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The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen

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Abstract Two aspen (*Populus tremuloides* Michx.) clones, differing in O₃ tolerance, were grown in a free-air CO₂ enrichment (FACE) facility near Rhinelander, Wisconsin, and exposed to ambient air, elevated CO₂, elevated O₃ and elevated CO₂+O₃. Leaf instantaneous light-saturated photosynthesis (P_S) and leaf areas (A) were measured for all leaves of the current terminal, upper (current year) and the current-year increment of lower (1-year-old) lateral branches. An average, representative branch was chosen from each branch class. In addition, the average photosynthetic rate was estimated for the short-shoot leaves. A summing approach was used to estimate potential whole-plant C gain. The results of this method indicated that treatment differences were more pronounced at the plant- than at the leaf- or branch-level, because minor effects within modules accrued in scaling to plant level. The whole-plant response in C gain was determined by the counteracting changes in P_S and A . For example, in the O₃-sensitive clone (259), inhibition of P_S in elevated O₃ (at both ambient and elevated CO₂) was partially ameliorated by an increase in total A . For the O₃-tolerant clone (216), on the other hand, stimulation of photosynthetic rates in elevated CO₂ was nullified by decreased total A .

Keywords Elevated carbon dioxide and ozone · Leaf area · Photosynthesis · *Populus tremuloides* (Michx.) · Potential carbon gain

Introduction

Concentrations of atmospheric CO₂ and O₃ are increasing (Bazzaz 1990; Chameides et al. 1995) as a result of fossil fuel consumption. The increases in CO₂ and O₃ concentrations have already been implicated in changes in terrestrial ecosystems (Miller 1973; Ciais et al. 1995; Keeling et al. 1996). Elevated atmospheric CO₂ is associated with increased photosynthetic rates, decreased stomatal conductance and transpiration, increased plant growth and potentially higher yields (Bowes 1993; Drake et al. 1997; Will and Ceulemans 1997; Pan et al. 1998; Tognetti et al. 1999). O₃, on the other hand, inhibits the growth of plants (Heath 1994; Pell et al. 1997) by decreasing stomatal conductance and photosynthesis, decreasing the content and activity of Rubisco, decreasing the content of chlorophyll and inducing accelerated senescence (Darrall 1989; Pell et al. 1992; Landry and Pell 1993; Pell et al. 1994; Karnosky et al. 1996; Nali et al. 1998). While the individual effects of CO₂ and O₃ on plants are rather well known, their interactive effect on plant growth and metabolism is still a matter of active discussion (Polle et al. 1993; Barnes et al. 1995; Rao et al. 1995; Kull et al. 1996; Lippert et al. 1997; McKee et al. 1997a; Dickson et al. 1998; Grams et al. 1999; Loats and Rebbeck 1999). It was first assumed that the contrasting effects of elevated CO₂ and O₃ might simply cancel each other out, and for several members of the *Gramineae* family there are experimental data to support this view (Rao et al. 1995; McKee et al. 1997b). Other studies on woody plants (Kull et al. 1996; Karnosky et al. 1998), in contrast, have shown that the combined effect of elevated CO₂ and O₃ does not always equal the sum of the individual effects of these gases.

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Most gas exchange research is performed on leaves at one particular developmental stage (Field et al. 1995) and the age-related variation is not addressed. While this approach is adequate for studying the mechanisms of regulation in response to changes in environmental conditions, it may not always characterize responses at the whole-plant level. Wait et al. (1999) showed that the expanding and expanded leaves in *Populus deltoides* respond differently to elevated CO₂ and that the ratio of expanding to expanded leaves determines the overall plant response. In fact, the age-related changes in light-saturated photosynthesis (P_S) are dynamic, and measuring this parameter for expanding and expanded leaves only may be too coarse. It is known that photosynthetic capacity dynamically increases as leaves develop, peaks at full expansion, and remains the same or decreases with maturity (Kozlowski et al. 1991).

While P_S provides a sound physiological estimate of biochemical leaf-level responses, it is not known how leaf-level responses compare to those at the plant level. For example, elevated CO₂ and O₃ may affect the size of individual leaves (Pääkkönen et al. 1996b; Jach and Ceulemans 1999), specific leaf area, the number of leaves/unit stem per branch (Pääkkönen et al. 1998) or the number of branches on the tree (Tognetti et al. 1999). Due to these potential plant-level allometric changes, that are not well documented, the changes in P_S may or may not be reflected at the whole-plant level.

The goal of our current study was to evaluate the relative role of assimilation rates and leaf area (A) in determining potential whole-plant C gain (C_{plant}) under elevated CO₂ and O₃ taking into account the leaf age-related variability in C assimilation. Detailed leaf-level measurements were made on representative branches of four major branch categories and potential C_{plant} was estimated based on the branch-level C gain and the number of branches in each category. The work was conducted on two field-grown trembling aspen (*Populus tremuloides* Michx.) clones, previously shown to have differential O₃ tolerance (Karnosky et al. 1996).

Materials and methods

Experimental site and plant material

Two aspen (*P. tremuloides*) clones (no. 216, O₃ tolerant and no. 259, O₃ sensitive), were grown in a free-air CO₂ enrichment (FACE) facility (Dickson et al. 2000) near Rhinelander, Wisconsin. The experimental site is located at 45°30'N and 89°30'W, on sandy loam soil. The differential O₃ tolerance of these two clones has been characterized based on physiological and growth responses as well as visual foliar symptoms (Coleman et al. 1995; Karnosky et al. 1996, 1998). The plant material was propagated from greenhouse-grown stock plants. The rooted cuttings were 6 months old by the time of planting in July 1997 and about 2.5 m tall by the time of measurement in 1999.

The treatments – control (ambient air), elevated CO₂, elevated O₃, and elevated CO₂+O₃ – were arranged in a randomized complete block design with three replicates. Each treatment ring is 30 m in diameter and spaced at least 100 m apart. The eastern half of each ring is planted at a density of 1 tree m⁻² with five aspen

clones with pairs of trees from the same clone arranged randomly. Around the ring, next to the vertical vent pipes that dispense CO₂ and O₃, is a 4-m buffer zone surrounding the core of 280–290 trees that are used for measurements. The detailed description of the experimental set-up and conditions can be found in Dickson et al. (2000).

Fumigation

Control plants were exposed to ambient air ([CO₂] averaged 360 µl l⁻¹ between 0700 hours and 1900 hours and 390 µl l⁻¹ between 1900 hours and 0700 hours; [O₃] averaged 34.6 and 36.9 nl l⁻¹ between 0700 hours and 1900 hours in 1998 and 1999, respectively). Elevated CO₂ and O₃ were applied from bud break (1 May in 1998 and 10 May in 1999) to bud set (15 October in 1998 and 30 September in 1999). Elevated CO₂-treated plants (alone and in combination with O₃) were exposed to 560 µl CO₂ l⁻¹ from 0700 hours to 1900 hours. The 1-min integrated CO₂ concentration was within 10% of the target concentration 80% (81% in 1998) of the time and within 20% of the target 96% (93% in 1998) of the time. Elevated O₃ treated plants (alone and in combination with CO₂) received 97.8 µl O₃ l⁻¹×h (sum 0 for 12-h fumigation) during the 1998 growing season and 89.0 µl O₃ l⁻¹×h during the 1999 growing season, with an average daytime (0700–1900 h) exposure concentration of 55 nl l⁻¹ in 1998 and 52 nl l⁻¹ in 1999 compared to the ambient seasonal O₃ dose of 65.3 and 62.8 µl l⁻¹×h for the 1998 and 1999 growing seasons, respectively. The daily peak concentration for the elevated O₃ treatment was calculated as twice the ambient concentration at 0700 h (the base value). The peak concentration was to be reached at noon and the [O₃] was held at the base level for the first and last hour of the daily fumigation. The transition from the base to peak level followed a sigmoidal function. This approach took into account the day-to-day variation and the natural seasonal changes in ambient O₃ levels and resulted in exposure to an O₃ level which was about 1.5 times that of ambient air. O₃ fumigation followed a typical diurnal curve (with peak concentrations in the early afternoon) and generally lasted from 0700 h to 1900 h. However, there were no O₃ fumigations during rain, fog, mist, dew or low-temperature ($t < 10^\circ\text{C}$) conditions, which occurred about 30% of the time.

Measurements

Gas exchange

P_S of the aspen clones was measured on 15–31 July 1999, with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, Neb.). Two plants per clone were sampled from each ring, totaling six plants per treatment. All measurements were made on intact leaves at the CO₂ concentration at which the plants were grown (360 µl l⁻¹ for control and elevated O₃ and 560 µl l⁻¹ for elevated CO₂ and CO₂+O₃), under saturating light of 1,200 µmol m⁻² s⁻¹ (controlled by the red/blue light-emitting diodes in the LI-6400), at ambient temperature (25–33°C), and air humidity (40–60%). P_S was measured for all leaves of the current terminal, for two lateral branches (one current year lateral [upper long-shoot (LS)] and the current-year increment of one 1-year-old lateral (lower LS)) and for the short shoots (SS) to account for differences in leaf age and branch position. Plants of at least two treatments were sampled each day to minimize the possibility of variations in weather conditions confounding treatment effects. Furthermore, plants from different treatments were sampled randomly to minimize the possibility of diurnal patterns confounding treatment effects. Measurements of diurnal trends in gas-exchange indicated an occasional mid-day depression in stomatal conductance but not in photosynthesis (unpublished data). The leaf plastochron index (LPI) (Larson and Isebrands 1971) was used as a measure of the physiological age of the leaf. Leaf development is a linear function of time (Ceulemans et al. 1988) and LPI provides an easy way to estimate relative leaf age on trees with indeterminate growth habit.

According to Larson and Isebrands (1971), the youngest leaf longer than 2.5 cm was assigned LPI=1, leaves older than that were assigned successively higher LPI values. The LPI numeration was applied independently to each LS. In order to discuss age-related physiological changes, we grouped the leaves into four age classes (young, LPI=1–8; recently mature, LPI 9–14; mature, LPI=15–25; old, LPI>25) (Larson and Isebrands 1971; Coleman et al. 1995). Leaves on SS emerge mostly as a single flush in spring and the LPI system cannot be applied to them. In addition, the lower LS had set bud by late July and had no developing leaves at the time of measurement.

Morphological parameters

Regression models were developed to describe the relationship between A (measured with an LI-3050A leaf area meter; Li-Cor) and the product of leaf length and width in each clone. The accuracy of the regression models was high ($R^2=0.988$ and $R^2=0.990$ for clones 216 and 259, respectively). The regression models were used to non-destructively estimate the area of individual leaves on three representative LS (current terminal, an upper LS and a lower LS) and on the SS. Tree height (H) and diameter (D) (at 3 cm from ground level) of the main stem were measured on all trees for which A was estimated (six plants per clone and treatment).

Scaling up

The P_S and A profiles by LPI were measured on different sets of plants to increase the robustness against plant-to-plant variation. The P_S and A profiles were matched based on the number of leaves per branch, aligned, and the product of these parameters at every given leaf position gave an estimated profile of potential leaf C gain for a particular branch (Coleman et al. 1995). It can be described as:

$$C_{\text{leaf}} = P_S \times A \quad (1)$$

where C_{leaf} is potential leaf C gain. The branch C gain (C_{br}) was estimated as a sum of the individual leaf estimates:

$$C_{\text{br}} = \sum C_{\text{leaf}} \quad (2)$$

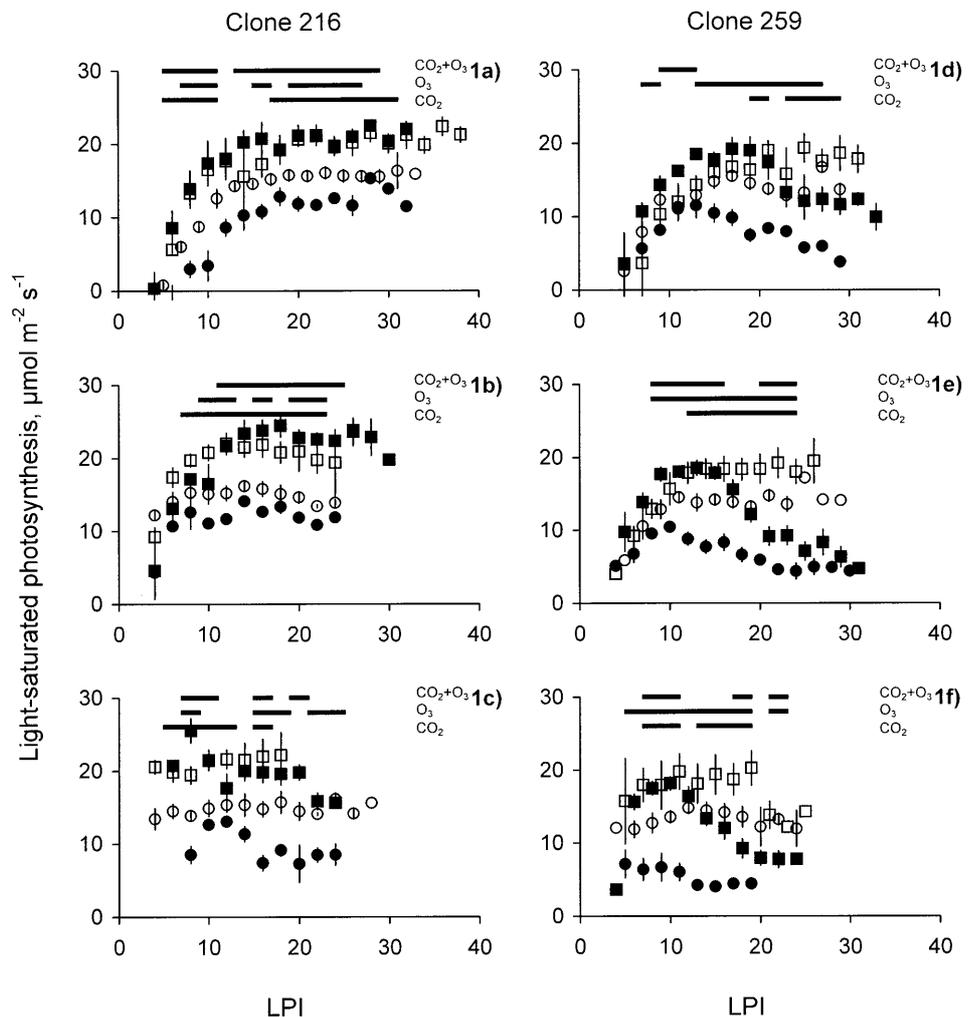
By multiplying C_{br} by the number of branches in a given category, we get an estimate for total branch-class C gain. The sum of all branch classes gives a potential C_{plant} (Isebrands et al. 1988):

$$C_{\text{plant}} = C_{\text{br-terminal}} + (a \times C_{\text{br-upper}}) + (b \times C_{\text{br-lower}}) + (c \times C_{\text{SS}}) \quad (3)$$

where $C_{\text{br-terminal}}$, $C_{\text{br-upper}}$, $C_{\text{br-lower}}$ and C_{SS} are potential C_{br} s for terminal, upper LS, lower LS and SS, respectively and a , b , and c are the number of branches in each respective category.

The average photosynthesis for each representative branch was calculated as the ratio of C_{br} to the A on the given branch and the average plant photosynthesis was calculated as the ratio of C_{plant} to total A . The leaf area ratio (LAR) was estimated for each individual branch class as the ratio of cumulative A on a given branch class

Fig. 1 Light-saturated photosynthesis (P_S) vs. leaf plastochron index (LPI) profiles on current terminals (a, d), upper (b, e) and lower (c, f) long-shoots (LS) of two aspen (*Populus tremuloides* Michx.) clones, 216 (a–c) and 259 (d–f). Control (○), elevated CO_2 (□), elevated O_3 (●) and $\text{CO}_2 + \text{O}_3$ (■). Mean \pm SE. The significant treatment effects compared to controls at a given leaf position were detected with two-tailed t -test at $P < 0.05$ using ring means at each LPI as the experimental unit ($n=3$). Significant difference of indicated treatment from control at a given LPI is indicated by horizontal bars at the top of the figure



to stem volume index. Branching index was calculated as the ratio of lateral branches to stem volume index.

Statistical analysis

The experiment was a randomized complete block design with four treatment rings in three blocks at the whole-plot level, with clone treated as a sub-plot factor for analyses comparing clones. The treatment differences in mean branch photosynthesis, A , LAR and the percent contribution of each branch class to the C_{plant} were analyzed separately by clone with the mixed-effects models procedure (PROC MIXED) of SAS (SAS Institute, Cary, N.C., 1996). Replicate and replicate \times CO₂ \times O₃ were random error terms, with levels of CO₂ and O₃ treated as fixed effects within ANOVA. Denominator df -s were calculated with Satterthwaite's approximation method for all ANOVAs. Treatment and clone differences in mean whole-plant parameters were analyzed with PROC MIXED partitioning the variation to the fixed effects of CO₂, O₃ and clone and their interactions, with replicate and replicate \times CO₂ \times O₃ used as random error terms. For all analyses, the patterns of variation attributable to replicate \times treatment effects justified the use of the pooled replicate \times CO₂ \times O₃ error term. Because plant size strongly influenced total area and C_{plant} , $\log(D^2 \times H)$ was included as a covariate in the models analyzing those parameters, and the resulting least squares means and SEs are shown in Fig. 4.

Results and discussion

Photosynthesis and leaf area

P_S increased in leaves until full expansion and remained at the same rate or decreased with leaf age (Fig. 1). Such age-related changes in photosynthesis are common in a variety of plants (Kozłowski and Pallardy 1997). In our current study, P_S decreased in the mature leaves on all branches of clone 259 when exposed to elevated O₃ and CO₂+O₃, whereas in clone 216 the decrease was observed only in the lower canopy. The O₃-induced inhibition of photosynthesis occurred at both ambient and elevated CO₂ in clone 259, but only at ambient CO₂ in clone 216. This is in contrast with the results of Kull et al. (1996), who reported greater age-related drop in P_S in CO₂+O₃-exposed trees of clone 216 than in O₃-exposed plants of the same clone. The different response of clone 216 in the two studies could be caused by differences in site fertility, as the study of Kull et al. (1996) was carried out in a nutrient-poor site whereas our current experiment is established on a fertile N-rich soil (Dickson et al. 2000).

Fig. 2 Individual leaf area (A) vs. LPI profiles on current terminals (a, d), upper (b, e) and lower (c, f) LS of two aspen clones, 216 (a–c) and 259 (d–f). Mean \pm SE. Symbols, abbreviations and presentation of the results of comparisons of means are the same as described for Fig. 1

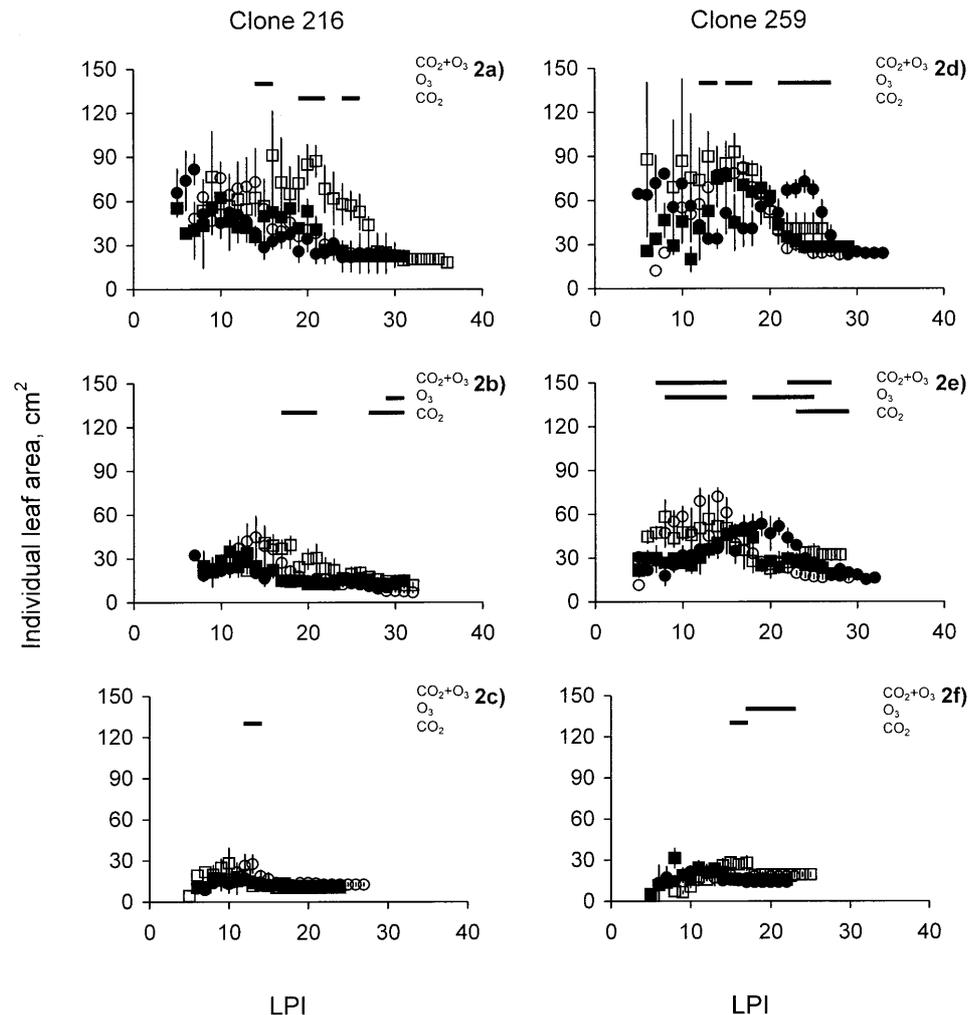
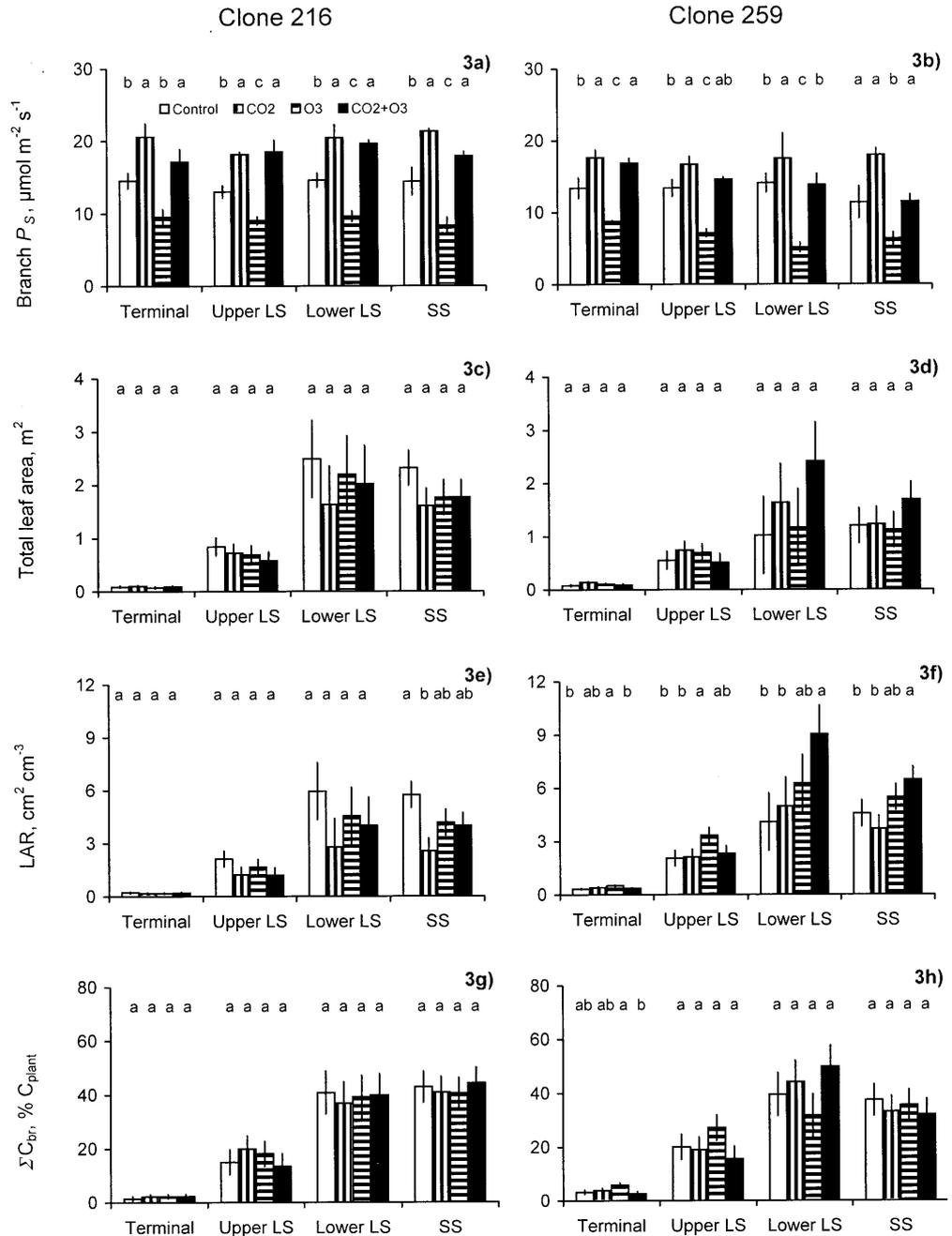


Fig. 3 Average branch P_s (Branch P_s ; **a, b**), total A per branch class (LAR) on stem volume basis [total A per diameter²×height; **e, f**] and the contribution of each branch class C gain (ΣC_{br}) to whole-plant C gain (% C_{plant} ; **g, h**) for the current terminal, upper LS, lower LS and SS of two aspen clones, 216 (**a, c, e**) and 259 (**b, d, f**). Mean±SE. Statistically significant differences ($P<0.05$) between treatments within clone and within a branch class are shown by different letters above the bars. For other abbreviations, see Figs. 1 and 2



Leaves from lower lateral branches matured sooner and reached maximum P_s at lower LPIs than leaves in the upper canopy. Across all leaf positions on the LS, elevated CO₂ increased P_s by an average of 34% in clone 216 and 25% in clone 259 compared to controls. These values are below the average of 50% stimulation reported for a range of species (reviewed by Curtis 1996) and are more conservative compared to those recorded in studies conducted under similar (FACE) conditions, where P_s increased 50–60% in the dominant canopy tree *Pinus taeda* (Ellsworth 1999) and 160–190% in four hardwood understory species (DeLucia and Thomas 2000). Under elevated O₃, P_s decreased by 29 and 40%, and under elevated CO₂+O₃ it increased by 15 and 19%

for clones 216 and 259, respectively. The amelioration effect of elevated CO₂ on O₃-induced inhibition supports similar observations across a number of species (e.g., Volin et al. 1998). The response to treatments was greater in the recently mature and mature leaves than in young leaves, probably because of lower P_s and non-functional stomata that are characteristic of expanding leaves (Choinski and Wise 1999). On average, all LS displayed a similar response to treatments regardless of their position in the canopy, although the magnitude varied. For example, the O₃-induced inhibition was greater in the lower LS than higher in the canopy, a phenomenon that has been related to higher stomatal conductance in shade compared to sun leaves (Tjoelker et al. 1995).

Treatment differences for individual leaf area were smaller than those in P_S (Fig. 2) and were often obscured by larger within-treatment variation. Individual leaf area was largest on the current terminal and smallest on the lower LS (Fig. 2). In general, both P_S and A peaked in recently mature leaves (LPI=9–14) making them the greatest producers of assimilates compared to other leaf age classes. In clone 259 the mature leaves of the O_3 -exposed plants were larger than under other treatments, partially compensating for the lower area-based P_S rates (Fig. 2).

Potential plant C gain

C gain calculations for branches (C_{br}) rather than individual leaves eliminate the issue of strong non-linear age-dependence between P_S and LPI, and thus make it easier to evaluate the treatment effects on assimilation. A significant CO_2 -induced increase and O_3 -induced decrease of average branch P_S were observed in both clones throughout the canopy (Fig. 3a, b). The combined exposure to elevated CO_2 and O_3 significantly stimulated photosynthesis compared to the control in all branch categories of clone 216, whereas in clone 259 significant stimulation occurred only in the current terminal. The average branch photosynthesis was very similar across branch classes. Overall, P_S did not differ between LS and SS leaves, in contrast to the report by Nelson and Michael (1982) who reported 30% lower values for SS of *Populus Tristis* no. 1. However, they hypothesized that the difference between LS and SS could reflect the greater age of SS compared to LS leaves, rather than differences in photosynthetic acclimation.

Total A for the individual branch classes was not significantly affected by treatment in either clone (Fig. 3c, d). About 40% of whole-plant A was on lower LS, about the same on SS, about 20% on upper LS and only a minor fraction on the current terminal. The large variation in data and limited treatment effects can be attributed to variation in plant size. In order to minimize the confounding effect of plant size, we calculated LAR for each branch class. While this presentation does not change the relative contribution of individual branch classes to the whole-plant A , the treatment differences become more obvious (Fig. 3e, f).

For clone 216, elevated CO_2 generally decreased LAR, although it was significant only in SS, whereas elevated O_3 and CO_2+O_3 did not affect LAR. In contrast, for clone 259, exposure to elevated CO_2 did not affect LAR, whereas elevated O_3 (in the upper LS) and CO_2+O_3 (in the lower LS and in SS) increased LAR compared to control. The increased A in the O_3 - and CO_2+O_3 -exposed plants of clone 259 may be an acclimation response compensating for decreased photosynthetic rates under O_3 stress. This hypothesis finds support in the works of Woodbury et al. (1994) and Pääkkönen et al. (1996a), who showed that plants ex-

Table 1 Average plant growth parameters of two aspen (*Populus tremuloides* Michx.) clones grown in ambient air (control), elevated CO_2 , elevated O_3 and elevated CO_2+O_3 . Arithmetic mean \pm SE ($n=6$). Different letters denote statistically significant difference between treatments within a clone at $P<0.05$ (mixed effects models procedure; SAS Institute, Cary, N.C., 1996). D Diameter, H height

	Clone 216	Clone 259
Plant diameter (cm)		
Control	3.4 \pm 0.3 b	3.3 \pm 0.2 ab
CO_2	4.6 \pm 0.3 a	3.7 \pm 0.2 a
O_3	3.7 \pm 0.2 b	2.9 \pm 0.1 b
CO_2+O_3	3.9 \pm 0.2 ab	3.3 \pm 0.2 ab
Plant height (cm)		
Control	293 \pm 111 a	237 \pm 11 a
CO_2	285 \pm 12 a	249 \pm 8 a
O_3	296 \pm 17 a	244 \pm 13 a
CO_2+O_3	302 \pm 16 a	228 \pm 7 a
Stem volume index (D^2H) (cm ³)		
Control	4,047 \pm 160 b	2,604 \pm 292 ab
CO_2	6,289 \pm 623 a	3,428 \pm 383 a
O_3	4,063 \pm 595 b	2,061 \pm 161 b
CO_2+O_3	4,734 \pm 586 ab	2,495 \pm 296 ab
Branching index ($\times 10^{-3}$)		
Control	8.7 \pm 0.9 a	8.9 \pm 0.6 ab
CO_2	5.1 \pm 0.3 b	6.6 \pm 0.8 b
O_3	7.9 \pm 0.7 a	9.9 \pm 1.0 ab
CO_2+O_3	7.0 \pm 0.4 ab	10.5 \pm 1.0 a

posed to chronic oxidative stress may preferentially invest in new foliage, and of Catovsky and Bazzaz (2000), who showed that such an increase in A could compensate for decreased photosynthetic rates. The greater O_3 -induced increase in LAR at elevated than at ambient CO_2 suggests that the two gases may have interactive effects on biomass allocation between foliage and stem.

An increase in A can, at equal branch size, result from larger individual leaves (Poorter and Remkes 1990) or from an increased number of leaves per branch (Pääkkönen et al. 1998). In our study, individual leaf size likely contributed more to the treatment differences, although statistically significant differences were observed only at a few leaf positions (Fig. 2). In addition, increases in A were closely paralleled by increased branching index (number of lateral branches per stem volume index, Table 1). The size of individual long-shoots per se (estimated with volume index D^2H), which could have affected branch A , did not vary significantly among treatments in any canopy position (data not shown). The treatment effects on average branch P_S and branch-class A counteracted one another, and the proportional contribution of branch classes to C_{plant} was similar across treatments for both clones (Fig. 3g, h). Because of uniform mean branch photosynthesis across different branch classes the relative contribution of each branch class to C_{plant} was closely proportional to the A of that class, with about 40% of whole-plant assimilation attributable to SS and lower LS, each, and about 20% to upper LS.

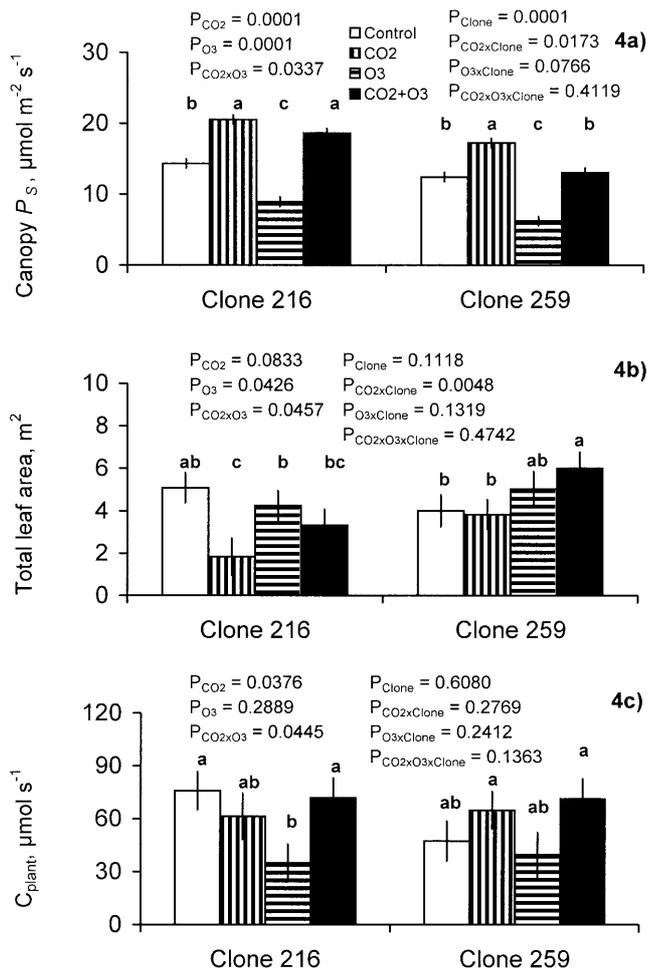


Fig. 4 Average canopy P_s (Canopy P_s ; a), total A (b) and C_{plant} (c) for two aspen clones, 216 and 259. Mean \pm SE. Total A and C_{plant} estimates are adjusted for treatment differences in plant size using the logarithm of stem volume index as a covariate. Statistically significant differences between treatments and clones are shown with different letters above the bars. The main and interactive effects of elevated CO₂, O₃ and clone are described with P values

Not surprisingly, average canopy photosynthesis (Fig. 4a) reflected the patterns observed in average photosynthesis for individual branch classes (with similar CO₂ and O₃ effects), because of little variation among branch classes. Treatment effects on total A , however, were more pronounced than those at the branch level (Fig. 4b). This indicates that small differences among modules can accrue in scaling and result in significant differences at the whole-plant level, as has been shown by Isebrands et al. (1988) in a similar scaling exercise. Furthermore, total A estimates are adjusted for differences in plant size among treatments and clones through covariance analysis, as treatment-related effects on plant size, which strongly influence A , would otherwise confound the interpretation of changes in total A .

The product of average canopy photosynthesis and total A is average plant C gain, adjusted for plant size (Fig. 4c). The yield of crop plants and the woody bio-

mass of trees have repeatedly been shown to be in good correlation with assimilation rates (Zelitch 1982; Wells et al. 1986; Isebrands et al. 1988), and we expected C_{plant} to reflect the treatment responses of P_s . To our surprise, the CO₂-induced increase in P_s was counteracted by decreases in A in clone 216. The average effect on C_{plant} of clone 216 under elevated CO₂, O₃ and CO₂+O₃ compared to the control was -28% , -55% and -8% , respectively, and the corresponding changes in leaf photosynthetic rates were $+43\%$, -38% and $+30\%$. For clone 259, there was a positive relationship between average P_s and C_{plant} , yet the response of C_{plant} to elevated O₃ and CO₂+O₃ was strongly affected by increased A . The average effect on C_{plant} in elevated CO₂, O₃ and CO₂+O₃ was $+26\%$, -10% and $+48\%$, respectively, whereas leaf P_s changed by $+39\%$, -50% and $+5\%$, respectively. The data suggest that the amelioration of the O₃ effect at the plant compared to at the leaf level of clone 259 was the result of increased total A at elevated O₃ levels. Furthermore, we conclude that the clonal difference in the response of A to elevated CO₂ could be responsible for the cardinally different response of C_{plant} to elevated CO₂ in clone 259 compared to clone 216 ($P=0.0017$), although the changes in average canopy photosynthesis were similar in the two clones. Our current observation of no CO₂ stimulation of C_{plant} is in contrast with our previous work with these and other *Populus* clones, where CO₂ significantly stimulated growth (Karnosky et al. 1996; Dickson et al. 1998).

In summary, the treatment effects on C_{plant} were influenced significantly by altered allometric relationships, moderating whole-plant responses compared to those at the leaf level. In clone 216 the CO₂-induced increase in leaf P_s was nullified by a decrease in A , and in clone 259 the O₃-induced inhibition of leaf P_s (at both ambient and elevated CO₂) was ameliorated by increased A at the plant level.

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References

- Barnes JD, Pfirmann T, Steiner K, Lütz C, Busch U, Küchenhoff H, Payer H-D (1995) Effects of elevated CO₂, elevated O₃ and potassium deficiency on Norway spruce [*Picea abies* (L.) Karst.]: seasonal changes in photosynthesis and non-structural carbohydrate content. *Plant Cell Environ* 18:1345–1357
- Bazzaz FA (1990) The response of natural ecosystems to the rising global CO₂ levels. *Annu Rev Ecol Syst* 21:167–196
- Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO₂. *Annu Rev Plant Physiol Mol Biol* 44:309–332
- Catovsky S, Bazzaz FA (2000) Contributions of coniferous and broad-leaved species to temperate forest carbon uptake: a bottom-up approach. *Can J For Res* 30:100–111

- Ceulemans R, Impens I, Steenackers V (1988) Genetic variation in aspects of leaf growth of *Populus* clones, using leaf plastochron index. *Can J For Res* 18:1069–1077
- Chameides WL, Kasibhatla PS, Yienger J, Levy IH (1995) Growth of continental-scale metro-agro-plexes, regional ozone pollution, and world food production. *Science* 264:74–77
- Choinski JS, Wise RR (1999) Leaf growth and development in relation to gas exchange in *Quercus marilandica* Muenchh. *J Plant Physiol* 154:302–309
- Ciais P, Tans PP, Trolier M, White JWC, Francey RJ (1995) A large northern hemisphere terrestrial CO₂ sink indicated by the ¹³C/¹²C ratio of atmospheric CO₂. *Science* 269:1098–1102
- Coleman MD, Isebrands JG, Dickson RE, Karnosky DF (1995) Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiol* 15:585–592
- Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environ* 19:127–137
- Darrall NM (1989) The effect of air pollutants on physiological processes in plants. *Plant Cell Environ* 12:1–30
- DeLucia EH, Thomas RB (2000) Photosynthetic responses to CO₂ enrichment of four hardwood species in a forest understory. *Oecologia* 122:11–19
- Dickson RE, Coleman MD, Riemenschneider DE, Isebrands JG, Hogan GD, Karnosky DF (1998) Growth of five hybrid poplar genotypes exposed to interacting elevated CO₂ and O₃. *Can J For Res* 28:1706–1716
- Dickson RE, Lewin KF, Isebrands JG, Coleman MD, Heilman WE, Riemenschneider DE, Söber J, Host GE, Zak DR, Hendrey GR, Pregitzer KS, Karnosky DF (2000) Forest atmosphere carbon transfer and storage (FACTS-II). The aspen free-air CO₂ and O₃ enrichment (FACE) project: an overview. USDA Forest Service, St. Paul, Minn.
- Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO₂. *Annu Rev Plant Physiol Mol Biol* 48:609–639
- Ellsworth DS (1999) CO₂ enrichment in a maturing pine forest: are CO₂ exchange and water status in the canopy affected? *Plant Cell Environ* 22:461–472
- Field CB, Jackson RB, Mooney HA (1995) Stomatal responses to increased CO₂: implications from the plant to the global scale. *Plant Cell Environ* 18:1214–1225
- Grams TEE, Anegg S, Häberle K-H, Langebartels C, Matyssek R (1999) Interactions of chronic exposure to elevated CO₂ and O₃ levels in the photosynthetic light and dark reactions of European beech (*Fagus sylvatica*). *New Phytol* 144:95–107
- Heath RL (1994) Possible mechanisms for the inhibition of photosynthesis by ozone. *Photosyn Res* 39:439–451
- Isebrands JG, Ceulemans R, Wiard B (1988) Genetic variation in photosynthetic traits among *Populus* clones in relation to yield. *Plant Physiol Biochem* 26:427–437
- Jach ME, Ceulemans R (1999) Effects of elevated atmospheric CO₂ on phenology, growth and crown structure of Scots pine (*Pinus sylvestris*) seedlings after two years of exposure in the field. *Tree Physiol* 19:289–300
- Karnosky DF, Gagnon ZE, Dickson RE, Coleman MD, Lee EH, Isebrands JG (1996) Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Can J For Res* 26:23–37
- Karnosky DF, Podila GK, Gagnon Z, Pechter P, Akkapeddi A, Sheng Y, Riemenschneider DE, Coleman MD, Dickson RE, Isebrands JG (1998) Genetic control of responses to interacting tropospheric ozone and CO₂ in *Populus tremuloides*. *Chemosphere* 36:807–812
- Keeling CD, Chine JFS, Whorf TP (1996) Increased activity of northern vegetation inferred from atmospheric CO₂ measurements. *Nature* 382:146–149
- Kozlowski TT, Pallardy SG (1997) Physiology of woody plants. Academic Press, San Diego, Calif.
- Kozlowski TT, Kramer PJ, Pallardy SG (1991) The physiological ecology of woody plants. Academic Press, New York
- Kull O, Söber A, Coleman MD, Dickson RE, Isebrands JG, Gagnon Z, Karnosky DF (1996) Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO₂. *Can J For Res* 26:639–648
- Landry LG, Pell EJ (1993) Modification of Rubisco and altered proteolytic activity in O₃-stressed hybrid poplar (*Populus maximowizii* × *trichocarpa*). *Plant Physiol* 101:1355–1362
- Larson PR, Isebrands JG (1971) The plastochron index as applied to developmental studies of cottonwood. *Can J For Res* 1:1–11
- Lippert M, Steiner K, Pfirrmann T, Payer H-D (1997) Assessing the impact of elevated O₃ and CO₂ on gas exchange characteristics of differently K supplied clonal Norway spruce trees during exposure and the following season. *Trees* 11:306–316
- Loats KV, Rebbeck J (1999) Interactive effects of ozone and elevated carbon dioxide on the growth and physiology of black cherry, green ash, and yellow-poplar seedlings. *Environ Pollut* 106:237–248
- McKee IF, Bullimore JF, Long SP (1997a) Will elevated CO₂ concentrations protect the yield of wheat from O₃ damage? *Plant Cell Environ* 20:77–84
- McKee IF, Eiblmeier M, Polle A (1997b) Enhanced ozone-tolerance in wheat grown at an elevated CO₂ concentration: ozone exclusion and detoxification. *New Phytol* 137:275–284
- Miller PR (1973) Oxidant-induced community change in a mixed conifer forest. Air pollution damage to vegetation. *Adv Chem* 122:101–107
- Nali C, Guidi L, Filippi F, Soldatini GF, Lorenzini G (1998) Photosynthesis of two poplar clones contrasting in O₃ sensitivity. *Trees* 12:196–200
- Nelson ND, Michael DA (1982) Photosynthesis, leaf conductance, and specific leaf weight in long and short shoots of *Populus* "Tristis no. 1" grown under intensive culture. *For Sci* 28:737–744
- Pääkkönen E, Metsärinne S, Holopainen T, Kärenlampi L (1996a) The ozone sensitivity of birch (*Betula pendula*) in relation to the developmental stage of leaves. *New Phytol* 132:145–154
- Pääkkönen E, Vahala J, Holopainen T, Karjalainen R, Kärenlampi L (1996b) Growth responses and related biochemical and ultrastructural changes of the photosynthetic apparatus in birch (*Betula pendula*) saplings exposed to low concentrations of ozone. *Tree Physiol* 16:597–605
- Pääkkönen E, Vahala J, Pohjola M, Holopainen T, Kärenlampi L (1998) Physiological, stomatal and ultrastructural ozone responses in birch (*Betula pendula* L.) are modified by water stress. *Plant Cell Environ* 21:671–684
- Pan Q, Wang Z, Quebedeaux B (1998) Responses of the apple plant to CO₂ enrichment: changes in photosynthesis, sorbitol, other soluble sugars, and starch. *Aust J Plant Physiol* 25:293–297
- Pell EJ, Eckardt N, Enyedi AJ (1992) Timing of ozone stress and resulting status of ribulose biphosphate carboxylase/oxygenase and associated net photosynthesis. *New Phytol* 120:397–405
- Pell EJ, Eckardt NA, Glick RE (1994) Biochemical and molecular basis for impairment of photosynthetic potential. *Photosyn Res* 39:453–462
- Pell EJ, Schlaghauser CD, Arteca RN (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. *Physiol Plant* 100:264–273
- Polle A, Pfirrmann T, Chakrabarti S, Rennenberg H (1993) The effects of enhanced ozone and enhanced carbon dioxide concentrations on biomass, pigments and antioxidative enzymes in spruce needles (*Picea abies* L.). *Plant Cell Environ* 16:311–316
- Poorter H, Remkes C (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia* 83:553–559
- Rao MV, Hale BA, Ormrod DP (1995) Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide. *Plant Physiol* 109:421–432

- Tjoelker MG, Volin JC, Oleksyn J, Reich PB (1995) Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. *Plant Cell Environ* 18:895–905
- Tognetti R, Longobucco A, Raschi A, Miglietta F, Fumagalli I (1999) Responses of two *Populus* clones to elevated atmospheric CO₂ concentration in the field. *Ann For Sci* 56:493–500
- Volin JC, Reich PB, Givnish TJ (1998) Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. *New Phytol* 138:315–325
- Wait DA, Jones CG, Wynn J, Woodward FI (1999) The fraction of expanding to expanded leaves determines the biomass response of *Populus* to elevated CO₂. *Oecologia* 121:193–200
- Wells R, Meredith WR, Williford JR (1986) Canopy photosynthesis and its relationship to plant productivity in near-isogenic cotton line differing in leaf morphology. *Plant Physiol* 82:635–640
- Will RE, Ceulemans R (1997) Effects of elevated CO₂ concentration on photosynthesis, respiration and carbohydrate status of coppice *Populus* hybrids. *Physiol Plant* 100:933–939
- Woodbury PB, Laurence JA, Hudler GW (1994) Chronic ozone exposure alters the growth of leaves, stems and roots of hybrid *Populus*. *Environ Pollut* 85:103–108
- Zelitch I (1982) The close relationship between net photosynthesis and crop yield. *BioScience* 32:796–802