Ann Sci For (1996) 53, 475-485

Original article

Water relations of oak species growing in the natural CO_2 spring of Rapolano (central Italy)

R Tognetti [1, 2], A Giovannelli [2], A Longobucco [3],
F Miglietta [3], A Raschi [3*]

[1] *Ce SIA, Accademia dei Georgofili, Logge degli Uffizi Corti, 50122;*
[2] *IMGPF, Consiglio Nazionale delle Ricerche, Via Atto Vannucci 13, 50134;*
[3] *IATA, Consiglio Nazionale delle Ricerche, Piazzale delle Cascine 18, 50144 Florence, Italy*

(Received 2 January 1995; accepted 3 October 1995)

Summary — The effect of elevated atmospheric carbon dioxide on water relations was examined on downy oak *(Quercus pubescens)* and holm oak *(Q ilex)* trees. The study was conducted on trees growing in a naturally enriched CO_2 spring. Sap velocity and sap flow were measured by the heat pulse technique. On the same trees, daily courses of xylem water potential, leaf conductance and transpiration were monitored. Plant water relations were evaluated by pressure–volume analysis method on shoots; on the same branches, relative conductivity of xylem was measured. Both species exhibited increased osmotic potential and decreased symplasmic fraction of water in trees adapted to increased CO_2. Downy oak showed lower stomatal conductance under elevated CO_2, but holm oak did not. Both species displayed higher sap flow in control trees. In both species, increased carbon dioxide did not influence xylem embolism formation.

drought / elevated CO_2 / embolism / *Quercus ilex* / *Quercus pubescens* / sap flow / water relations

Résumé — Relations hydriques de deux espèces de chênes poussant près d'une source enrichie en CO_2. *L'effet de l'enrichissement de l'atmosphère en CO_2 sur les relations hydriques du* Quercus pubescens *et du* Q ilex *a été étudié. Les mesures ont été réalisées au cours de l'état, sur des arbres poussant près d'une source enrichie naturellement en CO_2. Les flux de sève brute ont été mesurés par la technique de l'impulsion de chaleur ; sur les mêmes arbres, les cinétiques journalières de potentiel hydrique foliaire, de conductance foliaire et de transpiration ont été suivies. Les relations hydriques des plantes ont été évaluées par l'analyse de courbes pression–volume sur les bourgeons des mêmes branches prélevées pour les mesures de conductivité hydraulique du xylème. Les arbres des deux espèces ont présenté une augmentation du potentiel osmotique et une diminution de la fraction d'eau*

---

* Correspondence and reprints

symplasmique dans le milieu à plus forte concentration en CO_2 que dans le milieu ambiant. Au cours des deux journées de mesures, au contraine de Q ilex, Q pubescens *a présenté une conductance stomatique plus faible en forte concentration en CO_2 que dans un milieu ambiant. Dans les deux espèces le flux de sève brute des arbres témoins était plus élevé. L'augmentation de la concentration de CO_2 n'a pas influencé la formation d'embolie dans les deux espèces.*

embolie / enrichissement en CO_2 / flux de sève brute /* Quercus ilex / Quercus pubescens / *relations hydriques / sécheresse

INTRODUCTION

Due to the expected climate change it is likely that water stress conditions will occur more frequently in the next decades. This will interact with the effects that increasing global levels of atmospheric CO_2 will have on the anatomy and the physiology of plants. Most studies of these interactions have focused on gas exchange because of the direct relations between atmospheric carbon dioxide concentrations and rate of assimilation by the leaf (Eamus and Jarvis, 1989).

It has been shown that osmotic adjustment (lower solute potential) in leaves of plants exposed to elevated CO_2 allows them to maintain higher relative water content and turgor pressure (Morse et al, 1993). By maintaining positive turgor pressure and hydraulic efficiency, plants are able to sustain growth and metabolism during drought. High concentration of atmospheric carbon dioxide has been found to improve the response to water stress in most plants by inducing stomatal closure. This decreases transpiration and increases water-use eficiency (WUE) (Jarvis, 1989; Eamus, 1991). Elevated carbon dioxide may, in addition, induce changes in hydraulic architecture, thus possibly influencing the vulnerability to cavitation in the xylem (Tyree and Alexander, 1993). However, no studies have yet described water relations of adult trees subjected to elevated CO_2 over their entire life span.

It has been recently demonstrated (Miglietta and Raschi, 1993) that sites enriched naturally with CO_2 (termed CO_2 springs) may provide the opportunity for studying adult trees exposed throughout their development to an enriched carbon dioxide atmosphere. Several Mediterranean tree species growing in the Bossoleto CO_2 spring near Rapolano Terme (central Italy) (van Gardingen et al, 1995) offer the opportunity to better investigate the long-term response to concurrent CO_2 increase and water stress, as well as to compare the different species in their drought tolerance. The great realism of experiments carried out on plants in natural CO_2 springs compared to laboratory studies and/or manipulative experiments contributes to enhance the predictive value of observations made at these sites despite the lack of an exact control.

This study was undertaken with the aim of examining water relations of mature trees of holm oak and downy oak grown in elevated atmospheric carbon dioxide during a drought period in Mediterranean conditions. Trees sampled in this experiment have been exposed for generations to elevated CO_2 and have been subjected, during this time, to a large range of natural disturbances.

MATERIALS AND METHODS

Plant material and field site

The study took place in the natural CO_2 spring of Bossoleto, located near Rapolano Terme (Siena, central Italy); the site has been described elsewhere (Miglietta et al, 1993; van Gardingen et al, 1995). The CO_2 vents occur both at the bottom and on the flanks of a circular doline; concentration gradients are enhanced under stable (windless) atmospheric conditions. The CO_2 concentrations around the crown of the plants on which the experiment was performed ranged in daytime hours from 500 to 1 000 ppm with rapid fluctuations. The H_2S concentration in the spring is very low and cannot be considered harmful to plants (Polle, personal communication). The control site, 4 km from the gas vent, was chosen as being characterized by similar aspect, light exposure and vegetation. Measurements were conducted on trees of downy oak (*Quercus pubescens* Wild) and holm oak (*Quercus ilex* L), about 10 and 20 cm in diameter, and 4 and 7 m in height, respectively, on 8 June and 15 July 1993.

Shoot–water relations and embolism

Daily courses of xylem water potential (Ψ), leaf conductance (g_l) and transpiration (E) were measured at 2 hour intervals from predawn to sunset, using a pressure chamber (PMS 100, PMS Instrument Co, Corvallis, OR, USA) and a null-balance steady-state porometer (LI-1600,Li-Cor Inc, Lincoln, NE, USA), respectively. Six leaves per treatment and per species at a time, collected in the illuminated part of the crown, were sampled on six trees of the same dimension selected for the experiment both in the CO_2 spring and in the control site.

In July (just before the second day of measurements), the amount of xylem embolism was evaluated on ten terminal branch segments (similar in age and size) from the upper part of the crown for each treatment. Branches were collected early in the morning and placed in a sealed plastic container. In the laboratory, branches were recut under water. Hydraulic conductivity was measured on stem segments about 15 cm long, using the technique described by Sperry et al (1988). Distilled water was acidified (pH 1.8) by using oxalic acid (10 mol m^{-3}) and degassed by agitating it under vacuum for 45–60 min. This solution was stored in an air-free plastic bladder enclosed in a compressed gas tank. The perfusing solution was forced through the samples at constant low pressure (10 kPa), passing through a 0.2 μm in-line filter. The flow was measured with an analytical balance interfaced with a computer to automate the calculations. The initial conductivity (k_i), calculated from the flow-rate/pressure-gradient ratio, was recorded every 30 s and measured by averaging ten readings after steady state had been reached. The maximum conductivity (k_m) was calculated as previously described for k_i by repeating the measurements after flushing the solution through the stems at elevated pressure (180 kPa for 60 min). Embolism was expressed as the percent loss of hydraulic conductivity (LOSK = 1100 $(k_m - k_i)/k_m$).

Eight shoots per tree, from the branches sampled for conductivity measurements, were selected and pressure–volume curves established using the free transpiration method (Hinckley et al, 1980). Each shoot was recut in distilled water and rehydrated overnight in a dark refrigerator. During the next day, the braches were left to dry (transpiring freely) on the laboratory bench. Fresh weight (measured with an analytical balance), an average of two measurements (one before and one following the measurements of water potential) and water potential (measured with a pressure chamber) were recorded at regular intervals till the latter achieved about –5 MPa. Osmotic potential at saturation (π_{sat}), osmotic potential at turgor loss point (π_{tlp}), relative water content at turgor loss point (RWC_{tlp}) and symplastic water content (Θ_{sym}) were calculated according to Schulte and Hinckley (1985), and bulk modulus of elasticity (ε) was calculated from the actual data pairs as $(\Delta p/\Delta RWC)RWC$ where Δp is the change in turgor pressure. Many pressure–volume curves showed an initial plateau near full turgor, probably due to overhydration of the samples. Plateaus were eliminated and appropriate corrections were made to avoid errors in the parameters derived from the pressure–volume curves (Abrams and Menges, 1992). Statistical analyses of data were performed using analysis of variance methods followed by Duncan's multiple range test with $P < 0.05$.

Sap flux

Sap velocity and sap flow were measured on 8–9 June and 15–16 July by the thermoelectric 'heat pulse method' (HPV), using commercial HPV equipments (Custom HPV, Division of Fruit and Trees, DSIR, Private Bag, Palmerson North, New Zealand); one tree for control and one for spring site were sampled per species.

The basic sensor unit consists of a 2-mm-diameter stainless steel heating device and two thermistor probes (1.8 mm in diameter), situated 5 mm below and 10 mm above the heating device.

Four heaters were vertically installed at a height of 1 m and penetrated the xylem to a maximum depth of 35 mm, whereas the corresponding thermistor pairs were inserted at a depth ranged from 5 to 25 mm beneath the cambium. The probes and heaters were connected in a Wheatstone bridge configuration; a short (1 s) electrical pulse was applied to the heater. The heat pulse velocity (recorded at 30 min intervals) was calculated from the time taken by the re-equilibration of the bridge, ie, by the heat pulse to travel the distance from the midpoint of the two probes to the heat (2.5 mm) (Hüber and Schmidt, 1937); the conversion from heat velocity to sap flow was made according to Marshall's equation (Marshall, 1958), corrected to take into account the effect of sensor implantation wounds (Swanson and Whitfield, 1981). The sapwood components, represented by the volume fraction of gas, water and wood, were determined on increment borings by Archimedes's principle and dry weight. The area of sapwood was estimated from cores passing through the center of the trunks.

RESULTS

Both sampling days were hot and sunny; night to day air temperature ranged from 13–30 to 16–32 °C (relative humidity ranged from 40–50% and vapour pressure deficit up to 25 kPa), respectively, for June and July. No rain events occurred between the 2 measurement days.

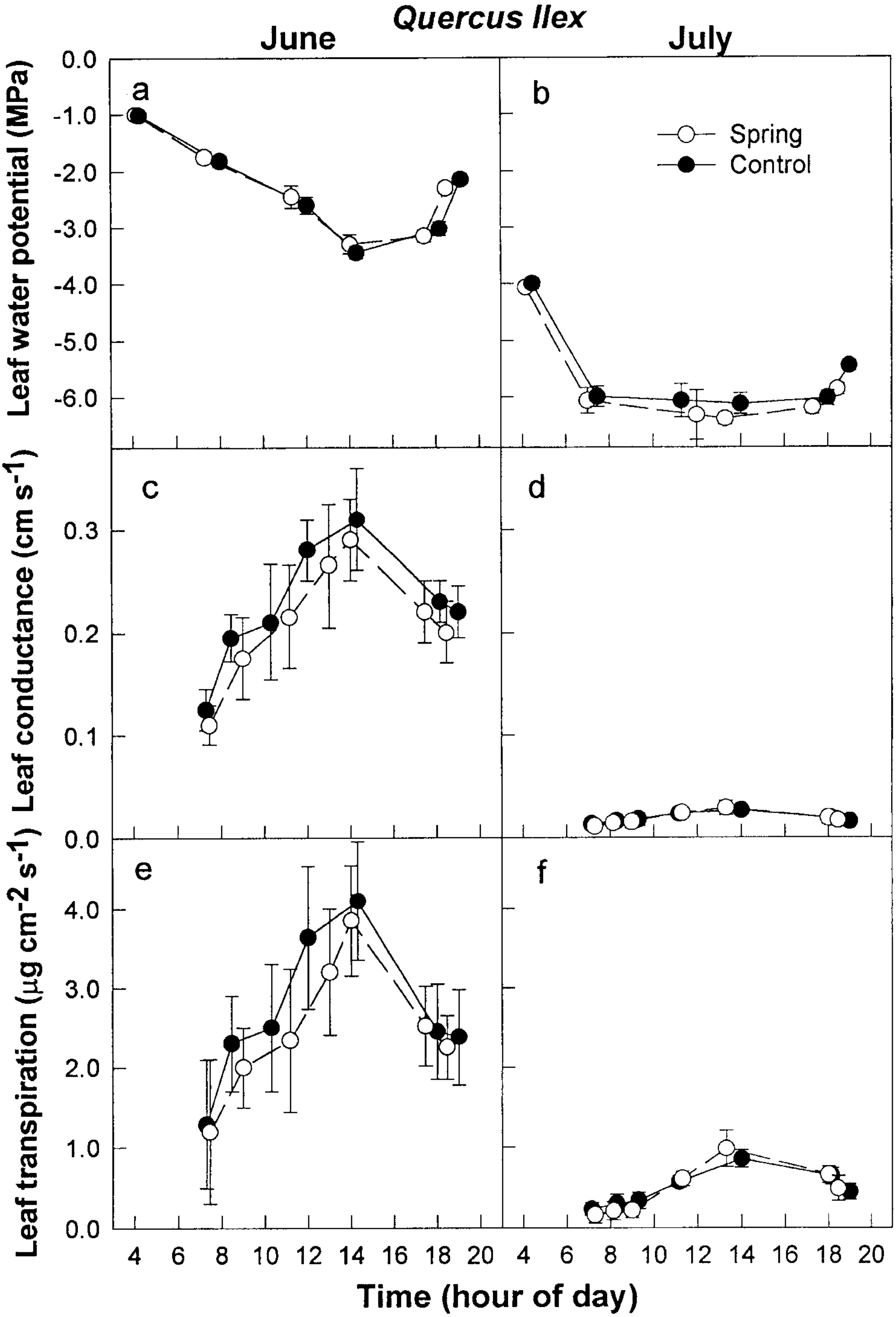
Q ilex underwent marked water stress. Predawn water potential from –1 MPa on 8 June reached –4 MPa on 15 July (fig 1a and b); on both days minimum potential was reached at about midday. On both measurement days, differences between control and spring site were not significant. On 8 June, leaf stomatal conductance and transpiration reached the maximum at midday (fig 1c); no midday depression was evidenced in either spring and control plants. Spring trees showed a tendency for lower transpiration (fig 1e), although the differences were not significant. The absolute values of g_l and E were much lower in July (fig 1d and f), and daily trends were much less evident. Again, no significant differences existed between the two treatments.

Leaf water potential in *Q pubescens* displayed higher absolute values than *Q ilex* on both measurement days (fig 2a and b). On 8 June, predawn values were about –0.3 MPa, without any differences between spring and control plants; daily minima were also similar in both treatments. Yet, during the day, spring plants showed a slower decrease of the values; minima of about –2.5 MPa were reached at about 1000 hours in control plants and much later in the spring plants. Afternoon recovery was quick and evident in both treatments. Leaf conductance and transpiration were lower in spring plants (fig 2c and e). Midday depression was more evident in control plants.

On 15 July, predawn water potential was lower in spring trees (fig 2b), although minima were similar for both treatments. On 15 July, g_l and E were much lower (fig 2d and f). Morning values were similar in both treatments, while in the afternoon spring trees were unable to recover. *Q ilex* displayed lower values than *Q pubescens* for Ψ, g_l and E in both June and July.

In *Q ilex* on both days of measurement, sap flow and velocity started to rise at 0530 hours, reaching the maximum values in the early hours of the afternoon, then both variably decreased to the night base line (fig 3a and b). The spring site tree showed lower absolute values than control trees. Measurements taken in July displayed lower sap velocity and flow than those in June.

Fig 1. Daily trends of leaf water potential, conductance and transpiration in *Quercus ilex* on 8 June (**a**, **c** and **e**) and 15 July (**b**, **d** and **f**). Solid lines and closed circles indicate control trees; broken lines and open circles indicate spring trees. Bars represent ± SE of the mean; bars not visible represent SE smaller than the symbol.


Quercus Ilex
June
July
a
b
c
d
e
f
Spring
Control
Leaf water potential (MPa)
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
Leaf conductance (cm s-1)
0.3
0.2
0.1
0.0
Leaf transpiration (µg cm-2 s-1)
4.0
3.0
2.0
1.0
0.0
4
6
8
10
12
14
16
18
20
Time (hour of day)

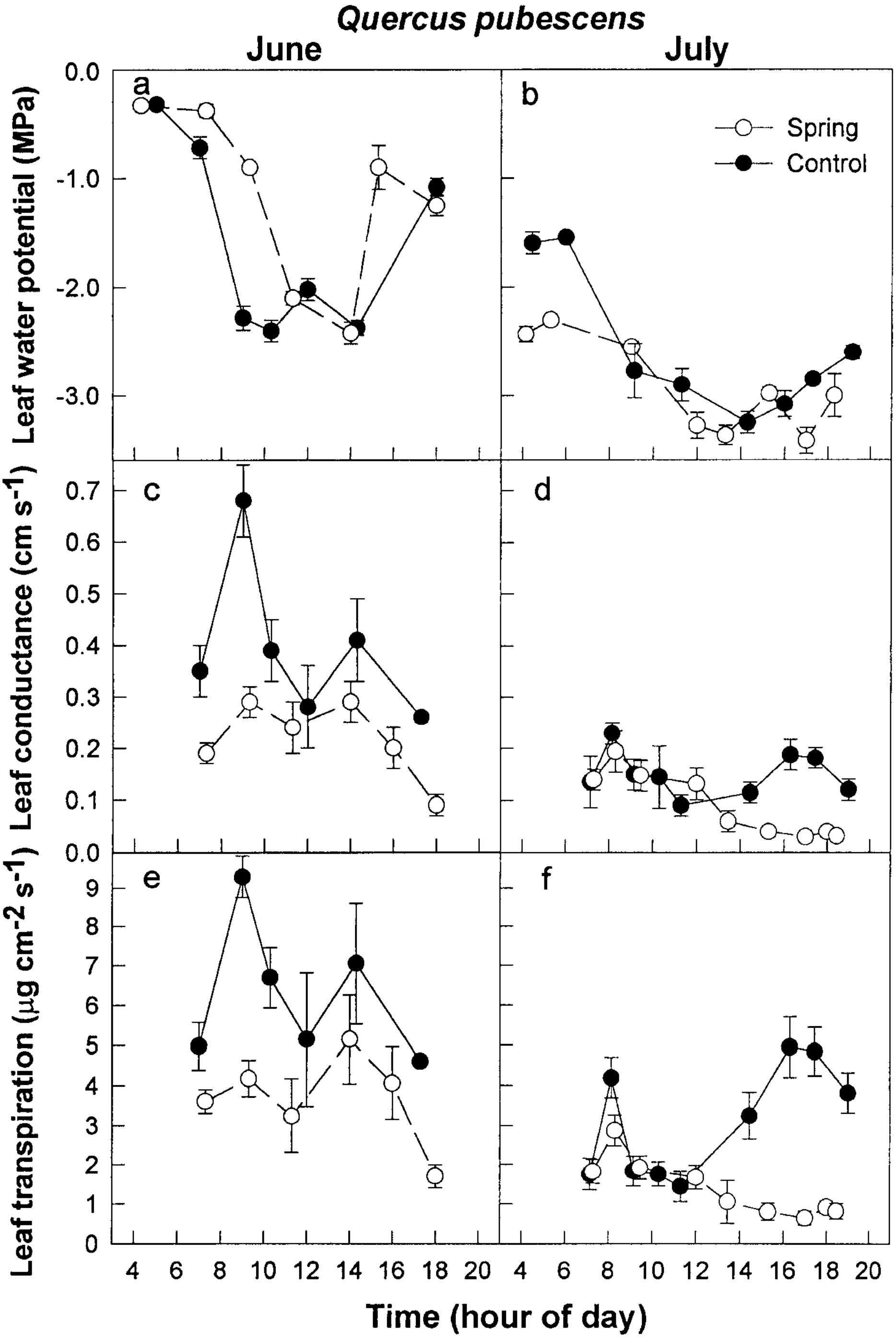

Quercus pubescens
June
July
a
b
c
d
e
f
Spring
Control
Leaf water potential (MPa)
0.0
-1.0
-2.0
-3.0
Leaf conductance (cm s-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Leaf transpiration (μg cm-2 s-1)
9
8
7
6
5
4
3
2
1
0
4 6 8 10 12 14 16 18 20
4 6 8 10 12 14 16 18 20
Time (hour of day)

Table I. Shoot tissue water relations, as derived from pressure–volume curves, and loss of hydraulic conductivity in the same branches, from the tested trees in two sites.

Site	*RWC_{tlp} (%)*	*π_{tlp} (MPa)*	*π_{sat} (MPa)*	*Θ_{sym} (MPa)*	*ε (MPa)*	*LOSK (%)*
Q ilex						
Control	84.0 ± 0.8^{a*}	–3.05 ± 0.09^{a*}	–1.82 ± 0.08^{a*}	0.85 ± 0.02^{a*}	14.48 ± 1.83^{a}	53.99 ± 8.04^{a}
Spring	81.3 ± 0.5^{b*}	–3.35 ± 0.07^{b*}	–2.23 ± 0.07^{b*}	0.71 ± 0.01^{b*}	16.00 ± 1.67^{a}	70.36 ± 4.68^{a}
Q pubescens						
Control	87.4 ± 1.0^{a*}	–2.04 ± 0.07^{a*}	–1.33 ± 0.06^{a*}	0.59 ± 0.03^{a*}	12.84 ± 1.78^{a}	68.18 ± 7.82^{a}
Spring	83.4 ± 0.8^{b*}	–2.39 ± 0.13^{b*}	–1.61 ± 0.04^{b*}	0.50 ± 0.02^{b*}	13.80 ± 1.08^{a}	65.94 ± 6.61^{a}

Means ± SE. Within species and columns, different letters indicate that means are significantly different at $P < 0.05$. * Significant differences ($P < 0.05$) between species. RWC_{tlp}: relative water content at turgor loss point; π_{tlp}: osmotic potential at turgor loss point; π_{sat}: osmotic potential at saturation; Θ_{sym}: symplastic water content; ε: bulk modulus of elasticity.

In *Q pubescens* sap flow was higher in June and in the control (in this case the difference was less evident than in holm oak) tree (fig 4a and b). In June, sap velocity started to rise earlier in the control tree (at 0530 hours). On both days of measurement, maximum sap flow was reached in the early afternoon, then it started decreasing until the night base line. The higher absolute values recorded in *Q ilex* are probably related to differences in crown architecture of the two species.

LOSK and parameters of shoot–water relations (π_{sat}, π_{tlp}, RWC_{tlp}, Θ_{sym} and ε) are presented in table I. LOSK was generally high: 70 and 65% in spring and 55 and 68% in control holm and downy oaks, respectively, without significant differences between sites and species. In both species, values of π_{sat}, π_{tlp}, RWC_{tlp} and Θ_{sym} were significantly lower in spring plants, while there were no site differences in ε. Under high CO_2, trees showed a decrease in osmotic potential of 0.3–0.4 MPa and an increase (although statistically not significant) in the bulk modulus of elasticity at full hydration of 1–1.5 MPa. *Q ilex* showed significantly higher absolute values of π_{sat}, π_{tlp}, RWC_{tlp} and Θ_{sym} than *Q pubescens* in both treatments.

DISCUSSION

Previous research in growth chambers has demonstrated that an increase in CO_2 may cause a reduction of stomatal density, but prolonged exposure to elevated CO_2 may have a different effect on different species (Woodward and Bazzaz, 1988; Idso, 1989).

Fig 2. Daily trends of leaf water potential, conductance and transpiration in *Quercus pubescens* on 8 June (**a**, **c** and **e**) and 15 July (**b**, **d** and **f**). Solid lines and closed circles indicate control trees; broken lines and open circles indicate spring trees. Bars represent ± SE of the mean; bars not visible represent SE smaller than the symbol.

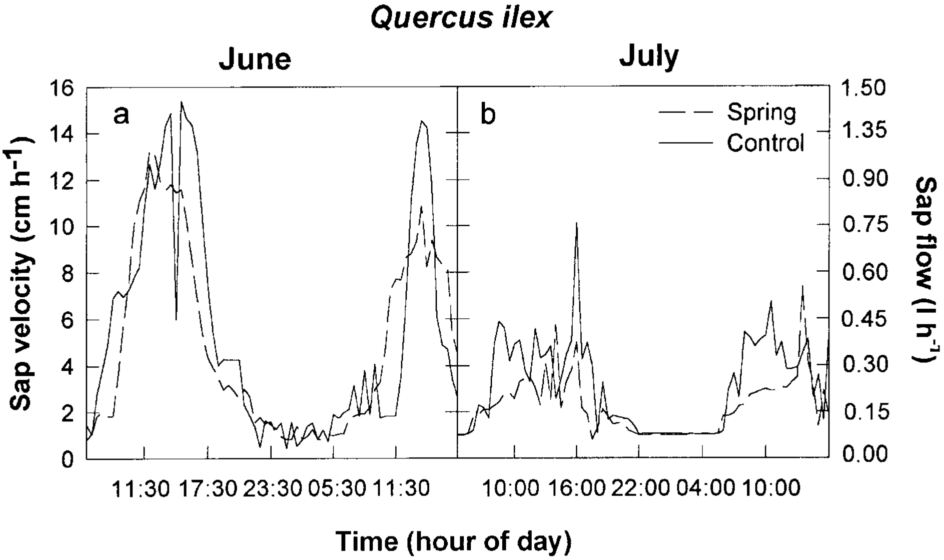


Fig 3. Daily course of sap velocity and sap flow in *Quercus ilex* on 8–9 June (**a**) and 15–16 July (**b**). Solid lines indicate control tree; broken lines indicate spring tree.

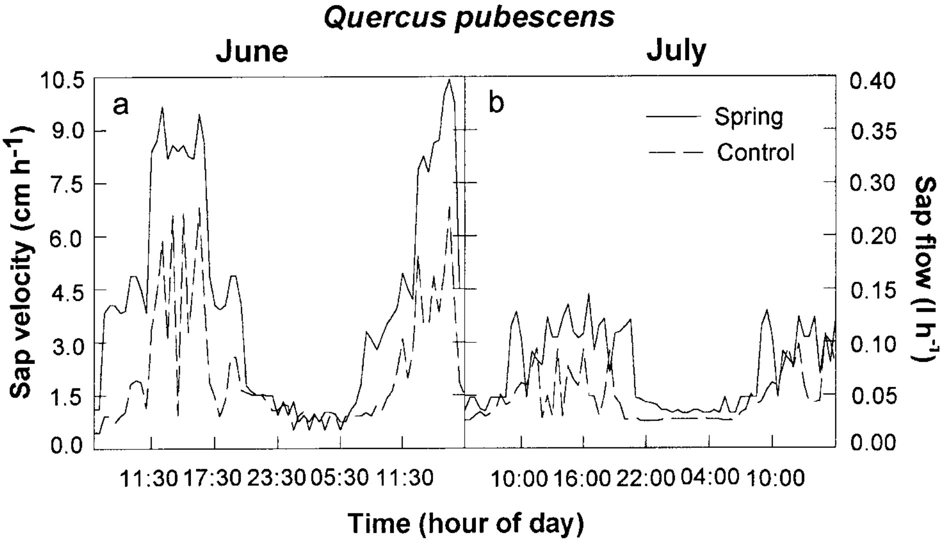


Fig 4. Daily course of sap velocity and sap flow in *Quercus pubescens* on 8–9 June (**a**) and 15–16 July (**b**). Solid lines indicate control tree; broken lines indicate spring tree.

Previous research in natural CO_2 spring did show some differences in stomatal density and dimension (Miglietta and Raschi, 1993; Paoletti, personal communication); however, the high variability and the presence of other environmental effects make it difficult to draw any conclusions. Most studies have shown that increased ambient carbon dioxide brings about a decrease in stomatal conductance (Eamus and Jarvis, 1989). On the other hand, stomatal sensitivity to increasing CO_2 varies with species and the effect is mediated by intercellular space CO_2 concentration rather than by ambient CO_2 concentration (Mott, 1988), and depends upon the degree of coupling between the leaf and the atmosphere above the leaf surface.

Holm oak trees growing in the carbon dioxide spring did not show significant differences in leaf conductance and transpiration with respect to the control trees. For the second day of measurements (in July), this may be attributed to the relative insensitivity of stomata to CO_2 concentration under severe drought; water stress conditions at the beginning of June, the first day of measurements, had not yet developed. In contrast, downy oak trees grown under high CO_2 displayed lower leaf conductance and transpiration than control trees on both measurement days. The differences (particularly in July) were exacerbated in the afternoon at higher vapour pressure deficit (Oechel and Strain, 1985). Leaf conductance and transpiration in downy oak showed a midday depression which was more evident in control trees and in June. At the same time, values of leaf water potential were higher in spring trees for a great part of the day. In this sense, a beneficial effect of elevated CO_2 on water relations was evident. This effect was not seen in holm oak; as water stress developed, daily water potential did not differ greatly between the two treatments, obscuring the importance of this parameter as an aspect of drought tolerance in oaks under elevated CO_2 (Tyree and Alexander, 1993).

In spite of being considered as more suitable for diffuse porous tree species, as it assumes wood is essentially homogeneous, the HPV technique has been applied successfully to ring-porous tree species (Miller et al, 1980; Borghetti et al, 1993; Raschi et al, 1995). In this experiment, despite limited dimension of the sample, sap velocity and sap flow values were consistent with stomatal behaviour. Spring trees showed a lower sap velocity and sap flow, but the difference was much more evident in downy oak, rather than in holm oak. The July values were much lower than the June values. The absolute values were consistent with those reported by other authors using the same technique (Visser et al, 1989; Borghetti et al, 1993). The peaks of sap flow from the night baseline may depend on nocturnal transpiration, common in Mediterranean environment, and/or on re-allocation of water in different parts of the plant subjected to water potential gradient. The phenomenon disappeared in July, under greater water-stress conditions. The differences in sap flow and sap velocity between the two species were consistent with the differences in the size of trees.

Estimated values of π_{sat}, π_{tlp}, RWC_{tlp}, Θ_{sym} and ε were similar to those found in the same oak species by other authors (Salleo and Lo Gullo, 1990; Dreyer et al, 1992; Sala and Tenhunen, 1994). The observed shift in osmotic potential (both at full turgor and zero turgor) in response to CO_2 in both species may enable plants to withstand lower water potentials (Morse et al, 1993). Osmotic adjustment in leaves of spring trees may contribute to maintain higher RWC and turgor pressure, then preventing full stomatal closure and allowing net photosynthesis to proceed during severe drought. In other experiments (Johnson et al, 1996; Johnson, Tognetti and Michelozzi, unpublished data), downy oak and holm oak growing in the CO_2 spring showed increased production of secondary compounds (tannins) and total nonstructural carbohydrates; this may provide an available source of osmoticum (Abrams, 1990). Despite the absence of significant differences in elastic modulus between treatments, the tendency to have higher tissue inelasticity may help trees in the CO_2 spring, particularly downy oak, to generate a favourable water potential gradient from the soil to the plant, at lower stomatal conductances. The decrease in symplasmic fraction of water, and the relative increase in apoplasmic fraction, found for the spring trees, could reflect an increase in xylem volume, a possible feature associated with growth in elevated CO_2 (Tyree and Jarvis, 1982). *Q ilex* showed greater absolute values of π_{sat}, π_{tlp}, RWC_{tlp} and Θ_{sym} than *Q pubescens* in both spring and control site. Such species differences are consistent with the leaf conductance and sap flow data, and may be related to the ability of *Q ilex* to tolerate lower water potentials. The possibility that the turgor response in *Q ilex* overrode the CO_2 effect cannot be ruled out. In *Q pubescens,* despite the osmotic adjustment showed by spring trees, leaf conductance was lower than control trees.

Despite big differences in vulnerability to xylem embolism (Raschi and Tognetti, unpublished data) and in xylem anatomy (holm oak being a diffuse-porous tree with vessels up to 150 µm in diameter and downy oak a ring-porous tree with vessels up to 500 µm in diameter), both species reached approximately the same levels of LOSK in branches: over 60%. The threshold-type relationship existing between decreasing water potential and embolism formation, and the very low leaf water potential reached at midday by both holm and downy oak in our experiment (over the value necessary to cause 60% of LOSK), could explain this similarity. However, holm oak spring trees showed a tendency for higher LOSK than control trees, while downy oak exhibited an opposite trend with may be attributed to stomatal regulation being more sensitive to high CO_2. Embolism formation may be partially responsible for the observed general decrease of sap flow in July; leaf water potentials reached levels for which cavitation rates can significantly increase.

In conclusion, elevated carbon dioxide can ameliorate the effects of drought on these two oak species by increasing osmotic potential and apoplasmic fraction of water. From this study, increased carbon dioxide does not show a clear influence on cavitation and embolism formation. Down oak exhibited lower stomatal conductance in spring tree, probably increasing WUE and enhancing the possibility to compete successfully in high CO_2 environment during dry periods. In another study, downy oak displayed similar performances in both mature trees and seedlings by increasing WUE and by fixing increased amount of carbon; this resulted in higher foliar tannin concentrations, increased specific leaf weight and enhanced isoprene emission (Johnson et al, 1996). Further studies on trees growing in the spring are needed to better understand species differences and the relative contributions of physiological, morphological and biochemical mechanims in adaption to high CO_2 and drought.

ACKNOWLEGMENTS

This work has been supported by EV Programme Environment. Contract EV5V CT 92-0093.

REFERENCES

Abrams MD (1990) Adaptions and responses to drought in *Quercus* species of North America. *Tree Physiol* 7, 227-238

Abrams MD, Menges ES (1992) Leaf ageing and plateau effects on seasonal pressure–volume relationships in three sclerophyllous *Quercus* species in Southeastern USA. *Funct Ecol* 6, 353-360

Borghetti M, Raschi A, Grace J (1989) Ultrasound emission after cycles of water stress in *Picea abies*. *Tree Physiol* 5, 229-237

Borghetti M, De Angelis P, Raschi A, Scarascia Mugnozza GE, Tognetti R, Valentini R (1993) Relations between sap velocity and cavitation in broad leaved trees. In: *Water Transport in Plants under Climatic Stress* (M Borghetti, J Grace, A Raschi, eds), Cambridge University Press, Cambridge, UK, 114-128

Dreyer E, Epron D, Yog Matig OE (1992) Photochemical efficiency of photosystem II in rapidly dehydrating leaves of 11 temperate and tropical tree species differing in their tolerance to drought. *Ann Sci For* 49, 615-625

Eamus D (1991) The interaction of rising CO_2 and temperatures with water use efficiency. *Plant Cell Environ* 14, 843-852

Eamus D, Jarvis PG (1989) The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. *Adv Ecol Res* 19, 1-55

Hinckley TM, Duhme F, Hinckley AR, Richter H (1980) Water relations of drought hardy shrubs: osmotic potential and stomata reactivity. *Plant Cell Environ* 3, 131-140

Hüber B, Schmidt E (1937) Eine Kompensationmethode zur thermoelektrischen Messung langsamer Luftstrom. *Ber Deut Bot Ges* 55, 514-529

Iolso SB (1989) Carbon dioxide and global change: earth in transition. IBR Press, Tempe, AZ, USA

Jarvis PG (1989) Atmospheric carbon dioxide and forests. *Philos Trans R Soc Lond (Biol]* 324, 369-392

Johnson JD, Michelozzi M, Tognetti R (1996) Carbon physiology of *Quercus pubescens* growing at the Bossoleto CO_2 spring in Central Italy. In: *Carbon Dioxide Springs and their Use in Biological Research* (A Raschi, F Miglietta, P Van Gardingen, eds), Cambridge University Press, Cambridge, UK (in press)

Marshall DC (1958) Measurement of sap flow in conifers by heat transport. *Plant Physiol* 33, 385-396

Miglietta F, Rashi A (1993) Studying the effect of elevated CO_2 in the open in a naturally enriched environment in central Italy. *Vegetatio* 104/105, 391-402

Miglietta F, Raschi A, Bettarini I, Resti R, Selvi F (1993) Natural CO_2 springs in Italy: a resource for examining long-term response of vegetation to rising atmospheric CO_2 concentrations. *Plant Cell Environ* 16, 873-878

Miller DR, Vavrina CA, Christensen TW (1980) Measurement of sap flow and transpiration in ring-porous oaks using a heat pulse velocity technique. *For Sci* 26, 485-489

Morse SR, Wayne P, Miao SL, Bazzaz FA (1993) Elevated CO_2 and drought after tissue water relations of birch (*Betula populifolia* Marsh) seedlings. *Oecologia* 95, 599-602

Mott KA (1988) Do stomata respond to CO_2 concentration other than intercellular? *Plant Physiol* 86, 200-203

Oechel WC, Strain BR (1985) Native species responses to increased atmospheric carbon dioxide concentration. In: *Direct Effects of Increasing CO_2 on Vegetation* (BR Strain, JD Cure, eds), United States Dept of Energy, DOE/ER-0238, 117-154

Raschi A, Tognetti R, Ridder HW, Beres C (1995) Water in the stems of sessile oak (*Quercus petraea*) assessed by computer tomography with concurrent measurements of sap velocity and ultrasound emision. *Plant Cell Environ* 18, 545-554

Sala A, Tenhunen JD (1994) Site-specific water relations and stomatal response of *Quercus ilex* in a Mediterranean watershed. *Tree Physiol* 14, 601-617

Salleo S, Lo Gullo MA (1990) Sclerophylly and plant water relations in three Mediterranean *Quercus* species. *Ann Bot* 65, 259-270

Schulte PJ, Hinckley TM (1985) A comparison of pressure–volume curve data analysis techniques. *J Exp Bot* 36, 1590-1602

Sperry JS, Donnelly R, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11, 35-40

Swanson RH, Whitfield DWA (1981) A numerical analysis of heat pulse velocity: theory and practice. *J Exp Bot* 32, 221-239

Tyree MT, Jarvis PG (1982) Water in tissues and cells. In: *Encyclopedia of Plant Physiology* (OL Lange, PS Nobel, CB Osmond, H Ziegler, eds), Springer-Verlag, Berlin, vol 12C, 35-77

Tyree MT, Alexander JD (1993) Plant water relations and the effects of elevated CO_2: a review and suggestions for future research. *Vegetatio* 104/105, 47-62

van Gardingen PR, Grace J, Harkness DD, Miglietta F, Raschi A (1995) Carbon dioxide emission at an Italian mineral spring: measurements of average CO_2 concentration and air temperature. *Agric For Meteorol* 73, 17-27

Visser H, Noppert F, van Wakeren H, Vaessen J (1989) Xylem sap velocity in relation to weather and air pollution. *IAWA Bull* 4, 427-439

Woodward FI, Bazzaz FA (1988) The responses of stomatal density to CO_2 partial pressure. *J Exp Bot* 39, 1771-1781