

## Growth dynamics, transpiration and water-use efficiency in *Quercus robur* plants submitted to elevated CO<sub>2</sub> and drought

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**Summary** — Seedlings of pedunculate oak (*Quercus robur* L) were grown for one growing season under ambient (350  $\mu\text{mol mol}^{-1}$ ) and elevated (700  $\mu\text{mol mol}^{-1}$ ) atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]) either in well-watered or in droughted (the water supply was 40% of the well-watered plants transpiration in both [CO<sub>2</sub>] conditions). In the droughted conditions, gravimetric soil water content (SWC) was on average 4  $10^{-2}$  g g<sup>-1</sup> lower under elevated [CO<sub>2</sub>]. In well-watered conditions, biomass growth was 39% higher in the elevated [CO<sub>2</sub>] treatment than under ambient [CO<sub>2</sub>]. However relative growth rate (RGR) was stimulated by the elevated [CO<sub>2</sub>] only for 17 days, in July, at the end of the stem elongation phase (third growing flush), which corresponded also to the phase of maximum leaf expansion rate. Both the number of leaves per plant and the plant leaf area were 30% higher in the elevated [CO<sub>2</sub>] treatment than under ambient [CO<sub>2</sub>]. In the droughted conditions, no significant enhancement in biomass growth and in plant leaf area was brought about by the elevated [CO<sub>2</sub>]. Transpiration rate was lower in the elevated [CO<sub>2</sub>] conditions, but whole plant water use was similar in the two [CO<sub>2</sub>] treatments, reflecting a compensation between leaf area and stomatal control of transpiration. Transpiration efficiency (W = biomass accumulation/plant water use) was improved by 47% by the elevated [CO<sub>2</sub>] in well-watered conditions but only by 18% in the droughted conditions. Carbon isotope discrimination ( $\Delta$ ) was decreased by drought and was increased by the elevated [CO<sub>2</sub>]. A negative linear relationship was found between transpiration efficiency divided by the atmospheric [CO<sub>2</sub>] and  $\Delta$ , as predicted by theory.

**elevated CO<sub>2</sub> / growth / leaf gas exchange / water-use efficiency / carbon isotope discrimination**

**Résumé** — Dynamique de croissance, transpiration et efficacité d'utilisation de l'eau de plants de *Quercus robur* soumis à une concentration élevée en CO<sub>2</sub> et à la sécheresse. Des semis de chêne pédonculé (*Quercus robur* L) ont été soumis, durant leur première saison de végétation, à des concentrations atmosphériques en CO<sub>2</sub> ([CO<sub>2</sub>]) ambiantes (350  $\mu\text{mol mol}^{-1}$ ) ou doublées (700  $\mu\text{mol mol}^{-1}$ )

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$\text{mol}^{-1}$ ) en conditions de bonne alimentation hydrique ou de sécheresse (fourniture d'eau égale à 40 % de la transpiration des plants bien irrigués pour chacune des conditions de  $[\text{CO}_2]$ ). L'humidité pondérale du sol (SWC) était en moyenne inférieure de  $4 \cdot 10^{-2} \text{ g g}^{-1}$  sous  $[\text{CO}_2]$  élevé comparativement à la  $[\text{CO}_2]$  ambiante. En conditions hydriques favorables, l'augmentation de la concentration en  $\text{CO}_2$  est à l'origine d'une stimulation de la croissance de 39 %. Cependant le taux de croissance relative (RGR) n'est stimulé par l'augmentation de la concentration en  $\text{CO}_2$  qu'au cours d'un intervalle de temps de 17 jours, en juillet, correspondant à la fin de la phase d'élongation de la tige (troisième flush de croissance) et à la phase de vitesse d'expansion foliaire maximale. Le nombre de feuilles ainsi que la surface foliaire par plant sont augmentés de 30 % par l'augmentation de la concentration en  $\text{CO}_2$ . En conditions de sécheresse, aucune stimulation de croissance pondérale ni de surface foliaire par plant ne sont observées en réponse à l'augmentation de la concentration atmosphérique en  $\text{CO}_2$ . Le taux de transpiration est réduit par l'augmentation de la concentration en  $\text{CO}_2$ , mais la transpiration totale par plant n'est pas affectée par la concentration atmosphérique en  $\text{CO}_2$ , traduisant une compensation entre augmentation de surface foliaire et fermeture stomatique. L'efficacité de transpiration ( $W = \text{accumulation de biomasse/eau transpirée}$ ) est augmentée de 47 % par l'augmentation de la concentration en  $\text{CO}_2$  en régime hydrique favorable et seulement de 18 % en régime hydrique limitant. La discrimination isotopique du carbone ( $\Delta$ ) des plants est diminuée par la sécheresse et augmentée par le doublement de la concentration en  $\text{CO}_2$ . Une relation linéaire négative entre l'efficacité de transpiration divisée par la concentration atmosphérique en  $\text{CO}_2$  et  $\Delta$  est observée conformément à la théorie (équation 5).

**enrichissement en  $\text{CO}_2$  / croissance / échanges gazeux foliaires / efficacité d'utilisation de l'eau / discrimination isotopique du carbone**

## INTRODUCTION

Because increasing atmospheric  $\text{CO}_2$  concentration ( $[\text{CO}_2]$ ) generally stimulates  $\text{CO}_2$  assimilation while reducing leaf transpiration rates in  $\text{C}_3$  plants, it is often thought that increasing  $[\text{CO}_2]$  will alleviate the impacts of drought constraints in this group of species (Chaves and Pereira, 1992; Tyree and Alexander, 1993). However, experimental data on the effects of elevated  $[\text{CO}_2]$  on plant transpiration and growth responses to drought remain scarce, particularly in forest tree species as it has been stressed by Ceulemans and Mousseau (1994) and Overdieck and Forstreuter (1994). Furthermore, existing data (Guehl et al, 1994; Picon et al, 1996) show that interspecific differences in these responses exist among forest trees.

In the present work, we have assessed the interactive effects of elevated  $[\text{CO}_2]$  and drought on aerial elongation growth, biomass accumulation, transpiration and water-use efficiency in pedunculate oak (*Quercus robur* L), a species of major area

representativity in western and central Europe. The objective of this study was to relate the biomass growth and water-use efficiency responses to elevated  $[\text{CO}_2]$  to the characteristics of elongation growth and leaf area expansion. In *Q. robur*, as in other *Quercus* species, aerial growth proceeds in successive flushes. It has been suggested by several authors (Kaushal et al, 1989; Norby and O'Neill, 1991; Ceulemans and Mousseau, 1994) that the growth pattern could constitute a relevant rationale for the interpretation of interspecific differences in the growth responses to elevated  $[\text{CO}_2]$ .

Another emphasis in this study was to assess the time-integrated water-use efficiency and its physiological determinants (eg, leaf gas exchange) by using the carbon isotope discrimination approach. Carbon isotope discrimination ( $\Delta$ ) – a dimensionless measure of plant  $^{13}\text{C}$  depletion as compared with atmospheric  $\text{CO}_2$  – provides time-integrated estimates of the ratio  $\text{CO}_2$  assimilation rate/leaf conductance (plant intrinsic water-use efficiency) (Farquhar and Richards, 1984; Farquhar et al, 1989). This

approach has been used in a few cases only (Guehl et al, 1994) in elevated CO<sub>2</sub> studies so far.

## MATERIALS AND METHODS

### *Plant material and experimental setup*

On 15 April (day of year 105), acorns of pedunculate oak (*Quercus robur* L., provenance Manoncourt, northeastern France) were germinated in 5 L (19.5 cm height, 20 cm diameter) cylindrical containers filled with a peat and sand mixture (1/1; v/v). At the same time, a complete fertilization (5 kg m<sup>-3</sup> of slow release fertilizer, Nutricote; N/P/K/13/13/13 + trace elements) was given to provide optimal nutrition conditions over all the experimental period. The plants were placed in two transparent (50 µm thick, 80% light transmission) polypropylene tunnels (5 x 3 x 2.3 m) located in a glasshouse. In the tunnels, [CO<sub>2</sub>] was maintained at 350 ± 30 µmol mol<sup>-1</sup> and 700 ± 50 µmol mol<sup>-1</sup> by an injection of CO<sub>2</sub> from a cylinder (100% CO<sub>2</sub>). [CO<sub>2</sub>] inside the tunnels was measured continuously by means of two infrared analysers (ADC-225-MK3, UK) and controlled by an automated regulation system. The tunnels were equipped with a fan that provided an outgoing airstream in order to remove i) excessive humidity due to the plant transpiration during the day and ii) excessive [CO<sub>2</sub>] due to the plant respiration during the night. The outgoing airstream was compensated by an ingoing airstream from the glasshouse. Each tunnel was also equipped with an air conditioner. Air temperature (*T*<sub>a</sub>), photosynthetic photon flux density (*I*<sub>p</sub>) and relative humidity (RH) inside the tunnels were measured continuously. Air temperatures ranged from 11 °C (minimum night temperature) to 30 °C (maximum diurnal temperature) during the experimental period. Air relative humidity ranged from 40 to 70% during the day. The plants were grown under natural photoperiod. In sunny conditions, *I*<sub>p</sub> was about 1 200 µmol m<sup>-2</sup> s<sup>-1</sup> at plant level (upper leaves). Linear regressions between the two tunnels were determined for *T*<sub>a</sub>, *I*<sub>p</sub> and RH and were not different (*P* < 0.05) from 1:1 lines.

From the beginning of the experiment, 43 plants of the ambient [CO<sub>2</sub>] treatment and 40 plants of the elevated [CO<sub>2</sub>] treatment were maintained well-watered by restoring soil water content

to field capacity twice a week. From d188 to d320, in each [CO<sub>2</sub>], ten plants were subjected to a drought treatment by reducing their water supply to 40% of the average amount of water used by the well-watered plants. Watering was performed every 3 or 4 days simultaneously in all treatments. In both watering regimes and [CO<sub>2</sub>], plant transpiration was assessed gravimetrically. Soil water evaporation was limited by covering the soil surface with waxed cardboard disks.

For both CO<sub>2</sub> treatments, eight to 12 plants were harvested for biomass determinations on days of the year 190 (9 July), 207 (26 July), 288 (15 October) and 320 (16 November).

Stem height and the length of all leaves were measured weekly. On the dates of the biomass determinations, linear regressions between total leaf length and actual plant leaf area were established. A unique relationship was obtained for all the experimental treatments and dates:

$$\text{Plant leaf area (cm}^2\text{)} = 0.0312 \times \text{total plant leaf length (cm)} - 13.90, r^2 = 0.85, P < 0.0001.$$

Daily leaf transpiration rate (g cm<sup>-2</sup> day<sup>-1</sup>) was calculated by dividing plant transpiration rate by the calculated leaf area.

For the four harvest dates, leaf, stem and root dry weights were measured. Relative growth rate (RGR, day<sup>-1</sup>) between two successive dates was determined as :

$$\text{RGR} = \frac{\ln DW_2 - \ln DW_1}{d_2 - d_1} \quad [1]$$

where DW<sub>2</sub> and DW<sub>1</sub> are the mean plant dry weights for two successive harvest dates (*d*<sub>1</sub> and *d*<sub>2</sub>). Plant specific leaf area (SLA) and leaf area ratio (LAR) were determined for the different harvest dates as the ratio leaf area/leaf dry weight and the ratio leaf area/plant dry weight, respectively.

Transpiration efficiency, defined on a mass basis (*W*, g g<sup>-1</sup>), was calculated at the end of the experiment by dividing the plant dry weight by the plant transpirational water consumption.

### *Gas-exchange measurements*

Carbon dioxide assimilation rate (*A*, µmol m<sup>-2</sup> s<sup>-1</sup>) and leaf conductance for water vapour (*g*,

mmol m<sup>-2</sup> s<sup>-1</sup>) were periodically measured in situ with a portable system (Li-Cor 6200, Lincoln, NE, USA). Inter-cellular [CO<sub>2</sub>] ( $c_i$ , μmol mol<sup>-1</sup>) was calculated by the Li-Cor software from  $A$  and  $g$  using the classical equations of CO<sub>2</sub> diffusion through the stomata. Plant intrinsic water-use efficiency was determined as the ratio of CO<sub>2</sub> assimilation rate to leaf conductance for water vapour ( $A/g$ , mmol mol<sup>-1</sup>). Gas-exchange was measured on 11 different dates in the well-watered treatments and on five different dates in the droughted treatments. During the measurements, one fully expanded leaf of the last developed flush was enclosed into the 4 L chamber of the Li-6200. Before gas exchange measurements, a print of the leaves was taken and leaf area was determined with a ΔT area meter (ΔT Devices, Cambridge, UK).

### Carbon isotope discrimination and leaf nitrogen concentration

Within each CO<sub>2</sub> treatment, all the leaves of the plants harvested on d320 were oven-dried (70 °C for 48 h) and finely ground for δ<sup>13</sup>C and total nitrogen concentration determinations. For the leaf δ<sup>13</sup>C measurements, about 3 mg of the powder were combusted in He + 3% O<sub>2</sub> at 1 050 °C and analysed by isotopic mass spectrometry (Finnigan Delta S mass spectrometer, Finnigan-Mat). Carbon isotope composition was expressed as the <sup>13</sup>C/<sup>12</sup>C ratio relative to that of the Pee Dee Belemnite standard. The resulting δ<sup>13</sup>C values were used to calculate isotopic discrimination as:

$$\Delta (\text{‰}) = \frac{\delta_a - \delta_p}{1\ 000 + \delta_p} \times 1\ 000 \quad [2]$$

where δ<sub>a</sub> and δ<sub>p</sub> refer to the isotopic compositions of atmospheric [CO<sub>2</sub>] and of the plant material, respectively. In our experimental conditions, δ<sub>a</sub> was different between the two tunnels due to the predominant industrial (CO<sub>2</sub> cylinder) origin of CO<sub>2</sub> in the elevated [CO<sub>2</sub>] tunnel (Guehl et al, 1994; Picon et al, 1996). In order to calculate Δ, the time-integrated δ<sub>a</sub> values of the two tunnels were assessed by measuring δ<sub>p</sub> in *Zea mays*, a C<sub>4</sub> plant which was grown in both [CO<sub>2</sub>] during the experimental period. According to Marino and McElroy (1991), δ<sub>p</sub> in *Zea mays* is linked to δ<sub>a</sub> by the following equation:

$$\delta_a = 3.276 + \delta_p \quad [3]$$

These measurements yielded δ<sub>a</sub> values of -14.2 and -29.8‰ under 350 and 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>], respectively.

Carbon isotope discrimination by the plant (Δ) is linearly related to the time-integrated value of the ratio of inter-cellular to ambient [CO<sub>2</sub>] ( $c_i/c_a$ ) and thus to plant intrinsic water-use efficiency ( $A/g$ ) (Farquhar et al, 1989):

$$\Delta = a + (b - a) \frac{c_i}{c_a} = 1 - \frac{1.6 \cdot 10^{-3} A}{c_a g} \quad [4]$$

where  $a$  and  $b$  are the discrimination coefficients against <sup>13</sup>CO<sub>2</sub> during diffusion into the leaf and carboxylation, respectively. The coefficients  $a$  and  $b$  are estimated to be 4.4 and 27, respectively (Farquhar et al, 1989).

Transpiration efficiency is related to Δ by (Farquhar and Richards, 1984):

$$W = \frac{c_a}{1.6 \cdot 10^{-3} v} \frac{b - \Delta}{b - a} \frac{12(1 - \Phi)}{18 k} \quad [5]$$

where  $c_a$  (μmol mol<sup>-1</sup>) is the mean ambient [CO<sub>2</sub>] during the growing period and  $v$  (mmol mol<sup>-1</sup>) is the mean value of leaf-to-air water vapor concentration difference during the growing period.

For leaf nitrogen concentration determinations, 200 mg of powdered material were oxidized in NH<sub>4</sub><sup>+</sup> with H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub> and a catalyser (K<sub>2</sub>SO<sub>4</sub> + S<sub>e</sub>) up to 330 °C (Kjeldahl oxidation) and determined by colorimetry with an autoanalyser II Technicon.

One- or two-way analysis of variance (ANOVA followed by Fisher's PLSD test) was used to assess the significance of treatment effects.

## RESULTS

### Seasonal course of transpiration and soil water content

The seasonal course of daily leaf transpiration rate and whole plant transpiration rate of the well-watered plants followed a rise-

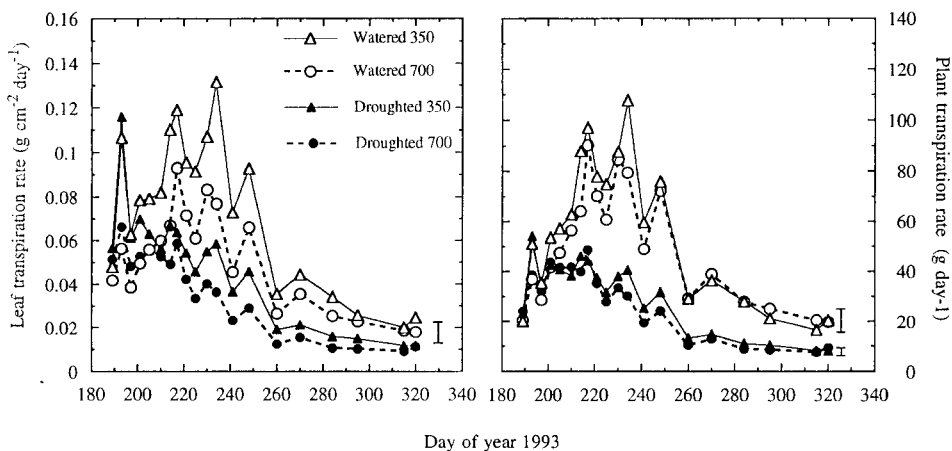
and-fall pattern (fig 1) primarily corresponding to the changes in day length and in daily potential evapotranspiration (data not shown). All during the measurement period, and in both watering regimes, leaf transpiration rate was reduced in the elevated [CO<sub>2</sub>] treatment (fig 1), whereas whole plant transpiration rate as well as time-integrated plant transpiration (table I) were not significantly affected by the [CO<sub>2</sub>] treatment.

The depressing effect of drought on leaf transpiration rate and plant transpiration rate appeared from d210 in both CO<sub>2</sub> treatments (fig 1) when gravimetric soil water content (SWC) had dropped below 35 10<sup>-2</sup> g g<sup>-1</sup> in the droughted plants of both CO<sub>2</sub> treatments (fig 2). From d225 to the end of the experiment, SWC in the droughted conditions was on average 4 10<sup>-2</sup> g g<sup>-1</sup> lower in the elevated [CO<sub>2</sub>] than in the ambient [CO<sub>2</sub>] treatment (fig 2). At the end of the growing season, on d320, predawn leaf water potential ( $\Psi_{wp}$ ) was also 0.4 MPa lower in the droughted and elevated [CO<sub>2</sub>] than in the droughted and ambient [CO<sub>2</sub>] conditions (table I). It must be emphasized that the more severe drought conditions observed here under elevated [CO<sub>2</sub>] are merely a con-

sequence of the type of control of water stress – in which transpiration and not soil water status was controlled – and do not reflect an effect of [CO<sub>2</sub>] per se.

### ***Stem elongation and leaf area expansion dynamics***

The plants generally produced three aerial growth flushes during the experimental period (table II). Only one plant in the well-watered and elevated [CO<sub>2</sub>] treatments produced four flushes. No significant CO<sub>2</sub> effect on stem elongation was observed for the first flush between d121 and d153 (table II), which probably reflects the predominant contribution of acorn carbon reserves mobilization. For the second (d151 to d173) and the third (d190 to d216) growth flushes, a clear stimulation of the stem elongation rate (fig 3) and of total flush length (table II) by elevated [CO<sub>2</sub>] was observed in the well-watered conditions. In the droughted conditions, stem elongation rate of the third flush was increased by the elevated [CO<sub>2</sub>] on d210 (fig 3). The drought treatment, which started on d188, decreased the elon-



**Fig 1.** Seasonal time course of daily leaf transpiration rate and plant transpiration rate determined gravimetrically. Vertical bars denote mean  $\pm$  1 SEM values ( $n = 8$  to 10). The onset of the drought treatment corresponded to d188.

**Table I.** Average values of season-integrated transpiration and leaf predawn water potential measured at the end of the growing season in *Quercus robur* plants grown in well-watered and droughted conditions under 350 and 700  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentrations.

Treatments	Watered		Droughted		ANOVA		
	350	700	350	700	$\text{CO}_2$	$\text{H}_2\text{O}$	x
Plant transpiration ( $\text{g plant}^{-1} \text{ season}^{-1}$ )	6 142	5 654	3 218	3 029	ns	**	ns
Leaf predawn water potential (MPa)	-0.52	-0.49	-1.19	-1.59	ns	**	*

The significance of  $\text{CO}_2$  or water supply regime effects is indicated for the different parameters; ns: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

**Table II.** Average number of growth flushes, stem length and leaf characteristics of *Quercus robur* plants harvested on day of year 320 and grown in well-watered or droughted conditions under 350 and 700  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentrations.

Treatments	Watered		Droughted		ANOVA		
	350	700	350	700	$\text{CO}_2$	$\text{H}_2\text{O}$	x
No of growth flushes	3	3.13	3	3	ns	ns	ns
Stem length (mm)							
1st flush	124	140	132	148	ns	ns	ns
2nd flush	116	224	112	228	**	ns	ns
3rd flush	220	301	205	210	*	*	*
4th flush	0	19	0	0	ns	ns	ns
Total	460	684	449	586	**	ns	ns
No of leaves $\text{plant}^{-1}$	26	33.7	24.3	31.5	**	ns	ns
Leaf area ( $\text{cm}^2 \text{ plant}^{-1}$ )	819	1 081	692	831	**	*	ns
Leaf nitrogen concentration ( $10^{-2} \text{ g g}^{-1}$ )	2.9	2.68	2.25	2.36	ns	**	ns

The significance of  $\text{CO}_2$  or water supply regime effects is indicated for the different parameters; ns: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

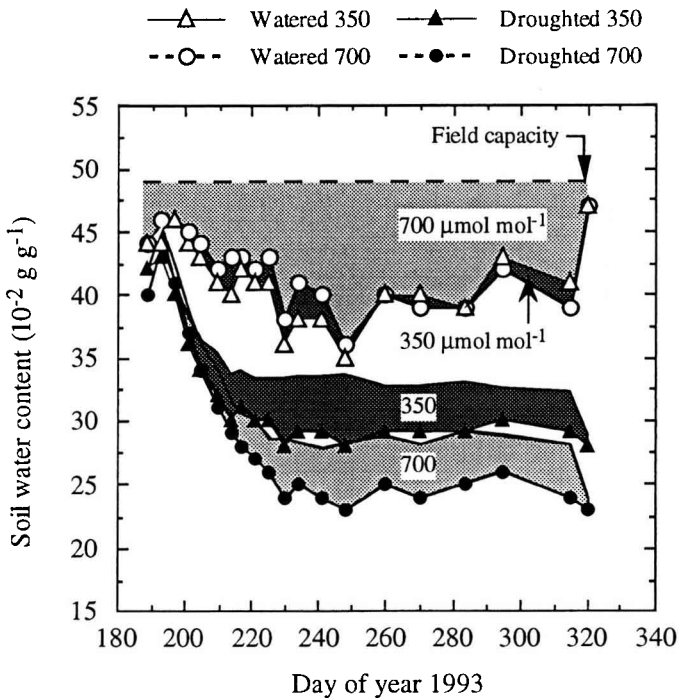
gation rate as well as the total length of the third flush only in the elevated [CO<sub>2</sub>] conditions (fig 3, table II). At the end of the growing season, the stem height of the plants grown under high [CO<sub>2</sub>] were 49 and 31% higher than those grown under ambient [CO<sub>2</sub>], in well-watered and droughted conditions, respectively (table II). No significant drought effect on total stem height was observed (table II).

Maximum leaf expansion rate occurred in all treatments between d160 and d200 (fig 4). Leaf expansion ceased on d210 in all treatments but, in the well-watered and elevated [CO<sub>2</sub>] treatments, it went on until d240. At the end of the season, the number of leaves per plant as well as plant leaf area were about 30% higher in the elevated [CO<sub>2</sub>] in well-watered conditions (fig 4, table II). In the droughted conditions, the number of leaves per plant was 30% higher under elevated than under ambient [CO<sub>2</sub>], whereas

plant leaf area was not significantly different between the [CO<sub>2</sub>] treatments (table II).

### Biomass growth

On d190, no CO<sub>2</sub> effect on plant dry weight was observed (fig 5). On d207, plant dry weight was 44% higher under elevated [CO<sub>2</sub>] than under ambient [CO<sub>2</sub>] (fig 5), which was associated with a two-fold higher value of RGR under elevated [CO<sub>2</sub>] between d190 and d207 (table III). This RGR stimulation was not associated with higher values of LAR (table III), the structural component of RGR (Hunt, 1982) and is therefore to be ascribed to a stimulation of net assimilation rate (NAR), the functional component of RGR. After d207, no difference in RGR was observed between the [CO<sub>2</sub>] treatments (table III). After 7 months in well-watered conditions (d320), the growth stimulation



**Fig 2.** Seasonal time course of soil water content (SWC) in the different experimental treatments. The dashed areas correspond to the range of fluctuations of SWC between successive waterings. For a given CO<sub>2</sub> concentration, the droughted plants were given 40% of the water used by the well-watered plants.

**Table III.** Average values of leaf area ratio, specific leaf area, root/shoot biomass ratio and mean relative growth rate of *Quercus robur* plants on the different harvest days, grown in well-watered and droughted conditions under 350 and 700  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentrations. The onset of drought treatment corresponded to d188.

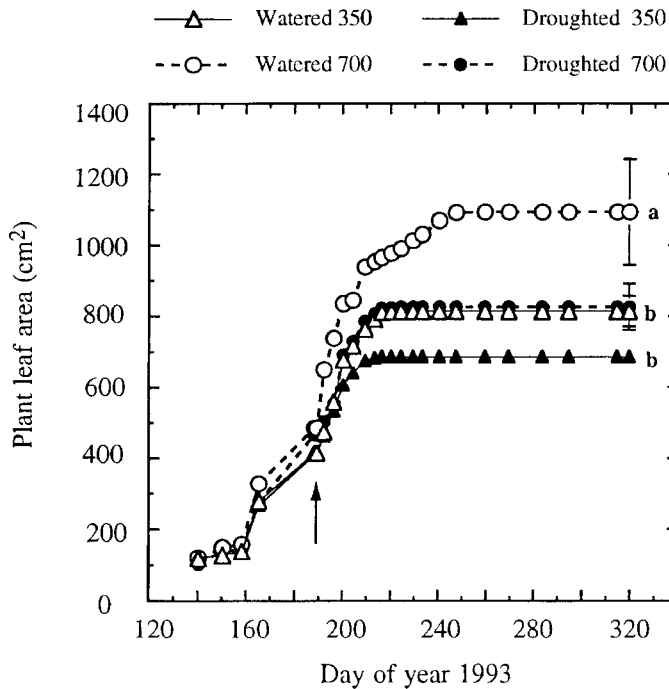
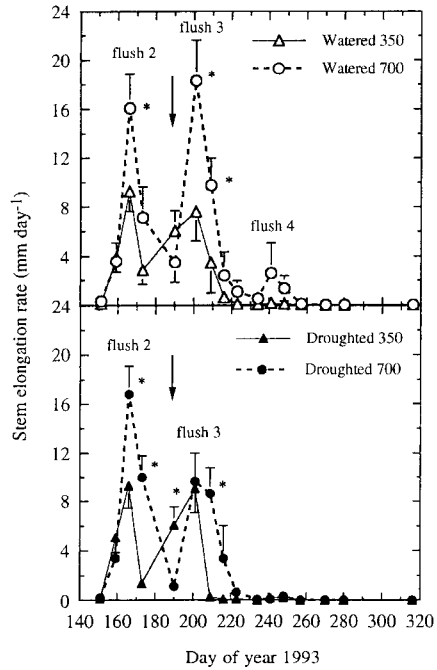
Day of year 1993	190			207			288			320						
	Watered		ANOVA	Watered		ANOVA	Watered		ANOVA	Droughted		ANOVA				
	350	700	$\text{CO}_2$	350	700	$\text{CO}_2$	350	700	$\text{CO}_2$	350	700	$\text{CO}_2 \text{ H}_2\text{O} \times$				
Leaf area ratio ( $\text{m}^2 \text{ kg}^{-1}$ )	5.68	5.07	ns	6.13	5.51	ns	2.71	2.82	ns	1.81	1.80	2.03	2.22	ns	**	ns
Specific leaf area ( $\text{m}^2 \text{ kg}^{-1}$ )	15.6	13.5	ns	16.7	16.7	ns	14.6	15.1	ns	12.2	12.5	13.4	13.3	ns	*	ns
Root/shoot biomass ratio	0.81	0.83	ns	0.83	0.83	ns	1.58	1.20	*	1.63	1.41	2.17	1.76	*	**	ns
Day of year 1993	190-207			207-288			288-320			190-320						
Treatments	Watered		ANOVA	Watered		ANOVA	Watered		ANOVA	Watered		ANOVA	Droughted			
	350	700		350	700		350	700		350	700		350	700		
Relative growth rate ( $\text{day}^{-1}$ )	0.0148	0.0301		0.0136	0.0112		0.0101	0.0108		0.0133	0.0152		0.0112	0.0113		

The significance of  $\text{CO}_2$  or water supply regime effects is indicated for leaf area ratio, specific leaf area and root/shoot biomass ratio; ns: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ .



promoted by elevated [CO<sub>2</sub>] was 30, 57, 33 and 39% at the leaf, stem, root and whole plant levels, respectively (fig 5). No CO<sub>2</sub> effect on plant biomass on d320 (fig 5) and on RGR between d190 and d320 (table III) was observed in the droughted treatments. The root/shoot biomass ratio increased steadily from d190 to d320. The R/S ratio was lower under elevated than under ambient [CO<sub>2</sub>] from d288 for the two watering conditions and R/S was higher in the droughted than in the well-watered conditions on d320.

**Fig 3.** Seasonal course of stem elongation rate in the different experimental treatments. Vertical bars denote 1 SEM. Asterisks indicate statistical significant ( $P < 0.05$ ,  $n = 8$  to 10) differences between CO<sub>2</sub> treatments. Data for the first growing flush have not been reported here but are given in table II. The onset of drought corresponded to d188 (arrows).



**Fig 4.** Seasonal time course of mean plant leaf area in the different experimental treatments. Vertical bars denote the mean treatment SEM values. Different letters indicate statistical significant ( $P < 0.05$ ,  $n = 8$  to 10) differences between treatments. The onset of drought corresponded to d188 (arrow).

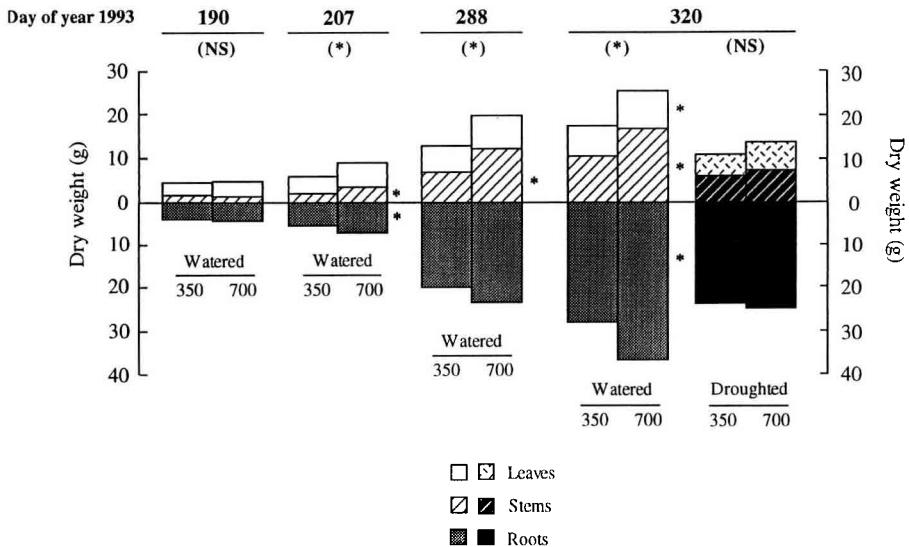
Average plant specific leaf area was not affected by  $[\text{CO}_2]$  in either watering regimes but was 10% higher in the droughted than in the well-watered conditions (table III) at the end of the season (d320). Leaf nitrogen concentration at the end of the season (d320) was reduced by drought by about 10% in both  $[\text{CO}_2]$ , but was not affected by  $[\text{CO}_2]$  (table II).

### Leaf gas exchange

In optimal watering conditions, despite slightly lower  $l_p$ ,  $A$  was stimulated in the elevated  $[\text{CO}_2]$  treatment as compared with the ambient  $[\text{CO}_2]$  treatment (fig 6) for the four first data sets (d160 to d180). A stimulation of  $A$  in the elevated  $[\text{CO}_2]$  treatment was also observed on d242, d244, d286, but not on d204, d216, d238 and d272. With

the exception of d244, d272 and d286,  $g$  was lower in the elevated  $[\text{CO}_2]$  than in the ambient  $[\text{CO}_2]$  treatment (fig 6). Plant intrinsic water-use efficiency was markedly higher (stimulation ranging between +56 and +121%) under elevated than under ambient  $[\text{CO}_2]$  on all measurement dates but not on d204, d216, d244 and d272. The mean values of  $A/g$  were positively linked with  $l_p$  ( $r^2 = 0.78$ ,  $P < 0.01$ ;  $r^2 = 0.71$ ,  $P < 0.01$  under 350 and 700  $\mu\text{mol mol}^{-1}$ , respectively) and the differences in  $A/g$  between the two  $[\text{CO}_2]$  treatments were highest for the days with high  $l_p$  values (fig 6).

Under the droughted conditions,  $A$  was significantly stimulated in the elevated  $[\text{CO}_2]$  treatment only on d244, while no significant  $\text{CO}_2$  effect was noticed for  $g$  (fig 6). Intrinsic water-use efficiency was higher under elevated than under ambient  $\text{CO}_2$  on d216 and d239 only.



**Fig 5.** Biomass of the different plant components on the successive harvest days. Asterisks indicate statistical significant ( $P < 0.05$ ,  $n = 8$  to 12) differences between  $\text{CO}_2$  treatments for the different plant components or at the whole plant level (between brackets). The onset of the drought treatment corresponded to d188.

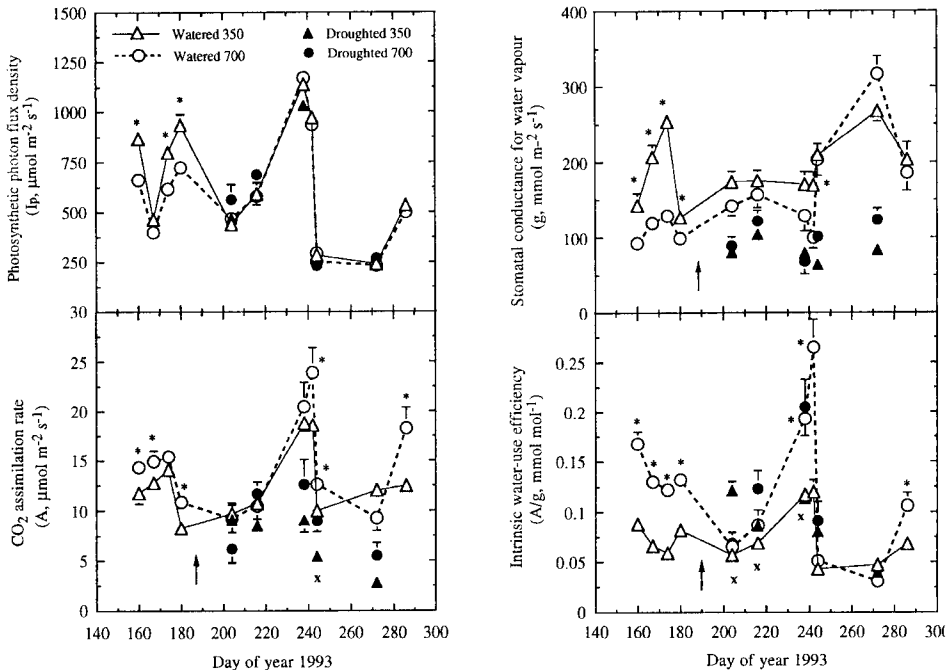
**Water-use efficiency and carbon isotope discrimination**

Water-use efficiency (W) was enhanced by 47% in the elevated [CO<sub>2</sub>] in the case of the well-watered plants and by only 18% in the droughted treatments (fig 7). Drought increased W by 43% under 350 μmol mol<sup>-1</sup> [CO<sub>2</sub>] but no significant drought effect on W arose under 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>] (fig 7). Leaf carbon isotope discrimination (Δ) was higher under elevated than under ambient [CO<sub>2</sub>] by 1.6 and 1.9‰ in the well-watered and droughted treatments, respectively (fig 7). In the droughted treatments, Δ was 1.7 and 1.5‰ lower as compared with the well-watered treatments under 350

and 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>], respectively (fig 7). Individual values of water-use efficiency were negatively linked with Δ in both CO<sub>2</sub> treatments (fig 8) with a clear difference between the two [CO<sub>2</sub>]. Dividing W by c<sub>a</sub> yielded a unique negative relationship with Δ (fig 8), as predicted by theory. The only outliers of this latter relationship (low W/c<sub>a</sub> values) were plants from the droughted and elevated CO<sub>2</sub> conditions (see also inset of fig 8).

**DISCUSSION**

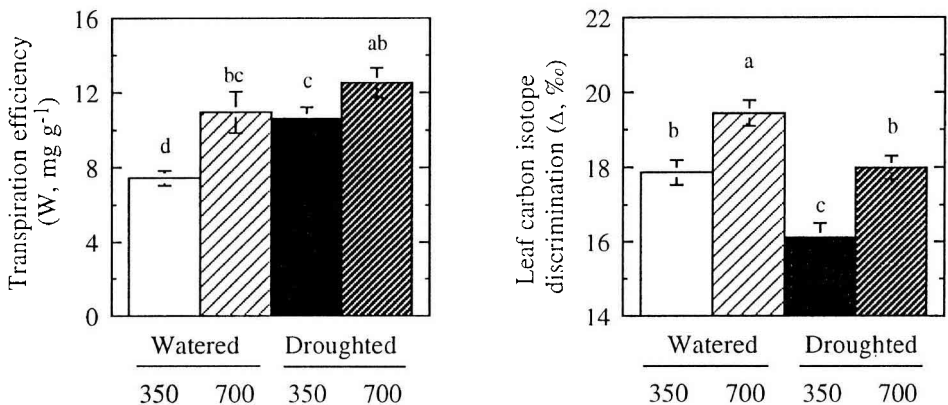
The stimulation in biomass growth (+39%) observed here by doubling [CO<sub>2</sub>] from the



**Fig 6.** Photosynthetic photon flux density at the leaf level (*I<sub>p</sub>*), CO<sub>2</sub> assimilation rate (*A*), leaf conductance for water vapour (*g*) and intrinsic water-use efficiency (*A/g*) in the different experimental treatments at different dates during the growing season. Vertical bars denote 1 SEM. Asterisks (well-watered treatment) and crosses (droughted treatment) indicate statistical significant (*P* < 0.05, *n* = 8 to 12) differences between CO<sub>2</sub> treatments. The onset of drought corresponded to d188 (arrows).

present atmospheric level (fig 5) is very close to the average dry weight increase of 41% reported by Poorter (1993) for 49 different temperate woody species, but is lower than the average value of biomass increase (+63%) reported by Ceulemans and Mousseau (1994) for deciduous trees. In the present study, the *Q robur* seedlings were grown under nonlimiting nutrient concentrations and no N (table II), P, K, Ca, Mg and S (data not shown) 'dilution' effect was observed during the growing season. At the end of the season, the root/shoot biomass ratio was decreased under elevated  $[CO_2]$ . Whether this result is linked, at least partly, with a more pronounced pot binding effect (Arp, 1991; Thomas and Strain, 1991; El Kohen et al, 1992; Morison, 1993) under elevated  $[CO_2]$ , remains an open question. The results available in the genus *Quercus* for the growth responses of young trees to elevated  $[CO_2]$  under nonlimiting nutritional conditions display a wide range of values: +22% (Norby and O'Neill, 1989) and +78% (Norby et al, 1986) in *Q alba*, +121% in *Q rubra* (Lindroth et al, 1993) and +138% in *Q petraea* (Guehl et al, 1994). These values are generally higher than the growth stimulation found in the present study.

The rather weak stimulation of biomass growth by elevated  $[CO_2]$  observed here in the well-watered conditions is to be related to the short time interval during which RGR was enhanced (table III); ie, about 17 days. It has been demonstrated in several species that RGR was stimulated by elevated  $[CO_2]$  at the beginning of the growing season only (Tolley and Strain, 1984; Norby et al, 1987; Coleman and Bazzaz, 1992; Poorter, 1993; Retuerto and Woodward, 1993; Vivin et al, 1995). In the present study, the period of RGR stimulation corresponded to the phase of maximum leaf expansion rate (fig 4) at the end of the stem elongation phase (fig 3) and led to an increased number of leaves and plant leaf area (table II) in the elevated  $[CO_2]$  treatment. It is noteworthy that no RGR stimulation occurred during the phase of intense biomass accumulation in the stems and roots after d207 (fig 5). This result highlights the role of the sensitivity of leaf area expansion to increasing  $[CO_2]$  in the determinism of the whole plant growth response (Gaudillère and Mousseau, 1989; Ferris and Taylor, 1994), at least under optimal nutrition. In the *Q robur* plants used here, the number of growth flushes was not increased in the elevated  $[CO_2]$  treatment



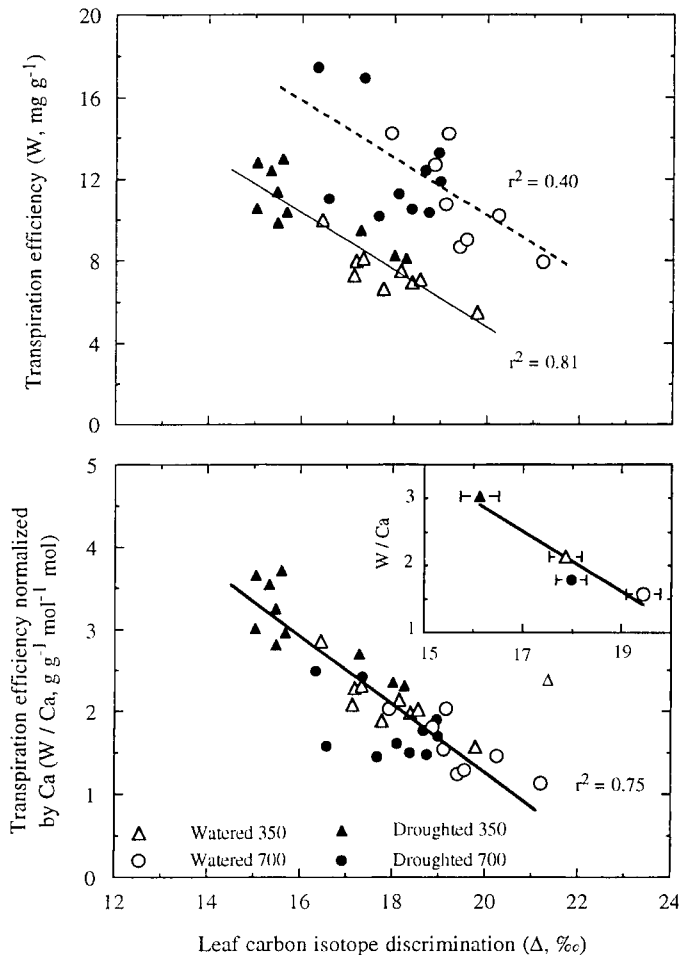
**Fig 7.** Transpiration efficiency (W) and leaf carbon isotope discrimination ( $\Delta$ ) in the different experimental treatments. Vertical bars denote  $\pm 1$  SEM. Different letters indicate statistical significant ( $P < 0.05$ ,  $n = 8$  to 10) differences between treatments.

(table II), which contrasts with previous findings obtained with *Q. petraea* (Guehl et al, 1994). In this latter study, the average number of growth flushes was 3.5 at 350  $\mu\text{mol mol}^{-1}$  [CO<sub>2</sub>] and 4.0 at 700  $\mu\text{mol mol}^{-1}$  [CO<sub>2</sub>] and plant leaf area was increased by 112% in the elevated [CO<sub>2</sub>] treatment, leading to a plant biomass increment of 138% at the end of the season. Whether the differences between both experiments – and in particular the difference in morphogenetic plasticity in relation to [CO<sub>2</sub>] – reflect specific differences or are linked to different annual climatic conditions (higher temperatures and

global radiation for the experiment with *Q. petraea*) remains an open question.

Substantial growth stimulation in response to increasing [CO<sub>2</sub>] has been associated with decreasing SLA and, in some species, with the existence of an additional palissadic parenchyma cell layer (Eamus and Jarvis, 1989; Ceulemans and Mousseau, 1994). In the present study, SLA was not affected by [CO<sub>2</sub>] (table III).

In the well-watered conditions, leaf conductance (fig 6) and leaf transpiration rates derived from plant water consumption mea-



**Fig 8.** Relationships between transpiration efficiency ( $W$ ) and leaf carbon isotope discrimination ( $\Delta$ ) and between water-use efficiency normalized by ambient CO<sub>2</sub> concentration ( $W/c_a$ ) and  $\Delta$ . The inset represents the relationship between the mean treatment values of  $W/c_a$  and  $\Delta$ . Vertical and horizontal bars denote  $\pm 1$  SEM unless smaller than the symbols.

surements (fig 1) were generally lower under elevated than under ambient  $[\text{CO}_2]$  as is commonly found in  $\text{C}_3$ , and namely woody species (Ceulemans and Mousseau, 1994). No straightforward interpretation of the  $\text{CO}_2$  effect on transpiration rates in the droughted plants is possible here since both SWC and  $\Psi_{\text{wp}}$  were lower in the elevated than in the ambient  $[\text{CO}_2]$ . The absence of significant  $\text{CO}_2$  effect on whole plant transpiration (fig 1, table I), reflects a compensation for increased plant leaf area by stomatal closure. Conroy et al (1988) observed the same result in *P radiata* plants in adequate P supply. According to Gifford (1988), the compensation between leaf area expansion and stomatal closure might be linked to root-shoot metabolic signalling in drought constrained situations. Do whole plant coordination mechanisms account for stomatal versus leaf area transpirational compensation also in nonconstrained conditions?

The absence of  $\text{CO}_2$  effect on biomass growth observed here for the droughted plants does not conform with the idea that elevated  $[\text{CO}_2]$  will alleviate the inhibitory effects of drought on growth (Tolley and Strain, 1984, 1985; Wray and Strain, 1986; Conroy et al, 1986, 1988; Marks and Strain, 1989; Johnsen, 1993; Townend, 1993; Samuelson and Seiler, 1994). The lack of  $\text{CO}_2$  effect is to be related here to the facts that i) the soil drought constraint (fig 2) developed concomitantly to the phase of potential RGR stimulation (fig 5) and maximum leaf expansion rate (fig 4) and ii) there was no release of the drought stress afterwards. Short drying cycles with rewatering periods might confer a higher response of the  $\text{CO}_2$ -enriched plants than a unique drying cycle (Tyree and Alexander, 1993). The lack of growth stimulation by elevated  $[\text{CO}_2]$  might also have been linked here with the slightly higher drought constraint (lower SWC induced by the type of drought application used) existing in the elevated  $[\text{CO}_2]$  as compared with the ambient  $[\text{CO}_2]$  conditions.

Water-use efficiency was increased by the elevated  $[\text{CO}_2]$  both at the leaf gas exchange (fig 6) and at the whole plant- and time-integrated (fig 7) levels as it is mostly found in  $\text{C}_3$  species (Morison, 1993; Tyree and Alexander, 1993) even in dense canopy conditions (Overdieck and Forstreuter, 1994). However, the increase in transpiration efficiency was less than the doubling that one would expect from the doubling of  $[\text{CO}_2]$  (eq [5]). This discrepancy is, at least in part, to be attributed to the fact that  $\Delta$  was increased by about 1.5–2.0‰ by the rising  $[\text{CO}_2]$  (fig 7), thus decreasing the second term of equation [5]. However, one has to be aware of the fact that some error (about 0.5‰) in the determination of  $\Delta$  was associated with the utilisation of a  $\text{C}_4$  plant for assessing  $\delta_a$  (eq [3]).

In the elevated  $[\text{CO}_2]$  conditions,  $W$  was not increased by drought despite decreasing  $\Delta$  values (figs 7, 8). To explain this discrepancy between  $W$  and  $\Delta$ , it may be suggested that, under elevated  $\text{CO}_2$ , the last term of equation [5] was decreased – and more precisely that the parameter  $\Phi$  was increased – by drought. Using  $^{13}\text{C}$  labelling techniques in the same species and experimental conditions as here, Vivin et al (1996) observed a decrease in the proportion of new carbon at the whole plant level in the elevated  $[\text{CO}_2]$  and droughted conditions 2 days after the labelling period. They attributed this decrease to possible carbon losses by root exudation or the emission of volatile compounds.

In conclusion, the experimental conditions used in the present study led to more pronounced soil drought under elevated  $[\text{CO}_2]$  accompanied by an absence of a  $\text{CO}_2$ -promoted growth stimulation. However, one has to be cautious for the extrapolation of these results to real forest conditions since in these conditions the growth response to  $\text{CO}_2$  will depend on the drought constraint level actually experienced by the trees. This level will be determined – among

other factors – by the trees ability to increase the soil prospection by the roots under elevated [CO<sub>2</sub>]. In our conditions, this effect was hindered by growing the seedlings in containers.

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