**Tree Physiology** 28, 243–254


# Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of $CO_2$ and $O_3$

JOHANNA RIIKONEN,[1,2] KATRE KETS,[3] JOSEPH DARBAH,[4] ELINA OKSANEN,[5] ANU SOBER,[3] ELINA VAPAAVUORI,[6] MARK E. KUBISKE,[7] NEIL NELSON[7] and DAVID F. KARNOSKY[4]

[1] *Department of Ecology and Environmental Science, University of Kuopio, PO Box 1627, FIN-70211, Kuopio, Finland*

[2] *Corresponding author (johanna.riikonen@uku.fi)*

[3] *Institute of Botany and Ecology, University of Tartu, Lai 40, 51005 Tartu, Estonia*

[4] *School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA*

[5] *Faculty of Biosciences, University of Joensuu, PO Box 111, FIN-80101 Joensuu, Finland*

[6] *Finnish Forest Research Institute, Suonenjoki Research Unit, FIN-77600 Suonenjoki, Finland*

[7] *USDA Forest Service, Forestry Sciences Laboratory, Rhinelander, WI 54501, USA*

Received June 26, 2007; accepted August 24, 2007; published online December 3, 2007

**Summary** Paper birch (*Betula papyrifera* Marsh.) and three trembling aspen clones (*Populus tremuloides* Michx.) were studied to determine if alterations in carbon gain in response to an elevated concentration of $CO_2$ ($[CO_2]$) or $O_3$ ($[O_3]$) or a combination of both affected bud size and carbohydrate composition in autumn, and early leaf development in the following spring. The trees were measured for gas exchange, leaf size, date of leaf abscission, size and biochemical characteristics of the overwintering buds and early leaf development during the 8th–9th year of free-air $CO_2$ and $O_3$ exposure at the Aspen FACE site located near Rhinelander, WI. Net photosynthesis was enhanced 49–73% by elevated $[CO_2]$, and decreased 13–30% by elevated $[O_3]$. Elevated $[CO_2]$ delayed, and elevated $[O_3]$ tended to accelerate, leaf abscission in autumn. Elevated $[CO_2]$ increased the ratio of monosaccharides to di- and oligosaccharides in aspen buds, which may indicate a lag in cold acclimation. The total carbon concentration in overwintering buds was unaffected by the treatments, although elevated $[O_3]$ decreased the amount of starch by 16% in birch buds, and reduced the size of aspen buds, which may be related to the delayed leaf development in aspen during the spring. Elevated $[CO_2]$ generally ameliorated the effects of elevated $[O_3]$. Our results show that both elevated $[CO_2]$ and elevated $[O_3]$ have the potential to alter carbon metabolism of overwintering buds. These changes may cause carry-over effects during the next growing season.

*Keywords: bud burst, bud carbohydrates, bud size, gas exchange, leaf abscission, leaf area index, leaf size, paper birch, phenology, photosynthesis, trembling aspen.*

## Introduction

Concentrations of atmospheric $CO_2$ ($[CO_2]$) and tropospheric $O_3$ ($[O_3]$) have increased by 30% since preindustrial times and are expected to increase by 0.52% per year (IPCC 2001, Vingarzan 2004). These greenhouse gases may cause alterations to the overwintering of perennials by affecting bud development and phenology, and annual growth cycles. Early bud burst in spring might increase the risk of frost damage; however, in several experiments the timing of bud burst was unaffected by elevated $[CO_2]$ (e.g. *Populus trichocarpa* T. & G. (Sigurdsson 2001), *Acer rubrum* L., *Acer saccharum* Marsh. (Norby et al. 2003) and *Populus tremuloides* Michx. (Karnosky et al. 2003)), whereas elevated $[O_3]$ delayed bud burst in *P. tremuloides, Betula papyrifera* Marsh. (Karnosky et al. 2003) and *Fagus crenata* Blume (Yonekura et al. 2004). These gases may affect the timing of bud set and the development of frost hardening in autumn (Jach et al. 2001). Bud development in autumn and early growth in spring, as well as carbon (C) accumulation in storage tissues, is dependent on C fixation occurring after growth cessation (Pollard 1970, Horwath et al. 1994, Ögren 2000).

There is little information on direct effects of elevated $[CO_2]$ and elevated $[O_3]$ on physiological processes associated with cold-hardening, even though the responses of leaf physiology are well known (Matyssek and Sandermann 2003, Long et al. 2004, Nowak et al. 2004, Oksanen et al. 2007). Moreover, few field studies have focused on the interactive effect of elevated $[CO_2]$ and elevated $[O_3]$ on the physiology of broad-leaved trees (Rebbeck and Scherzer 2002, Riikonen et al. 2004, Karnosky et al. 2005), even though almost 25% of the world's

forested area is currently exposed to $O_3$ concentrations above 60 ppb, as $CO_2$ concentrations are increasing (Fowler et al. 1999).

Elevated [$CO_2$] is known to enhance photosynthesis by increasing the carboxylation rate of Rubisco and by decreasing the rate of photorespiration (Drake et al. 1997, Long et al. 2004), although some acclimatory down-regulation in the amount and activity of Rubisco may occur, generally related to alterations in source–sink relationships and limitations in the supply of nitrogen (N) and water (Long et al. 2004). Seasonal variation in the response of photosynthesis to elevated [$CO_2$] has a large influence on the overwintering capacity of forest trees (Eamus and Ceulemans 2001). Photosynthesis is likely to be more responsive to elevated [$CO_2$] at high temperatures than at low temperatures (Drake et al. 1997), and because sink activity is highest in the middle of the growing season, it is generally assumed that the $CO_2$ response is highest during that period. The timing of leaf senescence has an important impact on seasonal forest C gain (Baldocchi et al. 2001). It has been hypothesized that elevated [$CO_2$] might improve the C balance of leaves, and thus increase leaf lifespan (Jach et al. 2001). Recent findings from free-air carbon dioxide enrichment (FACE) experiments with *Glycine max* (L.) Merr. (Dermody et al. 2006), *Populus tremuloides* (Karnosky et al. 2003) and *Populus* spp. (Tricker et al. 2004) indicate that elevated [$CO_2$] will delay autumnal senescence, whereas in some field experiments it had no effect on timing of leaf abscission (*Liquidambar styraciflua* L. (Herrick and Thomas 2003), *Acer saccharum*, *Liriodendron tulipifera* L. and *Quercus alba* L. (Gunderson et al. 1993, Norby et al. 2003).

Elevated [$CO_2$] has the potential to improve frost hardiness by increasing bud size and concentrations of cryoprotective soluble sugars in overwintering organs (Jach et al. 2001). However, little is known on whether the effect of elevated [$CO_2$] on late-season photosynthesis is reflected in the size and biochemical composition of developing buds. In an open-top chamber (OTC) study with *Betula pendula* Roth., elevated [$CO_2$] decreased bud dry mass, even though it enhanced photosynthetic rate and total leaf area without affecting individual leaf size (Riikonen et al. 2004, 2005, Peltonen et al. 2006).

Elevated [$O_3$] accelerates leaf senescence and induces early leaf fall (Pell et al. 1997, Karnosky et al. 2003, Riikonen et al. 2004). Ozone-induced early senescence is closely associated with decreased photosynthetic capacity, loss of Rubisco and chlorophyll and disturbances in carbohydrate metabolism (Pell et al. 1997, Dizengremel 2001). Elevated [$O_3$] may cause alterations in leaf carbohydrate metabolism by inhibiting phloem loading, or unloading or both, and by affecting the function of enzymes of carbohydrate metabolism, such as sucrose synthase and invertase (Einig et al. 1997, Dizengremel 2001). The altered assimilate transport capacity from leaves to sinks may induce down-regulation of photosynthesis and may reduce carbon allocation to storage organs and buds (Einig et al. 1997, Landolt et al. 1997, Lux et al. 1997, Dizengremel 2001). In studies with deciduous trees, elevated [$O_3$] reduced growth and shortened the growing season by decreasing the number of overwintering buds (*B. pendula*; Oksanen 2003), by reducing bud dry mass (*B. pendula*; Peltonen et al. 2006) and by delaying bud burst (Karnosky et al. 2003, Yonekura et al. 2004).

In earlier studies at the Aspen FACE site, elevated [$CO_2$] reduced the magnitude of the negative effect of elevated [$O_3$] on aspen growth, although the positive effect of elevated [$CO_2$] was negated, in part, by elevated [$O_3$] (King et al. 2005, Kubiske et al. 2006). However, in one study, the counteractive effect of elevated [$CO_2$] on gas exchange was found in an $O_3$-tolerant aspen clone but not in an $O_3$-sensitive clone (Noormets et al. 2001). In OTC studies with *B. pendula* and *L. tulipifera*, elevated [$CO_2$] counteracted some of the $O_3$ effects (Rebbeck and Scherzer 2002, Riikonen et al. 2004). It has been suggested that elevated [$CO_2$] may protect against $O_3$ effects by reducing stomatal conductance, and thus, decreasing $O_3$ uptake or by stimulating photosynthesis, leading to higher NADPH production and increased activity in enzymatic detoxification of active oxygen species (Rao et al. 1995, Podila et al. 2001).

In the present study, we examined three trembling aspen (*Populus tremuloides*) clones and paper birch (*Betula papyrifera*), grown at the Aspen FACE site with elevated [$CO_2$] and elevated [$O_3$] applied singly and in combination. We studied gas exchange throughout the 8th growing season; specific leaf area and leaf size in September; final size, carbohydrate, C, N and water concentrations of the buds in November; timing of leaf abscission in autumn; and bud burst and development of leaf area index the following spring. We sought answers to four questions. (1) Does elevated [$CO_2$] enhance the C gain of trees by stimulating photosynthesis throughout the growing season, by increasing leaf size and by delaying the timing of leaf abscission in autumn? (2) Does elevated [$O_3$] decrease photosynthetic rate and leaf size and shorten the growing season by accelerating leaf senescence? (3) Do these changes alter the size, carbohydrate and total C and N concentrations of the overwintering buds, the timing of bud burst and leaf area development in the following spring? (4) Does elevated [$CO_2$] ameliorate $O_3$ effects on leaf and bud physiology and phenology?

## Materials and methods

### *Aspen FACE site and plant material*

The Aspen FACE facility is located near Rhinelander, WI, USA (45°36′ N, 89°30′ W). In 1997, saplings of aspen (five clones), birch and maple (*Acer saccharum*) were planted in twelve 30-m diameter treatment rings, each representing either control (ambient [$CO_2$] and [$O_3$]), elevated [$CO_2$] (target 560 ppm), elevated [$O_3$] (target 1.5 × ambient) or a combination of elevated [$CO_2$] and elevated [$O_3$]. The experiment is a full-factorial design with three replicates of each treatment, blocked across northern, central and southern regions of the site. Our study utilizes vegetatively propagated aspen Clones 216, 271 (relatively $O_3$-tolerant clones) and 42E (a relatively $O_3$-sensitive clone) and birches originating from seeds col-

lected in Houghton County, MI (Dickson et al. 2000). The fumigation began in spring of 1998 and continued from bud break until leaf fall during daylight hours through the 8th growing season. In 2005, the fumigation was conducted from May 23 to October 12. Mean daytime ambient $CO_2$ and $O_3$ concentrations (8-h mean during June–October) were 384 ppm and 34.6 ppb, respectively, whereas mean elevated $CO_2$ and $O_3$ concentrations were 523 ppm and 48.2 ppb, respectively. The elevated [$CO_2$] treatment was within 20% of the target of 560 ppm, about 93% of the fumigation time. The mean value of the elevated [$O_3$] treatment in 2005 was 1.38 × ambient [$O_3$]. The cumulative $O_3$ exposure (AOT40, accumulated over the threshold of 40 ppb) from May to October was 9.8 ppb·h for ambient [$O_3$] and 29.8 ppb·h for elevated [$O_3$]. Daily temperature, soil water content and rainfall in growing season 2005 are presented in Figure 1. Further information about the field site, experimental design and technical details can be found in Dickson et al. (2000).

*Gas exchange*

Gas exchange was measured three times from June 15 to August 15 (referred to as midsummer measurements) and six times from August 26 to October 7, 2005 (referred to as late-season measurements). For the midsummer measurements, three branches in the middle third of the living crowns of aspen Clones 42E and 271 and two birch trees in each ring were selected, and six short shoot leaves for each tree were measured. The leaves used for measurements were selected near the tip of each branch, where buds were sampled on November 11. To examine the relationship between net photosynthesis ($P_n$) after shoot growth cessation (mid-to-late August in Rhinelander) and bud physiology, one tree (aspen Clone 216) and two additional birch trees in each ring were included in the study from August 26 onward. One short-shoot leaf and one long-shoot leaf were measured from selected branches in the middle canopy (six leaves per tree). Gas exchange was measured with a portable gas exchange apparatus Li-6400 (Li-Cor, Lincoln, NE), using growth $CO_2$ concentrations (360 ppm for control and elevated [$O_3$] treatments and 560 ppm for elevated [$CO_2$] and combination treatments) in the leaf chamber. To obtain maximal rate of $P_n$, measurements were made under saturating light conditions (1000–1600 µmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR)) between 0900 and 1200 h, when stomatal conductance ($g_s$) was maximal. Leaf temperature and relative humidity (RH) were not controlled. One measurement round was completed in 3–5 days. Data from the three branches were averaged for each tree.

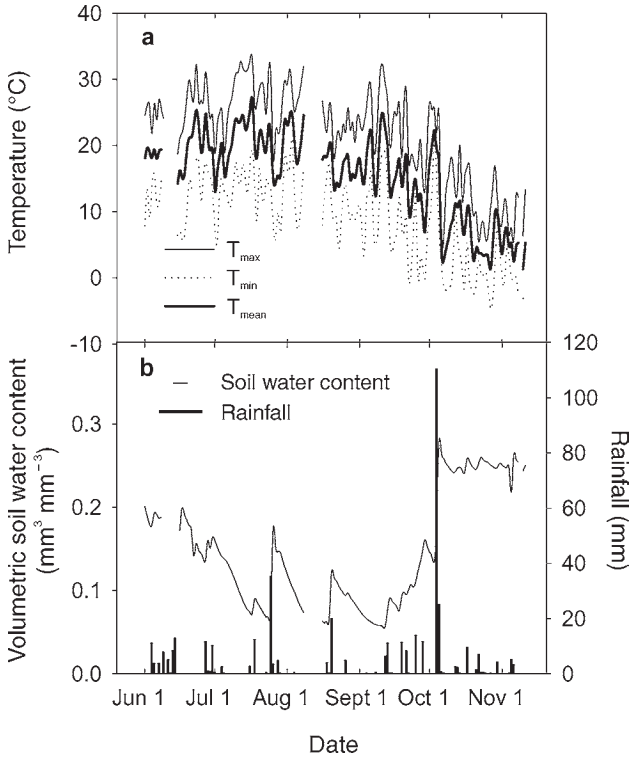


Figure 1. Maximum, minimum and mean air temperatures (a) volumetric soil water content (measured at 5–35 cm depth) and (b) rainfall measured daily at the Aspen FACE site during the 2005 growing season.

*Late-season specific leaf area and leaf size*

Specific leaf area (SLA; $cm^2$ $g_{DM}^{-1}$) and leaf size were determined on September 14–15, 2005. Fifteen leaves from each tree were collected from mid-canopy, avoiding branches used for other measurements. Leaf area (Li-Cor, LI-3050A) and dry mass (48 h at 60 °C) were measured.

*Timing of leaf fall*

Total number of leaves and all visible long-shoot buds on the three branches used for other measurements were counted on August 20–23, 2005 (about 200 leaves per tree). Timing of birch leaf fall was followed by counting leaves seven times from September 3 to October 7. Leaf abscission occurred later in aspen, and thus the number of leaves was counted only on October 6. Leaf abscission is expressed as the percentage of fallen leaves to total number of leaves. The ratio of long-shoot leaves to long-shoot buds was calculated to determine whether elevated [$CO_2$] or elevated [$O_3$] caused alterations in carbohydrate availability affected the number of developing buds.

*Bud size, total C, N and carbohydrate concentratios*

Buds were sampled on November 11, in a state of endodormancy, characterized by a minimal growth potential. Three branches used for other measurements were used for bud sampling. Twigs containing a terminal bud and five lateral buds in aspen, and three lateral buds in birch, were placed in liquid N and stored in a freezer. The buds were measured for fresh mass, and length and diameter of buds at the widest point were measured with a digital caliper. After freeze-drying in Eppendorf vials for 48 h, bud dry masses (DM) were determined. To ensure a representative sample for the CN analysis (CHN-900 Analyzer, Leco, St. Joseph, MI), one sample for each tree was prepared by sampling one bud from each branch

according to its position on the twig: one terminal bud and the first and second lateral buds. The rest of the buds were used for determining the concentrations of starch and soluble sugars, which were extracted by homogenizing the samples (100 mg DM) in 4 ml of 80% aqueous ethanol containing meso-erythritol as an internal standard. Samples were ultrasonicated for 45 min at room temperature. The next morning, extracts were centrifuged for 10 min at 1880 *g*. The pellet was retained for starch analysis. A 1-ml sample of the supernatant was dried in N gas (at 40–50 °C) and stored in a desiccator. The dry samples were silylated with *N*-trimethylsilyl-imidazole (500 µl) for 2 h and shaken. The concentrations of soluble sugars were determined by gas chromatography (Hewlett Packard, GC 6890, MSD type 5973) with a HP-5MS capillary column (250 µm ID × 30 m). The carbohydrates detected were fructose, galactose, glucose, sucrose, raffinose and stachyose.

The pellet remaining after extraction of the soluble sugars was vacuum-dried and stored for starch analysis. Starch was extracted from the residue by shaking for 20 h at room temperature with 10 ml of 30% perchloric acid. The extract was centrifuged for 13 min at 1880 *g*, and the supernatant was collected. Extraction was repeated twice with 5 ml of 30% perchloric acid each time. From the pooled supernatant, starch was determined colorimetrically at 625 nm by the anthrone method, with starch (soluble starch, Merck) in 30% perchloric acid as the standard (Hansen and Møller 1975).

Bud volumes were determined by the displacement method: for each species the buds were separated into ten size classes according to their fresh mass. Five buds for each class were immersed in water on a balance (Mettler, Toledo, AX 205). Based on the mass of water replaced by the buds (= the volume of the sunken buds), and the length ($l$) and diameter ($d$) of the buds, an equation approximating bud volumes was generated: $v = 0.57ld^2$ for birch ($r^2 = 0.92$) and $0.47ld^2$ for aspen ($r^2 = 0.95$).

### *Timing of bud burst and leaf area development*

Bud burst was monitored weekly, starting April 10, 2006. Bud burst was divided into four stages from closed to fully opened. To estimate leaf area index (LAI), the local light environment was assessed based on hemispheric photographs taken with a Nikon Coolpix 950 digital camera with an FC-2 fisheye converter. Three locations in aspen and aspen–birch sections were measured from each ring four times from May 2 to June 7, 2006. The images were analyzed with WinScanopy software (Regent Instruments, Québec City, Canada).

### *Data analysis*

The experimental design is a randomized complete block, with three replications of elevated [$CO_2$], elevated [$O_3$] and elevated [$CO_2$] + [$O_3$] treatments. The replication (block) was considered as a random effect. The main effects of whole-plot effects ($CO_2$, $O_3$) and split-plot effects (clone for aspen, and leaf type for birch) and their interactions were tested by linear mixed model analysis of variance (ANOVA). Data interpretation was simplified by conducting separate analyses for birch and aspen. In aspen, the responses of short-shoot and long-shoot leaves were similar, and thus, the data are presented with leaf types averaged. Measurement round was added as a fixed factor for repeated analysis when analyzing the gas exchange and LAI data in aspen and birch, and the leaf abscission data in birch. Statistical analysis for birch was performed on ring means (mean of two birches in midsummer and mean of four birches in late season). Data were ln-transformed to meet ANOVA requirements if necessary. The bud burst data were analyzed using the Kruskal-Wallis test. Values of $P < 0.05$ are significant.

## Results

### *Gas exchange*

Elevated [$CO_2$] increased $P_n$ in birch short-shoot leaves by 49% and in aspen Clones 42E and 271 by 73 and 52%, respectively, averaged over the growing season (Figure 2, Table 1). The magnitude of the absolute difference in $P_n$ between the el-

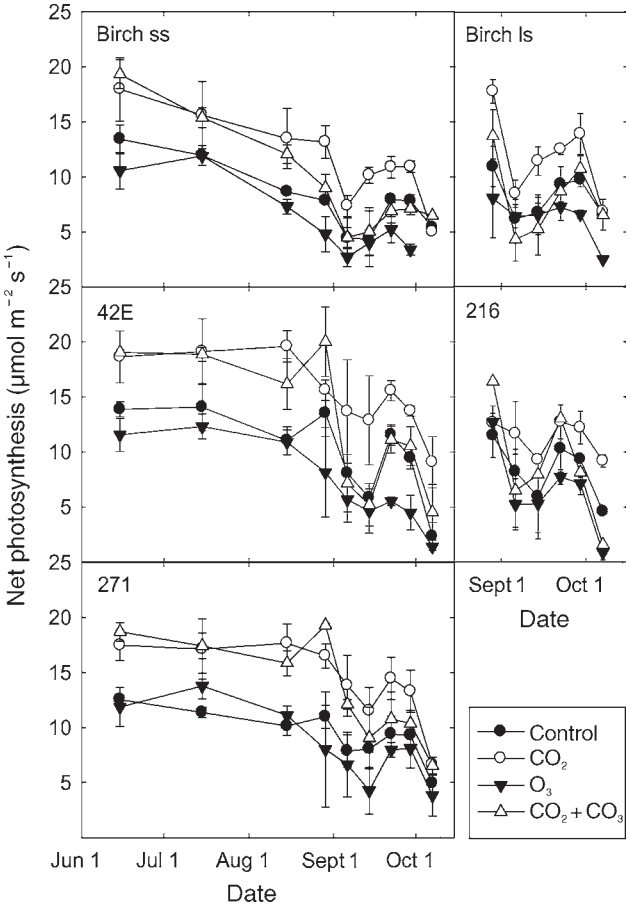


Figure 2. Light-saturated net photosynthesis in birch short-shoot (ss) and long-shoot (ls) leaves in aspen Clones 42E, 216 and 271, measured at the Aspen FACE site. Birch long-shoot leaves and aspen Clone 216 were included in the late-season measurements only. Measurements were made at the time of maximal stomatal conductance between 0900 and 1200 h. Data are means (± SE) of three replicates.

Table 1. Values of $P$ (where $P < 0.05$) for the main effects of [$CO_2$], [$O_3$], leaf type (short and long shoot leaves in birch), clone (Clones 42E, 271 and 216 in aspen) and measurement round and their interaction on net photosynthetic rate ($P_n$), stomatal conductance ($g_s$), $P_n$:$g_s$ ratio, leaf abscission, specific leaf area (SLA), leaf size and timing of leaf abscission during the 2005 growing season. Springtime leaf area index (LAI) was measured during the spring 2006. Abbreviations: ns = not significant; and – = not measured. Leaf size and SLA were determined on September 14–15, 2005. In birch, the short- and long-shoot leaves could not be separated when studying leaf abscission and LAI. Leaf abscission in each aspen clone was studied on October 6, 2005. The aspen clones could not be separated in the LAI measurement. The data were analyzed with linear mixed model ANOVA, with replication (block) as a random effect. The gas exchange, LAI and leaf abscission data in birch were analyzed as repeated measurements.

| | $P_n$ | $g_s$ | $P_n$:$g_s$ | SLA | Leaf size | Leaf abscission | LAI |
|---|---|---|---|---|---|---|---|
| *Birch* | | | | | | | |
| $CO_2$ | < 0.001 | 0.003 | < 0.001 | 0.006 | ns | 0.002 | 0.002 |
| $O_3$ | < 0.001 | 0.013 | ns | ns | ns | < 0.001 | 0.002 |
| Leaf | < 0.001 | ns | ns | < 0.001 | ns | – | – |
| Round | < 0.001 | < 0.001 | < 0.001 | – | – | < 0.001 | < 0.001 |
| $CO_2 \times O_3$ | ns | ns | 0.024 | < 0.001 | 0.028 | ns | 0.004 |
| Leaf × $CO_2$ | ns | ns | ns | ns | ns | – | – |
| Leaf × $O_3$ | ns | ns | ns | ns | ns | – | – |
| Round × $CO_2$ | ns | ns | ns | – | – | 0.040 | ns |
| Round × $O_3$ | ns | ns | 0.001 | – | – | 0.002 | 0.020 |
| Round × $CO_2$ × $O_3$ | 0.022 | ns | ns | – | – | ns | 0.014 |
| *Aspen* | | | | | | | |
| $CO_2$ | < 0.001 | ns | < 0.001 | ns | 0.047 | 0.029 | 0.020 |
| $O_3$ | < 0.001 | ns | 0.042 | ns | ns | ns | 0.000 |
| Clone | ns | 0.027 | < 0.001 | ns | ns | < 0.001 | – |
| Round | < 0.001 | < 0.001 | < 0.001 | – | – | – | 0.000 |
| $CO_2 \times O_3$ | ns | ns | ns | ns | ns | ns | 0.024 |
| Clone × $O_3$ | ns | ns | ns | ns | ns | ns | – |
| Round × $O_3$ | ns | ns | 0.006 | – | – | – | 0.005 |

evated and ambient [$CO_2$] treatments was relatively constant during the growing season. In birch long-shoot leaves and aspen Clone 216, measured in the late-season only, elevated [$CO_2$] enhanced $P_n$ by 42%. In aspen, stimulation of $P_n$ by elevated [$CO_2$] was found even on November 7, whereas in birch, the $CO_2$ effect was abolished after the first cold nights before the last measurements (Figure 2). During the summer, elevated [$CO_2$] reduced $g_s$ by 24% in birch, and it tended to be reduced also in aspen, but the $CO_2$ effect was highly variable in relation to the controls during late-season measurements, when $P_n$ and $g_s$ were low in all treatments (Figure 3, Table 1). The relationship between C gain and water loss, measured as the $P_n$:$g_s$ ratio, was significantly increased by elevated [$CO_2$] throughout the growing season in both species (Figure 4, Table 1).

Elevated [$O_3$] decreased $P_n$ in birch short-shoot leaves by 27%, aspen Clone 42E by 30% and Clone 271 by 13%, averaged over the growing season, and in birch long-shoot leaves by 23% and aspen Clone 216 by 42% in the late-season (Figure 2, Table 1). In both species, elevated [$O_3$] tended to lower $g_s$, especially during the summer (Figure 3, Table 1). The $P_n$:$g_s$ ratio was decreased by elevated [$O_3$] in the late-season in both species (Figure 4, Table 1).

In birch in the combination treatment, elevated [$CO_2$] overrode the $O_3$ effect on $P_n$ in midsummer, when $O_3$-induced reduction in $P_n$ was smaller than in late-season (12 versus 35%). In the late-season, however, the stimulatory effect of elevated [$CO_2$] on $P_n$ disappeared and $P_n$ of trees in the combination treatment remained at control rates (Figure 2, Table 1). In the combination treatment, $g_s$ tended to be lower than in other treatments (Figures 2 and 3, Table 1). However, in both species, the $P_n$:$g_s$ ratio in the combination treatment was similar to the ratio in the elevated [$CO_2$] treatment alone most of the time (Figure 4, Table 1).

### *Specific leaf area and leaf size*

Elevated [$CO_2$] decreased SLA in birch and elevated [$O_3$] tended to reduce SLA in both species (Tables 1 and 2). Leaf size was larger in the elevated [$CO_2$] treatment in aspen, and lower in the elevated [$O_3$] treatment in birch (Tables 1 and 2).

### *Leaf abscission*

Elevated [$CO_2$] delayed and elevated [$O_3$] accelerated leaf abscission in birch (Figure 6, Table 1). Birch trees grown in elevated [$O_3$] started to drop leaves in mid-August, whereas leaf abscission started in early September in control trees. By October 7, birch trees had lost 80% of leaves in the elevated [$O_3$] treatment compared with 69% in control trees. Because of the warm and sunny autumn in Wisconsin (Figure 1), most aspen leaves were retained on the branches until November and no

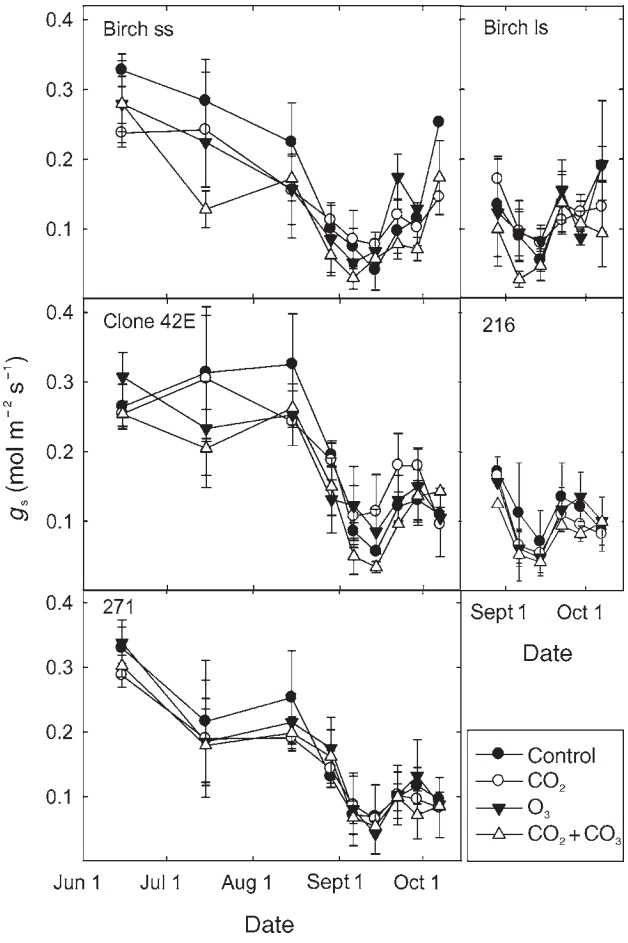


Figure 3. Stomatal conductance ($g_s$) in birch short-shoot leaves (ss) and long-shoot leaves (ls) and in aspen Clones 42E, 216 and 271, measured at the Aspen FACE site. Birch long-shoot leaves and aspen Clone 216 were included in the late-season measurements only. Measurements were made during maximal stomatal conductance between 0900 and 1200 h. Data are means (± SE) of three replicates.

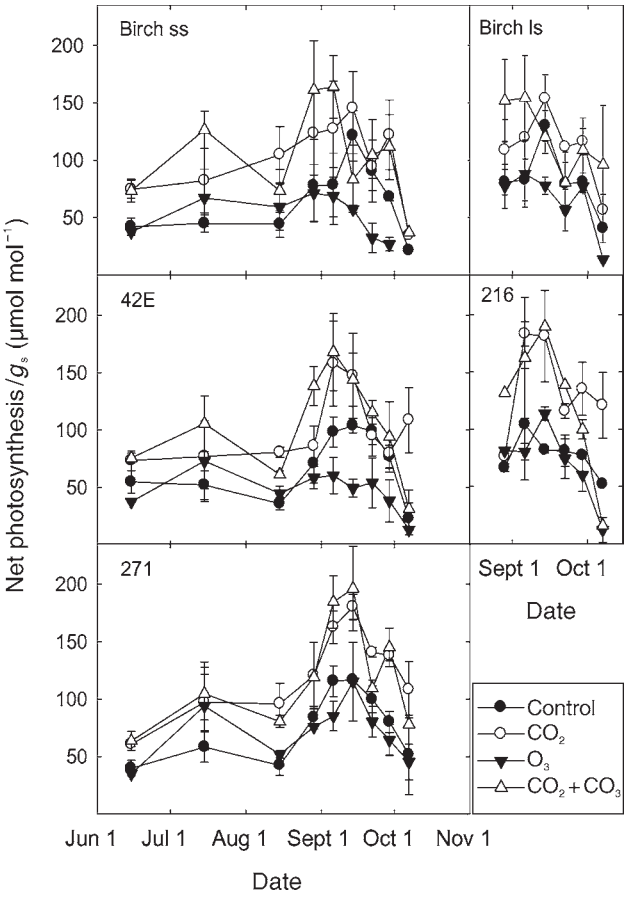


Figure 4. Ratio of light-saturated net photosynthesis and stomatal conductance ($g_s$) in birch short-shoot leaves (ss) and long-shoot leaves (ls) and in aspen Clones 42E, 216 and 271, measured at the Aspen FACE site. Birch long-shoot leaves and aspen Clone 216 were included in the late-season measurements only. Measurements were made between 0900 and 1200 h. Data are means (±SE) of three replicates.

treatment effects were found until early October. Elevated [$CO_2$] delayed leaf abscission in aspen. Leaf abscission in trees in the combination treatment was similar to that in control trees, except in Clone 271, where leaf abscission was delayed as long as in trees in the elevated [$CO_2$] treatment (Tables 2 and 3).

### *Buds*

The ratio of the number of long-shoot leaves to visible long-shoot buds was not significantly affected by the treatments in either species, although it was slightly lower in elevated [$CO_2$] (Tables 3 and 4). Dry mass of the birch buds was unaffected by the treatments (Tables 3 and 4). In birch buds, starch, soluble sugar and N concentrations were 45, 58 and 18 mg $g_{DM}^{-1}$, respectively, when averaged over all treatments (Tables 3 and 4). Major soluble sugar components were glucose and fructose (around 1.5% of DM). Foliar starch concentration was reduced by 16% in response to elevated [$O_3$], and by 5–10% in response to elevated [$CO_2$] and the combination treatment. Soluble sugars and total C concentration were unaffected by the treatments (Tables 3 and 5). Total N concentration was slightly lower and the C/N ratio higher in the elevated [$CO_2$] and elevated [$O_3$] treatments, but in the combination treatment total N was higher and the C/N ratio lower than control values. The interactive effect of elevated [$CO_2$] and elevated [$O_3$] resulted in a 3% lower bud water content in the elevated [$O_3$] treatment and 2% higher bud water content in the combination treatment (Tables 3 and 5) than in the control.

In aspen, bud volume was smaller and dry mass tended to be reduced in the elevated [$O_3$] treatment compared with the control ($P = 0.052$). Bud water content was unaffected by the treatments (Tables 3 and 4). Starch and soluble sugar concentrations of aspen buds were around 30 and 55 mg $g_{DM}^{-1}$, respectively (Tables 3 and 5). The major soluble sugars were sucrose and raffinose (around 2.2% of DM). Nitrogen concentration in buds was 11 mg $g_{DM}^{-1}$, when averaged over clones and treatments. The concentrations of starch and total soluble sugars,

Table 2. Mean values (± SE, $n = 3$) for specific leaf area ($cm^2\ g_{DM}^{-1}$) and leaf size ($cm^2$) in birch and aspen clones determined during September 14–15, 2005. Leaf abscission (%) in aspen clones was determined as number of leaves abscised by October 6 and is expressed as % of total number of leaves on three branches.

| | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
|---|---|---|---|---|
| *Specific leaf area* | | | | |
| Birch short-shoots leaves | 162.3 ± 1.6 | 155.5 ± 0.5 | 142.6 ± 3.2 | 166.8 ± 5.9 |
| long-shoot leaves | 140.0 ± 1.2 | 130.4 ± 1.5 | 120.4 ± 3.5 | 140.4 ± 3.8 |
| Aspen Clone 42E | 134.3 ± 4.8 | 127.3 ± 4.8 | 120.5 ± 6.4 | 123.1 ± 7.6 |
| 271 | 129.8 ± 10.0 | 116.2 ± 3.3 | 120.8 ± 3.5 | 116.3 ± 5.1 |
| 216 | 119.6 ± 12.3 | 122.7 ± 7.6 | 115.7 ± 3.3 | 115.9 ± 0.6 |
| *Leaf size* | | | | |
| Birch short-shoot leaves | 28.6 ± 3.2 | 24.8 ± 3.0 | 19.9 ± 2.5 | 29.9 ± 4.5 |
| long-shoot leaves | 22.9 ± 2.0 | 24.3 ± 2.9 | 20.0 ± 1.2 | 25.8 ± 1.7 |
| Aspen Clone 42E | 16.6 ± 0.3 | 18.3 ± 0.4 | 12.6 ± 0.6 | 14.9 ± 0.5 |
| 271 | 13.4 ± 1.6 | 16.5 ± 1.8 | 12.9 ± 0.7 | 13.6 ± 1.3 |
| 216 | 15.3 ± 0.3 | 17.6 ± 1.0 | 14.5 ± 0.4 | 17.2 ± 2.7 |
| *Leaf abscission* | | | | |
| Aspen Clone 42E | 61.6 ± 15.8 | 28.3 ± 8.0 | 75.9 ± 10.6 | 66.9 ± 11.9 |
| 271 | 31.6 ± 12.5 | 19.0 ± 4.4 | 23.0 ± 1.6 | 12.5 ± 4.5 |
| 216 | 37.8 ± 19.9 | 19.2 ± 5.0 | 43.9 ± 13.3 | 42.4 ± 3.1 |

total C and N, and C/N ratio were similar among the treatments (Tables 3–5). Elevated [$CO_2$] increased the ratio of monosaccharides to di- and oligosaccharides in aspen buds, reflecting a significantly higher concentration of fructose, a slightly higher concentration of glucose ($P = 0.053$) and a lower concentration of raffinose ($P = 0.061$). In Clone 216, the concentration of galactose was lower in the elevated [$O_3$] treatment (Tables 3 and 5).

*Timing of bud burst and spring leaf area index*

Bud burst occurred between April 17 and May 17, and was unaffected by the treatments in either species (data not shown). Elevated [$O_3$] decreased LAI in both species and elevated [$CO_2$] increased LAI in birch. In birch, LAI in the combination treatment was similar to the control value, and in aspen, elevated [$CO_2$] compensated, in part, for the inhibitory effect of elevated [$O_3$] on LAI. In aspen, development of LAI was de-

Table 3. Values of $P$ (where $P < 0.05$) for the main effects of $CO_2$, $O_3$ and aspen clone and their interaction on the bud/leaf ratio in long shoots, and final size and biochemical variables in long-shoot buds in birch and aspen Clones 42E, 271 and 216 growing at the Aspen FACE site. The buds were collected on November 11, 2005. Data were analyzed by mixed model ANOVA, with replication (block) as a random effect. Non-significant interactions were omitted from the table, ns = not significant.

| | Birch | | | Aspen | | | |
|---|---|---|---|---|---|---|---|
| | $CO_2$ | $O_3$ | $CO_2 \times O_3$ | $CO_2$ | $O_3$ | Clone | Clone × $O_3$ |
| Bud/leaf | ns | ns | ns | ns | ns | 0.001 | ns |
| Bud volume | ns | ns | ns | ns | 0.043 | 0.036 | ns |
| Dry mass | ns | ns | ns | ns | ns | ns | ns |
| Water content | 0.043 | ns | 0.028 | ns | ns | < 0.001 | ns |
| Total N | ns | ns | 0.022 | ns | ns | ns | ns |
| Total C | ns | ns | ns | ns | ns | ns | ns |
| C/N ratio | ns | ns | 0.034 | ns | ns | ns | ns |
| Starch | ns | ns | 0.005 | ns | ns | 0.001 | ns |
| Soluble sugars | ns | ns | ns | ns | ns | < 0.001 | ns |
| Mono/di- and oligosaccharides | ns | ns | ns | 0.034 | ns | < 0.001 | ns |
| Glucose | ns | ns | ns | ns | ns | < 0.001 | ns |
| Fructose | ns | ns | ns | 0.028 | ns | < 0.001 | ns |
| Galactose | ns | ns | ns | ns | ns | 0.001 | 0.004 |
| Sucrose | ns | ns | ns | ns | ns | < 0.001 | ns |
| Raffinose | ns | ns | ns | ns | ns | < 0.001 | ns |
| Stachyose | ns | ns | ns | ns | ns | < 0.001 | ns |

Table 4. Mean values (± SE, $n = 3$) for the ratio of visible buds to leaves in long shoots, and volume ($mm^3$), dry mass (mg), water content (mg), total C (% DM) and N concentrations (% DM)and C/N ratio in the buds of birch and aspen Clones 42E, 271 and 216, collected on November 11, 2005.

| | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
|---|---|---|---|---|
| *Bud/leaf* | | | | |
| Birch | 0.81 ± 0.05 | 0.74 ± 0.05 | 0.82 ± 0.03 | 0.73 ± 0.01 |
| Aspen Clone 42E | 0.33 ± 0.02 | 0.22 ± 0.02 | 0.30 ± 0.03 | 0.22 ± 0.04 |
| 271 | 0.44 ± 0.04 | 0.45 ± 0.06 | 0.35 ± 0.07 | 0.38 ± 0.05 |
| 216 | 0.37 ± 0.09 | 0.29 ± 0.05 | 0.36 ± 0.02 | 0.38 ± 0.01 |
| *Bud volume* | | | | |
| Birch | 27.4 ± 2.6 | 29.1 ± 2.1 | 28.6 ± 1.3 | 28.3 ± 2.3 |
| Aspen Clone 42E | 14.2 ± 0.9 | 15.1 ± 2.0 | 11.9 ± 1.4 | 13.1 ± 0.1 |
| 271 | 11.4 ± 0.3 | 11.8 ± 1.5 | 11.9 ± 1.2 | 12.1 ± 0.5 |
| 216 | 12.9 ± 0.2 | 12.5 ± 1.2 | 10.6 ± 1.1 | 10.3 ± 1.5 |
| *Dry mass* | | | | |
| Birch | 18.3 ± 0.5 | 17.9 ± 1.1 | 19.4 ± 1.2 | 17.3 ± 1.0 |
| Aspen Clone 42E | 8.9 ± 0.4 | 10.0 ± 1.8 | 8.0 ± 0.7 | 8.5 ± 0.5 |
| 271 | 7.5 ± 0.4 | 9.0 ± 1.2 | 7.4 ± 0.9 | 7.7 ± 0.5 |
| 216 | 10.1 ± 0.1 | 9.5 ± 0.8 | 8.5 ± 0.5 | 8.8 ± 1.6 |
| *Water content* | | | | |
| Birch | 39.9 ± 0.1 | 39.8 ± 0.4 | 38.8 ± 0.5 | 40.6 ± 0.6 |
| Aspen Clone 42E | 41.9 ± 1.2 | 40.3 ± 0.2 | 40.9 ± 0.4 | 41.7 ± 1.8 |
| 271 | 43.9 ± 2.3 | 41.3 ± 0.2 | 45.3 ± 0.7 | 44.5 ± 2.2 |
| 216 | 37.1 ± 0.7 | 40.1 ± 0.6 | 37.3 ± 0.5 | 37.3 ± 0.1 |
| *Total N* | | | | |
| Birch | 1.82 ± 0.04 | 1.76 ± 0.03 | 1.75 ± 0.02 | 1.92 ± 0.07 |
| Aspen Clone 42E | 1.21 ± 0.15 | 1.10 ± 0.14 | 1.09 ± 0.04 | 1.15 ± 0.04 |
| 271 | 1.28 ± 0.11 | 1.13 ± 0.11 | 1.16 ± 0.05 | 1.08 ± 0.10 |
| 216 | 1.07 ± 0.02 | 1.06 ± 0.04 | 1.09 ± 0.00 | 1.03 ± 0.05 |
| *Total C* | | | | |
| Birch | 57.1 ± 0.6 | 57.2 ± 0.5 | 57.8 ± 0.2 | 56.9 ± 0.6 |
| Aspen Clone 42E | 55.9 ± 0.6 | 55.6 ± 0.2 | 55.7 ± 0.4 | 54.5 ± 1.1 |
| 271 | 56.7 ± 0.7 | 56.9 ± 0.4 | 56.5 ± 0.1 | 56.9 ± 0.5 |
| 216 | 56.4 ± 0.3 | 56.6 ± 0.3 | 56.5 ± 0.1 | 56.9 ± 0.4 |
| *C/N ratio* | | | | |
| Birch | 31.7 ± 0.9 | 32.8 ± 0.7 | 33.3 ± 0.3 | 30.1 ± 1.3 |
| Aspen Clone 42E | 54.7 ± 0.8 | 54.5 ± 0.4 | 54.6 ± 0.4 | 53.3 ± 1.1 |
| 271 | 55.4 ± 0.8 | 55.7 ± 0.5 | 55.3 ± 0.2 | 55.8 ± 0.6 |
| 216 | 55.4 ± 0.3 | 55.6 ± 0.2 | 55.4 ± 0.1 | 55.9 ± 0.5 |

layed in the elevated [$O_3$] treatment, whereas in birch, LAI developed faster in elevated [$CO_2$], and slightly more slowly in elevated [$O_3$] compared with control trees (Table 1, Figure 6).

## Discussion

Despite the persistent increase in $P_n$ (49–73%) during the growing season, an enhanced ratio of photosynthetic gain to water loss, delayed leaf abscission and greater leaf size in aspen, elevated [$CO_2$] did not affect final bud size or the concentration of starch, total soluble sugars or C and N in overwintering buds. Although trees growing in elevated [$CO_2$] were bigger (King et al. 2005) than in other treatments, the number of visible long-shoot buds per long-shoot leaves (acting as a carbohydrate source) was unaltered by elevated [$CO_2$]. Thus, the carryover effect of altered C acquisition did not result from an increased number of buds. Because only a small amount of fixed C is needed for bud development, it has been suggested that availability of photosynthate does not alone regulate bud development (Sprugel et al. 1991). It is also possible that bud development and accumulation of carbohydrates were not restricted by carbohydrate deficiency in control trees or trees in the elevated [$CO_2$] treatment. However, in aspen, the ratio of monosaccharides to di- and oligosaccharides increased in response to elevated [$CO_2$]. This indicates slightly higher concentrations of glucose and fructose, and lower concentrations of raffinose and sucrose, which are important in stabilizing cellular structures during freeze dehydration (Hirsh 1987, Crowe et al. 1990), in addition to a general solute response (Sakai and Larcher 1987). Cox and Stushnoff (2001) found that raffinose and stachyose accumulated late during aspen bud hardening. We speculate that, in aspen trees grown in ele-

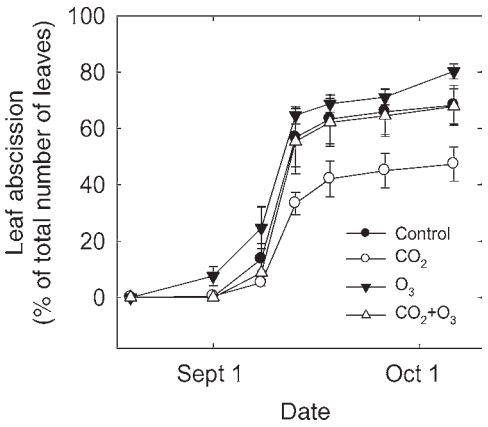


Figure 5. Leaf abscission in paper birches at the Aspen FACE site during autumn 2005. Number of abscised leaves was calculated weekly and is expressed as a percentage of total number of leaves. Data are means (± SE) of three replicates.

vated [$CO_2$], the acclimation process occurred later than in control trees, and sucrose and raffinose were synthesized later from glucose, fructose and galactose. Studies with *Eucalyptus pauciflora* Sieb. ex Spreng. revealed a lag in acclimation as winter approached, and a greater susceptibility to freezing damage in elevated [$CO_2$] (Loveys et al. 2006). These authors concluded that reduced $g_s$ and increased leaf temperature possibly delay the signal transduction required for cold acclimation. According to earlier observations in the Aspen FACE facility, the timing of growth cessation and bud set, an important determinant of development of freezing tolerance, was delayed in aspen (Karnosky et al. 2005). Isebrands et al. (2001) reported that shoot dieback on aspen grown in elevated [$CO_2$] was related to delayed senescence.

Elevated [$O_3$] reduced C gain of the birches by decreasing $P_n$ by 27%, by reducing leaf size and LAI (Karnosky et al. 2003) and by inducing accelerated leaf abscission. Although responses were not reflected in total C concentration in birch buds, bud starch concentration was reduced by 16% in elevated [$O_3$]. Reductions in bud starch concentration in autumn were likely linked to conversion of starch to soluble sugars in

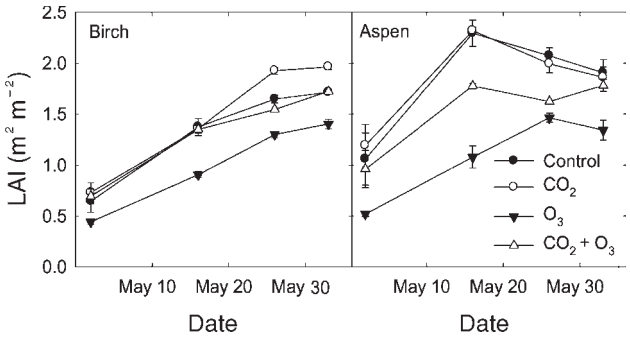


Figure 6. Leaf area index (LAI) in aspen and aspen–birch sections at the Aspen FACE site in spring 2006. Measurements were made at three locations in each section and ring, using the hemispherical fisheye approach. Data are means (± SE) of three replicates.

the development of freezing tolerance. The unaltered concentration of total C, despite reduction in starch concentration, may indicate $O_3$-induced alterations in C allocation in buds. Ozone induces an accumulation of several C-based secondary compounds in aspen and birch leaves at the Aspen FACE that may be associated with $O_3$ defense (Liu et al. 2005). Peltonen et al. (2006) found increased concentrations of several phenolic compounds in surface exudates on *B. pendula* buds collected in October.

The reduced size of aspen buds in the elevated [$O_3$] treatment may be related to impaired springtime LAI development. Maini (1966) reported a correlation between long-shoot growth capacity and bud size in a within-canopy comparison of *Populus* spp. Although elevated [$O_3$] did not alter total C, N, carbohydrate and water concentrations in aspen buds, galactose concentration was reduced in Clone 216. Galactose, present at low concentrations in aspen buds, is a structural unit of raffinose and stachyose, and also a constituent of cell wall polysaccharides. The unresponsiveness of bud carbohydrate concentration to elevated [$O_3$] in aspen is in accordance with other studies. Lux et al. (1997) found that starch and total soluble sugar concentrations were similar in ambient [$O_3$] and in filtered air in *Fagus sylvatica* L. buds, although the concentration of sucrose increased and concentrations of raffinose and glucose decreased in ambient [$O_3$]. Yonekura et al. (2004) found reduced carbohydrate concentrations in *Fagus crenata* leaves, but not in buds, during winter after exposure to elevated [$O_3$]. They concluded that buds may have priority as a sink for accumulating carbohydrates compared with other organs, because the dry mass of other organs was reduced. Our aspen data support this theory.

The timing of bud burst in spring 2006 was unaffected by elevated [$CO_2$] or elevated [$O_3$] or a combination of both. In 2006, effective temperature sum started to accumulate late in spring, and bud burst was completed within 17 days in aspen compared with 26 days in 2005, which might have masked any treatment effects on bud burst. This contrasts with earlier observations in the Aspen FACE experiment, when aspen bud burst was delayed by elevated [$O_3$] (Karnosky et al. 2003).

The combination of elevated [$CO_2$] and elevated [$O_3$] induced some alterations in birch bud development because N concentration and water content were higher, and dry mass and C/N ratio lower than in the other treatments. Similarly, Peltonen et al. (2006) concluded that elevated [$CO_2$] and elevated [$O_3$] did not affect *B. pendula* buds independently, because the combination treatment reversed the negative single treatment effects on bud size. In our experiment, elevated [$CO_2$] generally ameliorated the effect of elevated [$O_3$]. In both species, $g_s$ was usually lowest in the combination treatment, which probably caused a reduction in $O_3$ uptake. Also, the ratio of C gain to water loss and $O_3$ uptake were higher in trees in the combination treatment than in control trees throughout the growing season. In birch, ameliorative effect of elevated [$CO_2$] on $O_3$-induced inhibition of photosynthesis seemed to disappear in mid-August. Elevated [$O_3$] may limit sink activity in the combination treatment, resulting in an accumulation of carbohydrates in leaves, leading to down-regu-

Table 5. Mean values (± SE, $n = 3$) for carbohydrate concentration (mg $g_{DM}^{-1}$) in the buds of birch and aspen Clones 42E, 271 and 216, collected on November 11, 2005 at the Aspen FACE site.

| Carbohydrate | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
|---|---|---|---|---|
| *Starch* | | | | |
| Birch | 49.2 ± 0.9 | 44.4 ± 1.7 | 41.5 ± 0.3 | 46.8 ± 1.8 |
| Aspen Clone 42E | 30.2 ± 1.9 | 25.4 ± 0.7 | 25.6 ± 2.0 | 28.2 ± 2.7 |
| 271 | 32.0 ± 6.0 | 32.3 ± 3.6 | 31.9 ± 0.4 | 34.3 ± 3.2 |
| 216 | 26.4 ± 0.02 | 26.9 ± 1.2 | 24.3 ± 0.2 | 26.2 ± 2.1 |
| *Soluble sugars* | | | | |
| Birch | 58.9 ± 3.7 | 57.2 ± 1.0 | 56.8 ± 1.0 | 57.6 ± 4.6 |
| Aspen Clone 42E | 63.1 ± 2.6 | 56.1 ± 3.9 | 60.6 ± 1.8 | 60.0 ± 1.2 |
| 271 | 53.7 ± 5.0 | 53.0 ± 2.0 | 56.8 ± 2.2 | 53.5 ± 3.5 |
| 216 | 51.2 ± 2.8 | 52.1 ± 1.1 | 50.3 ± 1.0 | 53.4 ± 0.5 |
| *Mono/di- and oligosaccharides* | | | | |
| Birch | 1.43 ± 0.18 | 1.42 ± 0.28 | 1.28 ± 0.05 | 1.65 ± 0.20 |
| Aspen Clone 42E | 0.09 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.01 | 0.09 ± 0.01 |
| 271 | 0.10 ± 0.01 | 0.24 ± 0.02 | 0.14 ± 0.03 | 0.16 ± 0.03 |
| 216 | 0.22 ± 0.02 | 0.35 ± 0.09 | 0.28 ± 0.11 | 0.28 ± 0.05 |
| *Glucose* | | | | |
| Birch | 13.57 ± 1.27 | 12.91 ± 0.77 | 12.95 ± 0.43 | 14.27 ± 0.61 |
| Aspen Clone 42E | 2.71 ± 0.31 | 2.36 ± 0.22 | 2.81 ± 0.27 | 2.82 ± 0.24 |
| 271 | 2.81 ± 0.04 | 5.41 ± 0.76 | 3.88 ± 0.58 | 3.86 ± 0.17 |
| 216 | 4.18 ± 0.14 | 6.04 ± 1.16 | 5.40 ± 1.64 | 6.16 ± 0.75 |
| *Fructose* | | | | |
| Birch | 15.30 ± 1.13 | 14.86 ± 0.71 | 14.67 ± 0.48 | 15.99 ± 0.69 |
| Aspen Clone 42E | 1.72 ± 0.18 | 1.60 ± 0.08 | 1.77 ± 0.20 | 1.82 ± 0.15 |
| 271 | 1.72 ± 0.01 | 3.86 ± 0.67 | 2.67 ± 0.36 | 2.53 ± 0.12 |
| 216 | 3.22 ± 0.15 | 4.63 ± 0.76 | 4.37 ± 1.45 | 4.92 ± 0.70 |
| *Galactose* | | | | |
| Birch | 5.58 ± 0.55 | 5.13 ± 0.64 | 4.22 ± 0.35 | 5.11 ± 0.83 |
| Aspen Clone 42E | 0.48 ± 0.07 | 0.29 ± 0.02 | 0.40 ± 0.08 | 0.38 ± 0.06 |
| 271 | 0.32 ± 0.46 | 0.97 ± 0.53 | 0.43 ± 0.07 | 0.93 ± 0.46 |
| 216 | 1.68 ± 0.23 | 2.63 ± 0.76 | 0.71 ± 0.29 | 0.67 ± 0.01 |
| *Sucrose* | | | | |
| Birch | 9.3 ± 1.0 | 9.0 ± 1.0 | 9.5 ± 0.5 | 8.2 ± 1.7 |
| Aspen Clone 42E | 26.8 ± 1.1 | 25.2 ± 1.2 | 24.8 ± 0.7 | 24.8 ± 1.2 |
| 271 | 22.2 ± 3.2 | 20.0 ± 0.3 | 24.2 ± 1.9 | 24.3 ± 4.1 |
| 216 | 18.9 ± 2.3 | 16.6 ± 0.4 | 18.0 ± 2.0 | 17.4 ± 0.0 |
| *Raffinose* | | | | |
| Birch | 12.0 ± 1.3 | 12.0 ± 1.6 | 12.4 ± 0.7 | 10.9 ± 1.2 |
| Aspen Clone 42E | 26.6 ± 2.2 | 22.5 ± 2.5 | 26.3 ± 1.1 | 26.0 ± 0.8 |
| 271 | 24.2 ± 1.9 | 20.1 ± 0.6 | 22.9 ± 3.2 | 18.6 ± 0.6 |
| 216 | 19.0 ± 0.4 | 18.4 ± 0.7 | 17.5 ± 1.5 | 19.0 ± 1.7 |
| *Stachyose* | | | | |
| Birch | 3.21 ± 0.37 | 3.25 ± 0.54 | 3.08 ± 0.28 | 3.00 ± 0.34 |
| Aspen Clone 42E | 4.82 ± 0.34 | 4.15 ± 0.27 | 4.52 ± 0.48 | 4.19 ± 0.44 |
| 271 | 2.41 ± 0.07 | 2.76 ± 0.24 | 2.76 ± 0.38 | 3.25 ± 0.75 |
| 216 | 4.24 ± 0.09 | 3.82 ± 0.82 | 4.36 ± 0.28 | 5.25 ± 0.31 |

lation of photosynthesis. This suggestion is supported by significant decreases in growth in birch (14–18%) in the combination treatment compared with elevated [$CO_2$] alone (Karnosky et al. 2005), and by a 38% increase in starch concentration in the leaves of 4-year-old aspen in the combination treatment, but only 19 and 15% increases in elevated [$CO_2$] and [$O_3$] alone, respectively (Kopper and Lindroth 2003). However, SLA measured in September 2005 tended to be lower in the combination treatment only in aspen, which may indicate sugar accumulation in aspen leaves but not in birch leaves.

We conclude that altered C gain in response to elevated [$CO_2$] or elevated [$O_3$] or both did not result in changes in the total amount of C allocated to buds. However, both gases have the potential to alter C metabolism in buds. Elevated [$CO_2$] increased the ratio of monosaccharides to di- and oligosaccharides in aspen buds, which may be related to delayed development of frost tolerance. Elevated [$O_3$] reduced starch concentration in birch buds and decreased bud size in aspen. These changes, together with altered C reserves for maintenance respiration, cryoprotection and spring growth support, may cause carryover effects during the subsequent spring and summer, shown here as an $O_3$-induced delay in LAI development in spring 2006 in aspen.

**Acknowledgments**

Aspen FACE is principally supported by the U.S. Department of Energy's Office of Biological and Environmental Research, Grant no. DE-FG02-95ER62125 to Michigan Technological University, and Contract no. DE-AC02-98CH10886 to Brookhaven National Laboratory, the U.S. Forest Service Northern Global Change Program and Northern Research Station, Michigan Technological University, and Natural Resources Canada – Canadian Forest Service. This research was supported by The Finnish Cultural Foundation, Emil Aaltonen Foundation and Saastamoinen Foundation. We thank the personnel of the Aspen FACE site, especially Wendy Jones and David B. Karnosky for taking and analyzing the fisheye photos for the LAI estimations. We are grateful to Hanna Ruhanen and Marja-Leena Jalkanen for technical assistance and to Dr. Jaakko Heinonen for statistical guidance.

**References**

Baldocchi, D., E. Falge, L.H. Gu et al. 2001. FLUXNET: a new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor and energy flux densities. Bull. Am. Meteorol. Soc. 82:2415–2434.

Cox, S.E. and C. Stushnoff. 2001. Temperature-related shifts in soluble carbohydrate content during dormancy and cold acclimation in *Populus tremuloides*. Can. J. For. Res. 31:730–737.

Crowe, J.H., J.F. Carpenter, L.M. Crowe and T.J. Anchordoguy. 1990. Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. Cryobiology 27:219–231.

Dermody, O., S.P. Long and E.H. DeLucia. 2006. How does elevated $CO_2$ or ozone affect the leaf-area index of soybean when applied independently? New Phytol. 169:145–155.

Dickson, R.E., K.F. Lewin, J.G. Isebrands et al. 2000. Forest–atmosphere carbon transfer storage-II (FACTS II). The aspen free-air $CO_2$ and $O_3$ enrichment (FACE) project: an overview. USDA Forest Service, North Central Research Station, Gen. Tech. Report NC-214, 68 p.

Dizengremel, P. 2001. Effects of ozone on the carbon metabolism of forest trees. Plant Physiol. Biochem. 39:729–742.

Drake, B.G., M.A. Gonzàlez-Meler and S.P. Long. 1997. More efficient plants: a consequence of rising atmospheric $CO_2$? Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:609–639.

Eamus, D. and R. Ceulemans. 2001. The effects of greenhouse gases on the gas exchange of forest trees. *In* The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems. Eds. D.F. Karnosky, R. Ceulemans, G.E. Scarascia-Mugnozza and J.L. Innes. CABI Publishing, Oxford, pp 17–56.

Einig, W., U. Lauxmann, B. Hauch, R. Hampp, W. Landolt, S. Maurer and R. Matyssek. 1997. Ozone-induced accumulation of carbohydrates changes enzyme activities of carbohydrate metabolism in birch leaves. New Phytol. 137:673–680.

Fowler, D., J.N. Cape, M. Coyle, C. Flechard, J. Kuylenstierna, K. Hicks, D. Derwent, C. Johnson and D. Stevenson. 1999. The global exposure of forests to air pollutants. Water Air Soil Pollut. 116:5–32.

Gunderson, C.A., R.J. Norby and S.D. Wullschleger. 1993. Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated $CO_2$: no loss of photosynthetic enhancement. Plant Cell Environ. 16:797–807.

Hansen, J. and I.B. Møller. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantative determination with anthrone. Anal. Biochem. 68:87–94.

Herrick, J.D. and R.B. Thomas. 2003. Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations. Tree Physiol. 23:109–118.

Hirsh, A.G. 1987. Vitrification in plants as a natural form of cryoprotection. Cryobiology 24:214–228.

Horwath, W.R., K.S. Pregizer and E.A. Paul. 1994. $^{14}C$ Allocation in tree–soil systems. Tree Physiol. 14:1163–1176.

Intergovernmental Panel on Climate Change. 2001. Climate change 2001: the scientific basis. Technical summary, 63 p. Available at: http://www.ipcc.ch/pub/wg1TARtechsum.pdf

Isebrands, J.G., E.P. McDonald, E. Kruger, G. Hendrey, K. Pregitzer, K. Percy, J. Sober and D.F. Karnosky. 2001. Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. Environ. Pollut. 115:359–371.

Jach, M.E., R. Ceulemans and M.B. Murray. 2001. Impact of greenhouse gases on the phenology of forest trees. *In* The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems. Eds. D.F. Karnosky, R. Ceulemans, G.E. Scarascia-Mugnozza and J.L. Innes. CABI Publishing, Oxford, pp 193–223.

Karnosky, D.F., D.R. Zak, K.S. Pregitzer et al. 2003. Tropospheric $O_3$ moderates responses of temperate hardwood forests to elevated $CO_2$: a synthesis of molecular to ecosystem results from the Aspen FACE project. Funct. Ecol. 17:289–304.

Karnosky, D.F., K.S. Pregitzer, D.R. Zak, M.E. Kubiske, G.R. Hendrey, D. Weinstein, M. Nosal and K. Percy. 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant Cell Environ. 28:965–981.

King, J.S., M.E. Kubiske and K.S. Pregizer. 2005. Tropospheric $O_3$ compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric $CO_2$. New Phytol. 168:4–12.

Kopper, B.J. and R.L. Lindroth. 2003. Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. Oecologia 134:95–103.

Kubiske, M., V.S. Quinn, W.E. Heilman, E.P. Mcdonald, P.E. Marquardt, R.M. Teclaw, A.L. Friend and D.F. Karnosky. 2006. Interannual climatic variation mediates elevated $CO_2$ and $O_3$ effects on forest growth. Glob. Change Biol. 12:1054–1068.

Landolt, W., M.S. Günthardt-Goerg, I. Pfenninger, W. Einig, R. Hampp, S. Maurer and R. Matyssek. 1997. Effects of fertilization on ozone-induced changes in the metabolism of birch (*Betula pendula*) leaves. New Phytol. 137:389–397.

Liu, L., J.S. King and C.P. Giardina. 2005. Effects of elevated concentrations of atmospheric $CO_2$ and tropospheric $O_3$ on leaf litter production and chemistry in trembling aspen and paper birch communities. Tree Physiol. 25:1511–1522.

Long, S., E.A. Ainsworth, A. Rogers and D.R. Ort. 2004. Rising atmospheric carbon dioxide: plants FACE the future. Annu. Rev. Plant Biol. 55:591–628.

Loveys, B.R., J.G. Egerton and M.C. Ball. 2006. Higher daytime leaf temperatures contribute to lower freeze tolerance under elevated $CO_2$. Plant Cell Environ. 29:1077–1086.

Lux, D., S. Leonardi, J. Müller, A. Wiemken and W. Flückiger. 1997. Effects of ambient ozone concentrations on contents of non-structural carbohydrates in young *Picea abies* and *Fagus sylvatica*. New Phytol. 137:399–409.

Maini, J.S. 1966. Apical growth of *Populus* spp. I. Sequential pattern of internode, bud and branch length of young individuals. Can. J. Bot. 44:615–622.

Matyssek, R. and H. Sandermann, Jr. 2003. Impact of ozone on trees: an ecophysiological perspective. Prog. Bot. 64:349–404.

Noormets, A., A. Sôber, E.J. Pell, R.E. Dickson, G.K. Podila, J. Sôber, J.G. Isebrands and D.F. Karnosky. 2001. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated $CO_2$ and/or $O_3$. Plant Cell Environ. 24:327–336.

Norby, R.J., J.S. Hartz-Rubin and M.J. Verbrugge. 2003. Phenological responses in maple to experimental atmospheric warming and $CO_2$ enrichment. Glob. Change Biol. 9:1792–1801.

Nowak, R.S., D.S. Ellsworth and S.D. Smith. 2004. Functional responses of plants to elevated atmospheric $CO_2$—do photosynthetic and productivity data from FACE experiments support early predictions? Tansley Review. New Phytol. 162:253–280.

Ögren, E. 2000. Maintenance respiration correlates with sugar but not nitrogen concentration in dormant plants. Physiol. Plant. 108: 295–299.

Oksanen, E. 2003. Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed to six growing seasons and potted seedlings exposed for one season. Tree Physiol. 23:603–614.

Oksanen, E., S. Kontunen-Soppela, J. Riikonen, J. Uddling and E. Vapaavuori. 2007. Northern environment predisposes birches to ozone damage. Plant Biol. 56:191–196.

Pell, E.J., C.D. Schlagnhaufer and R.N. Arteca. 1997. Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol. Plant. 100:264–273.

Peltonen, P.A., R. Julkunen-Tiitto, E. Vapaavuori and J.K. Holopainen. 2006. Effects of elevated carbon dioxide and ozone on aphid oviposition preference and birch bud exudate phenolics. Glob. Change Biol. 12:1670–1679.

Podila, G.K., A.R. Paolacci and M. Badiani. 2001. The impact of greenhouse gases on antioxidants and foliar defence compounds. *In* The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems. Eds. D.F. Karnosky, R. Ceulemans, G.E. Scarascia-Mugnozza and J.L. Innes. CABI Publishing, Oxford, pp 57–125.

Pollard, D.F.W. 1970. Leaf area development on different shoot types in a young aspen stand and its effect upon production. Can. J. For. Res. 48:1801–1804.

Rao, M.V., B.A. Hale and D.P. Ormrod. 1995. Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide. Plant Physiol. 109:421–432.

Rebbeck, J. and A.J. Scherzer. 2002. Growth responses of yellow-*poplar (Liriodendron tulipifera* L.) exposed to 5 years of $O_3$ alone or combined with elevated $CO_2$. Plant Cell Environ. 25:1527–1537.

Riikonen, J., M.-M. Lindsberg, T. Holopainen, E. Oksanen, J. Lappi, P. Peltonen and E. Vapaavuori. 2004. Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. Tree Physiol. 24:1227–1237.

Riikonen, J., T. Holopainen, E. Oksanen and E. Vapaavuori. 2005. Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated $CO_2$ and $O_3$ in the field. Tree Physiol. 25:621–632.

Sakai, A. and W. Larcher. 1987. Frost survival of plants: responses and adaptation to freezing stress. Springer-Verlag, New York, 94 p.

Sigurdsson, B.D. 2001. Elevated [$CO_2$] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. Trees 15:403–413.

Sprugel, D.G., T.M. Hinckley and W. Schaap. 1991. The theory and practice of branch autonomy. Annu. Rev. Ecol. Syst. 22:309–334.

Tricker, P.J., C. Calfapietra, E. Kuzminsky et al. 2004. Long-term acclimation of leaf production, development, longevity and quality following 3 year exposure to free-air $CO_2$ enrichment during canopy closure in *Populus*. New Phytol. 162:413–426.

Vingarzan, R. 2004. A review of surface ozone background levels and trends. Atmos. Environ. 38:3431–3442.

Yonekura, T., M. Yoshidome, M. Watanabe, Y. Honda, I. Ogiwara and T. Izuta. 2004. Carry-over effects of ozone and water stress on leaf phenological characteristics and bud frost hardiness of *Fagus crenata* seedlings. Trees 18:581–588.