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\textsubscript{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\textsubscript{s} and A. For example, in the O3-sensitive clone (259), inhibition of P\textsubscript{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.
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 (P₅) 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 P₅ 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; Jacob 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 P₅ 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 (C₅) 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 C₅ 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 1⁻¹ between 00700 hours and 19000 hours and 390 µl 1⁻¹ between 19000 hours and 0700 hours; [O₃] averaged 3.4 µl 1⁻¹ between 0700 hours and 19000 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 1⁻¹ from 0700 hours to 19000 hours. The 1-min integrated CO₂ concentration was within 10% of the target concentration 85% (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 1⁻¹ h⁻¹ (sum 0 for 12-h fumigation) during the 1998 growing season and 89.0 µl O₃ 1⁻¹ h⁻¹ during the 1999 growing season, with an average daytime (0700–1900 h) exposure concentration of 55 µl 1⁻¹ in 1998 and 52 µl 1⁻¹ in 1999 compared to the ambient seasonal O₃ dose of 68.5 µl 1⁻¹ and 62.8 µl 1⁻¹ 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 in 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 (<10°C) conditions, which occurred about 30% of the time.

Measurements

Gas exchange

P₅ 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 1⁻¹ for control and elevated O₃, and 560 µl 1⁻¹ 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%). P₅ was measured for all leaves of the current terminal, for two lateral branches (one current-year lateral upper long-shoot (LSI) 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 photosynthesis index (LPI) (Larson and Heberd 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 (R²=0.988 and R²=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 Pₛ and A profiles by LPI were measured on different sets of plants to increase the robustness against plant-to-plant variation. The Pₛ 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:

\[ C_{leaf} = P_{s} \times A \]  (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:

\[ C_{br} = \sum C_{leaf} \]  (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):

\[ C_{plant} = C_{br-terminal}(a \times b \times c) + C_{br-upper}(b \times c) + C_{br-lower}(c \times a) \]  (3)

where \( C_{br-terminal}, C_{br-upper}, C_{br-lower} \) and \( C_{SS} \) are potential \( C_{br} \) 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 (Pₛ) vs. leaf plasto-chron 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 CO₂ (□), elevated O₃ (●) and CO₂+O₃ (■). Means±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.](image-url)
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 $C_{\text{plan}}$ were analyzed separately by clone with the mixed-effects models procedure (PROC MIXED) of SAS (SAS Institute, Cary, N.C., 1996). Replicates and replicates×CO$_2$×O$_3$ were random error terms, with levels of CO$_2$ and O$_3$ 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 CO$_2$, O$_3$ and clone and their interactions, with replicate and replicate×CO$_2$×O$_3$ used as random error terms. For all analyses, the patterns of variation attributable to replicate×treatment effects justified the use of the pooled replicate×CO$_2$×O$_3$ error term. Because plant size strongly influenced total area and $C_{\text{plan}}$, log($D^2$×$H$) 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 (Kozlowski and Pallardy 1997). In our current study, $P_s$ decreased in the mature leaves on all branches of clone 259 when exposed to elevated O$_3$ and CO$_2$+O$_3$, whereas in clone 216 the decrease was observed only in the lower canopy. The O$_3$-induced inhibition of photosynthesis occurred at both ambient and elevated CO$_2$ in clone 259, but only at ambient CO$_2$ 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 CO$_2$+O$_3$-exposed trees of clone 216 than in O$_3$-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. 3 Average branch $P_s$ (Branch $P_s$: a, b), total A per branch class (c, d), leaf area ratio (LAR) on stem volume basis [total A per diameter x height, e, f] and the contribution of each branch class C gain ($\Sigma C_{bin}$) 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). Means+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 CO$_2$ 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 O$_3$, $P_o$ decreased by 29 and 40%, and under elevated CO$_2$+O$_3$ it increased by 15 and 19% for clones 216 and 259, respectively. The amelioration effect of elevated CO$_2$ on O$_3$-induced inhibition supports similar observations across a number of species (e.g., Voin 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 O$_3$-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 exposed 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 (Poore 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.

### Table 1

<table>
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<tr>
<th></th>
<th>Clone 216</th>
<th>Clone 259</th>
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Fig. 4 Average canopy $P_c$ (Canopy $P_c$; a), total A (b) and $C_{plant}$ (c) for two aspen clones, 216 and 259. Means±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 CO$_2$, O$_3$ 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 CO$_2$ and O$_3$ 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 $P_c$. To our surprise, the CO$_2$-induced increase in $P_c$ was counteracted by decreases in A in clone 216. The average effect on $C_{plant}$ of clone 216 under elevated CO$_2$, O$_3$ and CO$_2$+O$_3$ 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 $P_c$ and $C_{plant}$ yet the response of $C_{plant}$ to elevated O$_3$ and CO$_2$+O$_3$ was strongly affected by increased A. The average effect on $C_{plant}$ in elevated CO$_2$, O$_3$ and CO$_2$+O$_3$ was +26%, −10% and +48%, respectively, whereas leaf $P_c$ changed by +39%, −50% and +5%, respectively. The data suggest that the amelioration of the O$_3$ effect at the plant compared to at the leaf level of clone 259 was the result of increased total A at elevated O$_3$ levels. Furthermore, we conclude that the clonal difference in the response of A to elevated CO$_2$ could be responsible for the cardinaly different response of $C_{plant}$ to elevated CO$_2$ 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 CO$_2$ stimulation of $C_{plant}$ in contrast with our previous work with these and other Populus clones, where CO$_2$ 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 CO$_2$-induced increase in leaf $P_c$ was nullified by a decrease in A, and in clone 259 the O$_3$-induced inhibition of leaf $P_c$ (at both ambient and elevated CO$_2$) was ameliorated by increased A at the plant level.

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References


Field CB, Jacob RR, Moorey HA (1995) Stomatal responses to increased CO2: implications for the plant to the global scale. Plant Cell Environ 18:1214–1225


