indianapolis, IN, USA) and incubated for 1 h at 55 °C. For Douglas fir a 20 μL sample aliquot was used with 360 μL of sodium-acetate buffer and 20 μL amyloglucosidase. The reaction was stopped by incubation at 100 °C and the resulting solution analysed for glucose using the procedures of Ngo & Lenhoff (1980). These procedures used a 15 μL sample aliquot added to 35 μL of each 3-methyl, 2-benzo thiazolinone hydrazine (MBTH, Sigma #M-8006, St. Louis, MO, USA) and 3-dimethylaminobenzoic acid (DMAB, Sigma #D-0787, St. Louis, MO, USA) and 15 μL of peroxidase and glucose oxidase (Sigma #510–6, St. Louis, MO, USA) incubated for 25 min at 37 °C and read on a microplate reader at 595 nm. Nitrogen analyses were performed at the Boston University Stable Isotope Laboratory using a Heraeus carbon-nitrogen analyser.

Measurement of monoterpene concentration in tissues and cartridges

Needle samples weighing \approx 1 g fresh mass were collected, immediately wrapped in foil and immersed in liquid nitrogen where they were stored until analysis.

Table 1 Gas exchange rates of *Pinus ponderosa* in June (1996 and 1995 needles) and September 1996 (1995 needles only) for carbon assimilation (*A*, $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (*g*, $\text{mmol m}^{-2} \text{s}^{-1}$) and intercellular CO_2 concentration (*c_i*, $\mu\text{mol mol}^{-1}$). Trees were grown at either 350 or 700 $\mu\text{mol mol}^{-1} \text{CO}_2$ and either control (CN) or elevated (HN) soil nitrogen. Values are means \pm standard error (*n* = 3).

	350/CN	350/HN	700/CN	700/HN
1996 needles in June 1996				
A	6.0 \pm 0.3 ^a	7.1 \pm 0.5 ^a	13.6 \pm 0.4 ^b	11.6 \pm 0.6 ^b
g	99 \pm 18 ^a	134 \pm 22 ^a	123 \pm 14 ^a	92 \pm 4 ^a
c _i	240 \pm 20 ^a	256 \pm 12 ^a	506 \pm 13 ^b	483 \pm 8 ^b
1995 needles in June 1996				
A	6.9 \pm 0.6 ^a	8.0 \pm 0.3 ^a	12.6 \pm 1.2 ^b	11.2 \pm 2.7 ^b
g	119 \pm 23 ^a	140 \pm 19 ^a	105 \pm 5 ^a	90 \pm 20 ^a
c _i	245 \pm 4 ^a	249 \pm 9 ^a	492 \pm 11 ^b	488 \pm 7 ^b
1995 needles in September 1996				
A	4.4 \pm 0.7 ^a	4.7 \pm 0.2 ^a	7.4 \pm 1.2 ^b	6.0 \pm 0.9 ^b
g	84 \pm 6 ^a	80 \pm 5 ^a	64 \pm 8 ^b	49 \pm 12 ^b
c _i	263 \pm 11 ^a	253 \pm 4 ^a	509 \pm 12 ^b	487 \pm 25 ^b

Means were tested using a two-way fixed effect ANOVA, significant differences ($P < 0.05$) are identified by different letters within a row.

Subsequently, samples were ground in liquid nitrogen using a mortar and pestle and extracted with 20 mL of pentane for at least 5 days prior to analysis. All samples were analysed using a gas chromatograph (model 5890 Series II, Hewlett Packard Inc., Palo Alto, CA, USA) equipped with a flame ionization detector (FID). Samples were separated on a DB-wax capillary column (J and W Scientific Inc., Folsom, CA, USA) using the temperature ramping described by Litvak & Monson (1998). Sample identification was based upon retention times of known monoterpenes and quantified using an internal fenchone standard as previously described (Lerdau *et al.* 1997).

In the laboratory, cartridge samples were analysed using a gas chromatograph (model 5890 Series II, Hewlett-Packard Inc., Palo Alto, CA, USA) connected to a thermal desorption unit (model ATD 400, Perkin Elmer Inc., Buckinghamshire, UK). Samples were thermally desorped off the collection cartridge for 10 min at 275 °C and trapped on Tenax TA maintained at -30 °C. The trap was flash-heated to 300 °C to release the sample into the gas chromatograph. To test for trapping and desorption efficiency, monoterpene samples of known quantity were generated with a permeation tube and loaded onto the cartridges at the same rates as those used in the field measurements. Subsequent desorption and analysis demonstrated that 95–100% of the generated sample was measured. Sample components were separated on a DB-1 capillary column

(J and W Associates, Folsom, CA, USA) with a temperature programme of 2 min at 50 °C followed by a 4 °C min⁻¹ ramp to 150 °C suitable for detection of $\geq \text{C}_6$ non-methane hydrocarbons. After all compounds had been eluted, the column was heated at 30 °C min⁻¹ to 250 °C and held for 4 min to remove possible contaminants. Compounds were detected with an FID and quantified based upon injections of a decane standard and identified by (a) comparison of retention times with known monoterpenes, and (b) comparison with retention indices of samples analysed using a GC/MS at the National Center for Atmospheric Research.

Statistical procedures

The effects of the CO_2 and N treatments on Ponderosa pine and Douglas fir were analysed using a two-way fixed effect ANOVA (SAS Institute 1990). The potential effects of the growth chambers were analysed using a one-way ANOVA that compared Ch'less trees to their chambered counterparts.

Results

Gas exchange

Differences in gas exchange parameters between the different growth treatments are expressed relative to the baseline treatment of 350/CN. Gas exchange characteristics of both 1996 and 1995 needles of Ponderosa pine in June 1996 exhibited no statistical chamber effects on *A*, *g*, or *c_i*. The possible exception was a slightly higher *c_i* in the Ch'less trees over the 350/CN trees in 1995 needles ($F = 9.93$; d.f. = 1; $P = 0.051$). In the second field campaign in September 1996 there were also no significant chamber effects on the measured gas exchange parameters. Increasing growth CO_2 concentration from 350 $\mu\text{mol mol}^{-1}$ to 700 $\mu\text{mol mol}^{-1}$ at CN and HN in 1996 needles measured in June caused significant increases in *A* ($F = 183.22$; d.f. = 1; $P = 0.0001$) and *c_i* ($F = 316.12$; d.f. = 1; $P = 0.0001$), whereas *g* was not affected (Table 1). Changing N availability at either of the two growth CO_2 concentrations produced no change in gas exchange characteristics (Table 1). The 1996 needles displayed a statistically significant CO_2 -N interaction ($F = 183.22$; d.f. = 1; $P = 0.0097$) on *A* that was not apparent in any other gas exchange comparison. In 1995 needles, increasing the growth CO_2 concentration from 350 $\mu\text{mol mol}^{-1}$ to 700 $\mu\text{mol mol}^{-1}$ at CN and HN caused a significant increase in *A* ($F = 16.99$; d.f. = 1; $P = 0.0033$) and *c_i* ($F = 793.25$; d.f. = 1; $P = 0.0001$), while *g* was not affected (Table 1). When N availability was increased at 350 $\mu\text{mol mol}^{-1} \text{CO}_2$ or 700 $\mu\text{mol mol}^{-1} \text{CO}_2$ there

Table 2 Gas exchange rates of *Pseudotsuga menziesii* in June (1996 and 1995 needles) and October 1996 (1996 needles only) for carbon assimilation (*A*, $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (*g*, $\text{mmol m}^{-2} \text{s}^{-1}$) and intercellular CO_2 concentration (*c_i*, $\mu\text{mol mol}^{-1}$). Trees were grown at either 350 $\mu\text{mol mol}^{-1}$ or 550 $\mu\text{mol mol}^{-1}$ CO_2 . Values are means \pm standard error (*n* = 3).

	350	550
1996 needles in June 1996		
<i>A</i>	1.8 \pm 0.3 ^a	2.3 \pm 0.3 ^a
<i>g</i>	40 \pm 13 ^a	26 \pm 4 ^a
<i>c_i</i>	266 \pm 19 ^a	394 \pm 16 ^b
1995 needles in June 1996		
<i>A</i>	2.4 \pm 0.5 ^a	3.8 \pm 0.2 ^b
<i>g</i>	38 \pm 11 ^a	39 \pm 4 ^a
<i>c_i</i>	238 \pm 11 ^a	371 \pm 25 ^b
1996 needles in September 1996		
<i>A</i>	1.9 \pm 0.2 ^a	2.2 \pm 0.5 ^a
<i>g</i>	27 \pm 3 ^a	17 \pm 4 ^a
<i>c_i</i>	227 \pm 5 ^a	326 \pm 20 ^b

Means were tested using a two-way fixed effect ANOVA, significant differences ($P < 0.05$) are identified by different letters within a row.

were no changes in gas exchange parameters (Table 1). Differences in needle age had no effect on any gas exchange parameter. In September, increasing the growth CO_2 concentration from 350 $\mu\text{mol mol}^{-1}$ –700 $\mu\text{mol mol}^{-1}$ significantly increased *A* ($F = 14.45$; d.f. = 1; $P = 0.0274$) and *c_i* ($F = 263.55$; d.f. = 1; $P = 0.0001$) and decreased *g* ($F = 9.36$; d.f. = 1; $P = 0.0156$) in 1995 needles (Table 1). There was no influence of soil N on any gas exchange parameter of 1995 needles in September. During the second campaign in September, the gas exchange characteristics of 1995 needles significantly declined in *A* ($F = 36.61$; d.f. = 1; $P = 0.0001$) and *g* ($F = 20.93$; d.f. = 1; $P = 0.0003$) relative to the June campaign (Table 1), whereas *c_i* was not altered.

The effects of the growth treatments on Douglas fir trees are also expressed relative to the chambered trees grown at 350 $\mu\text{mol mol}^{-1}$ CO_2 . There was no effect of the Terracosms in June or September on *A*, *g*, or *c_i* when comparing the Ch⁻less trees to the 350/+0 trees in either needle age class. There were some changes in *A* and *g* when grown at 550 $\mu\text{mol mol}^{-1}$ CO_2 , but only the change in *c_i* was significant for 1996 needles ($F = 25.65$; d.f. = 1; $P = 0.0072$) (Table 2). The 1995 needles exhibited significant effects of elevated growth CO_2 on *A* ($F = 8.23$; d.f. = 1; $P = 0.0455$) and *c_i* ($F = 23.93$; d.f. = 1; $P = 0.0081$) (Table 2). The 1995 needles of Douglas fir had greater carbon assimilation rates ($F = 10.23$; d.f. = 1; $P = 0.0127$) than the 1996 needles, but had similar *g* and *c_i* at 350 $\mu\text{mol mol}^{-1}$ CO_2 (Table 2). Douglas fir demonstrated no change in *A* between the June and

October campaigns (Table 2). Although *g* was not different between the two field campaigns for 1996 needles, a significant decline in *c_i* ($F = 10.82$; d.f. = 1; $P = 0.0110$) occurred at both growth CO_2 concentrations.

Needle starch and nitrogen

Needle starch content in 1996 needles of Ponderosa pine was unchanged by growth in the chambers, but starch content in the 1995 needles was decreased significantly ($F = 772.87$; d.f. = 1; $P = 0.0001$) by growth in the chambers. The starch content of the 1996 needles was marginally increased by growth at elevated CO_2 ($F = 4.26$; d.f. = 1; $P = 0.0728$), and needle starch was not influenced by soil N level (Fig. 1a). In contrast, growth at elevated CO_2 for 1995 needles significantly increased starch levels ($F = 19.17$; d.f. = 1; $P = 0.0024$), while there was no effect of soil N (Fig. 1b). Needle N content of Ponderosa pine in both age classes was not altered by growth in the chambers. There was a significant reduction in needle N in 1996 needles when grown at elevated CO_2 ($F = 6.34$; d.f. = 1; $P = 0.0359$), but there was no effect in 1995 needles (Fig. 1c,d). The alteration of soil N level in Ponderosa pine did not alter the needle N content of either 1996 or 1995 needles (Fig. 1c,d). The ratio of needle starch to N (starch/N) was reduced significantly in both 1996 ($F = 33.10$; d.f. = 1; $P = 0.0104$) and 1995 ($F = 46.22$; d.f. = 1; $P = 0.0065$) needles by growth in the chambers. In both needle age classes, growth at elevated CO_2 significantly increased the starch/N ratio (1996: $F = 15.55$; d.f. = 1; $P = 0.0043$. 1995: $F = 9.27$; d.f. = 1; $P = 0.0160$) whereas there was no effect of soil N (Fig. 1e,f). Total needle starch in the 1996 needles of Ponderosa pine was significantly greater than in 1995 needles ($F = 16.17$; d.f. = 1; $P = 0.0009$); additionally 1996 needles had a greater starch/N ratio than 1995 needles ($F = 6.14$; d.f. = 1; $P = 0.0240$). Needle age did not affect needle N level.

Growth in the Terracosms for both 1996 and 1995 needles of Douglas fir did not alter needle starch level, N content, or the starch/N ratio. The effects of growth CO_2 caused no differences in needle starch (Fig. 2a), N content (Fig. 2b), or the starch/N ratio (Fig. 2c) in both 1996 and 1995 needles of Douglas fir. Needle age in Douglas fir did not affect needle N content, but 1995 needles had significantly lower starch content ($F = 19.70$; d.f. = 1; $P = 0.0441$) and starch/N ratio ($F = 57.23$; d.f. = 1; $P = 0.0422$) than found in 1996 needles.

Needle monoterpene concentration

Monoterpene concentrations within the 1996 and 1995 needle tissues of Ponderosa pine were dominated by β -pinene, which accounted for 55–75% of the total monoterpene pool (Fig. 3); α -pinene accounted for 20–32%,

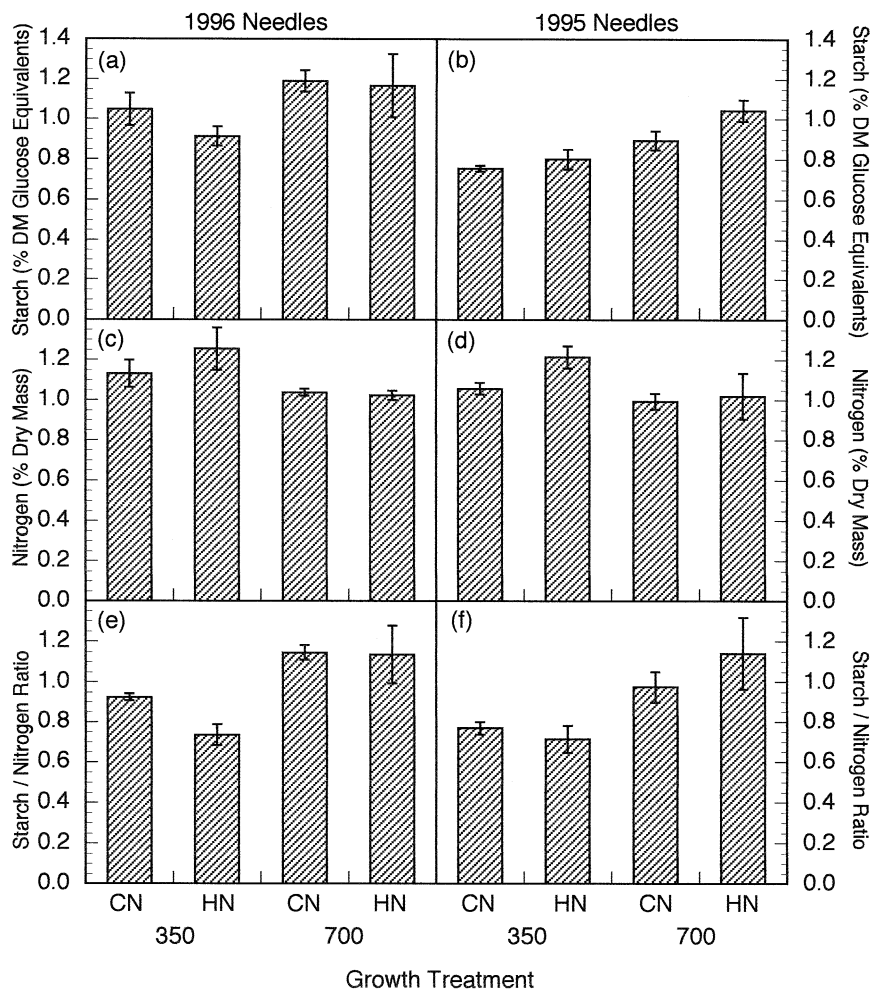


Fig. 1 Starch content (% dry mass glucose equivalents), nitrogen (% dry mass), and starch to nitrogen ratio for 1996 (current year) needles (a, c, e) and 1995 (previous year) needles (b, d, f) of *Ponderosa pine*. Values are means \pm SE ($n = 3$).

with the remainder being Δ -3-carene. Neither the total needle concentration nor the concentrations of individual monoterpenes in 1996 needles were significantly different between the Ch'less trees and the 350/CN trees. In 1995 needles the α -pinene concentration did not differ between the Ch'less trees and the 350/CN trees, though the 350/CN trees exhibited a significant increase in β -pinene ($F = 28.02$; d.f. = 1; $P = 0.0132$). The increase in β -pinene concentration in the 1995 needles in the 350/CN trees did not increase the total monoterpene concentration over the Ch'less trees.

Under CN and HN conditions, growth at 700 $\mu\text{mol mol}^{-1}$ CO₂ as compared to 350 $\mu\text{mol mol}^{-1}$ CO₂ in 1996 needles exhibited no change in the total monoterpene concentration (Fig. 3a). Increasing the soil N availability at 350 or 700 $\mu\text{mol mol}^{-1}$ CO₂ had no effect on total needle monoterpene concentration (Fig. 3a). A similar relationship between growth treatment and total needle monoterpene concentration as described for 1996 needles was observed in the 1995 needles (Fig. 3b).

In 1995 needles, increasing the growth CO₂ concentration in CN or HN conditions caused no change in the

total needle monoterpene concentration (Fig. 3b). Growth at elevated CO₂ concentration did significantly reduce the concentration of β -pinene in the 1995 needles ($F = 6.56$; d.f. = 1; $P = 0.0336$), (Fig. 3b). The addition of soil N at 350 $\mu\text{mol mol}^{-1}$ CO₂ had no effect on the total needle monoterpene concentration in 1995 needles. The combination of growth at 700 $\mu\text{mol mol}^{-1}$ CO₂ and the addition of soil N caused no change in the total needle monoterpene concentration (Fig. 3b). Total needle monoterpene concentration was significantly greater in the 1995 needles than in the 1996 needles ($F = 30.12$; d.f. = 1; $P = 0.0001$). This difference was due to greater concentrations of α -pinene ($F = 9.73$; d.f. = 1; $P = 0.0062$) and β -pinene ($F = 61.82$; d.f. = 1; $P = 0.0001$).

During September 1996, the monoterpene concentration in 1995 needles was again not influenced by the chambers. The trends observed in June were repeated with neither the CO₂ nor N treatments having an influence on needle monoterpene concentrations (Fig. 3c). A significant reduction in total monoterpene concentration occurred in 1995 needles between the September and June campaigns ($F = 36.68$; d.f. = 1; $P = 0.0001$). The

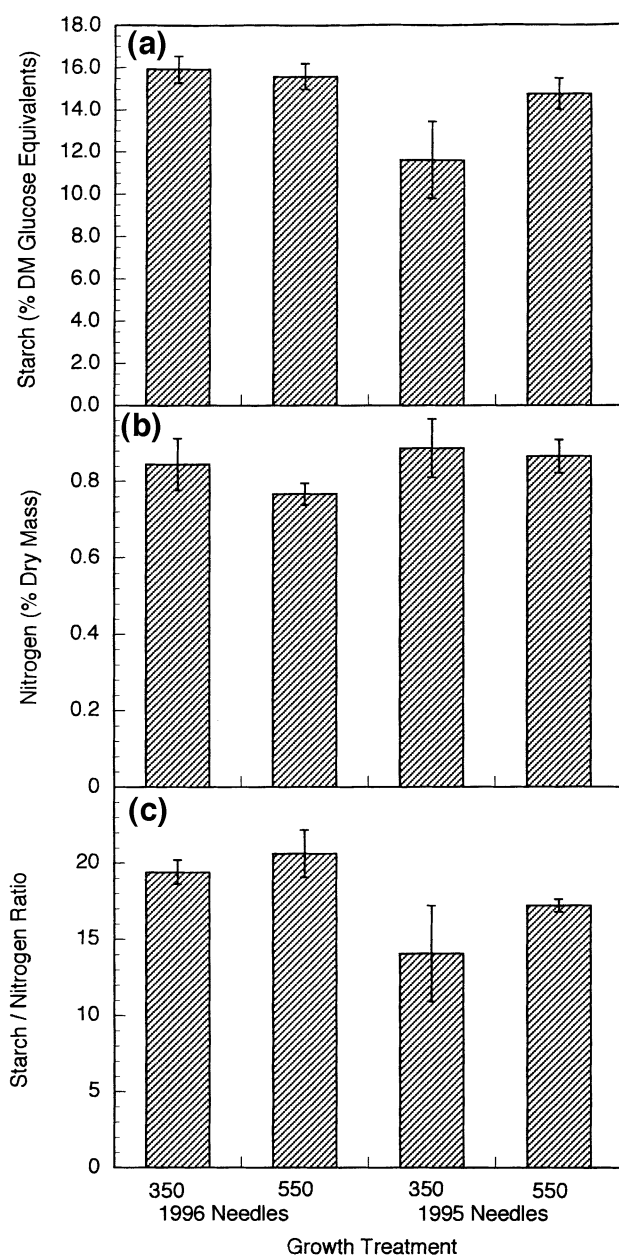


Fig. 2 Starch content (% dry mass glucose equivalents, a), nitrogen (% dry mass, b), and starch to nitrogen ratio (c) for 1996 (current year) and 1995 (previous year) needles of Douglas fir. Values are means \pm SE ($n = 3$).

reduction in total monoterpene concentration was due to reductions in α -pinene ($F = 49.60$; d.f. = 1; $P = 0.0001$), β -pinene ($F = 36.55$; d.f. = 1; $P = 0.0001$), and Δ -3-carene ($F = 5.15$; d.f. = 1; $P = 0.0365$).

Seven monoterpenes were identified within the needle tissue of both 1996 and 1995 needles of Douglas fir (Fig. 4). In both needle age classes 83–97% of the total needle monoterpene concentration was accounted for by α - and β -pinene, but other monoterpenes at low concentra-

tions were also detected including Δ -3-carene, myrcene, terpinene, phellandrene, and limonene. Comparison of 1996 needles in the Ch'less and 350/+0 treatments indicated a statistically significant increase in α -pinene ($F = 152.5$; d.f. = 1; $P = 0.0065$) and β -pinene ($F = 132.43$; d.f. = 1; $P = 0.0075$) that caused an increase in the total monoterpene concentration over the Ch'less trees ($F = 748.58$; d.f. = 1; $P = 0.0013$). There were no effects of the Terracosms on monoterpene concentrations in the 1995 needles. Additionally, the needle monoterpene concentration in the 350/+0 trees was dominated by β -pinene as opposed to α -pinene as found in the Ch'less trees. Increasing the growth CO_2 concentration from 350 $\mu\text{mol mol}^{-1}$ CO_2 to 550 $\mu\text{mol mol}^{-1}$ CO_2 caused no change in the total monoterpene concentration in 1996 needles (Fig. 4). As observed in the 1996 needles the increased growth CO_2 concentration had no effect on the total needle monoterpene concentration or that of individual monoterpenes in 1995 needles. The decline in β -pinene observed in 550 $\mu\text{mol mol}^{-1}$ CO_2 in 1995 needles was not significant. The 1995 needles contained lower total needle monoterpene concentration than found in the 1996 needles ($F = 5.97$; d.f. = 1; $P = 0.0404$) (Fig. 4). This difference was attributable to lower concentrations of α -pinene ($F = 8.06$; d.f. = 1; $P = 0.0218$) and β -pinene ($F = 8.08$; d.f. = 1; $P = 0.0217$) alone, as concentrations of all other monoterpenes were unchanged.

Measurements during October revealed a higher concentration of camphene in the 1996 needles for trees grown in the Terracosms, compared to the Ch'less trees ($F = 12.61$; d.f. = 1; $P = 0.0381$), but there was no CO_2 treatment effects on monoterpene concentrations (Fig. 5). Comparing the monoterpene concentrations in 1996 needles between the June and October campaigns indicated no alteration of concentration of the major monoterpenes (α -pinene, β -pinene, Δ -3-carene). In contrast, the concentrations of the minor monoterpenes were elevated in October as compared to June including myrcene ($F = 8.52$; d.f. = 1; $P = 0.0193$), camphene ($F = 35.72$; d.f. = 1; $P = 0.0003$), phellandrene ($F = 7.73$; d.f. = 1; $P = 0.0239$), and terpinolene ($F = 7.23$; d.f. = 1; $P = 0.0275$) (Fig. 5).

Monoterpene emission rate

Monoterpene emissions from the 1996 and 1995 needles of Ponderosa pine were not altered by the presence of chambers in June; however, in September the chambers caused significant increases in the emission of α -pinene ($F = 10.52$; d.f. = 1; $P = 0.0477$) and Δ -3-carene ($F = 14.93$; d.f. = 1; $P = 0.0307$). These increases are due to two extremely high emission measurements in the Ch'less trees and removal of these values from the data set produced similar emission rates from Ch'less and 350/CN trees. No differences in the emission rates of 1996

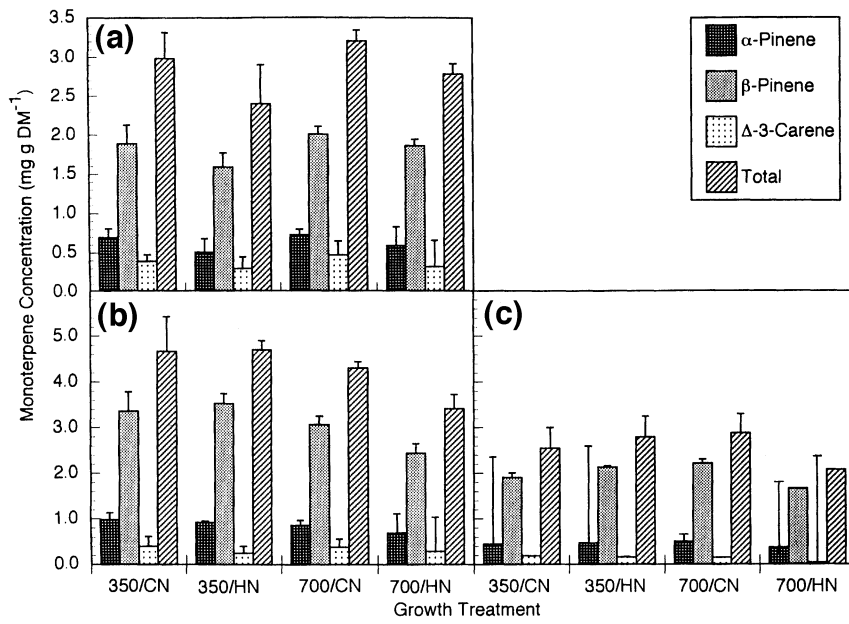


Fig. 3 Monoterpene concentration (mg/g dry mass) in 1996 (current year, a) and 1995 (previous year, b) needles of Ponderosa pine in June 1996 and 1995 (previous year) needles in September 1996 (c). Values are means \pm SE ($n = 3$).

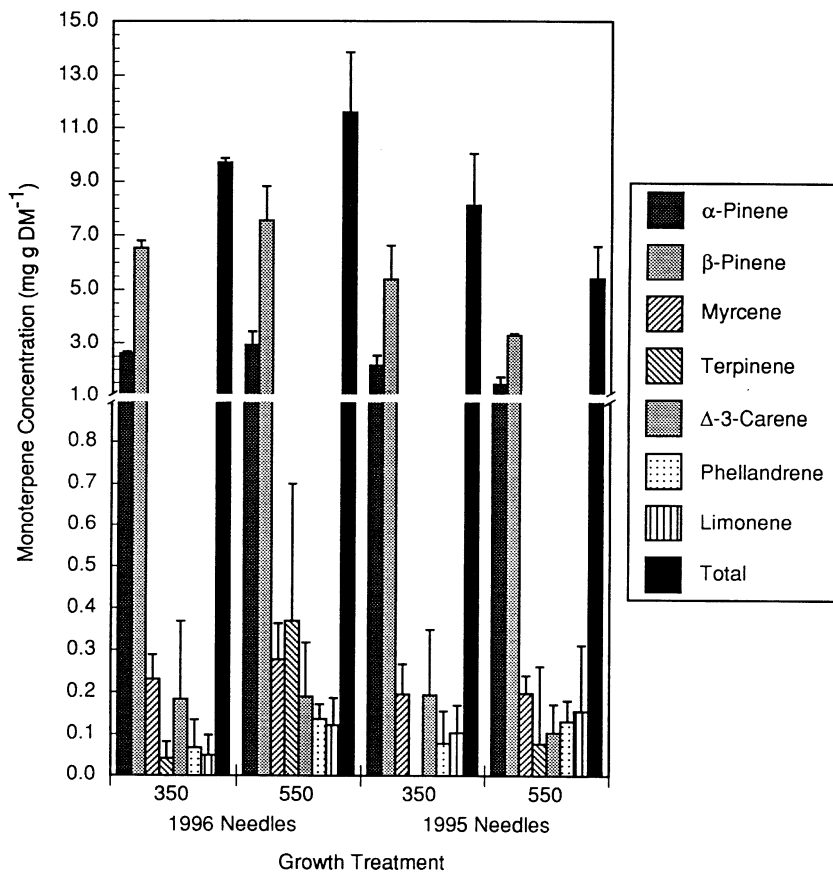


Fig. 4 Monoterpene concentration (mg/g dry mass) in 1996 (current year) and 1995 (previous year) needles of Douglas fir in June 1996. Values are means \pm SE ($n = 3$).

(Fig. 6a) and 1995 (Fig. 6b) needles among growth treatments were apparent in June. The high emission rates of 1995 needles in the 700/HN treatment (Fig. 6b) was caused by a single high emission measurement. In June, the monoterpene emission rates between 1996 and 1995

needles did not differ (Fig. 6a,b). Emissions in September for 1995 needles, however, were confounding and indicated a variety of CO₂ and N effects (Fig. 6c). Statistically significant declines in α -pinene emission occurred because of CO₂ ($F = 6.74$; d.f. = 1; $P = 0.0318$), N ($F =$

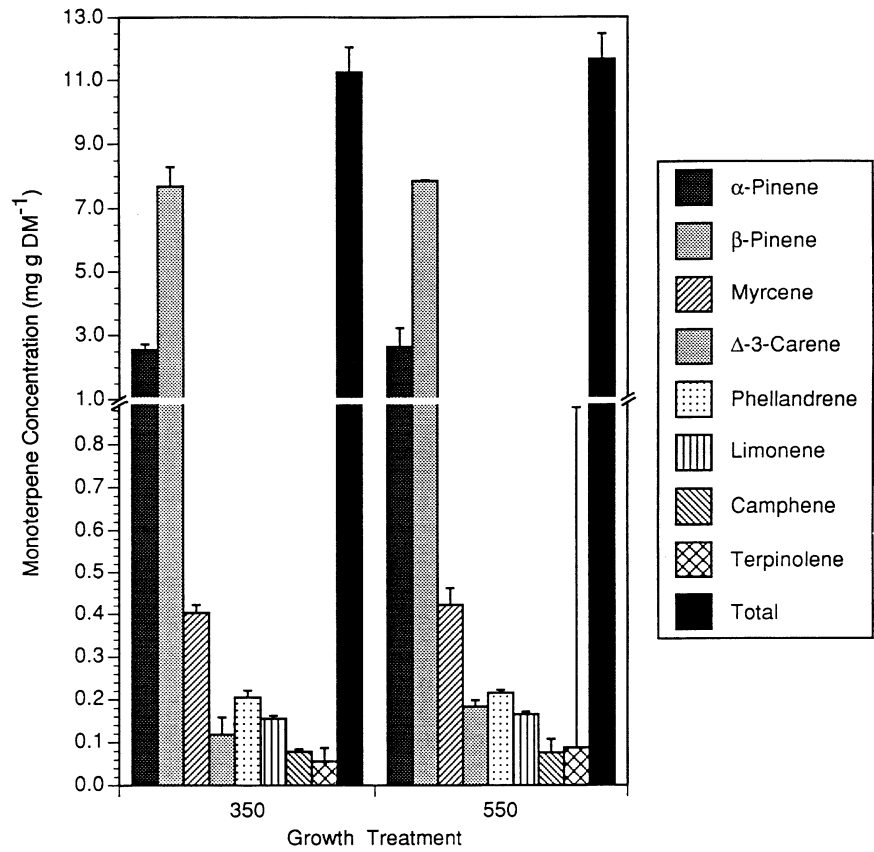


Fig. 5 Monoterpene concentration (mg/g dry mass) in 1996 (current year) needles of Douglas fir in October 1996. Values are means \pm SE ($n = 3$).

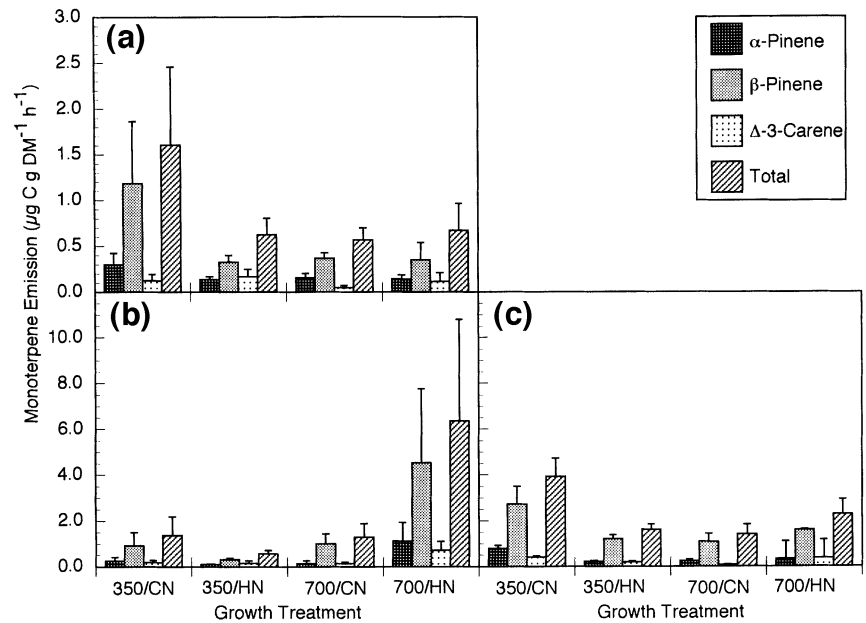


Fig. 6 Monoterpene emission rate ($\mu\text{g C g DM}^{-1} \text{h}^{-1}$) from 1996 (current year, a) and 1995 (previous year) needles in June of 1996 and 1995 (previous year, b) needles in September of 1996 (c) of Ponderosa pine. Values are means \pm SE ($n = 2$ or 3).

7.83; d.f. = 1; $P = 0.0233$) and the $\text{CO}_2\text{-N}$ interaction ($F = 14.06$; d.f. = 1; $P = 0.0056$), but there was no change in β -pinene emission rate (Fig. 6c). Additionally, there were significant declines in the emission rates of both Δ -3-carene ($F = 6.52$; d.f. = 1; $P = 0.0339$) and total mono-

terpenes ($F = 7.21$; d.f. = 1; $P = 0.0277$) due to a $\text{CO}_2\text{-N}$ interaction (Fig. 6c). The emission rate for 1995 needles exhibited no change between the June and September field campaigns for total monoterpenes or individual monoterpenes. There was a single significant interaction

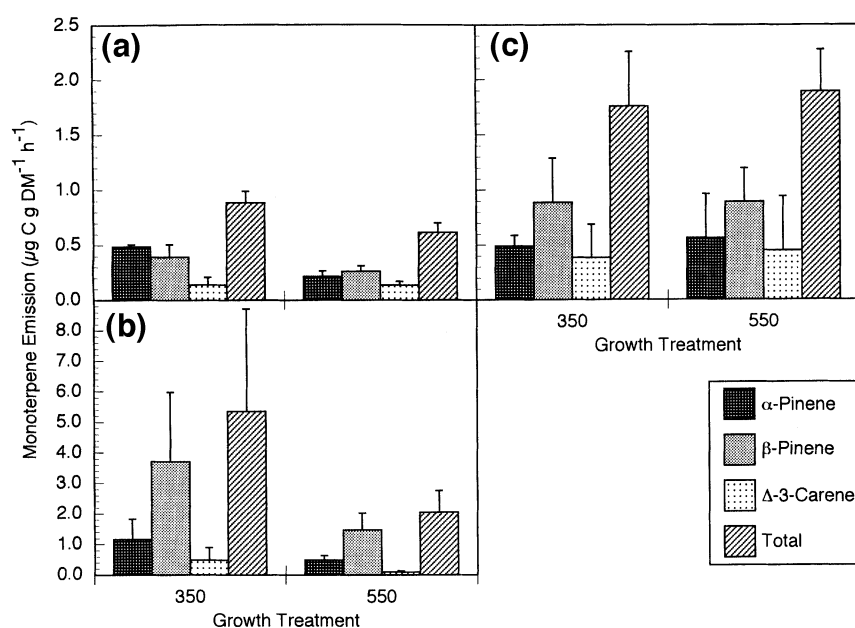


Fig. 7 Monoterpene emission rate ($\mu\text{g C g dry mass}^{-1} \text{h}^{-1}$) from 1996 (current year, a) and 1995 (previous year, b) needles in June of 1996 and 1996 (current year) needles in October of 1996 (c) of Douglas fir. Values are means \pm SE ($n = 2$ or 3).

effect of campaign \times CO₂ \times N that increased the emission of Δ -3-carene ($F = 5.15$; d.f. = 1; $P = 0.0365$).

In the 1996 and 1995 needles of Douglas fir there were no effects of growth in the Terracosms on the emission of monoterpenes in June or October. In the 1996 needles growth at 550 $\mu\text{mol mol}^{-1}$ CO₂ as compared to growth at 350 $\mu\text{mol mol}^{-1}$ CO₂ had a significant negative effect on the emission of α -pinene ($F = 16.78$; d.f. = 1; $P = 0.0263$), but emission of other monoterpenes and the total monoterpene emission rate did not change (Fig. 7a). There was no effect of CO₂ on the emission from 1995 needles for any individual monoterpene or for the total monoterpene emission rate partially due to the high emission variability in the 350 $\mu\text{mol mol}^{-1}$ CO₂ treatment (Fig. 7b). In October, as in June, there were no effects of growth at elevated CO₂ on the total monoterpene emission rate or emissions of individual monoterpenes from 1996 needles (Fig. 7c). The monoterpene emission rate of 1996 needles and 1995 needles did not differ for individual monoterpenes or total monoterpenes. The October emission rate from 1996 needles significantly increased for α -pinene ($F = 9.90$; d.f. = 1; $P = 0.0162$), compared to June emission rates, but emission of β -pinene, Δ -3-carene, and total monoterpenes did not change.

Needle monoterpene concentration at variable growth temperature in Douglas fir

The total needle monoterpene concentration, and the concentration of individual monoterpenes, in 1996 needles of Douglas fir was not affected by growth in the Terracosms. The concentration of α -pinene, β -pinene, Δ -3-carene, and total monoterpene concentration were

not changed by an increase of 4 °C in growth temperature (Fig. 8). A CO₂-temperature interaction explained increased concentrations of Δ -3-carene ($F = 6.42$; d.f. = 1; $P = 0.0351$). Interestingly, in these chambers there was a significant CO₂ effect that caused concentrations of α -pinene ($F = 12.49$; d.f. = 1; $P = 0.0077$), β -pinene ($F = 13.33$; d.f. = 1; $P = 0.0065$), and total monoterpene concentration ($F = 12.94$; d.f. = 1; $P = 0.0070$) to decline at elevated CO₂ at both growth temperatures (Fig. 8).

Discussion

The influence of growth at elevated CO₂ concentration on monoterpene emissions per unit biomass

The results of this study do not support the hypothesis that needle monoterpene production or emission is linked to needle carbon balance. In 1996 (current year) and 1995 (previous year) needles from trees of Ponderosa pine, growth at elevated CO₂ concentration caused an increase in net photosynthesis rate per unit of needle area and in the ratio of starch to N. In neither age class of needles, however, were tissue monoterpene concentrations nor emission rates observed to increase. These relationships were less certain for data collected from Douglas fir needles. However, for the one case where needle photosynthesis rates were higher at elevated CO₂ (1995 needles), there was no change in monoterpene concentration or emission rate.

These results do not bode well for proposed efforts to use resource acquisition and allocation models to predict monoterpene emission rates (e.g. Lerda *et al.* 1995; Monson *et al.* 1995). Two of the most prominent allocation

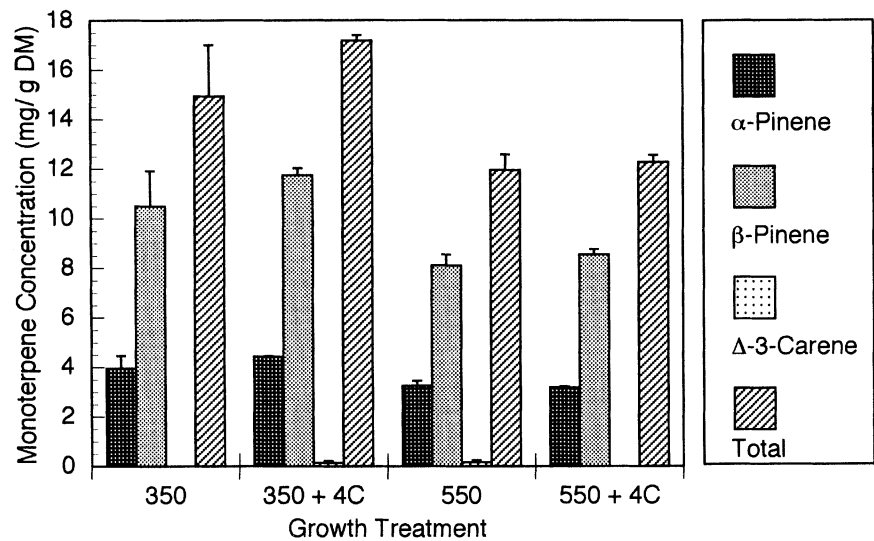


Fig. 8 Total monoterpene concentration (mg g^{-1} dry mass) in 1996 (current year) needles of Douglas fir. Values are means \pm SE ($n = 3$).

models (the Growth-Differentiation Balance Model and the Carbon Nutrient Balance Model) are built on the premise that carbon in excess of that required for immediate growth should be allocated to carbon-based defenses (Lerdau *et al.* 1994b). Excess carbon could arise because of the growth-constraining influence of other nutrients (e.g. N) or a decreased growth sink activity (due to environmental stress or seasonal influences on phenology). In a broad review of the literature, Gershenson (1994) found support for these relationships, specifically noting that monoterpene synthesis appeared to be linked to dynamics in tissue carbon balance. Lerdau *et al.* (1995) determined that in greenhouse grown Douglas fir, changes in monoterpene concentration and emission reflected phenologically driven changes in carbon demand. Thus, we hypothesized that growth in elevated CO_2 , especially in the presence of low N availability, would result in the accumulation of excess carbon, and promote the synthesis and emission of defensive compounds such as monoterpenes. This hypothesis was not supported by the observations.

Past studies have determined that the monoterpene emission rate from coniferous needles is a function of (i) temperature, through its influence on terpene vapour pressure, and (ii) tissue monoterpene concentration (a dependence that can be explained through Henry's Law) (Lerdau *et al.* 1994a, 1995, 1997). The relationship between tissue monoterpene concentration and emission rate could not be verified in the current studies. Data from none of the independent or pooled treatment classes revealed a significant correlation between these two variables (data not shown). It is likely that the lack of observed correlation is due to the limited range of tissue concentrations that were obtained for analysis in the current study. In past studies tissue concentrations varied over an 8–10 fold range (Lerdau *et al.* 1994a, 1995, 1997).

In the present study, tissue monoterpene concentrations were limited to only a 2–3 fold range. Although there was not an obvious correlation between monoterpene concentration and emission, the basic patterns of needle concentration did reflect the types of monoterpenes emitted.

The influence of growth at elevated CO_2 concentration and climate warming on monoterpene emissions per unit ground area

Despite no significant change in the rate of monoterpene emission per unit needle mass, growth at elevated CO_2 could still influence emission rate per unit ground area. Increased total needle area is a common result of growth at elevated CO_2 in conifers (Larigauderie *et al.* 1994; Griffin *et al.* 1995; Ineichen *et al.* 1995; Prior *et al.* 1997). For the Ponderosa pine trees used in this study, growth at elevated CO_2 for 39 months caused a 60% increase in leaf area index (LAI) (Tingey *et al.* 1996). For the Douglas fir trees, LAI was not influenced by growth CO_2 in December of 1996 after 2.5 years of growth in the Terracosms (D.M. Olszyk, Environmental Protection Agency, Corvallis, pers. comm.). Using these results from younger trees for extrapolation, one might predict increases in the emission of monoterpenes from Ponderosa pine canopies in a regime of elevated CO_2 , due solely to the presence of more emitting needle area per unit of ground area. Emissions from Douglas fir canopies would not be sensitive to this effect. These contrasting responses make it clear that species-specific differences must be taken into account when trying to predict the response of monoterpene emissions over broad geographical areas to environmental change.

In a future world of elevated tropospheric CO_2 concentrations, enhancement of monoterpene emissions due to

increased canopy needle area will likely be augmented by the direct effects of increased temperature. Thus, high-CO₂ induced increases in the mean global surface temperature could translate into increased regional summertime temperatures, and consequently increased forest monoterpene emissions. In the current study, the effect of temperature on emissions was estimated using the model of Guenther *et al.* (1993) and a hypothesized increase in the local temperature regime of 4 °C. The model predicted increases in emission of 52%, 46%, and 41% for α -pinene, β -pinene, and Δ -3-carene, respectively.

The elevated emission rates predicted for an increase in temperature can only be sustained given a sufficient monoterpene source within the needle. Based on the data presented in Fig. 8, it is clear that tissue monoterpene concentration does not change when Douglas fir trees are grown at 4 °C above ambient as compared to trees grown at ambient temperature. The maintenance of a steady monoterpene pool size in the face of predicted higher emissions when grown at elevated temperature could only occur if monoterpene synthesis (the mevalonic acid pathway and/or monoterpene cyclase activity) keeps pace with emissions. When combined, the measured effects of elevated CO₂ on LAI dynamics and the simulated effect of increased temperature on volatilization, are predicted to cause an 80% increase in the emissions of monoterpenes from Ponderosa pine canopies, and a 50% increase in the emissions from Douglas fir canopies. The response of LAI to growth at elevated CO₂ is likely quite variable for different forest canopies and dependent upon the degree of canopy closure. Thus, the quantitative response we have calculated should be interpreted with caution when applied to other canopies.

When placed within the context of atmospheric chemistry, increased emission of monoterpenes can potentially impact the oxidative state of the troposphere. In the atmosphere monoterpenes react in the gas phase with both ozone (O₃) and hydroxyl radical (OH·) during the day, and with nitrate radical (NO₃) during the night. The most commonly emitted monoterpenes (e.g. α -pinene and β -pinene) have tropospheric lifetimes of 2–3 h, reflecting the relatively high rate constants for these oxidative reactions (Fehsenfeld *et al.* 1992). Depending on the assumptions regarding the global concentrations of tropospheric nitrogen oxides (NO_x), the oxidation of α - and β -pinene by OH· or O₃ yields, on average, 15–20% of the carbon being converted to carbon monoxide (CO) (Hatakeyama *et al.* 1991). The oxidation of CO to CO₂ exerts an additional influence on tropospheric OH· concentration. The carbon that remains in the partially oxidized monoterpene molecules is either deposited back to the earth's surface, or partitions into organic aerosols in the troposphere (Andreae & Crutzen 1997). Thus, the oxidative chemistry of monoterpenes in the atmosphere

has potentially strong influences on the state and reactive dynamics of odd oxygen compounds and aerosol formation.

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