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The effects of elevated CO_2 and water stress on whole plant CO_2 exchange, carbon allocation and osmoregulation in oak seedlings

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Summary — Seedlings of *Quercus robur* L grown under present (350 µmol mol^{-1}) or twice the present (700 µmol mol^{-1}) atmospheric CO_2 concentrations, were either maintained well-watered or subjected to a drought constraint late in the growing season (25 August 1993). Despite an initial stimulation of biomass growth (+44%) by elevated CO_2, there was no significant difference in plant dry weight at the end of the growing season (15 October 1993) between the two CO_2 treatments, irrespective of watering regime. Under drought conditions, although there was no growth increase in response to elevated CO_2 concentration, there was a stimulation in net photosynthesis. In addition, the respiration rate of the root + soil system (root dry matter basis) was slightly lower in the elevated than in the ambient CO_2 concentration. These results, together with the results from short-term ^{13}C labelling, suggest enhanced plant carbon losses through processes not assessed here (aerial respiration, root exudation, etc) under elevated CO_2 concentration. In the droughted conditions, new carbon relative specific allocation values (RSA) were greater under elevated CO_2 than under ambient CO_2 concentration in both leaf and root compartments. Osmotic potentials at full turgor (π_o) were lowered in response to water stress in leaves by 0.4 MPa for the elevated CO_2 treatment only. In roots, osmotic adjustment (0.3 MPa) occurred in both the CO_2 treatments.

elevated CO_2 / water stress / osmoregulation / carbon allocation / *Quercus robur*

Résumé — Effets de l'augmentation de la concentration atmosphérique en CO_2 et d'un déficit hydrique sur les échanges gazeux, la répartition carbonée et l'osmorégulation de semis de chêne. *Des semis de chêne pédonculé (*Quercus robur *L) cultivés sous des concentrations atmosphériques en CO_2 de 350 ou 700 µmol mol^{-1} ont été, pour moitié, soit bien alimentés en eau, soit soumis à une sécheresse appliquée tardivement dans la saison de végétation (25 août 1993). En dépit d'une première phase de stimulation de la production de biomasse (+44 %, 30 juillet 1993) par le CO_2, aucune différence significative dans la biomasse des plants entre les deux traitements CO_2 n'a été obser-*

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vée à la fin de la saison de végétation (15 octobre 1993), ceci quel que soit le régime hydrique. En conditions de sécheresse, l'assimilation nette de CO_2 fut stimulée par le CO_2, malgré l'absence de stimulation sur la croissance. Par ailleurs, le taux de respiration du système racine-sol (rapportée à la matière sèche racinaire) était légèrement plus faible sous CO_2 élevé que sous CO_2 ambiant. Ces résultats, ajoutés aux résultats de marquages $^{13}CO_2$ à court terme suggèrent des pertes carbonées augmentées sous CO_2 élevé, par l'intermédiaire de processus non étudiés ici (respiration aérienne, exudation racinaire,...). En conditions de sécheresse, les valeurs de répartition relative spécifique du nouveau carbone étaient plus importantes sous CO_2 élevé que sous CO_2 normal, à la fois dans les compartiments foliaire et racinaire. Les potentiels osmotiques à pleine turgescence (π_0) étaient diminués en réponse au stress hydrique dans les feuilles de 0,4 MPa uniquement pour le traitement CO_2 à 700 µmol mol^{-1}. Dans les racines, un ajustement osmotique (0,3 MPa) était observé pour les deux traitements CO_2.

***CO_2 / sécheresse / osmorégulation / répartition carbonée* / Quercus robur**

INTRODUCTION

Osmoregulation, ie, the lowering of osmotic potential by the net increase in intracellular organic and mineral solutes in response to water deficit, is one of the processes by which changes in atmospheric CO_2 can interfere with drought adaptation features of C_3 plants (Conroy et al, 1988; Chaves and Pereira, 1992; Tschaplinski et al, 1993; Tyree and Alexander, 1993).

Under drought conditions, osmotic adjustment on the one hand and growth and metabolic processes on the other may compete for a limited supply of carbon (Munns and Weir, 1981). Thus, it might be hypothesized that increasing atmospheric CO_2 concentration favours osmotic adjustment through enhanced carbon supply to the different plant components and increased organic solute concentrations. However, elevated CO_2 concentrations often lead to reduced total mineral ion concentrations in the plant tissues (Conroy, 1992; Overdieck, 1993). The responses of mineral solute concentrations to elevated CO_2 have not yet been addressed in tree species. The question whether, in response to elevated CO_2 concentration, reduced mineral solute concentrations may offset the increase in organic solute remains open.

In the present study, we investigated the responses of pedunculate oak (*Quercus robur* L) seedlings to elevated atmospheric CO_2 concentration and water stress. More precisely, i) carbon allocation ($^{13}CO_2$ labelling) to the different plant components was assessed in relation to the whole plant CO_2 exchange and ii) the relationships between alterations in carbon allocation and in osmoregulation were investigated.

MATERIALS AND METHODS

Plant material

Quercus robur L acorns were collected in the Forêt Domaniale de Manoncourt (Meurthe et Moselle, eastern France) during autumn 1992 and kept overwinter in a cold chamber at –1 °C. From March 1993, acorns were planted in 5 000 cm^3 cylindrical plastic containers (20 cm deep) filled with a sphagnum peat–sand mixture (1:1, v:v) and fertilized with delayed release Nutricote 100 (NPK 13-13-13 + trace elements; 5 kg m^{-3}). Pots were placed in two transparent tunnels located in a glasshouse at INRA Champenoux. Seedlings were exposed to either ambient (350 ± 30 µmol mol^{-1} CO_2) or elevated carbon dioxide concentration (700 ± 50 µmol mol^{-1} CO_2), and were watered weekly. The CO_2 control and monitoring system as well as the growth conditions have been described previously by Guehl et al (1994) and Vivin et al (1995). Irradiance was

about 60% of the outside conditions. Average daily temperatures were 26 °C (maximum) and 11 °C (minimum); relative humidity was 70%.

From 25 August 1993, 15 seedlings were randomly assigned to well-watered or water-stressed treatments, and water supply was withheld in the latter treatment. Direct evaporation from the containers was prevented by covering the substrate with waxed cardboard disks and the transpirational water use of the seedlings was determined gravimetrically. Whole plant water use did not differ among the CO_2 treatments (fig 1) during the soil drying cycle. At the end of the experiment, the water-stressed seedlings of both CO_2 concentration conditions displayed water use values amounting to 25% of the nonstressed treatments. For a given date during the drying cycle, a transpiration index – considered as a measure of internal plant drought constraint – was calculated at the individual plant level as the ratio actual water use rate/maximum water use rate (julian day 241, fig 1).

On 15 October (julian day 288), the following factors were assessed: the allocation of recently fixed carbon, whole plant CO_2 exchange, growth, water relations and mineral solute concentrations.

Water relations

Predawn leaf water potential (Ψ_{wp}, MPa) was determined with a Scholander pressure chamber. In order to assess osmotic adjustment, osmotic potentials of the sap expressed from leaves or root tips in the actual plant conditions (π) and at full turgor (π_o) were measured. To achieve the full turgor state, one to three leaves, or some root tips, were saturated in distilled water for 8 h in darkness. After blotting with filter paper, the plant material was transferred into 1 mL syringes and immediately frozen in liquid nitrogen. Samples were then kept deep frozen. Before the sap was expressed in the syringes, the leaves or root tips were thawed out 30 min at room temperature. Osmotic potential of the sap (10 µl) was measured with a calibrated vapour pressure osmometer (Wescor 5500, Logan, UT, USA). Assuming the invariability of the nonosmotic water fraction during drought, relative water content (RWC) was calculated using the following formula:

$$RWC = \pi/\pi_o \quad [1]$$

Growth and biomass

Leaf area was measured using an area meter (ΔT Devices, UK). Leaves, stems and roots were separated, weighed and oven dried at 60 °C for 48 h before dry mass determination. Water content (g H_2O per g dry mass) of the plant compartments was calculated from the fresh and dry masses.

Biomass partitioning between the plant compartments was assessed by determining i) the leaf mass ratio (LMR, leaf dry mass/whole plant dry mass, g g^{-1}), ii) the stem mass ratio (SMR, stem dry mass/whole plant dry mass, g g^{-1}), iii) the root mass ratio (RMR, root mass/whole plant mass, g g^{-1}) and iv) the root:shoot ratio (root mass/[leaf mass + stem mass]). Specific leaf mass ratio (SLA, dm^2 g^{-1}) and leaf area ratio (LAR, dm^2 g^{-1}) were calculated as the leaf area to leaf mass and the leaf area to plant mass, respectively.

Carbon allocation and whole plant CO_2 exchange

The CO_2 exchange and $^{13}CO_2$ labelling experiments were conducted in a climatized phytotronic chamber using a semi-closed ^{13}C labelling system

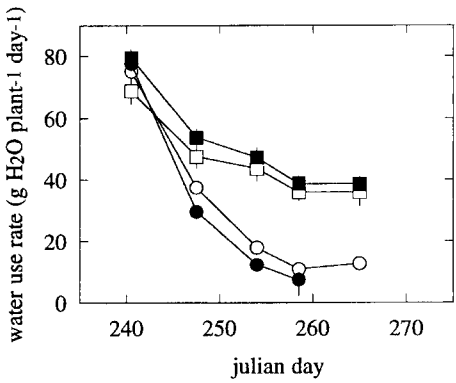

Fig 1. Time course of water use rates (g H_2O $plant^{-1}$ day^{-1}) in *Quercus robur* seedlings grown in different CO_2 concentrations and water supply combinations. (■) 350 µmol mol^{-1} and well-watered; (●) 350 µmol mol^{-1} and droughted; (□) 700 µmol mol^{-1} and well-watered; (○) 700 µmol mol^{-1} and droughted. Data represent the mean values and SEM of 12 to 13 replicates per treatment.

described in detail elsewhere (Vivin et al, 1995). Total CO_2 concentration in the chamber was constantly maintained at either 350 or 700 μmol mol^{-1} CO_2. The short-term (8 h duration) $^{13}CO_2$ labelling (1.5% ^{13}C) was performed using eight plants. To ensure that most of the $^{13}CO_2$ injected was absorbed by the plants (Mordacq et al, 1986) and to avoid effects on air $\delta^{13}C$ due to carbon isotope discrimination by the plants (Farquhar et al, 1989), plants were left in the chamber after the cessation of CO_2 injection until the CO_2 compensation point was reached. The incorporation of ^{13}C into individual plant parts was determined 12 h (three plants) and 48 h (five plants, 2 nights and 1 day) after the beginning of $^{13}CO_2$ assimilation. Four to six unlabelled plants were also harvested to assess natural ^{13}C abundances. Relative abundance of ^{13}C in plant samples was determined using an isotope ratio mass spectrometer (Finnigan MAT, Delta S). Powdered plant tissues were combusted before analysis (He + 3% O_2, 1 050 °C) and their carbon as well as nitrogen concentrations were measured using an elemental analyser.

Carbon isotope ratio data were expressed in terms of the conventional δ notation according to the relationship:

$$\delta = (R_S - R_{PDB}) / R_{PDB} \times 1\,000‰ \quad [2]$$

where R_S and R_{PDB} refer to the $^{13}C/^{12}C$ ratio in the sample and in the Pee-Dee Belemnite standard, respectively. They were also converted into atom percent (Atom%) defined as:

$$\text{Atom} = {}^{13}C / ({}^{13}C + {}^{12}C) \times 100\% \quad [3]$$

To appreciate the incorporation in a pool relative to a maximum possible value, we used relative specific allocation (RSA) defined as:

$$RSA = (\text{Atom}\%_{SL} - \text{Atom}\%_{SC}) / (\text{Atom}\%_{AL} - \text{Atom}\%_{AC}) \quad [4]$$

where subscripts SL and SC refer to samples from labelled and from nonlabelled plants, respectively; subscripts AL and AC refer to air samples taken in the exposure chamber and in the CO_2 tunnels, respectively.

Simultaneously to the $^{13}CO_2$ labelling experiment, carbon dioxide exchange was separately measured on the below-ground and the above-ground compartments of the plant–soil system. The diurnal course of net CO_2 assimilation rates was determined as the time course of CO_2 flow rates entering the chamber; the below-ground CO_2 efflux rates were calculated from the slope of the linear regression between time and CO_2 concentration in the root compartment (Vivin et al, 1995). For technical reasons, CO_2 efflux from the aerial plant parts during the night could not be measured.

Soluble minerals analysis

Soluble inorganic ion concentrations (K, Mg, Mn, Na, Ca, P, S) were determined by ICP spectrophotometry. Five hundred mg of powdered tissue were extracted twice with 25 + 25 mL of ultrapure water for 1 h at room temperature. Solutions were analyzed on plasma torch (JY38 Plus). Results were expressed on a water volume basis (mmol L^{-1}) either in the actual plant water status, or at full turgor.

Data analysis

Statistical differences between treatments were analysed by one- or two-way analyses of variance (ANOVA) followed by Fisher's PLSD test.

RESULTS

Water relations

At the end of the experiment, the plants in the well-watered treatments had similar leaf Ψ_{wp} values (–0.93 MPa) under ambient and elevated CO_2 concentration (table I). In contrast, the late season soil water stress applied here decreased Ψ_{wp} in both CO_2 treatments, and this effect was more pronounced under elevated CO_2 (–2.5 MPa) than under ambient CO_2 concentration (–1.7 MPa). The π_0 values were about twice more negative in leaves than in roots. In leaves, water stress only lowered π_0 (by approximately 0.4 MPa) in the elevated CO_2 treatment (table I). At the individual plant

Table I. Predawn leaf water potential (Ψ_{wp}), water content, relative water content (RWC) and total and inorganic solute concentrations (actual plant conditions and at full turgor) in leaves and roots of *Quercus robur* seedlings grown for 6 months in factorial treatments of 350 or 700 µmol mol^{-1} CO_2 concentrations and well-watered or droughted conditions.

	Leaf							*Root tip*						
	Well-watered		*Droughted*		P < F			*Well-watered*		*Droughted*		P < F		
	350	*700*	*350*	*700*	*CO_2*	*H_2O*	*x*	*350*	*700*	*350*	*700*	*CO_2*	*H_2O*	*x*
Ψ_{wp} (MPa)	-0.93^{a}	-0.93^{a}	-1.72^{b}	-2.46^{c}	ns	**	ns							
Water content (g g^{-1})	1.39^{bc}	1.43^{c}	1.23^{a}	1.32^{ab}	ns	**	ns	8.26^{c}	5.59^{b}	3.26^{a}	3.58^{a}	*	**	*
RWC (g g^{-1})	0.93^{c}	0.83^{b}	0.81^{ab}	0.77^{a}	**	**	ns	0.80^{b}	0.63^{a}	0.67^{a}	0.60^{a}	**	*	ns
Solute concentration (mM)	518^{a}	543^{ab}	588^{b}	713^{c}	**	**	*	243^{a}	341^{b}	404^{c}	460^{c}	**	**	ns
Total minerals (mM)	225^{a}	241^{a}	274^{b}	306^{c}	*	**	ns	40^{a}	60^{ab}	102^{bc}	151^{c}	*	**	ns
K	141^{a}	141^{a}	177^{b}	199^{c}	*	**	*	24^{a}	36^{ab}	65^{b}	98^{c}	ns	**	ns
Mg	43^{a}	56^{ab}	52^{ab}	60^{b}	ns	ns	ns	5^{a}	8^{ab}	12^{bc}	15^{c}	ns	**	ns
Na, Ca, Mn, PO_4, SO_4	41^{a}	36^{a}	45^{a}	46^{a}	ns	ns	ns	11^{a}	17^{ab}	26^{bc}	39^{c}	ns	ns	ns
Solute concentration at full turgor (mM)	482^{a}	451^{a}	472^{a}	547^{b}	ns	*	**	191^{a}	212^{a}	267^{b}	276^{b}	ns	**	ns
Total minerals at full turgor (mM)	210^{a}	205^{a}	221^{ab}	235^{b}	ns	*	ns	35^{a}	46^{a}	68^{b}	64^{b}	ns	**	ns
K	131^{a}	119^{a}	143^{b}	154^{b}	ns	**	*	21^{a}	28^{a}	43^{b}	42^{b}	ns	**	ns
Mg	40^{a}	50^{a}	42^{a}	46^{a}	ns	ns	ns	4^{a}	5^{a}	8^{b}	7^{ab}	ns	*	ns
Na, Ca, Mn, PO_4, SO_4	38^{a}	36^{a}	36^{a}	35^{a}	ns	ns	ns	10^{a}	13^{ab}	17^{b}	16^{b}	ns	ns	ns

The significance of CO_2 or water supply regime effects is indicated for the different variables; ns: not significant; * $P < 0.05$; ** $P < 0.01$.

level, significant positive correlations were only found under elevated CO_2 between π_o and either transpiration index or Ψ_{wp} (fig 2). In roots, there was osmotic adjustment (π_o decrease of about 0.3 MPa) in response to drought, and this response was not affected by the CO_2 concentration (table I).

Growth and biomass

At the end of the growing season (15 October 1993), all the plants were in a rest phase. Under ambient CO_2, 92 and 8% of the plants had produced three and four growth flushes, respectively, whereas under elevated CO_2, these proportions were 71 and 29% (data not shown, Vivin et al, 1995).

Despite an initial stimulation of biomass growth stimulation (+44%) by elevated CO_2 until 30 July, there was no significant difference in plant dry weight at the end of the growing season ($P = 0.402$, October 15) between the two CO_2 treatments, whatever the watering regime. Drought reduced whole plant biomass accumulation in both elevated and ambient CO_2 treatments by a factor of 0.82 and 0.73, respectively. Stem mass ratio was increased by elevated CO_2 in both watering regimes ($P = 0.003$), whereas RMR and the R:S ratio were significantly decreased ($P < 0.001$). Drought did not

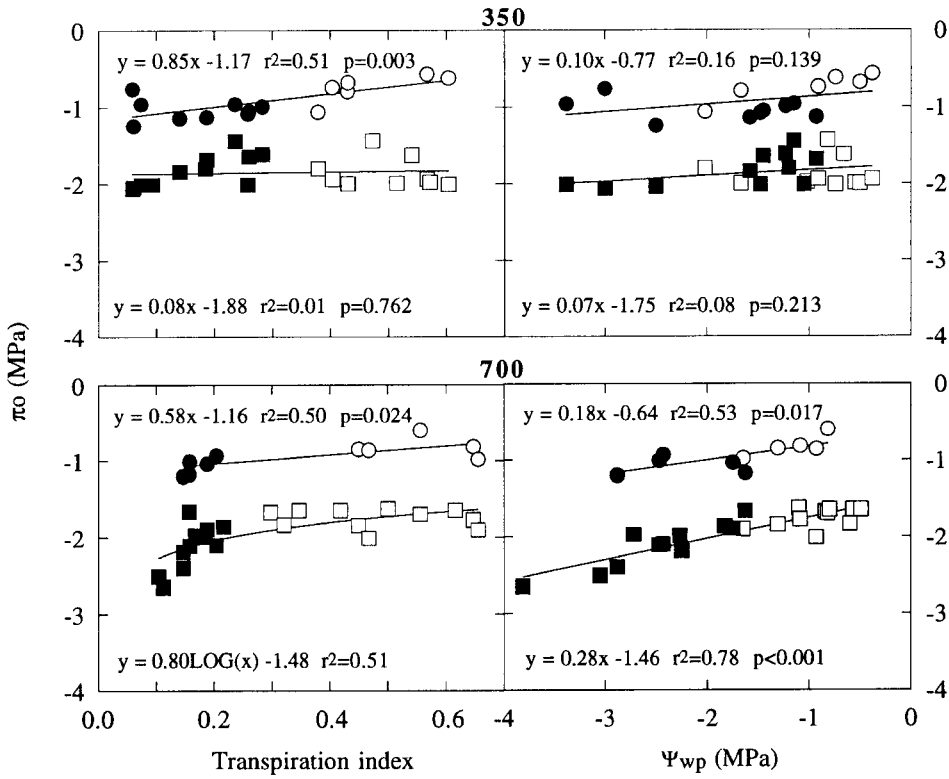

Fig 2. Relationships between leaf and root tip osmotic potential at full turgor (π_o) and predawn leaf water potential (Ψ_{wp}) or transpiration index in *Quercus robur* seedlings grown in different CO_2 concentrations and water supply combinations. (□) Well-watered leaves; (■) droughted leaves; (○) well-watered roots; (●) droughted roots.

affect the different biomass partitioning parameters. On 15 October, plant leaf area ($P = 0.043$), SLA ($P = 0.018$) and LAR ($P = 0.029$; table II) were significantly increased by elevated CO_2.

In both watering regimes, the elevated CO_2 treatment had no significant effect on the whole plant N concentration ($P = 0.340$; table II). However, on leaf area basis, nitrogen content was significantly decreased ($P = 0.008$) by elevated CO_2 (–8 and –10% under well-watered and droughted treatments, respectively). The whole plant C:N ratio was unaffected by water stress or increasing CO_2 ($P = 0.726$).

CO_2 gas exchange

On 15 October, in the well-watered treatments, net CO_2 assimilation rate (A, μmol m^{-2} s^{-1}) was not stimulated by increasing CO_2 (fig 3). On a plant basis, the respiratory CO_2 evolution of the root–soil compartment was quite similar in ambient and elevated CO_2 treatments (fig 4). However, on a root dry mass basis, slightly lower values were exhibited in the elevated CO_2 treatment. The water stress resulted in a decrease in A in both CO_2 treatments, but the decrease was less underelevated than underambient CO_2 (fig 3). Apparently, elevated CO_2 stimulated net assimilation rate in the droughted plants. Root–soil respiration, on a plant basis, was slightly decreased by drought irrespective of the CO_2 treatment (about –30%). On a root dry mass basis, mean root–soil respiration values were slightly lower under 700 than under 350 μmol mol^{-1} CO_2.

Table II. Growth and biomass allocation variables in *Quercus robur* seedlings grown for 6 months in factorial treatments of 350 or 700 μmol mol^{-1} CO_2 concentrations and well-watered or droughted conditions.

	30 July			*15 October*						
	Well-watered		P < F	*Well-watered*		*Droughted*		P < F		
	350	*700*	*CO_2*	*350*	*700*	*350*	*700*	*CO_2*	*H_2O*	*x*
Whole plant dry mass (g $plant^{-1}$)	11.1	16.0	*	30.6	35.7	25.2	26.1	ns	*	ns
Leaf mass ratio (g g^{-1})	0.37	0.34	ns	0.20	0.22	0.22	0.24	ns	ns	ns
Stem mass ratio (g g^{-1})	0.19	0.22	*	0.21	0.26	0.22	0.24	*	ns	ns
Root mass ratio (g g^{-1})	0.44	0.44	ns	0.59	0.52	0.56	0.52	**	ns	ns
Root:shoot ratio (g g^{-1})	0.83	0.83	ns	1.52	1.11	1.30	1.09	**	ns	ns
Leaf area (dm^2)	6.73	8.83	*	8.47	10.16	7.69	9.15	*	ns	ns
Specific leaf area (dm^2 g^{-1})	1.67	1.63	ns	1.49	1.58	1.42	1.57	*	ns	ns
Leaf area ratio (dm^2 g^{-1})	0.61	0.55	ns	0.30	0.36	0.31	0.39	*	ns	ns
Whole plant nitrogen content (%)	2.23	2.06	ns	1.82	1.92	1.95	1.99	ns	ns	ns
Leaf nitrogen content (mg cm^{-2})	2.08	2.13	ns	2.38	2.17	2.63	2.40	**	**	ns
Whole plant C:N ratio (g g^{-1})	19.7	21.5	*	24.3	23.6	22.8	22.9	ns	ns	ns

The significance of CO_2 or water supply regime effects is indicated for the different variables; ns: not significant; * $P < 0.05$; ** $P < 0.01$.

Carbon isotope composition and new carbon allocation

Carbon isotope composition of all nonlabelled plants was on average 17‰ more negative in plants in elevated CO_2 than in ambient CO_2 (fig 5). Such a large difference can only be accounted for by differences in source air isotopic composition between the two tunnels and not by differences in isotope discrimination by the plants (Guehl et al, 1994; Picon et al, 1996; Vivin et al, 1995). Carbon isotope composition of the labelled plants was significantly higher than that of the nonlabelled plants whatever the CO_2 concentration or water treatment ($P < 0.001$; fig 5).

Four hours after the end of labelling, leaf $\delta^{13}C$ was significantly increased in all treatments as compared with the control plants ($P < 0.001$). However, less new carbon was incorporated in the leaf compartment of the droughted plants grown in ambient CO_2 concentration as reflected by the lower RSA values displayed in this treatment. In the drought treatments and 40 h after the end of labelling, the difference in leaf $\delta^{13}C$ between the labelled and control plants, and RSA, were still higher in the elevated than in the ambient CO_2 concentration ($p < 0.001$).

In the roots of the droughted plants grown under ambient CO_2 concentration, no significant ^{13}C labelling ($P = 0.608$) was found, whereas in the droughted plants from the

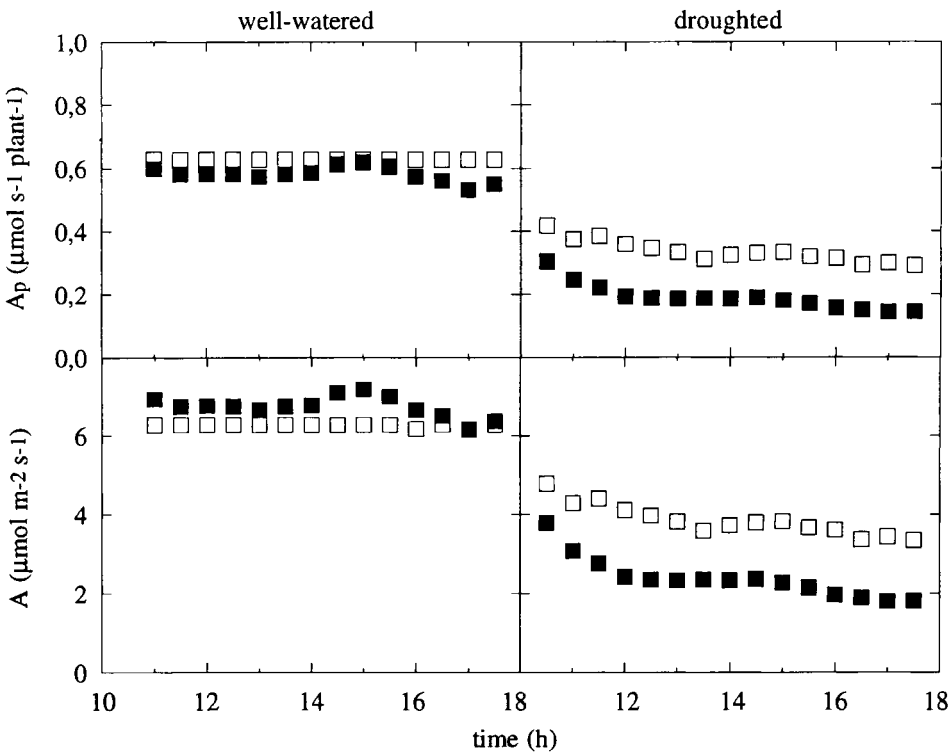

Fig 3. Diurnal course of net CO_2 assimilation rate expressed on a plant basis (A_p) or on a leaf area basis *(A)* in *Quercus robur* seedlings grown in different CO_2 concentrations and water supply combinations. Measurements were made during the 8 h of ^{13}C labelling on 15 October. (■) 350 µmol mol^{-1}; (□) 700 µmol mol^{-1}. $n = 8$.

elevated CO_2 treatment $\delta^{13}C$ was less negative in both 4 and 40 h after the labelling ($P = 0.030$).

At the whole plant level, a clear discrepancy existed between the two CO_2 treatments: i) For the drought treatments, the labelling was only effective in the elevated CO_2 treatment ($P < 0.001$). ii) In the elevated CO_2 treatments, a significant decrease in $\delta^{13}C$ and RSA was found between 4 and 40 h after the labelling ($P = 0.031$), whereas in the ambient CO_2 treatments no decrease was observed ($P = 0.941$).

Soluble mineral concentrations

In the leaves of the well-watered plants, total soluble mineral concentration accounted for about 45% of osmotic potential at full turgor irrespective of the CO_2 concentration (table I). Potassium and magnesium were the most important analyzed osmotic solutes. In the roots of the well-watered plants, soluble minerals contributed less to the osmotic potential at full turgor (18 and 22% in the 350 and 700 μmol mol^{-1} CO_2 treatments, respectively). In root tips, total concentration of mineral ions at full turgor

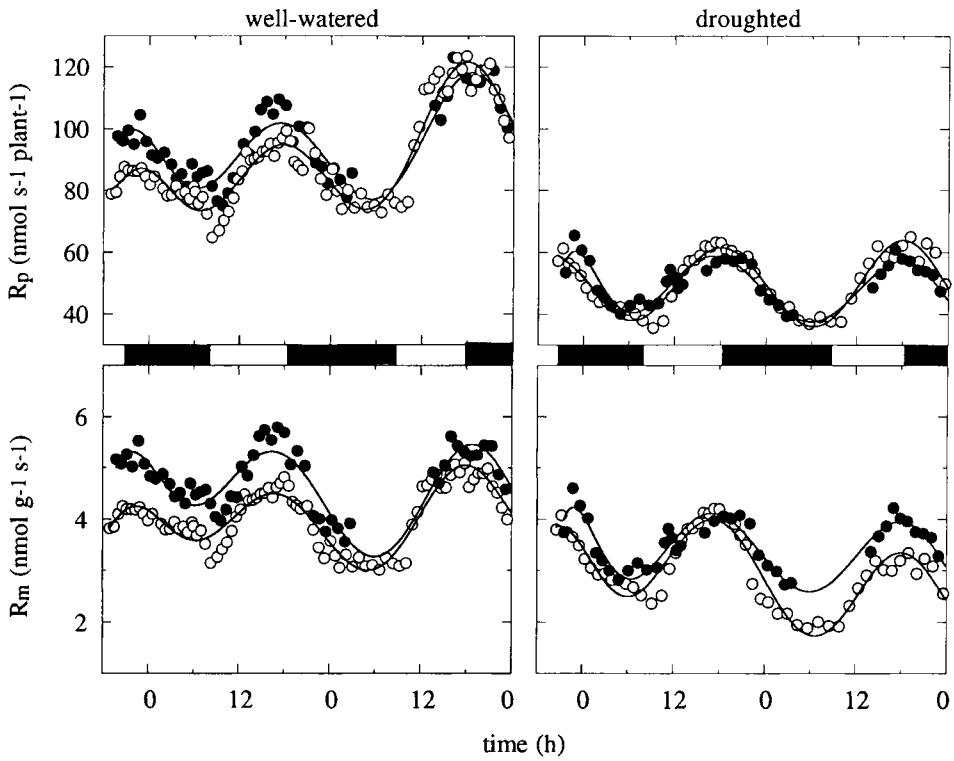

Fig 4. Time course of below-ground respiration rate expressed on a plant basis (R_p) or on a root dry mass basis (R_m) in *Quercus robur* seedlings grown in different CO_2 concentrations and water supply combinations. Measurements were begun the night preceding ^{13}C labelling. (●) 350 μmol mol^{-1}; (○) 700 μmol mol^{-1}. $n = 8$.

was significantly increased by water stress in both CO_2 treatments ($P = 0.001$), whereas in the leaves this effect occurred in the elevated CO_2 treatment only ($P = 0.049$). The respective contributions of the mineral solutes to osmotic potential were not significantly affected by drought (table I).

DISCUSSION

Despite the initial biomass stimulation (+44%) in July, there was no significant enhancement of plant biomass due to a doubling of the ambient atmospheric CO_2 concentration in well-watered pedunculate oak seedlings at the end of the growing period (table II). This lack of response is in contrast with the general trend (+68%) observed in tree species under optimal nutrition and water supply (Ceulemans and Mousseau, 1994). In the genus *Quercus*, a wide range of growth stimulation values has been reported in the literature: 1.22 (Norby and O'Neill, 1989) and 1.86 (Norby et al, 1986) in *Q alba*, 2.21 in *Q rubra* (Lindroth et al, 1993), 2.38 in *Q petraea* (Guehl et al, 1994).

Harvest dates may affect the interpretations of elevated CO_2 experiments (Coleman and Bazzaz, 1992). The strong initial

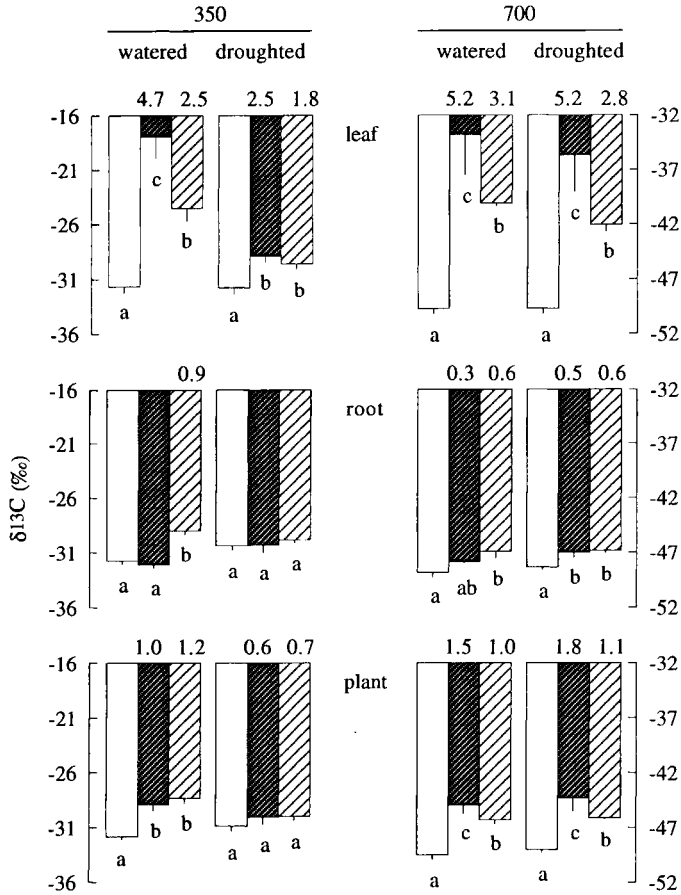

Fig 5. Carbon isotopic composition ($\delta^{13}C$) and relative specific allocation (RSA, average treatment values) in leaves and roots, and at the whole plant level in *Quercus robur* seedlings grown in different CO_2 concentrations and water supply combinations. (□) Unlabelled plants; (■) labelled plants harvested 4 h after the end of the labelling; (☑) labelled plants harvested 40 h after the end of the labelling. For a given plant compartment and treatment, mean $\delta^{13}C$ values not sharing common letters are significantly different ($P < 0.05$).

enhancement of growth observed in response to long-term CO_2 enrichment has been shown to decline in time (Tolley and Strain, 1985; Norby et al, 1987; Bazzaz et al, 1989; Retuerto and Woodward, 1993; Vivin et al, 1995). It has often been suggested that pot size, pot shape, concentrations and total amounts of nutrients in the pots may affect the assessment of the responses of plants (Arp, 1991; Idso and Kimball, 1991; Thomas and Strain, 1991) and cause the growth-stimulating effect to be transient (Poorter, 1993). In the present study, the *Q robur* seedlings were grown under nonlimiting nutrient concentrations on a well-aerated growing substrate and roots had not completely filled the pots at the end of the growing season, leading us to consider it unlikely that oak root growth could have been constrained by pot size and nutrient availability.

In terms of global growth analysis, the growth-stimulating effect of elevated CO_2 observed over a growing season will depend on the duration of the period during which relative growth rate (RGR) is stimultated (Coleman and Bazzaz, 1992; Poorter, 1993). It has already been demonstrated that RGR was often stimulated by elevated CO_2 only during the early stages of the growing period, and that after there was no effect or sometimes an inhibition of RGR (Neales and Nicholls, 1978), as observed in the present species (Vivin et al, 1995).

At the end of the growing period no significant difference in *A* was found between 350 and 700 μmol mol^{-1} CO_2 concentrations under well-watered conditions (fig 3). The lack of a stimulation of *A* reflects a long-term downward acclimation of photosynthetic capacity under the elevated CO_2, a response already found in *Quercus robur* by Bunce (1992). Moreover, below-ground (root + soil) respiration rates expressed on a root dry mass basis were slightly lower under elevated than ambient CO_2 treatment, as often reported on whole plant tree species: *Quercus prinus* (Bunce, 1992), *Castanea sativa* (Mousseau, 1993) or *Acer saccharum* (Reid and Strain, 1994).

For the droughted plants, the lack of growth response to elevated CO_2 was in contrast with the stimulation in both *A* at the leaf and whole plant levels (fig 3) and the intensity of entry of new carbon in the leaves 4 h after the end of the labelling (fig 5). This discrepancy is reinforced by the fact that below-ground respiration (R_m) was slightly lower at the high CO_2 level than under ambient CO_2 (fig 4). As it is suggested by the decrease in whole plant $\delta^{13}C$ values between 4 and 40 h after the end of the labelling, greater new carbon losses at high CO_2 concentration could have contributed to the lack of growth response to elevated CO_2. Further investigations, including aboveground respiration and direct root exudation measurements (Norby et al, 1987; Rouhier et al, 1994), are needed to substantiate this hypothesis.

Plants grown with limiting water supply generally allocate relatively more dry matter to the root compartment (Poorter, 1993); this effect would be advantageous for the acquisition of water under field conditions (Gifford, 1979; Tyree and Alexander, 1993). Thus, it is relevant to assess whether plant water status can be improved by a greater carbon allocation to the roots in high CO_2 conditions (Morison, 1993). In the present study, drought had no apparent effect on carbon partitioning parameters (R:S ratio, RMR) calculated from the final biomass results. This could be explained by the fact that water limitation was applied late in the growing season.

However, an original result of this study is that 40 h after the end of the labelling period, the proportion of new carbon in both leaves and roots of the droughted plants was significantly higher under 700 μmol mol^{-1} CO_2 than under 350 μmol mol^{-1} CO_2. This suggests that the amount of carbon available for growth and osmoregulation

were greater under elevated CO_2 treatment, as suggested by Masle (1992).

Indeed, osmoregulation was only observed in leaves under high CO_2 and was not entirely accounted for by the K^+ concentrations (table I). A stimulation of osmoregulation by elevated CO_2 was also reported in *Pueraria lobata* leaves (Sasek and Strain, 1989); however, no significant effect of the CO_2 concentration was found in several tropical trees (Reekie and Bazzaz, 1989) or in *Pinus taeda* (Tschaplinski et al, 1993). Further investigations are needed to characterize the other solutes involved in osmoregulation. Osmotic adjustment is commonly associated with starch breakdown and concomittant increase in low molecular weight organic solutes (Tyree and Jarvis, 1982; Morgan, 1984). Preliminary ^{13}C NMR spectrometry analyses (data not shown) revealed that soluble carbohydrates (glucose, fructose, sucrose), organic acids (quinic acid, malic acid), free amino acids (arginine, glutamine) were main solutes in the *Q robur* seedlings.

Despite the stimulating effect of elevated CO_2 on leaf osmoregulation, the depressing effect of drought on growth was not alleviated as has been proposed (Chaves and Pereira, 1992; Stulen and Den Hertog, 1993; Tyree and Alexander, 1993). This shows that osmoregulation was not a crucial factor to consider in our situation. Whether this conclusion can be extended to situations with drought constraints applied early in the growing season remains an open question.

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