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# Stem wood properties of *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* saplings after 3 years of treatments to elevated carbon dioxide and ozone

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**Abstract**

**The aim of this study was to examine the effects of elevated carbon dioxide [$CO_2$] and ozone [$O_3$] and their interaction on wood chemistry and anatomy of five clones of 3-year-old trembling aspen (*Populus tremuloides* Michx.). Wood chemistry was studied also on paper birch (*Betula papyrifera* Marsh.) and sugar maple (*Acer saccharum* Marsh.) seedling-origin saplings of the same age. Material for the study was collected from the Aspen Free-Air $CO_2$ Enrichment (FACE) experiment in Rhinelander, WI, USA, where the saplings had been exposed to four treatments: control (C; ambient $CO_2$, ambient $O_3$), elevated $CO_2$ (560 ppm during daylight hours), elevated $O_3$ ($1.5 \times$ ambient during daylight hours) and their combination ($CO_2 + O_3$) for three growing seasons (1998–2000). Wood chemistry responses to the elevated $CO_2$ and $O_3$ treatments differed between species. Aspen was most responsive, while maple was the least responsive of the three tree species. Aspen genotype affected the responses of wood chemistry and, to some extent, wood structure to the treatments. The lignin concentration increased under elevated $O_3$ in four clones of aspen and in birch. However, elevated $CO_2$ ameliorated the effect. In two aspen clones, nitrogen in wood samples decreased under combined exposure to $CO_2$ and $O_3$. Soluble sugar concentration in one aspen clone and starch concentration in two clones were increased by elevated $CO_2$. In aspen wood, α-cellulose concentration changed under elevated $CO_2$, decreasing under ambient $O_3$ and slightly increasing under elevated $O_3$. Hemicellulose concentration in birch was decreased by elevated $CO_2$ and increased by elevated $O_3$. In aspen, elevated $O_3$ induced statistically significant reductions in distance from the pith to the bark and vessel lumen diameter, as well as increased wall thickness and wall percentage, and in one clone, decreased fibre lumen diameter. Our results show that juvenile wood properties of broadleaves, depending on species and genotype, were altered by atmospheric gas concentrations predicted for the year 2050 and that $CO_2$ ameliorates some adverse effects of elevated $O_3$ on wood chemistry.**

*Keywords:* aspen, birch, cell wall, α-cellulose, climate change, fibre, hemicellulose, lignin, maple, vessel

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## Introduction

The rise in the atmospheric concentrations of greenhouse gases increases the radiative forcing of the climate system that tends to warm the Earth's surface (IPCC, 2001). Carbon dioxide ($CO_2$) is the dominant human-influenced greenhouse gas the concentration of which has risen by 31% between years 1750 and 1999 (IPCC, 2001). After $CO_2$ and $CH_4$, the third most important greenhouse gas enhancing the radiative forcing is tropospheric ozone ($O_3$), which is estimated to have increased by about 35% since the Pre-industrial

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Era (IPCC, 2001). Because of the changing climate, the concern for the future of forest ecosystems is increasing. In general, $CO_2$ and $O_3$ have opposite impacts on vegetation. Increase in [$CO_2$] enhances photosynthesis, growth and productivity of trees (Saxe *et al*., 1998; Norby *et al*., 1999; Gielen & Ceulemans, 2001) but increasing tropospheric [$O_3$] is the main atmospheric pollutant that negatively affects forests (Broadmeadow, 1998; Skärby *et al*., 1998; Matyssek & Innes, 1999; Krause *et al*., 2002). The interaction of these two gases is complex and the combined effects have been far less studied (Allen, 1990; Kull *et al*., 1996, 2003; Karnosky *et al*., 1999; Broadmeadow & Jackson, 2000, Olszyk *et al*., 2000, Rebbeck & Scherzer, 2002) than single effects. A recent report on aspen suggests that genotype has an important role on the extent of the effects and elevated [$CO_2$] will not ameliorate the negative effects of elevated [$O_3$] on growth (Isebrands *et al*., 2001), but opposite results have also been published (e.g. Karnosky *et al*., 1999).

In wood formation, several consecutive phases follow each other (i.e., division in the cambial zone, differentiation, cell expansion, secondary wall deposition and autolysis of xylem elements) (e.g. Puech *et al*., 2000). These different phases are potential targets for disturbances due to different stressors. Wood properties vary within and between species and within a tree from base to apex and from pith to bark apart from influence of the growth environment. Wood quality is affected by chemical composition and structure, which, from an economical point of view, affect the end use possibilities of wood as raw material in mechanical and chemical forest industry. Knowledge on the effects of atmospheric greenhouse gases on wood properties is still very limited, and studies on deciduous species are rare. Some of the studies have been made on branch wood that greatly differs from stem wood (Haygreen & Bowyer, 1996). The effects of combined elevated [$CO_2$] and [$O_3$] on stem wood properties are totally lacking, and only few reports on $O_3$ impacts have been published. Responses of coniferous trees to elevated [$CO_2$] are contradictory in terms of wood strength (Beismann *et al*., 2002; Ceulemans *et al*., 2002) and tracheid wall thickness (Yazaki *et al*., 2001; Atwell *et al*., 2003). In general, the responses vary between species and genotypes (Beismann *et al*., 2002).

The effects of a 6-month exposure to elevated [$O_3$] on silver birch (*Betula pendula* L.) stem anatomy included a decrease of xylem width, in particular, in low-fertilized plants and also a reduction of single cell area of tracheids in potted cuttings (Matyssek *et al*., 2002). In 3-year-old Norway spruce (*Picea abies* L. Karst) saplings, effects of one-season exposure to elevated [$O_3$] on stem wood structure depended on nutrient availability, and an increase in tracheid wall thickness and a decrease or increase in latewood tracheid diameter were detected under elevated $O_3$ (Kurczyńska *et al*., 1998). Regarding $O_3$ effects on stem wood chemistry, results of non-structural carbohydrates are variable, and no data on cell wall structural components are available. In sugar maple (*Acer saccharum* Marsh.) seedlings, elevated [$O_3$] had no effect on sucrose and starch concentrations of stems (Bertrand *et al*., 1999). However, in 40-year-old and mature ponderosa pines (*Pinus ponderosa* Laws.) starch concentrations of stems were highest at most polluted sites along a long-term $O_3$ and N deposition gradient (Grulke *et al*., 2001).

Increased wood volume growth has been reported under elevated [$CO_2$] in 6-year-old loblolly pine (*Pinus taeda* L.) trees (Telewski *et al*., 1999), in longleaf pine (*Pinus palustris* Mill.) seedlings (Runion *et al*., 1999), and in 6-year-old Scots pine (*Pinus sylvestris* L.) trees (Ceulemans *et al*., 2002). However, diameter growth was not affected by elevated [$CO_2$] in young Siberian larch (*Larix sibirica* Ledeb.) seedlings (Yazaki *et al*., 2001). Regarding wood density, an increase has been observed in 6-year-old Norway spruce stem wood (Hättenschwiler *et al*., 1996) and in stems of radiata pine (*Pinus radiata* D. Don) seedlings (Atwell *et al*., 2003) while in loblolly pine (Telewski *et al*., 1999) and Scots pine (Ceulemans *et al*., 2002) no change in density was detected under elevated [$CO_2$]. Wood strength increased in 6–7-year-old Norway spruce under a 4-year treatment to elevated [$CO_2$] while beech (*Fagus sylvatica* L.) showed no such response (Beismann *et al*., 2002), and in Scots pine, wood compression strength decreased (Ceulemans *et al*., 2002). Reported changes in wood anatomy include an increase in tracheid diameter in Scots pine (Ceulemans *et al*., 2002), and an increase in tracheid lumen diameter in Siberian larch (Yazaki *et al*., 2001) or no change in radiata pine (*P. radiata* D. Don) seedlings (Atwell *et al*., 2003). Tracheid wall thickness increased in radiata pine (Atwell *et al*., 2003), and decreased in Siberian larch (Yazaki *et al*., 2001). Regarding chemical composition, no effects of elevated [$CO_2$] on stem wood of longleaf pine (Runion *et al*., 1999), radiata pine (Atwell *et al*., 2003) and Douglas-fir (Tingey *et al*., 2003) were detected but, on branch wood of Norway spruce an increase in starch and a decrease in nitrogen were reported (Hättenschwiler *et al*., 1996). Decreased lignin was observed in beech stem wood after a 3-year exposure of elevated [$CO_2$] (Blaschke *et al*., 2002) and in branch wood after a 5-year fumigation period (Cotrufo & Ineson, 2000).

We determined the interactive effects of elevated [$CO_2$] and [$O_3$] on stem wood chemistry and anatomy of trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.) and sugar maple (*A.*

*saccharum* Marsh.) after 3 years of exposure in the Aspen FACE experiment. We hypothesized that the opposite effects of elevated [$CO_2$] and [$O_3$] on tree growth would have a differential impact on wood properties because of differences in substrate availability for wood formation. We assumed that elevated [$CO_2$] would increase radial growth but would not affect cell wall chemistry because the young saplings were well supplied with nutrients. In contrast, elevated [$O_3$], as a strong oxidative agent, was assumed to have complex effects both on structure and chemistry of wood. Further, we assumed that elevated [$CO_2$] will ameliorate the effects of [$O_3$]. Five aspen genotypes, paper birch and sugar maple were included in the study to examine the differences between genotypes and species to climate change.

## Materials and methods

### *Plant material*

Trembling aspen (*P. tremuloides* Michx.) clones that originated from the Great Lakes Region were vegetatively propagated from green wood cuttings (Dickson *et al*., 2000). Paper birch (*B. papyrifera* Marsh.) and sugar maple (*A. saccharum* Marsh.) originated from seed collected in Houghton County, Michigan (Dickson *et al*., 2000). Plant material was planted in the ring areas in early June 1997. Plants were exposed to elevated $CO_2$ and $O_3$ yearly from the beginning of May to the end of September, starting in 1998. Five aspen clones were included in the experiment. Three of the clones were selected according to their differing sensitivity to $O_3$: 216 and 271 = relatively tolerant, and 259 = relatively sensitive (Karnosky *et al*., 1996); and two clones, 8L and 42E, were selected according to their leaf phenology and differing response to elevated $CO_2$ (Kubiske *et al*., 1998).

### *Experimental design and sampling*

The long-term Aspen Free-Air $CO_2$ Enrichment (FACE) experiment is located in Rhinelander, WI, USA. The study contains 12 individual rings that are 30 m in diameter. The experiment is a full-factorial design with three control rings (no added $CO_2$ or $O_3$), three $CO_2$ rings, three $O_3$ rings, and three $CO_2 + O_3$ rings. The exposure system has been previously described by Dickson *et al*. (2000) and Karnosky *et al*. (2003). Since 1998, trees were exposed during daylight hours from budbreak to budset to four treatments: control, C (ambient $CO_2$, ambient $O_3$); elevated $CO_2$ (560 μmoL $mol^{-1}$ vs. ambient $CO_2$ of 360 μmol $mol^{-1}$); elevated $O_3$ (1.5 × ambient) and their combination ($CO_2 + O_3$). Daytime ambient $CO_2$ concentrations averaged 360, 360, and 350 μmol $mol^{-1}$; elevated $CO_2$ concentrations averaged 530, 548, and 545 μmol $mol^{-1}$ for the three growing seasons. Elevated $O_3$ exposures averaged for 54.5, 51.1 and 48.9 nL $L^{-1}$ (12 h daytime mean during growing season) compared with control ring $O_3$ which averaged for 34.6, 36.9, and 36.0 nL $L^{-1}$ for the same period. For the seasonal and hourly $O_3$ concentrations see Karnosky *et al*. (2003). The total seasonal exposures (SUM 00) for daylight hours were 97 900, 87 900, and 78 800 nL $L^{-1}$ h for elevated $O_3$, compared with control values at 59 100, 62 800 and 58200 nL $L^{-1}$ h.

Material for the study was collected randomly from a pool of candidate trees within ± 10% of the mean height of that clone or species in that ring. All trees were chosen from the edge of the core area of each ring in August 2000 after 3 years of fumigation from 84 aspen, 12 birch and 12 maple trees. Stem samples were collected 30 cm above the ground level. Stem sections were transported in dry ice (−80 °C) to Suonenjoki Research Station, Finland and stored at −20 °C until processing. Wood of aspen, birch, and maple was examined for concentrations of acetone-soluble extractives, gravimetric and acid-soluble lignin, α-cellulose, hemicellulose, soluble sugars, starch, and nitrogen. Microscopical analysis of aspen wood included the measurements of lumen diameters of vessels and fibres and fibre wall thickness and the percentages of vessels and cell walls.

### *Wood chemistry*

Disks were further processed for chemical analyses. Bark, phloem, and cambium were removed from the samples. Secondary xylem, consisting of pith and growth rings of 1998–2000, was fractionated into picks (approximately 2–3 mm in thickness) that were freeze-dried, milled (Polymix A10, Kinematica AG, Switzerland) at −25 °C and stored at −20 °C.

Milled stem wood (2 g) was extracted in acetone (150 mL) by Soxhlet method according to the SCAN-CM (1994) standard for gravimetric measurement of acetone-soluble extractives and to yield extractive-free samples. α-cellulose, hemicellulose, gravimetric lignin and acid-soluble lignin were analysed from the extractive-free samples as described by Anttonen *et al*. (2002).

Soluble sugars were extracted from milled samples (30–100 mg of dry weight, DW) with a total volume of 15 mL of 80% aqueous ethanol. Starch was extracted from the residue with 20 mL of 30% perchloric acid. Soluble sugars and starch were measured by the anthrone method (Hansen & Møller, 1975). Total nitrogen concentration of the secondary xylem was

analysed from milled aspen samples with a CHN-1000 Analyzer (Leco Co., St Joseph, MI, USA).

### *Wood structure*

Distance from the pith to the bark (radial growth) was measured with a digital calliper on the stem disks. Microscopical samples were taken from the disks for measuring percentages of cell types, diameter of vessel and fibre lumen and fibre wall thickness in radial and tangential axis. The above parameters were measured from the third annual ring formed in year 2000. For microscopic sectioning, 45° sectors of stem disks were boiled in water in a microwave oven for 2 min to soften the wood. Then the samples were frozen and 16 μm thick cross-sections were cut at −14 °C with a Leitz 1516 (Ernst Leitz, Ontario, Canada) cryomicrotome. The sections were stained with safranin, rinsed with water, dehydrated with ascending alcohol series, rinsed with xylene, and mounted in Canada balsam.

Vessel and fibre lumen diameter and the cell wall thickness of fibres were measured using an Olympus BX60 (Olympus Optical, Tokyo, Japan) microscope connected to a Spot insight B/W video camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA) and Image-Pro plus 4.1 for Windows (Media Cybernetics, Silver Spring, MD, USA) program. The image area was 1600 × 1200 pixels. Ten-fold magnification was used for analysing the percentage of cell types and vessel and fibre lumen diameter and 40-fold magnification was used for analysing the fibre wall thickness. In the 10-fold magnification, one pixel corresponded to 0.74 μm and in 40-fold magnification to 0.19 μm. Three images of the transverse sections of annual rings formed in the year 2000 were taken and analysed, per plant. The cells that were either located partly in the image or whose cell walls were broken by cross-section preparation, were excluded from analysis. The lumen diameter of vessels and fibres was measured from cells larger than 1000 $\mu m^2$ or equal to 15–500 $\mu m^2$, respectively. For measurements of lumen diameters, the mean number of vessels and fibres measured per image was ca. 100 and 2500, respectively. In analysis of fibre wall thickness, 50–60 cell walls were included in one sample.

### *Statistical analyses*

The data were analysed using descriptive statistics (normality and homogeneity of variance) and the GLM procedure of SPSS-Win 11.0 package (SPSS, Chicago, IL, USA). For analysis, the experiment was considered a randomized complete block design with three replicates of exposure treatments. In aspen, for statistical differences between the treatments, data were analysed (SPSS-Win 11.0 GLM procedure), based on ring means ($n = 3$), using a mixed-model design of analysis of variance (ANOVA) with the average responses of $CO_2$, $O_3$, clone, and ring. Fixed effects were $CO_2$, $O_3$, clone, $CO_2 \times O_3$ interaction, $CO_2 \times$ clone, $O_3 \times$ clone, and $CO_2 \times O_3 \times$ clone. Random effects included ring. In birch and maple, the design was similar but without the clone. In statistical tests, arcsin transformations were used for the wall and vessel percentages. Data are presented as means with standard errors. Because of the low number of replicates ($n = 3$) for the treatments, and the corresponding risk of type II statistical errors, we report $P$-values $< 0.10$ as significant.

## Results

### *Wood chemistry of aspen, birch and maple*

The average α-cellulose, total lignin, and nitrogen concentrations in aspen, birch, and maple were 50.1%, 36.2%, 41.4% DW; 21.7%, 22.2%, 23.1% DW and 0.17%, 0.13%, 0.13% DW, respectively. The average concentrations in aspen, birch, and maple wood were 5.3%,

**Table 1** Summary of $P$-values for the effects of treatments on stem wood chemistry (% of dry weight) in aspen

| Source of variation | df | α-cellulose | Hemicellulose | Total lignin | Gravimetric lignin | Acid-soluble lignin | Extractives | Soluble sugars | Starch | C | N | C/N ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ | 1 | 0.934 | 0.575 | 0.378 | 0.442 | 0.460 | 0.145 | 0.086 | 0.022 | 0.002 | 0.025 | 0.035 |
| $O_3$ | 1 | 0.220 | 0.526 | 0.286 | 0.188 | 0.202 | 0.139 | 0.809 | 0.180 | 0.904 | 0.006 | 0.002 |
| $CO_2 \times O_3$ | 1 | 0.051 | 0.286 | 0.064 | 0.104 | 0.123 | 0.621 | 0.629 | 0.443 | 0.432 | 0.050 | 0.072 |
| Clone | 4 | 0.491 | 0.969 | 0.061 | 0.006 | 0.000 | 0.000 | 0.002 | 0.000 | 0.128 | 0.000 | 0.000 |
| Ring | 2 | 0.081 | 0.499 | 0.757 | 0.911 | 0.172 | 0.879 | 0.161 | 0.890 | 0.598 | 0.006 | 0.028 |
| $CO_2 \times$ clone | 4 | 0.771 | 0.892 | 0.650 | 0.751 | 0.517 | 0.268 | 0.015 | 0.041 | 0.454 | 0.134 | 0.066 |
| $O_3 \times$ clone | 4 | 0.902 | 0.599 | 0.047 | 0.027 | 0.491 | 0.514 | 0.616 | 0.180 | 0.198 | 0.048 | 0.070 |
| $CO_2 \times O_3 \times$ clone | 4 | 0.369 | 0.273 | 0.806 | 0.858 | 0.820 | 0.982 | 0.360 | 0.099 | 0.854 | 0.440 | 0.518 |

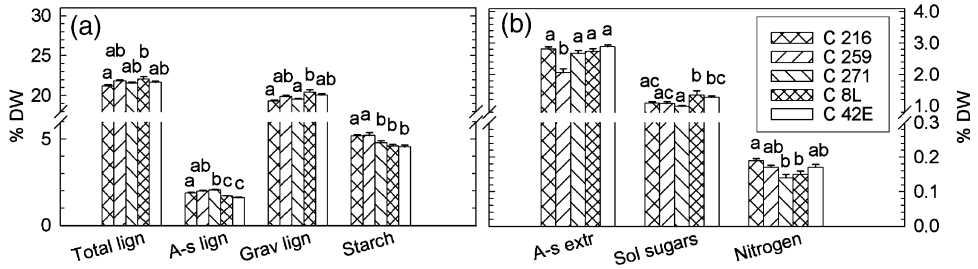


**Fig. 1** Overall responses on (a) total lignin, acid-soluble lignin, gravimetric lignin and starch, and (b) acetone-soluble extractives, soluble sugars and nitrogen for each aspen clone at the end of the third treatment season. Error bars indicate +1 SE. Values with different letters are significantly different ($P<0.1$) among the clones.

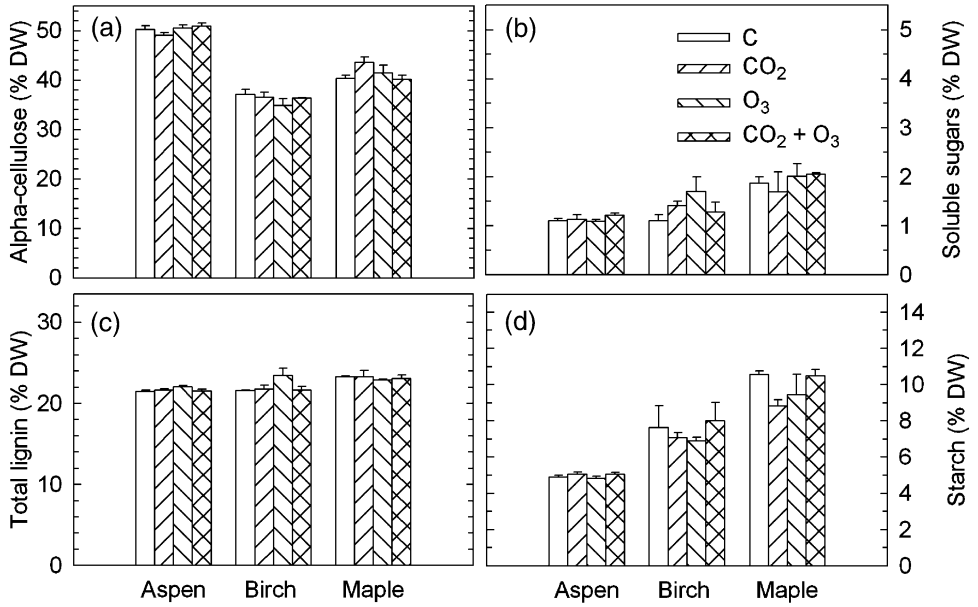


**Fig. 2** Overall responses on (a) α-cellulose, (b) soluble sugars, (c) total lignin, and (d) starch in stem wood of aspen, paper birch and sugar maple for each treatment, summarizing clone averages for aspen at the end of the third treatment season. Error bars indicate +1 SE.

10.7%, 10.5% DW for hemicellulose; 19.7%, 20.0%, 21.6 % DW for gravimetric lignin; 1.8%, 2.4%, 1.5% DW for acid-soluble lignin; 2.7%, 3.9%, 1.5% DW for acetone-soluble extractives; 1.1%, 1.4%, 1.9% for soluble sugars; 5.0%, 7.4% and 9.8% DW for starch and 49.1%, 48.1%, 48.1% DW for carbon, respectively.

*Genetic variation.* Genetic variation was remarkable on certain compounds of aspen wood (Table 1). Total lignin concentration was 4% (relatively) higher in clone 8L than in clone 216 (Fig. 1a). Clones 216, 259, and 271 had higher acid-soluble lignin than clones 8L and 42E (Fig. 1a). Clone 8L had higher concentration of gravimetric lignin than clones 216 and 271 (Fig. 1a). Starch concentration was slightly, but significantly higher in clones 216 and 259 as compared with other clones (Fig. 1a). The concentration of acetone-soluble extractives, which include a variety of different compounds (e.g., lipids, terpenes, and resin acids) was 26% lower in clone 259 than in other clones (Fig. 1b). Clone 8L had 19–27% higher concentration of soluble sugars than clones 271, 259, or 216, respectively (Fig. 1b). Clone 42E had 23% higher concentration of soluble sugars than clone 271. Nitrogen concentration was 21–27% higher in clone 216 as compared with clones 271 and 8L, respectively (Fig. 1b). The concentrations of α-cellulose, hemicellulose and carbon did not vary by clone (Table 1).

*Treatment effects.* In aspen stem wood, increases in soluble sugars (Fig. 2b) and starch (Fig. 2d) were detected under the $CO_2$ exposure, but due to the interaction between clone and the $CO_2$ treatment (Table 1), the effect was significant only in clone 8L for soluble sugars (Table 2) and in clones 259 and 271 for starch (Table 2). Interactions between the $O_3$

**Table 2** Summary of the effects of treatments for each aspen clone, paper birch and sugar maple on stem wood chemistry (% of dry weight) at the end of the third treatment season

| Treatment | Clone/Species | α-cellulose | Hemicellulose | Total lignin | Gravimetric lignin | Acid-soluble lignin | Extractives | Soluble sugars | Starch | Carbon | Nitrogen | C/N ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 216 | 49.4 ± 1.2 | 6.0 ± 1.0 | 21.3 ± 0.4 | 19.4 ± 0.3 | 1.9 ± 0.1 | 2.7 ± 0.1 | 1.1 ± 0.1 | 5.0 ± 0.1 | 49.1 ± 0.1 | 0.192 ± 0.013 | 263.6 ± 15.6 |
| | 259 | 51.2 ± 1.3 | 3.6 ± 2.1 | 21.5 ± 0.2 | 19.6 ± 0.3 | 1.9 ± 0.1 | 2.2 ± 0.3 | 1.1 ± 0.2 | 5.4 ± 0.1 | 49.1 ± 0.2 | 0.170 ± 0.006 | 289.3 ± 10.2 |
| | 271 | 50.3 ± 1.4 | 6.0 ± 1.5 | 21.5 ± 0.3 | 19.4 ± 0.3 | 2.1 ± 0.1 | 2.8 ± 0.1 | 1.0 ± 0.0 | 4.5 ± 0.1 | 49.2 ± 0.1 | 0.167 ± 0.003 | 295.4 ± 5.8 |
| | 8L | 50.1 ± 0.7 | 6.0 ± 1.2 | 21.3 ± 0.6 | 19.6 ± 0.5 | 1.7 ± 0.1 | 2.8 ± 0.3 | 1.0 ± 0.1 | 4.6 ± 0.3 | 49.3 ± 0.1 | 0.153 ± 0.015 | 327.0 ± 30.0 |
| | 42E | 52.3 ± 3.0 | 5.2 ± 1.9 | 21.7 ± 0.4 | 20.1 ± 0.3 | 1.6 ± 0.1 | 2.8 ± 0.1 | 1.3 ± 0.0 | 4.7 ± 0.2 | 49.1 ± 0.2 | 0.193 ± 0.019 | 258.1 ± 22.4 |
| | Birch | 37.1 ± 0.9 | 10.8 ± 0.5 | 21.6 ± 0.1 | 19.4 ± 0.1 | 2.7 ± 0.5 | 3.7 ± 0.3 | 1.1 ± 0.1 | 7.6 ± 1.2 | 48.0 ± 0.3 | 0.136 ± 0.009 | 355.5 ± 25.8 |
| | Maple | 40.4 ± 0.6 | 11.2 ± 1.1 | 23.3 ± 0.1 | 21.8 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.9 ± 0.1 | 10.6 ± 0.2 | 48.1 ± 0.2 | 0.122 ± 0.012 | 401.6 ± 36.5 |
| $CO_2$ | 216 | 49.0 ± 1.1 | 5.9 ± 0.9 | 20.9 ± 0.3 | 19.0 ± 0.2 | 1.9 ± 0.1 | 2.9 ± 0.1 | 1.0 ± 0.1 | 5.2 ± 0.2 | 49.0 ± 0.1 | 0.197 ± 0.010 | 253.3 ± 10.8 |
| | 259 | 48.1 ± 0.1 | 5.9 ± 0.7 | 21.6 ± 0.2 | 19.6 ± 0.2 | 2.0 ± 0.1 | 2.3 ± 0.3 | 1.0 ± 0.1 | 5.5 ± 0.4 | 48.9 ± 0.2 | 0.157 ± 0.009 | 313.9 ± 17.5 |
| | 271 | 50.9 ± 1.0 | 3.6 ± 1.9 | 21.7 ± 0.3 | 19.5 ± 0.2 | 2.1 ± 0.1 | 2.5 ± 0.0 | 0.8 ± 0.0 | 5.4 ± 0.4 | 49.1 ± 0.2 | 0.147 ± 0.020 | 349.1 ± 51.6 |
| | 8L | 48.9 ± 1.1 | 6.0 ± 0.5 | 21.8 ± 0.4 | 20.0 ± 0.4 | 1.8 ± 0.1 | 2.9 ± 0.3 | 1.8 ± 0.5 | 4.6 ± 0.2 | 48.9 ± 0.1 | 0.187 ± 0.012 | 264.1 ± 16.5 |
| | 42E | 48.5 ± 2.9 | 7.5 ± 2.9 | 22.3 ± 0.1 | 20.7 ± 0.1 | 1.6 ± 0.1 | 3.0 ± 0.1 | 1.3 ± 0.2 | 4.4 ± 0.0 | 49.1 ± 0.1 | 0.180 ± 0.015 | 276.2 ± 21.3 |
| | Birch | 36.6 ± 1.0 | 9.7 ± 0.3 | 21.8 ± 0.5 | 19.9 ± 0.8 | 2.5 ± 0.6 | 4.1 ± 0.5 | 1.4 ± 0.1 | 7.1 ± 0.3 | 48.2 ± 0.1 | 0.136 ± 0.007 | 356.4 ± 17.7 |
| | Maple | 43.6 ± 1.1 | 10.0 ± 0.8 | 23.3 ± 0.8 | 21.7 ± 0.9 | 1.6 ± 0.1 | 1.5 ± 0.3 | 1.7 ± 0.4 | 8.8 ± 0.3 | 48.2 ± 0.3 | 0.115 ± 0.012 | 428.3 ± 46.0 |
| $O_3$ | 216 | 49.8 ± 1.0 | 4.3 ± 0.9 | 21.7 ± 0.3 | 19.7 ± 0.2 | 2.0 ± 0.1 | 2.6 ± 0.2 | 1.1 ± 0.1 | 5.3 ± 0.2 | 49.4 ± 0.1 | 0.209 ± 0.017 | 247.8 ± 20.3 |
| | 259 | 50.8 ± 1.8 | 6.1 ± 1.8 | 22.0 ± 0.4 | 20.0 ± 0.4 | 1.9 ± 0.1 | 1.7 ± 0.2 | 1.1 ± 0.1 | 4.4 ± 0.2 | 48.9 ± 0.1 | 0.197 ± 0.020 | 254.5 ± 27.6 |
| | 271 | 50.5 ± 1.2 | 6.3 ± 2.3 | 22.0 ± 0.2 | 19.9 ± 0.1 | 2.1 ± 0.1 | 2.8 ± 0.4 | 1.0 ± 0.1 | 4.4 ± 0.0 | 49.1 ± 0.2 | 0.150 ± 0.017 | 336.0 ± 38.7 |
| | 8L | 48.8 ± 1.9 | 3.9 ± 2.0 | 23.0 ± 0.4 | 21.3 ± 0.4 | 1.7 ± 0.1 | 2.5 ± 0.1 | 1.1 ± 0.1 | 4.7 ± 0.1 | 49.4 ± 0.1 | 0.143 ± 0.020 | 358.7 ± 50.1 |
| | 42E | 50.4 ± 3.8 | 7.8 ± 2.9 | 21.4 ± 0.5 | 19.8 ± 0.5 | 1.6 ± 0.1 | 2.8 ± 0.0 | 1.2 ± 0.1 | 4.4 ± 0.3 | 49.2 ± 0.1 | 0.150 ± 0.015 | 334.1 ± 31.2 |
| | Birch | 34.9 ± 1.4 | 12.0 ± 0.7 | 23.5 ± 0.9 | 21.0 ± 0.8 | 2.4 ± 0.1 | 4.2 ± 0.2 | 1.7 ± 0.3 | 6.9 ± 0.2 | 48.2 ± 0.1 | 0.142 ± 0.010 | 342.3 ± 23.8 |
| | Maple | 41.4 ± 1.6 | 10.3 ± 0.5 | 22.9 ± 0.1 | 21.3 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 | 2.0 ± 0.3 | 9.4 ± 1.2 | 48.1 ± 0.1 | 0.172 ± 0.018 | 286.1 ± 27.0 |
| $CO_2 + O_3$ | 216 | 49.3 ± 2.1 | 5.2 ± 0.5 | 20.9 ± 0.3 | 19.0 ± 0.3 | 1.8 ± 0.1 | 2.9 ± 0.2 | 1.2 ± 0.1 | 5.3 ± 0.1 | 49.0 ± 0.1 | 0.179 ± 0.009 | 279.7 ± 14.2 |
| | 259 | 50.3 ± 4.5 | 4.6 ± 3.1 | 22.2 ± 0.5 | 20.1 ± 0.5 | 2.0 ± 0.1 | 2.1 ± 0.2 | 1.2 ± 0.1 | 5.5 ± 0.1 | 48.8 ± 0.1 | 0.153 ± 0.003 | 318.6 ± 7.1 |
| | 271 | 50.8 ± 1.6 | 6.2 ± 1.3 | 21.2 ± 0.1 | 19.3 ± 0.1 | 1.9 ± 0.1 | 2.6 ± 0.0 | 1.1 ± 0.1 | 4.9 ± 0.1 | 48.8 ± 0.3 | 0.103 ± 0.019 | 501.0 ± 80.4 |
| | 8L | 52.5 ± 3.7 | 3.8 ± 0.9 | 22.2 ± 0.9 | 20.6 ± 0.9 | 1.6 ± 0.1 | 2.7 ± 0.1 | 1.5 ± 0.1 | 4.4 ± 0.3 | 49.0 ± 0.1 | 0.130 ± 0.021 | 395.5 ± 57.7 |
| | 42E | 54.8 ± 2.6 | 2.3 ± 1.0 | 21.1 ± 0.5 | 19.5 ± 0.4 | 1.6 ± 0.1 | 2.9 ± 0.2 | 1.2 ± 0.0 | 4.7 ± 0.3 | 49.1 ± 0.1 | 0.157 ± 0.018 | 321.2 ± 33.9 |
| | Birch | 36.4 ± 0.1 | 10.3 ± 0.6 | 21.6 ± 0.4 | 19.5 ± 0.4 | 2.1 ± 0.1 | 3.5 ± 0.2 | 1.3 ± 0.2 | 8.0 ± 1.0 | 48.1 ± 0.2 | 0.122 ± 0.005 | 395.9 ± 19.0 |
| | Maple | 40.2 ± 0.8 | 10.4 ± 0.9 | 23.0 ± 0.5 | 21.5 ± 0.5 | 1.5 ± 0.1 | 1.7 ± 0.1 | 2.1 ± 0.0 | 10.5 ± 0.3 | 48.2 ± 0.2 | 0.128 ± 0.018 | 389.7 ± 49.7 |

Values are means ± SE, $n = 3$.

 

exposure and clone for gravimetric lignin and nitrogen were detected (Table 1). The $O_3$ treatment increased lignin concentrations slightly in all clones except clone 42E (Table 2) but decreased nitrogen concentrations in clones 271 ($P = 0.040$) and 42E ($P = 0.087$).

Significant interactive effects of $CO_2$ and $O_3$ were detected on α-cellulose, total lignin (= gravimetric + acid-soluble lignin) and nitrogen concentrations and C/N ratio (Table 1). The interaction for α-cellulose was due to the fact that in the $CO_2$ treatment its concentration decreased while in the $CO_2 + O_3$ treatment the concentration slightly increased (Fig. 2a). For total lignin, the $O_3$ exposure alone increased the concentration, but under the $CO_2$ treatment the effect was nullified (Fig. 2c). The interaction for nitrogen was because the $CO_2$ treatment alone did not have an influence while the $CO_2 + O_3$ treatment decreased the nitrogen concentration (Table 2).

In birch (Table 3), the $O_3$ exposure increased hemicellulose (Table 2), while the $CO_2$ treatment had an opposite effect. Interaction between the $CO_2$ and $O_3$ treatments for total lignin and gravimetric lignin was similar as the interaction for total lignin in aspen (Fig. 2c). In maple wood, statistically significant interaction between the $CO_2$ and $O_3$ treatments were found for starch and carbon concentrations (Table 4). The interaction for starch was due to decreased concentrations in the $CO_2$ and $O_3$ treatments singly while in the combination treatment no effect on starch concentration was detected (Fig. 2d). Carbon concentrations increased in the elevated $CO_2$ and $O_3$ treatments alone while the combined exposure nullified the effect (Table 2).

*Wood structure of aspen*

In the secondary xylem of aspen the average value for distance from the pith to the bark was 13.3 mm. The average vessel and fibre lumen diameters were 74.6 and 11.9 μm, respectively. Fibre wall radial and tangential thicknesses were 3.0 and 2.8 μm, respectively. The vessel and cell wall percentages in aspen stem wood were 28.1% and 41.9%, respectively.

*Genetic variation.* The structural parameters (distance from the pith, vessel and fibre lumen diameter, cell wall thickness, vessel and cell wall percentage) varied significantly by clone (Tables 5 and 6, Fig. 3a, b). Clones 271 and 216 had the largest distance from the pith that differed significantly from the other clones (Fig. 3a). Clone 271 had the largest fibre lumen diameter that differed significantly from clones 8L and 216 having the smallest diameters (Fig. 3a). Wall thickness of fibres was highest in clones 8L and 271 (Fig. 3a). Clone 216 had the smallest vessel lumen diameter and the lowest vessel percentage (Fig. 3b) differing significantly from the other clones. Clone 216 also had the highest cell wall percentage differing significantly from clones 259 and 42E with the lowest values (Fig. 3b).

*Treatment effects.* The $CO_2$ treatment increased distance from the pith (Table 5, Fig. 4a) but due to the interaction between clone and $CO_2$ treatment, the increase was significant in clones 216 and 259 only (Tables 5 and 6). The $CO_2$ treatment had no statistically significant effect on cell structure of the secondary xylem although the diameters of vessel and fibre lumen tended to be

**Table 3** Summary of *P*-values for the effects of treatments on stem wood chemistry (% of dry weight) in paper birch

| Source of variation | df | α-cellulose | Hemicellulose | Total lignin | Gravimetric lignin | Acid-soluble lignin | Extractives | Soluble sugars | Starch | C | N | C/N ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ | 1 | 0.688 | 0.011 | 0.151 | 0.297 | 0.487 | 0.670 | 0.779 | 0.772 | 0.899 | 0.270 | 0.286 |
| $O_3$ | 1 | 0.277 | 0.061 | 0.089 | 0.139 | 0.425 | 0.959 | 0.272 | 0.925 | 0.824 | 0.655 | 0.593 |
| $CO_2 \times O_3$ | 1 | 0.371 | 0.562 | 0.098 | 0.083 | 0.880 | 0.133 | 0.108 | 0.408 | 0.448 | 0.285 | 0.300 |
| Ring | 2 | 0.587 | 0.052 | 0.113 | 0.093 | 0.174 | 0.346 | 0.442 | 0.981 | 0.516 | 0.616 | 0.617 |

**Table 4** Summary of *P*-values for the effects of treatments on stem wood chemistry (% of dry weight) in sugar maple

| Source of variation | df | α-cellulose | Hemicellulose | Total lignin | Gravimetric lignin | Acid-soluble lignin | Extractives | Soluble sugars | Starch | C | N | C/N ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ | 1 | 0.466 | 0.590 | 0.845 | 0.911 | 0.684 | 0.635 | 0.788 | 0.625 | 0.874 | 0.180 | 0.210 |
| $O_3$ | 1 | 0.388 | 0.841 | 0.462 | 0.468 | 0.967 | 0.710 | 0.373 | 0.699 | 0.946 | 0.110 | 0.148 |
| $CO_2 \times O_3$ | 1 | 0.129 | 0.527 | 0.851 | 0.805 | 0.744 | 0.579 | 0.677 | 0.084 | 0.044 | 0.314 | 0.439 |
| Ring | 2 | 0.899 | 0.767 | 0.123 | 0.096 | 0.417 | 0.494 | 0.447 | 0.624 | 1.000 | 0.753 | 0.925 |

**Table 5** Summary of *P*-values for the effects of treatments on stem wood anatomy in aspen

| Source of variation | df | Dfp | Vessel (%) | Wall (%) | Ld vessel | Ld fibre | Fwt (rad) | Fwt (tan) |
|---|---|---|---|---|---|---|---|---|
| $CO_2$ | 1 | 0.018 | 0.469 | 0.758 | 0.135 | 0.451 | 0.612 | 0.413 |
| $O_3$ | 1 | 0.003 | 0.133 | 0.018 | 0.043 | 0.036 | 0.022 | 0.065 |
| $CO_2 \times O_3$ | 1 | 0.360 | 0.509 | 0.662 | 0.511 | 0.774 | 0.390 | 0.814 |
| Clone | 4 | 0.000 | 0.000 | 0.009 | 0.000 | 0.009 | 0.000 | 0.002 |
| Ring | 2 | 0.151 | 0.344 | 0.459 | 0.829 | 0.553 | 0.639 | 0.957 |
| $CO_2 \times$ clone | 4 | 0.043 | 0.399 | 0.874 | 0.933 | 0.523 | 0.582 | 0.837 |
| $O_3 \times$ clone | 4 | 0.101 | 0.817 | 0.231 | 0.417 | 0.044 | 0.102 | 0.091 |
| $CO_2 \times O_3 \times$ clone | 4 | 0.997 | 0.534 | 0.829 | 0.332 | 0.983 | 0.971 | 0.908 |

Dfp, distance from the pith; Ld, lumen diameter; Fwt, fibre wall thickness.

**Table 6** Summary of the effects of treatments for each aspen clone on structural parameters in annual ring 2000 of stem wood at the end of the third treatment season

| Treatment | Clone/Species | Dfp (mm) | Vessel (%) | Wall (%) | Ld vessel (μm) | Ld fibre (μm) | Fwt ± rad (μm) | Fwt ± tan (μm) |
|---|---|---|---|---|---|---|---|---|
| Control | 216 | 14.5 ± 0.9 | 24.4 ± 1.0 | 42.5 ± 0.2 | 63.5 ± 1.7 | 11.7 ± 0.3 | 2.8 ± 0.1 | 2.5 ± 0.1 |
| | 259 | 11.1 ± 0.3 | 30.0 ± 0 | 38.3 ± 0 | 70.2 ± 0 | 11.4 ± 0 | 2.7 ± 0.1 | 2.6 ± 0.1 |
| | 271 | 16.0 ± 0.6 | 31.7 ± 0.7 | 37.2 ± 0.5 | 75.3 ± 1.8 | 13.3 ± 0.1 | 2.9 ± 0.1 | 2.6 ± 0.1 |
| | 8L | 11.7 ± 1.5 | 31.9 ± 2.8 | 40.4 ± 5.2 | 81.7 ± 2.1 | 11.5 ± 0.8 | 3.5 ± 0.3 | 3.1 ± 0.3 |
| | 42E | 12.9 ± 0.9 | 27.0 ± 1.0 | 40.8 ± 1.6 | 81.6 ± 4.3 | 12.1 ± 0.1 | 2.8 ± 0.1 | 2.8 ± 0.1 |
| $CO_2$ | 216 | 16.9 ± 0.8 | 23.2 ± 2.7 | 45.8 ± 4.5 | 65.2 ± 3.1 | 11.5 ± 0.6 | 2.9 ± 0.1 | 2.8 ± 0.1 |
| | 259 | 13.2 ± 0.6 | 29.0 ± 0.9 | 36.3 ± 4.6 | 80.6 ± 4.8 | 12.4 ± 0.8 | 2.7 ± 0.2 | 2.6 ± 0.2 |
| | 271 | 18.1 ± 0.9 | 28.7 ± 1.5 | 38.0 ± 3.6 | 79.5 ± 1.9 | 13.8 ± 0.2 | 2.9 ± 0.1 | 2.8 ± 0.1 |
| | 8L | 10.6 ± 1.4 | 32.7 ± 0.3 | 38.3 ± 2.5 | 82.8 ± 5.5 | 11.8 ± 0.4 | 3.2 ± 0.1 | 3.0 ± 0.1 |
| | 42E | 14.1 ± 0.8 | 31.4 ± 1.2 | 38.5 ± 0.1 | 81.2 ± 3.3 | 12.0 ± 0.3 | 2.8 ± 0.1 | 2.8 ± 0.2 |
| $O_3$ | 216 | 12.8 ± 0.5 | 22.9 ± 1.0 | 47.7 ± 7.2 | 66.6 ± 0.1 | 12.0 ± 1.2 | 3.1 ± 0.3 | 3.0 ± 0.4 |
| | 259 | 9.6 ± 0.8 | 29.5 ± 0.8 | 37.0 ± 0.8 | 70.3 ± 2.5 | 11.6 ± 0.3 | 2.6 ± 0.1 | 2.5 ± 0.1 |
| | 271 | 14.8 ± 0.7 | 29.8 ± 1.5 | 48.1 ± 3.0 | 71.5 ± 1.6 | 11.4 ± 0.2 | 3.3 ± 0.1 | 3.1 ± 0.1 |
| | 8L | 12.9 ± 1.0 | 30.0 ± 1.3 | 43.9 ± 0.6 | 79.0 ± 0.6 | 11.0 ± 0.2 | 3.4 ± 0.2 | 3.2 ± 0.2 |
| | 42E | 11.8 ± 0.6 | 28.4 ± 2.2 | 38.1 ± 4.4 | 73.3 ± 3.6 | 12.0 ± 1.1 | 2.8 ± 0.1 | 2.6 ± 0.1 |
| $CO_2 + O_3$ | 216 | 14.2 ± 1.0 | 25.2 ± 0.7 | 50.4 ± 1.0 | 65.6 ± 0.8 | 11.3 ± 0.3 | 3.2 ± 0.1 | 3.0 ± 0.1 |
| | 259 | 11.2 ± 1.0 | 26.9 ± 5.9 | 40.1 ± 4.5 | 68.2 ± 7.1 | 11.5 ± 0.6 | 2.9 ± 0.2 | 2.7 ± 0.1 |
| | 271 | 15.7 ± 1.0 | 26.4 ± 1.2 | 44.7 ± 1.8 | 70.3 ± 2.3 | 12.1 ± 0.4 | 3.5 ± 0.1 | 3.3 ± 0.1 |
| | 8L | 11.1 ± 1.0 | 28.2 ± 1.7 | 43.9 ± 2.2 | 81.8 ± 5.1 | 11.4 ± 0.3 | 3.3 ± 0.2 | 3.1 ± 0.1 |
| | 42E | 12.6 ± 0.2 | 27.7 ± 1.4 | 39.8 ± 2.6 | 81.3 ± 4.0 | 12.4 ± 0.5 | 2.9 ± 0.3 | 2.8 ± 0.2 |

Dfp, distance from the pith; Ld, lumen diameter; Fwt, fibre wall thickness. Values are means ± SE, $n = 3$, except in clone 259 controls, $n = 1$–3.

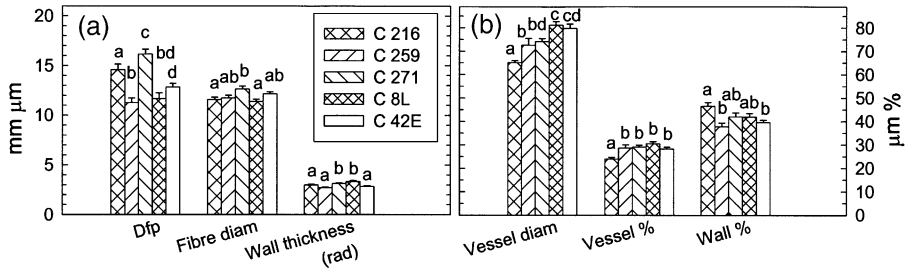


**Fig. 3** Overall responses for each aspen clone on (a) distance from the pith (Dfp), fibre lumen diameter and fibre wall thickness (radial), and (b) vessel lumen diameter, vessel percentage and cell wall percentage in annual ring 2000 of stem wood at the end of the third treatment season. Error bars indicate + 1 SE. Values with different letters are significantly different ($P < 0.1$) among the clones.

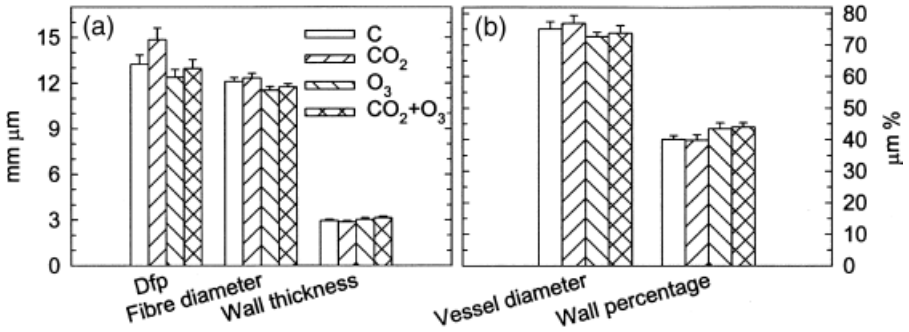


**Fig. 4** Overall responses on (a) distance from the pith (Dfp, mm), fibre lumen diameter and radial fibre wall thickness (µm), and (b) vessel lumen diameter (µm) and cell wall percentage in annual ring 2000 of stem wood for each treatment summarizing clone averages for aspen at the end of the third treatment season. Error bars indicate +1 SE.

increased (Table 5, Fig. 4). The $O_3$ treatment significantly affected wood anatomy (Table 5); vessel and fibre lumen diameter decreased while the percentage of cell walls and the thickness of radial fibre walls increased (Fig. 4a, b, Table 6). The decrease in fibre lumen diameter was significant only in clone 271 due to the interaction between clone and $O_3$ treatment (Tables 5 and 6). Treatments did not have significant effects on vessel percentage (Tables 5 and 6).

## Discussion

### *Does elevated $CO_2$ and $O_3$ affect wood properties?*

In deciduous trees, xylem is more diverse than in softwoods. Vessels function as a transport route for water and different inorganic and organic substances. Fibres provide mechanical support for the stem and crown and ray parenchyma cells store reserves. The chemical composition of cell walls and the architecture of xylem affect wood properties that differ from pith to bark and from base to apex. Our results showed that environmental impact (i.e., elevated $CO_2$ and $O_3$ treatments) as well as genotype and species had statistically significant effects on wood properties in the Aspen FACE experiment. The most general finding was the increase in lignin under elevated $O_3$ in both aspen and birch, but this was nullified by elevated $CO_2$. Regarding aspen wood structure, elevated $O_3$ induced statistically significant reductions in cellular dimensions and increases in wall thickness and percentage indicating a denser xylem structure and reduced radial growth, as has been reported by Isebrands *et al*. (2001).

### *Impacts of elevated $CO_2$*

Elevated $CO_2$ alone did not have any statistically significant effects on structure of aspen wood, although the diameters of vessel and fibre lumen tended to increase. This is in line with our observation of the significant increase in distance from the pith that agrees with previous reports on increase in volume growth at this site (Isebrands *et al*., 2001; Karnosky *et al*., 2003).

Chemical composition of wood was affected by $CO_2$. In aspen, elevated $CO_2$ induced an increase in nonstructural carbohydrates, soluble sugars, and starch, but the effect depended on genotype. This is in agreement with Norway spruce branch wood where an increase in starch was found (Hättenschwiler *et al*., 1996). Nonstructural carbohydrates represent an important pool for stored carbon (Hoch *et al*., 2003) and substrates for growth processes (Höll, 2000) and wood formation (Oribe *et al*., 2003). Within the xylem, nonstructural carbohydrates are situated in ray parenchyma cells (Krabel, 2000; Puech *et al*., 2000). Rays compose a tissue system serving as efficient radial translocation route and storage site for reserves (Sauter, 2000). The increase in nonstructural carbohydrate production under $CO_2$ treatment was most likely due to increased photosynthesis that enhanced aboveground growth of aspen (Karnosky *et al*., 2003).

Regarding structural carbohydrates in aspen, a significant interaction between $CO_2$ and $O_3$ indicated that the $CO_2$ treatment reduced α-cellulose by 1.6% under ambient $O_3$ and increased it by 0.9% under elevated $O_3$. The interaction may suggest that under elevated $CO_2$ concentration, $O_3$ affected carbon allocation by diverting the enhanced carbohydrate production to cellulose synthesis. Cellulose is synthesized on plasma membranes (Higuchi, 1997; Haigler *et al*., 2001) and its synthesis precedes the accumulation of lignin in cell wall. In contrast, previous studies show no changes in holocellulose of *F. sylvatica* twig wood (Cotrufo & Ineson, 2000), in cellulose of *P. palustris* stem wood (Runion *et al*., 1999), or in cellulose fraction of

*Pseudotsuga menziesii* woody tissue (Tingey *et al*., 2003) under elevated $CO_2$.

In birch, the $CO_2$ treatment decreased the concentration of hemicelluloses that are structural components of cell wall (Brett & Waldron, 1996) representing the amorphous material that is encrusted among cellulose microfibrils (Higuchi, 1997). Hemicelluloses, apart from their structural role (Yoshida *et al*., 2000) in the wall and middle lamella (Hafrén *et al*., 2000), represent a potential carbon reserve in trees (Bollmark *et al*., 1999; Cherbuy *et al*., 2001). In softwoods, hemicellulose deposition is active at the stages when inner ($S_3$) and outer ($S_1$) cell wall layers are formed (Higuchi, 1997). An open question is how and if different cell wall layers and/or thickness of layers are related to the observed changes in concentrations of cell wall components.

Elevated $CO_2$ alone had no effect on wood nitrogen concentration in any of the species while the combined exposure to elevated $CO_2$ and $O_3$ caused a relative decrease of 15% in aspen. The lack of impact of elevated $CO_2$ alone is consistent with observations for *P. menziesii* woody tissue (Tingey *et al*., 2003). However, this is in contrast to many studies where elevated $CO_2$ has been reported to decrease nitrogen concentration in wood of softwood and hardwood species (Kohen *et al*., 1992; Hättenschwiler *et al*., 1996; Runion *et al*., 1999; Cotrufo & Ineson, 2000). The Aspen FACE experiment was established on fertile site that, several decades ago, was used for agriculture. The soil nitrogen levels, while still relatively high, are within the range previously reported for natural aspen stands (Dickson *et al*., 2000).

### *Impacts of elevated $O_3$*

Elevated $O_3$ alone affected the chemistry of secondary xylem, but this was dependent on genotype in aspen. The gravimetric lignin concentration increased in all aspen clones except clone 42E, and in birch. Lignin gives mechanical strength to stems and assists in solute transportation by decreasing the permeability of walls, and impacts resistance to attack of micro-organisms (Higuchi, 1997). However, elevated $CO_2$ nullified the increase in lignin. The tendency of increasing lignin concentration under elevated $O_3$ is interesting since up to now no data exist on $O_3$ effects on cell wall chemistry of wood. Our results of increases in lignin may indicate changes in carbon allocation leading to enhanced activity of the phenylpropanoid biosynthetic pathway. This has been shown in previous research at this FACE site where PAL transcripts increased under elevated $O_3$ (Wustman *et al*., 2001). In birch, hemicellulose increased under elevated $O_3$, but on the contrary, decreased under elevated $CO_2$. The changes in hemicellulose concentration may suggest that elevated $O_3$ and $CO_2$ affected the allocation of carbon leading to changes in hemicellulose biosynthesis and/or deposition (Higuchi, 1997).

Elevated $O_3$ alone affected the structure of wood. Decrease in distance from the pith is in agreement with previous reports from this site (Isebrands *et al*., 2001). Accordingly, in *B. pendula*, elevated [$O_3$] has been reported to decrease xylem width (Matyssek *et al*., 2002). In agreement with reduced distance from the pith, the diameter of vessel lumen decreased while the percentage of cell walls and thickness of radial fibre walls increased. Reduced diameter of vessel lumen could decrease the efficiency of water transport in xylem (Tyree *et al*., 1994) under $O_3$ exposure. In the present study, the decrease in lumen diameter induced by the $O_3$ treatment was less pronounced under elevated $CO_2$, although this was not statistically significant. It remains to be seen if $CO_2$ will ameliorate the negative effects of $O_3$ on wood structure of aspen in mature trees.

### *Intraspecies and interspecies variation and effects on responses to treatments*

Our study demonstrated that wood chemistry and its responses to atmospheric gas concentrations were affected by genotype as reported by Costa e Silva *et al*. (1998) and King *et al*. (1998) and differed between species. Aspen genotype significantly affected the concentrations of total lignin, gravimetric, and acid-soluble lignin and acetone-soluble extractives as well as soluble sugars, starch, and nitrogen, but did not affect structural carbohydrates (α-cellulose and hemicellulose) of cell walls. Interestingly, clone 8L with small radial growth (Fig. 3a) had the highest total lignin concentrations, and another small clone, 259 (Isebrands *et al*., 2001) had the greatest starch concentrations and the lowest concentration of acetone-soluble extractives. These results suggest that photosynthates were used for cell wall lignification and storage rather than growth. The lowest extractive concentration and the smallest size in clone 259 are in line with the suggestion that growth rate and tree vigour correlate positively with extractive concentration (Kramer & Kozlowski, 1979).

Genotypic differences in wood structure showed that the clone 271 (Isebrands *et al*., 2001) had the largest fibre lumen diameters. The intermediate clone 216 (Isebrands *et al*., 2001) clearly differed in wood structure from the other clones. Clone 216 had the smallest fibre and vessel lumen diameters with largest wall percentage and smallest vessel percentage. Reduced vessel size could be hypothesized to reduce water transport ability.

Interspecies differences were evident. Aspen had the greatest α-cellulose concentrations while birch had the

lowest α-cellulose concentrations and maple had the greatest lignin and starch concentrations. Regarding the high α-cellulose concentration, aspen would be the best candidate for end-use purposes in pulp and paper production. The largest starch concentration is in line with the maple syrup production in sugar maple.

Wood chemistry in aspen was most responsive to the elevated $CO_2$ and $O_3$ treatments, while birch was less prone to changes. Maple was the least responsive of the three tree species to the treatments, which is in agreement with other findings from the site (Karnosky *et al*., 2003). In aspen, the observed interactions between clone and treatments in wood chemistry (gravimetric lignin, nitrogen, soluble sugars, starch) give support to earlier findings of clonal differences in responses to elevated $CO_2$ and $O_3$ at the FACE site (Isebrands *et al*., 2001). On the contrary, genotype had only little effect on responses of wood structure to treatments. Fibre lumen diameter was the only structural parameter showing differing responses in clones to $O_3$ exposure.

## Conclusions

Results from this work suggest that juvenile wood properties of deciduous trees were altered by atmospheric gas concentrations that are predicted in the future. However, genotype and species affected the responses, and interactive effects of elevated $CO_2$ and $O_3$ on wood chemistry were observed. We want to emphasize that the trees in the Aspen FACE experiment were saplings when most of the annual biomass growth is allocated to foliage and less to the growth of stem (Gower *et al*., 1994). Structure and chemical composition of the wood differs in the juvenile phase from that in mature trees and most wood properties change quickly with a distance from the pith (Zobel & Buijtenen, 1989) and therefore care should be taken in predicting the response of mature trees to future climate.

More long-term impact studies covering different growth phases of trees on the effects of elevated $CO_2$ and $O_3$ on wood properties are needed before we can predict the characteristics of wood produced under future climate.

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