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Original article

Responses of two *Populus* clones to elevated atmospheric CO_2 concentration in the field

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Abstract – Two poplar clones, hybrid *Populus deltoides* Bartr. Ex Marsh × *Populus nigra* L. (*Populus* × *euramericana*), clone I-214, and *Populus deltoides*, clone Lux, were grown from clonal hardwood cuttings for one growing season in either ambient (360 μmol mol^{-1}) or elevated (560 μmol mol^{-1}) [CO_2] in FACE-system rings at Rapolano Terme (Siena, Italy). Both clones I-214 and Lux exhibited a higher above-ground biomass, photosynthesis at light saturation and instantaneous transpiration efficiency (ITE) in CO_2-enriched air. The elevated [CO_2]-induced responses of clone I-214 included increased investment in branch and leaf biomass, and enhanced stem volume. The elevated [CO_2]-induced responses of clone Lux included an increase in the number of branches and leaf area (which might result in a higher leaf area index, LAI). Photosynthetic acclimation under elevated [CO_2] was found only during the early morning and only in clone I-214. Stomatal conductance and transpiration (on a leaf area basis) decreased under elevated [CO_2] particularly in clone Lux and at the end of the experiment. The effects of elevated [CO_2] on leaf osmotic potential were limited, at least in conditions of non-limiting water availability. Clonal differences in response to elevated [CO_2] should be taken in account when planning future poplar plantations in the forecast warmer and drier Mediterranean sites.(© Inra/Elsevier, Paris.)

biomass / elevated [CO_2] / FACE-system / gas exchange / *Populus* / volume index / water relations

Résumé – Réponses de deux clones de peuplier à l'augmentation de la concentration atmosphérique en CO_2 en conditions extérieures. Deux clones de peuplier, l'hybride *Populus deltoides* Bartr. Ex Marsh × *Populus nigra* L. (*Populus* × *euramericana*), clone I-214, et *Populus deltoides*, clone Lux, ont été cultivés à partir de boutures ligneuses pendant une saison de croissance soit sous la concentration en CO_2 ([CO_2]) ambiante (360 μmol mol^{-1}), soit sous une [CO_2] élevée (560 μmol mol^{-1}) dans des systèmes d'enrichissement en CO_2 à l'air libre (FACE) près de Rapolano Terme (Sienne, Italie). Pour les deux clones, on a observé une stimulation de la croissance en biomasse aérienne, de la photosynthèse en conditions d'éclairement saturant ainsi que de l'efficience de transpiration instantanée (ITE, rapport vitesse d'assimilation CO_2/vitesse de transpiration) en réponse à l'augmentation de [CO_2]. Dans le cas du clone I-214, on a observé une augmentation très marquée de la biomasse des branches et des feuilles ainsi que du volume des tiges en réponse à l'augmentation de [CO_2]. Dans le cas du clone Lux, l'augmentation de la [CO_2] a induit une augmentation du nombre des branches et de la surface foliaire, impliquant une augmentation de l'index foliaire (LAI). Un ajustement négatif de la capacité photosynthétique sous [CO_2] élevé a été observé durant la matinée et uniquement dans le cas du clone I-214. On a noté une diminution de la conductance stomatique pour la diffusion gazeuse et de la transpiration foliaire en réponse à l'augmentation de la [CO_2], en particulier dans le cas du clone Lux et à la fin de l'expérience. Les effets de la [CO_2] élevée sur le potentiel osmotique foliaire étaient très faibles, du moins en conditions de disponibilité en eau non limitante. Nos résultats montrent que les différences de la réponse à l'augmentation de la [CO_2] entre clones doivent être prises en considération pour les plantations futures de peupliers en zone Méditerranéenne.(© Inra/Elsevier, Paris.)

biomasse / échange de gaz / élevé [CO_2] / FACE- système / incrément de volume / *Populus* / relations de l'eau

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1. Introduction

The stimulation of tree growth by short-term exposure to elevated atmospheric CO_2 concentration ($[CO_2]$) has been well documented [1, 3, 8]. This growth enhancement is the result of the stimulation of a number of basic processes underlying overall plant growth and development. Amongst the primary effects of elevated $[CO_2]$ on trees, an increase in photosynthetic rates [6], a reduction of stomatal conductance and decreased leaf transpiration rates [17] are also generally reported for *Populus*, although this is not always the case [1]. Trees grown in elevated $[CO_2]$ can show evidence of downward acclimation of photosynthesis (see [11]), i.e. a decrease in photosynthetic performance as compared with trees grown in ambient $[CO_2]$, when measured under the same conditions, due to intrinsic changes in the photosynthetic machinery. Secondary effects may include growth and several morphological and developmental effects [4, 5]. In Mediterranean environments, the mechanisms for turgor maintenance are particularly important for growth and survival of plants. Osmotic adjustment in leaves of plants exposed to elevated $[CO_2]$ due to enhanced concentrations of soluble sugars [3] might allow them to maintain higher relative water content and turgor pressure [15], thus being able to sustain growth and metabolism during drought [20]. Contrasting results are, however, reported in the literature [21].

Amongst different tree species and genotypes within the same genus and species, physiological and morphological responses to elevated $[CO_2]$ may vary considerably (e.g. [4–7, 9, 16]). Because of the steadily increasing demand for biomass as a renewable energy source [12], there is a need to obtain more information on the likely consequences of the predicted global $[CO_2]$ change on growth, development and productivity of highly productive, short-rotation tree crops such as *Populus* spp. and hybrids. Poplar species and hybrids generally show a large positive response to CO_2 enrichment [2, 4, 5, 10, 16] under more or less controlled environmental conditions (glasshouse cabinets, growth chambers and open-top chambers). There has been discussion of problems associated with interpreting plant responses to elevated $[CO_2]$ when grown in manipulated environments [1]; however, the effects on poplar species in the field have not been elucidated.

The concept of response specificity among tree genera to an increase in $[CO_2]$ [3, 9] has been extended to within genera [4, 5]. The aim of this study was to examine the effects of an increase in $[CO_2]$ on growth characteristics, gas exchange and leaf water relations of two *Populus* clones, differing in crown architecture, plant branchiness, leaf morphology, and resistance to climatic and biotic factors. We exposed clonal cuttings to elevated $[CO_2]$ for one growing season in the field by means of a free air CO_2 enrichment facility, FACE-system.

2. Materials and methods

2.1. Plant materials and planting conditions

Two poplar clones, hybrid *Populus deltoides* Bartr. Ex Marsh × *Populus nigra* L. (*Populus* × *euramericana*) clone I-214 which is relatively resistant to wind, susceptible to *Marssonina brunnea* (Ell. and Ev.) P. Magn. and has a light crown, and *Populus deltoides* clone Lux which is moderately drought resistant and characterized by an open crown with large branches and leaves, were raised from clonal hardwood cuttings (25 cm long) in two FACE-system rings (one CO_2-enriched, 560 μmol mol^{-1}, and one at ambient $[CO_2]$, 360 μmol mol^{-1}) at Rapolano Terme (Siena, Italy). Each ring was divided into four sectors. On 11 April 1997, the cuttings, 52 per ring (i.e. 13 per clone and per sector), were planted at a spacing of 1 m (1 × 1 m). The distance between the two rings was 30 m, and to reduce the boundary effect, each ring was surrounded by several spare plants. CO_2 enrichment started 3 weeks after planting at bud break. Each ring was manually weeded, and all plants were daily drip irrigated throughout the experiment. Because nutrient conditions were near optimal at the start of the experiment, fertilizer was only applied once during the spring.

2.2. FACE-system design

The FACE-system consists of a perforated circular annulus, CO_2 supply components, $[CO_2]$ monitoring components and a PC-based control program. The circular array of multiple emitter port points is a 8-m-diameter toroidal distribution PVC plenum with an internal diameter of 20 cm. A high volume blower injects air into the plenum. Pure CO_2 is mixed with ambient air by placing the outlet immediately after the blower at the level of a flexible pipe which connects the blower to the plenum. The CO_2 injection rate is controlled by a motorized metering valve (Zonemaster, Satchwell Control System, Milano, Italy). CO_2 was supplied 24 h per day. The height of the plenum may be increased by means of extensive legs. This has permitted us to follow the growth of plants and allowed for CO_2 fumigation of the plant canopy up to 2 m in height. A detailed description of the FACE-system can be found in Miglietta et al. [14].

2.3. Growth and biomass measurements

Total plant height (H), basal (D) and apical stem diameter, number of leaves and branches were monitored

throughout the experiment. Stem volume index was estimated for each plant as D^2H and as $(\pi/3)H(R_1^2+R_1R_2+R_2^2)$, where R_1 and R_2 are the radii at the bottom and the top of the stem, respectively.

At the end of August 1997, plants were harvested for analysis of above-ground biomass (stem, branches and leaves). All leaves, branches and stems were oven-dried at 70 °C until constant weight was reached. Leaf weight ratio (LWR) was calculated as the ratio of total leaf biomass to total plant biomass. Leaf area (stem and branches) was determined using an area meter (Li-cor, Lincoln, NE, USA). Specific leaf area (SLA) was calculated as the ratio of total leaf area to total leaf biomass. Leaf area index was estimated from total leaf area per plant and number of plants per clone and per ground area of the ring (50 m^2). Stem diameters of harvested plants were measured at 1-m intervals. For each 1-m stem segment, the volume was calculated based on the formula for the truncated cone as above, but where R_1 and R_2 are the radii at the bottom and the top of each segment, respectively, and H is the length of the segment. Total stem volume was obtained by summing the volumes of all individual stem segments. Branch length per plant was also determined.

2.4. Gas exchange measurements

Gas exchange measurements (light-saturated photosynthesis, stomatal conductance and leaf transpiration) were made using a portable, open-system gas analyser (CIRAS, PP-systems, Hitchin, UK), on intact attached leaves at the same developmental stage. Mature, fully expanded leaves (sixth from the apex) of three plants per sector were sampled. Maximum photosynthetic rate, stomatal conductance and instantaneous transpiration efficiency (ITE, calculated as photosynthesis/transpiration) were measured at about 2-week intervals on sunny days, from 9 to 14 h, under saturating PPFD conditions of about 1 500 µmol m^{-2} s^{-1}. On several occasions, gas exchange was monitored throughout the day. At the end of the experiment, two identical open gas exchange systems (previously cross-calibrated) were used for reciprocal photosynthetic rate determination. The reference [CO_2] was set at 360 and 560 µmol mol^{-1}, and measurements performed in the two rings (two CO_2 treatments) simultaneously (measuring the same leaf at both reference [CO_2] alternately). The measurements were made in the morning at 2-h intervals on labelled leaves; the measurements were first made on setting the measurement [CO_2] at the plant growth [CO_2]; subsequently the measurement [CO_2] was switched from low to high in the case of plants grown at ambient [CO_2] or vice versa in the case of plants exposed to elevated [CO_2].

2.5. Pressure–volume curves

Determination of pressure–volume relationships followed the method of Roberts and Knoerr [18]. Six trees per clone, per treatment were selected for pressure–volume curves. One fully expanded leaf at the same stage of development per tree was sampled on different dates during the summer, recut under distilled water and rehydrated overnight in the dark. During the next day, the leaves were progressively dehydrated by the sap expression method using a pressure chamber. Water was expressed and collected into vials filled with a wad of tissue, which were attached to the exposed petiole, until water no longer emerged from the cut surface. Successive points on the pressure–volume curve (the cumulative volume of expressed water and the corresponding water potential required to express that volume from the tissue) were measured at increments of about 0.1–0.2 MPa. After the pressure chamber readings, leaves were oven-dried at 70 °C to determine their relative water content (RWC, fresh weight – dry weight/saturated weight – dry weight). Leaves were weighed immediately before and after the pressure–volume measurements in order to confirm that more than 90 % of the water removed from the tissue during the experiment was recovered. Water potential components (osmotic potential at saturation and turgor loss point, RWC at turgor loss point) were calculated according to Schulte and Hinckley [19]. Weight-averaged bulk modulus of elasticity was calculated after Wilson et al. [22].

2.6. Statistical analysis

Results were subjected to either a one-way or two-way analysis of variance (ANOVA) to statistically examine the effects of clone and CO_2 treatment.

3. Results

Heights of clone I-214 were significantly ($P < 0.05$) greater in the elevated [CO_2] treatment than in the ambient [CO_2] treatment only during the second half of July and the first week of August (from day of year 196 to 217), but by the end of the experimental treatment the difference in average plant height was small (*figure 1*, upper panel, and *table I*). Clone Lux did not show any difference in height between treatments throughout the experimental period. Clone I-214 was overall taller than clone Lux ($P < 0.05$). Clonal differences in plant height were more pronounced in the elevated [CO_2] treatment. Clone Lux showed a strong ($P < 0.05$) and positive effect of the elevated [CO_2] treatment on the number of branches produced during the growing season (*table I*), and clonal differences were evident only under elevated

Table I. The effect of atmospheric CO_2 concentration (ambient and elevated [CO_2]) on overall plant growth and on leaf area and biomass characteristics of two poplar clones (I-214 and Lux) grown under field conditions for 5 months. All data refer to the end of experiment.

Parameter	Clone I-214 CO_2 treatment 360 µmol mol^{-1}	Clone I-214 CO_2 treatment 560 µmol mol^{-1}	% Δ	Clone Lux CO_2 treatment 360 µmol mol^{-1}	Clone Lux CO_2 treatment 560 µmol mol^{-1}	% Δ
Overall plant growth						
Final height (cm)	212.17 (5.08)	220.33 (5.56)	+ 4 ns	198.25 (5.35)	209.33 (5.30)	+ 6 ns
Stem volume index (cm^3)	918.48 (114.09)	1285.70 (173.85)	+ 40 *	948.10 (106.62)	1003.81 (117.00)	+ 6 ns
Total stem volume (cm^3)	477.87 (33.55)	570.12 (30.93)	+ 19 ns[1]	511.91 (40.53)	541.61 (34.66)	+ 6 ns
Number of branches	12.50 (1.62)	12.58 (1.33)	+ 0.6 ns	13.73 (1.69)	18.91 (2.18)	+ 38 *
Branch lenght (cm)	60.88 (4.50)	68.84 (4.21)	+ 13 ns	55.48 (3.53)	51.62 (3.49)	– 7 ns
Biomass (DW)						
Stem (g)	76.41 (4.92)	101.78 (10.77)	+ 33 *	82.37 (7.85)	108.45 (10.67)	+ 32 ns[1]
Branches (g)	38.83 (6.48)	67.33 (13.20)	+ 73 ns[1]	42.45 (7.03)	56.47 (10.55)	+ 33 ns
Stem + branches (g)	115.24 (10.89)	169.11 (23.24)	+ 47 *	124.82 (13.85)	164.92 (21.02)	+ 32 ns[1]
Leaves (g)	108.48 (13.17)	159.58 (19.43)	+ 47 *	172.69 (17.39)	226.54 (16.14)	+ 31 *
Above-ground biomass (g)	223.72 (18.81)	328.69 (42.41)	+ 47 *	297.51 (26.35)	391.46 (43.52)	+ 32 *
LWR (g g^{-1})	0.48 (0.01)	0.49 (0.01)	+ 0 ns	0.58 (0.01)	0.58 (0.01)	+ 0 ns
Leaf area characteristics						
Number of leaves per plant	64.25 (1.45)	63.92 (1.44)	– 0.5 ns	59.92 (1.44)	58.33 (1.45)	– 3 ns
Leaf area of main stem (m^2)	0.63 (0.04)	0.65 (0.04)	+ 5 ns	0.68 (0.04)	0.83 (0.04)	+ 23 *
Leaf area of branches (m^2)	1.03 (0.21)	1.18 (0.19)	+ 14 ns	1.21 (0.19)	1.46 (0.21)	+ 21 ns
Total leaf area (m^2)	1.66 (0.24)	1.83 (0.22)	+ 10 ns	1.89 (0.22)	2.296 (0.23)	+ 21 ns
SLA (cm^2 g^{-1})	152.10 (9.22)	114.68 (8.41)	– 25 *	109.44 (8.41)	101.09 (8.79)	- 8 ns
Leaf area index	1.60	1.82	+ 14	1.90	2.30	+ 21

Values are the means (± 1 SE, n = 12). Percent change = [(elevated – ambient) / ambient] × 100. Significance is also reported ($P < 0.05$, ns[1] $P < 0.06$).

[CO_2] (clone Lux had a higher number of branches than clone I-214). Branch length was not significantly affected by the CO_2 treatment (*table I*), though under elevated [CO_2] branches tended to be longer ($P < 0.05$) in clone I-214 and shorter in clone Lux.

We observed consistent ($P = 0.055$) increases in stem volume per plant in clone I-214 but weak in clone Lux (*table I*). Stem volume index (both equations) was constantly and significantly ($P < 0.05$) greater in the elevated [CO_2] treatment throughout the growing season only in clone I-214 (*figure 1*, lower panel). The increased stem volume production in the elevated [CO_2] treatment was explained not only by stimulated height growth but also by increased stem diameters. Clone I-214 showed a significantly ($P < 0.05$) larger stem volume index than clone Lux only under elevated [CO_2].

At the end of the experiment there was a significant ($P < 0.05$) treatment difference in above-ground biomass (stem + branches + leaves) in both clones. The increase in above-ground biomass caused by the elevated [CO_2] treatment was proportionally larger for clone I-214 (*table I*). Clone Lux showed consistently ($P < 0.05$) greater total above-ground biomass than clone I-214, regardless of treatment, because of a much greater leaf dry weight. A significant ($P < 0.05$) and positive effect of the CO_2 enrichment was observed on the biomass of all plant parts in clone I-214 (*table I*); the largest effect of elevated [CO_2] was found on branch biomass increase (despite not much change in number or length of branches). Biomass of branches of clone Lux did not increase significantly under elevated [CO_2] despite their increase ($P < 0.05$) in number. The relative stimulation in biomass of other plant parts of clone Lux was smaller compared to that observed for clone I-214. LWR was not affected by elevated [CO_2] in both clones. LWR was higher ($P < 0.05$) for clone Lux than for clone I-214, regardless of the treatment.

The number of leaves per plant did not differ between treatments throughout the study period (time course not shown, *table I*). Leaf area (of both main stem and branches) increased under elevated [CO_2] but not significantly in clone I-214 (*table I*). Such a stimulation was more pronounced in clone Lux and significant ($P < 0.05$) for leaves of the main stem. LAI increased in the CO_2-enriched ring and more evidently for clone Lux. Elevated [CO_2] significantly ($P < 0.05$) decreased SLA in clone I-214 but not in clone Lux (*table I*). Clonal differences were generally evident ($P < 0.05$) in both treat-

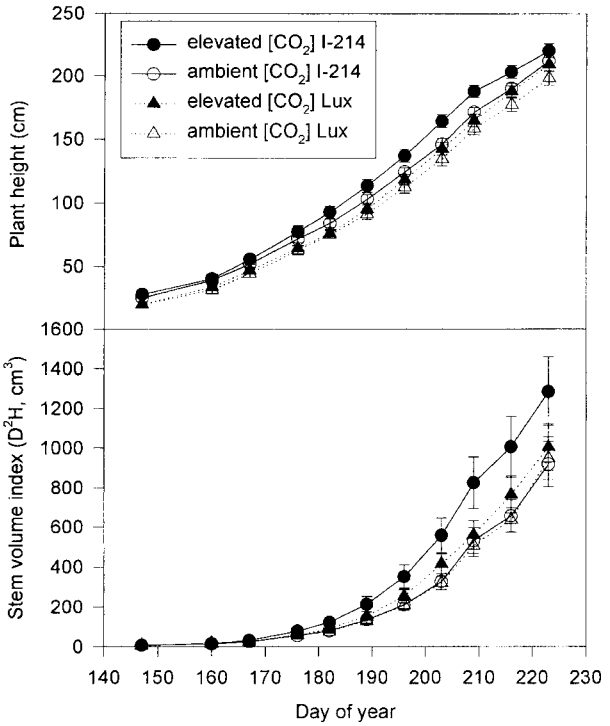

Figure 1. Time course of average plant height (upper panel) and stem volume index (lower panel) for two poplar clones (I–214 and Lux) grown in FACE-systems in the field in ambient or elevated [CO_2]. Vertical bars represent the standard error of the mean (12 replications). Clones and treatments are referred to by symbols in the legend.

ments (relatively more pronounced in ambient [CO_2] for SLA and in elevated [CO_2] for leaf area of main stem).

Photosynthetic rates at light saturation were strongly and similarly enhanced by the elevated [CO_2] treatment in both clones (*table II*). During the course of the summer, photosynthetic rates at light saturation remained stable in the elevated [CO_2] treatment, while at ambient [CO_2] there was a decrease towards the end of the experiment (August). Stomatal conductance and leaf transpiration were generally lower in clone Lux, and were significantly decreased in the elevated [CO_2] treatment, particularly in clone Lux and at the end of the experiment (*table II*). During the course of the summer, stomatal conductance and leaf transpiration decreased regardless of the treatment. As a result of the strong increase in photosynthetic rates and, secondarily, decrease in leaf transpiration, ITE was significantly enhanced by the elevated [CO_2] treatment in both clones (*table II*). The ratio of internal [CO_2] (C_i) to ambient (i.e. external) [CO_2] (C_a) did not change with CO_2 enrichment in both clones (*table I*).

The reciprocal photosynthesis measurements at high [CO_2] (560 μmol mol^{-1}), were significantly ($P < 0.01$) lower for plants grown at elevated [CO_2] only in the early morning and for clone I-214 (*figure 2*); the growth treatment had less of an effect, as for clone Lux, but the interaction between growth treatment and measurement [CO_2] was significant ($P < 0.01$). Photosynthetic rates tended to decrease more steeply during the course of the morning when measurements were made at low [CO_2] (360 μmol mol^{-1}). However, net photosynthesis measured under high [CO_2] was always found to be at least twice ($P < 0.001$) that measured under low [CO_2]. This was true for both growth treatments and for both clones.

There was no significant effect of the elevated [CO_2] treatment on osmotic potentials (at turgor loss point and at saturation) in both clones (*table III*). Elevated [CO_2] significantly reduced the RWC at turgor loss point and increased the weight-averaged bulk modulus of elasticity only in July, and particularly in clone Lux. Clonal differences were generally small, except for the weight-averaged bulk modulus of elasticity. Osmotic potentials (at turgor loss point and at saturation) were lower in August than in July, while the bulk modulus of elasticity increased in August, except for clone Lux in elevated [CO_2].

4. Discussion

Clone I-214 responded positively to elevated [CO_2] by increasing stem volume. The increase was much less evident in clone Lux, indicating that the stimulation by elevated [CO_2] might be affected by the genotype. The increase in stem volume in clone I-214 was primarily associated with increases in stem diameter and secondarily connected with increases in stem height. In fact, height growth stimulation in response to elevated [CO_2] tended to level off by the end of the experiment, while stem volume was still increasing and was significantly larger than control trees. Many experiments conducted in manipulated environments report stimulated height growth in response to elevated [CO_2] for poplar [2, 4, 5, 16]. Our experiment conducted in field conditions confirms the need for extreme caution in extrapolating results obtained in studies in controlled conditions to the real world.

The higher responsiveness of clone I-214 than clone Lux to elevated [CO_2] was also indicated by the relatively larger increase in total branch and leaf biomass (and total above-ground biomass), though clone Lux showed more branches (though tendentially shorter) in response to elevated [CO_2], while clone I-214 did not. However, LWR did not vary much in response to elevated [CO_2] in both clones, so under elevated [CO_2] trees did not

Table II. Light-saturated photosynthesis (A_{max}), stomatal conductance (g_s), leaf transpiration (E), instantaneous transpiration efficiency (ITE) and the ratio of internal [CO_2] to external [CO_2] (C_i/C_a) measured over the summer at the growth [CO_2] on leaves of two poplar clones (I-214 and Lux).

Parameter	Day 1997	Clone I-214 CO_2 treatment 360 µmol mol^{-1}	Clone I-214 CO_2 treatment 560 µmol mol^{-1}	Clone Lux CO_2 treatment 360 µmol mol^{-1}	Clone Lux CO_2 treatment 560 µmol mol^{-1}	*P*-level Clone (C)	*P*-level Treatment (T)	*P*-level C × T
A_{max}	17/06	18.60(1.22)	25.99(1.03)	17.93(0.86)	25.00(0.39)	ns	***	ns
(µmol m^{-2} s^{-1})	10/07	17.19(0.39)	23.28(0.26)	17.53(1.32)	23.21(2.45)	ns	***	ns
	11/07	18.30(0.35)	26.68(0.54)	19.27(0.25)	27.64(0.03)	*	***	ns
	30/07	19.87(0.55)	23.90(0.20)	19.20(2.19)	22.50(0.95)	ns	**	ns
	12/08	06.61(1.93)	27.05(1.34)	6.89(2.55)	23.48(3.80)	ns	***	ns
g_s	17/06	0.65(0.05)	0.68(0.08)	0.63(0.02)	0.47(0.01)	*	ns	*
(mol m^{-2} s^{-1})	10/07	0.50(0.04)	0.50(0.04)	0.45(0.02)	0.38(0.05)	***	*	*
	11/07	0.63(0.06)	0.63(0.05)	0.55(0.02)	0.48(0.04)	***	ns	ns
	30/07	0.31(0.08)	0.17(0.01)	0.21(0.01)	0.17(0.05)	ns	*	ns
	12/08	0.35(0.06)	0.15(0.02)	0.25(0.05)	0.13(0.03)	*	***	ns
E	17/06	7.27(0.37)	7.33(0.62)	7.10(0.42)	6.12(0.29)	ns	ns	ns
(mmol m^{-2} s^{-1})	10/07	9.26(0.08)	9.13(0.13)	9.02(0.01)	8.08(0.35)	***	**	*
	11/07	9.23(0.08)	9.02(0.01)	8.92(0.16)	8.39(0.03)	***	***	ns
	30/07	4.18(0.61)	2.90(0.05)	3.32(0.04)	2.64(0.30)	ns	*	ns
	12/08	5.29(0.13)	3.47(0.15)	4.77(0.35)	2.97(0.27)	*	***	ns
ITE	17/06	2.71(0.38)	3.69(0.25)	2.69(0.32)	4.35(0.19)	ns	***	ns
	10/07	1.86(0.02)	2.57(0.10)	1.95(0.13)	2.86(0.16)	*	***	ns
	11/07	1.98(0.02)	2.97(0.07)	2.17(0.08)	3.30(0.01)	***	***	ns
	30/07	5.08(0.72)	8.43(0.15)	6.05(0.58)	9.10(1.23)	ns	***	ns
	12/08	1.09(0.32)	8.00(0.10)	1.28(0.37)	7.93(0.52)	ns	***	ns
C_i/C_a	17/06	0.72(0.03)	0.75(0.01)	0.72(0.02)	0.70(0.01)	ns	ns	ns
	10/07	0.71(0.01)	0.73(0.01)	0.69(0.01)	0.70(0.00)	**	ns	ns
	11/07	0.70(0.01)	0.74(0.00)	0.70(0.00)	0.69(0.01)	*	ns	ns
	30/07	0.66(0.03)	0.62(0.03)	0.67(0.04)	0.69(0.04)	ns	ns	ns
	12/08	0.73(0.01)	0.72(0.02)	0.75(0.00)	0.70(0.03)	ns	ns	ns

Values are the means (± 1 SE, n = 6–30), and significance (P) (LSD test, $P < 0.05$) is shown by the symbols: ns = not significant, * = 0.05, ** = 0.01, *** = 0.001.

Table III. Leaf tissue water relation parameters, as derived from pressure–volume curves on leaves of two poplar clones (I-214 and Lux) grown under field conditions for five months at elevated and ambient atmospheric [CO_2].

Day 1997	Parameter	Clone I-214 CO_2 treatment 360 µmol mol^{-1}	Clone I-214 CO_2 treatment 560 µmol mol^{-1}	Clone Lux CO_2 treatment 360 µmol mol^{-1}	Clone Lux CO_2 treatment 560 µmol mol^{-1}	*P*-level Clone (C)	*P*-level Treatment (T)	*P*-level C × T
05/07	RWC_{tlp} (%)	89.90(0.80)	90.80(0.10)	86.60(0.40)	88.50(0.70)	***	**	ns
	π_{tlp} (MPa)	−1.14(0.05)	−1.11(0.05)	−1.07(0.09)	−1.05(0.05)	ns	ns	ns
	π_{sat} (MPa)	−0.79(0.02)	−0.76(0.03)	−0.77(0.04)	−0.90(0.07)	ns	ns	ns
	ε (MPa)	8.08(0.72)	8.46(0.45)	5.72(0.21)	7.95(0.76)	**	**	ns
07/08	RWC_{tlp} (%)	91.30(0.50)	90.10(1.10)	90.20(0.60)	89.20(1.10)	ns	ns	ns
	π_{tlp} (MPa)	−1.26(0.03)	−1.25(0.07)	−1.23(0.04)	−1.16(0.04)	ns	ns	ns
	π_{sat} (MPa)	−1.00(0.04)	−1.04(0.11)	−0.94(0.08)	−0.82(0.03)	*	ns	ns
	ε (MPa)	11.79(1.06)	10.65(0.90)	9.85(1.19)	7.81(0.76)	**	ns	ns

Values are the means (± 1 SE, n = 6), and significance (P) (LSD test, $P < 0.05$) is shown by the symbols: ns = not significant, * = 0.06, ** = 0.05, *** = 0.001.

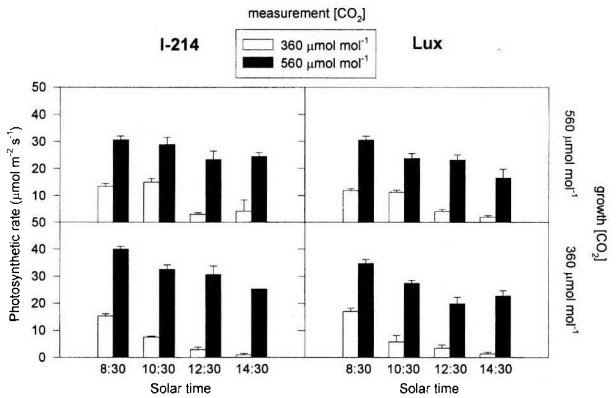

Figure 2. Time course of photosynthesis during morning hours (mid-August) of two poplar clones (I–214 and Lux) grown in FACE-systems in the field in ambient or elevated [CO_2]. Measurements were made in low [CO_2] (360 µmol mol^{-1}) and under high [CO_2] (560 µmol mol^{-1}). Vertical bars represent the standard error of the mean (6–8 replications).

become more efficient in terms of the amount of biomass produced per unit leaf. Nevertheless, clone I-214 showed a pronounced and significant decrease in SLA under the CO_2 enrichment.

The total number of leaves per plant did not vary between treatments in both clones; contrasting results are reported in the literature for poplar clones [4, 16]. Although net photosynthesis per unit leaf area was significantly and similarly increased in both clones in the elevated [CO_2] treatment, there was a clonal difference with respect to the effects of the CO_2 enrichment on total leaf area. Increases in leaf area (main stem leaves and secondarily branch leaves) under elevated [CO_2] were more consistent for clone Lux, and this was reflected in a higher LAI. Differences between clones in leaf area increases under elevated [CO_2] were reported by Ceulemans et al. [5].

Increases in the photosynthetic rate under elevated [CO_2] have been reported in poplar [13], as well as decreases in stomatal conductance and leaf transpiration [17]. As a result of the increase in assimilation rate and decrease in leaf transpiration, ITE of leaves increased at elevated [CO_2] in clone Lux. ITE also increased in clone I-214, despite not much reduction in leaf transpiration by elevated [CO_2]. This increase is a common response in woody species exposed to elevated [CO_2] [3], but differences between genotypes can be important in planning future poplar plantations in Mediterranean environments. In particular, the proportionally lower leaf transpiration in clone Lux under elevated [CO_2] may allow this genotype to endure drought by better modulating water usage; but this advantage may be offset by the increased foliage area. The observed decreased stomatal conductance and leaf transpiration, regardless of the treatment, during the course of the summer may be related to the increase in VPD (vapour pressure deficit). The ratio of internal [CO_2] to external [CO_2] was not affected by CO_2 enrichment, even though at elevated [CO_2] intercellular [CO_2] should rise if stomata close consistently [8], suggesting that there was little or no stomatal acclimation to elevated [CO_2] in these poplar clones.

The decrease in photosynthesis in control trees of both clones in August was not observed in trees under elevated [CO_2]. Because gas diffusion through stomata was not responsible for this difference, it is possible to hypothesize that the photosynthetic machinery of leaves under elevated [CO_2] can maintain its efficiency for longer either under optimal or stress conditions (e.g. heat stress). Kalina and Ceulemans [13] observed, under non-limiting conditions of N and P content, an increased photochemical efficiency of PSII and a build up of light-harvesting complexes of PSII in two poplar clones in response to elevated [CO_2].

There is evidence in many tree species for acclimation (or down-regulation) of photosynthesis when grown long term in elevated [CO_2] [11]. We found an indication of acclimation only during the early morning and only in clone I-214. Gaudillère and Mousseau [10] observed a lack of early acclimation in clone I-214 which was attributed to its high sink strength (i.e. roots). Similarly, no down regulation of photosynthesis was found by Kalina and Ceulemans [13] in two hybrid poplar clones (Beauprè and Robusta). Ceulemans et al. [6], studying poplar hybrids, observed some acclimation of photosynthesis in a glasshouse experiment but not in open-top chambers. This experiment shows that negative acclimation to elevated [CO_2] can hardly be observed in the field. Nevertheless, down-regulation of photosynthesis may sometimes be observed depending on the time of day and the genotype selected for measurements.

The effect of elevated [CO_2] on leaf osmotic potentials was limited. There was a significant increase in weight-averaged bulk modulus of elasticity and RWC at turgor loss point in response to elevated [CO_2], but only in July and consistently only in clone Lux. A lack of marked responses to elevated [CO_2] is also reported by Tschaplinski et al. [21] for American sycamore and sweetgum, and some effects for sugar maple seedlings. In contrast, Morse et al. [15] and Tognetti et al. [20] reported a lowering of osmotic potential in grey birch seedlings, and holm and downy oak trees, respectively, growing under elevated [CO_2]. The rapid growth rate of the two poplar clones in well-watered conditions might have avoided the solute accumulation under high [CO_2].

More inelastic tissue (higher bulk modulus of elasticity) in leaves of clone Lux in July may help trees in elevated [CO_2] to generate a favourable water potential gradient from the soil to the plant, at lower stomatal conductance in mid-summer.

We conclude that the two clones responded positively to elevated [CO_2], both exhibiting a higher above-ground biomass, photosynthesis at light saturation and ITE in CO_2-enriched air, but that the degree of the response varied with the clone and the parameter considered. Indeed, stomatal conductance and transpiration decreased under elevated [CO_2] particularly in clone Lux and at the end of the experiment. The CO_2-induced responses of clone I-214 included increased investment in branch and leaf biomass, and enhanced stem volume. The CO_2-induced responses of clone Lux included an increase in the number of branches and leaf area (which might result in a higher LAI). We found an indication of photosynthetic acclimation under elevated [CO_2] only during the early morning and only in clone I-214. CO_2 enrichment did not induce osmotic adjustment in both clones, at least in well-watered conditions. Clonal differences in response to elevated [CO_2] should be taken into account when planning future poplar plantations in warmer and drier Mediterranean sites as foreseen by the Global Circulation Model.

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