

Seasonal embolism and xylem vulnerability in deciduous and evergreen Mediterranean trees influenced by proximity to a carbon dioxide spring

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Summary We investigated how proximity to natural CO₂ springs affected the seasonal patterns of xylem embolism in *Quercus ilex* L., *Quercus pubescens* Willd., *Fraxinus ornus* L., *Populus tremula* L. and *Arbutus unedo* L., which differ in leaf phenology and wood anatomy. Xylem embolism was evaluated in both artificially dehydrated branches and in hydrated apical branches collected at monthly intervals during a 20-month sampling period. Initial specific hydraulic conductivity was also evaluated. We found species-dependent differences in xylem hydraulic properties in response to elevated CO₂ concentration. *Populus tremula* was the most embolized and *A. unedo* was the least embolized of the species examined. Effects of elevated CO₂ were significant in *Q. pubescens*, *P. tremula* and *A. unedo*, whereas the overall response to elevated CO₂ was less evident in *F. ornus* and *Q. ilex*. Specific hydraulic conductivity differed among species but not between sites, although the interaction between species and site was significant. Differences in xylem vulnerability between trees growing near to the CO₂ spring and those growing in control areas were small. Although differences in hydraulic properties in response to elevated CO₂ concentration were small, they may be of great importance in determining future community composition in Mediterranean-type forest ecosystems. The possible causes and ecological significance of such differences are discussed in relation to elevated CO₂ concentration and other environmental conditions.

Keywords: cavitation, CO₂ springs, diffuse-porous, global change, hydraulic conductivity, Mediterranean-type ecosystems, ring-porous, semiring-porous.

Introduction

Mediterranean forests are potentially sensitive to climate change because present rainfall barely meets potential evapotranspiration. Because predicted climate change is likely to

increase the frequency of water stress conditions in the next few decades (Wigley et al. 1984), the effects of elevated CO₂ concentration on plant water relations may be critical for Mediterranean forest tree species.

High concentrations of atmospheric CO₂ can improve plant response to water stress by inducing stomatal closure (see Tyree and Alexander 1993). Although a variety of stomatal responses to changes in CO₂ concentration has been reported (e.g., Beadle et al. 1979, Higginbotham et al. 1985, Nijs et al. 1988, Ellsworth et al. 1995, Teskey 1995, Körner and Würth 1996), a decrease in transpiration and an increase in water use efficiency and drought avoidance (Jarvis 1989, Eamus 1991) are expected to take place. Tognetti et al. (1998, 1999) reported that *Quercus* species growing near a natural CO₂ spring in Bossoleto (Siena, Italy) displayed decreased sensitivity of leaf conductance to vapor pressure deficit (VPD) compared with trees at a control site, and this decrease in sensitivity was less at high VPD than at low VPD. In late spring and at the end of summer, leaf conductance was higher at the control site, particularly in the coolest hours of the day, than at the CO₂ spring. Tognetti et al. (1996) also found increased (i.e., more negative) osmotic potential and decreased symplasmic fraction of water in *Quercus* trees adapted to elevated CO₂ concentration. By maintaining positive turgor pressure, plants were able to sustain growth and metabolism during drought at the natural CO₂ spring.

Stomata play an important role in limiting cavitation (Jones and Sutherland 1991). Because xylem sap is usually under high tension during the growing season, cavitation may be nucleated by entry of air through conduit wall pores leading to disruption of the continuous column of water (Zimmermann 1983, Sperry and Tyree 1988). Freeze-thaw cycles during winter cause xylem embolism by nucleation of gaseous bubbles formed within the xylem conduits during the transition of water from the liquid to the ice phase (Tyree and Sperry 1989, Sperry and Sullivan 1992). Extensive xylem embolism has

been observed in several species during the growing season even under conditions of limited stress (Cochard and Tyree 1990, Tyree et al. 1992, Kolb and Davis 1994, Sperry et al. 1994, Tyree et al. 1994, Magnani and Borghetti 1995), particularly in older xylem (Sperry et al. 1988a). Elevated concentrations of atmospheric CO₂ may cause higher growth rates during spring when water resources are adequate, but this response might make plants more vulnerable to cavitation and thus less likely to survive static drought stresses as a result of soil drying later in the growing season.

We measured hydraulic conductivity in branches of five Mediterranean tree species differing in growth habit and wood structure. *Quercus ilex* L. is a diffuse-porous evergreen tree, and a climax species in Mediterranean forests. *Quercus pubescens* Willd. is a ring-porous deciduous tree, and a co-dominant species (with *Q. ilex*) in many mature communities over large areas of the Mediterranean. *Fraxinus ornus* L. is a ring-porous deciduous tree characteristic of mixed forests on bedrock in the Mediterranean region. *Populus tremula* L. is a semiring-porous deciduous tree widespread throughout Europe. *Arbutus unedo* L. is a semi-ring-porous evergreen shrub or tree characteristic of Mediterranean coastal scrub vegetation. We took advantage of a natural CO₂ experiment (Amthor 1995) in which forest trees surround natural CO₂ springs to determine how elevated CO₂ concentration affects xylem transport capacity in co-occurring tree species. We hypothesized that long-term differences in atmospheric CO₂ availability between CO₂ springs and a neighboring control site would be reflected in improved water use and hydraulic architecture modifications in trees growing by the CO₂ spring.

Materials and methods

Site description

The natural CO₂ springs of Bossoleto and Armaiolo (43°17' N, 11°35' E and 350 m.a.s.l.) are located near Rapolano Terme (Siena, central Italy); detailed information about the sites is given elsewhere (Miglietta and Raschi 1993, Miglietta et al. 1993, Körner and Miglietta 1994, Jones et al. 1995, van Gardingen et al. 1995). The CO₂ vents occur both at the bottom and on the flanks of a circular doline (Bossoleto) and on the banks of a creek (Armaiolo); concentration gradients are enhanced under stable (windless) atmospheric conditions. Daytime CO₂ concentrations around the crown of the experimental plants ranged from 500 to 1000 μmol mol⁻¹ with rapid fluctuations. The H₂S and SO₂ concentrations at the springs are low and cannot be considered harmful to plants (H. Rennenberg, Universitaet, Freiburg, Germany, personal communication). Large parts of both sites are forested with a coppice in which *Q. ilex* and *Q. pubescens* are the main species. The control sites were chosen for their similarity to the CO₂ spring sites with respect to tree age and association, morphology, light exposure, and soil nutrient availability and depth (van Gardingen et al. 1997).

Measurements were carried out (summer and autumn) over two consecutive years, 1993 and 1994, on mature trees of *Q. pubescens*, *Q. ilex*, *F. ornus*, *P. tremula* (Bossoleto) and *A. unedo* (Armaiolo). Trees were 6–15 cm in diameter, and

3–9 m in height. The climate is typically sub-Mediterranean with rainfall occurring mainly during autumn and winter. The dry period extends from mid-June to mid-September. Frost is common in winter, particularly in January and February. In December 1993 and February and March 1994 (Figure 1), the minimum daily temperature dropped below 0 °C frequently (minimum –6 °C), whereas in January 1994 minimum daily temperature was rarely below zero. In all months, maximum daily temperature was always above zero. The microclimate did not differ between the control and CO₂ spring sites.

Monitoring seasonal occurrence of embolism

Xylem embolism in apical branches was quantified by determining the hydraulic conductivity (mass flow rate/pressure gradient) of the xylem before and after the removal of air emboli from cavitated vessels by the flushing method described by Sperry et al. (1988b). Five to six apical branches (1–1.2 m long) were collected at dawn monthly from the upper crown of each of six trees per species. Branches were immediately enclosed in polyethylene bags, stored in a cooler in the dark and brought to the laboratory within 2 hours of collection. Branches were then soaked for 15–30 min before measurement to release tension in the xylem and to prevent artifacts in the measurement of hydraulic conductivity resulting from tissue capacitance (Sperry et al. 1988b). From each branch, a segment (1–2 years old) 150–200 mm long and about 5 mm in diameter was cut under water and then, while still submerged, recut to avoid further embolism. Each segment was located far enough from the original branch base to avoid including conduits embolized during branch collection. Both ends of the stem section were fitted with rubber gaskets, trimmed with a razor blade in distilled water and attached to rubber tubes. The perfusing solution was 10 mM (10 mol m⁻³) oxalic acid in distilled water (pH 1.8) to minimize microbial occlusions of xylem vessels. The perfusing solution was degassed by agitating under vacuum for 60 min, and then introduced into an air-free plastic ball enclosed in a compressed gas tank. The solution, passed through a 0.2-μm inline filter (POLYCAP-36AS, Arbor Technologies, Inc., Ann Arbor, MI), perfused the samples under a constant pressure gradient maintained by a pressure regulator. Stopcocks allowed selective influx for

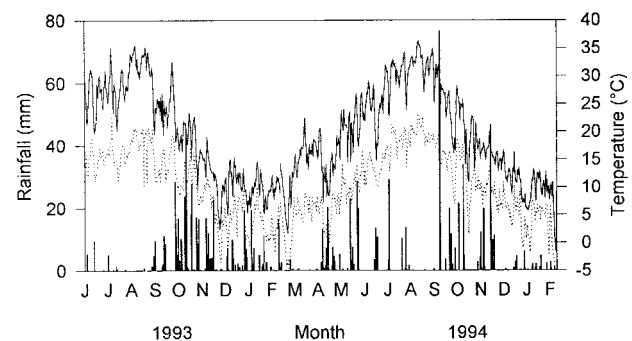


Figure 1. Seasonal changes in daily precipitation, and maximum and minimum daily air temperatures at the study sites over the sampling period.

measuring the initial conductivity (K_i) on one sample at a time under a pressure gradient of 9–10 kPa. Maximum conductivity (K_m) was determined by pressurizing the solution through all the segments at 150 kPa for 60–90 min. The permeating solution flowed from the pressurizing reservoir across the samples and into a container on an analytical balance connected to a computer that processed the data.

Measurements of hydraulic conductivity were recorded every 30 s and calculated by averaging ten readings after steady state had been achieved. Percent loss of hydraulic conductivity (Loss K) was determined as: $\text{Loss } K = 100 (K_m - K_i) / K_m$. Initial specific hydraulic conductivity (K_S), which was used as a measure of the porosity of the xylem on a cross-sectional area basis, was calculated from: $K_S = K_i / A_w$, where A_w is the cross-sectional area of xylem tissue.

Xylem embolism in dehydrated branches

The vulnerability of xylem to embolism was determined as percent embolism expressed as a function of the minimum water potential reached by a dehydrating branch. In spring, several branches (3–5 years old at the base and 1.5–2 m long, longer than all or most of the conduits in each species) were cut in the field. Because native loss in hydraulic conductivity was high throughout the year, branches were wrapped in plastic bags and allowed to equilibrate (and to promote air diffusion into cavitated conduits) overnight with the basal ends in water. Branches were then recut under water and flushed with distilled degassed water at 150 kPa for 1.5 h and allowed to dehydrate on the laboratory bench. During dehydration, xylem water potential (Ψ) was measured at regular intervals with a pressure chamber on three excised shoots from each branch. Simultaneously, the extent of xylem embolism was measured on another three excised shoots from each branch. Values of water potential and embolism were plotted on the same graph to produce a vulnerability curve.

Results

The summers of 1993 and 1994 were both characterized by droughts (Figure 1). Predawn and minimum shoot water potentials were generally correlated with rainfall (see Jones et al. 1995, Tognetti et al. 1996, 1998, 1999). At the onset of drought (late June), a sharp decrease in water potential was observed. In midsummer 1993 (drier than 1994), minimum water potential dropped to low values and predawn values were only slightly above minimum water potential values. These values were well below the threshold values inducing cavitation and also below the osmotic potential at turgor loss point. Full recovery of water potentials was observed after the first major rainfall in September. At that time, predawn water potential approached spring values.

Branch segments were severely embolized throughout the year. *Populus tremula* was the most embolized and *A. unedo* was the least embolized of the species examined (Table 1). Throughout the 1993 and 1994 summers, embolism fluctuated between 50 and 70% depending on the species. Percent loss of hydraulic conductivity was lowest—between 30 and 60%, depending on the species—after the rains in early autumn of both years (Table 1). Of the species examined, *P. tremula* showed the most consistent recovery of hydraulic conductivity. Coinciding with freezing-thawing events during the 1993–1994 winter (December 1993 to February 1994), embolism increased to about 60–70% in each species (regardless of site), except in *P. tremula* where it was 30–50%.

Effects of elevated CO₂ on percent loss of hydraulic conductivity were significant ($P < 0.05$, ANOVA) in *Q. pubescens*, *P. tremula* and *A. unedo* (Table 1), but the overall response to elevated CO₂ was less evident in *F. ornus* ($P = 0.08$) and *Q. ilex*. Seasonal differences in the effects of elevated CO₂ on percent loss of hydraulic conductivity were observed. In *Q. pubescens*, the percentage loss of hydraulic conductivity was lower in trees at the CO₂ spring site than in trees at the control site throughout the year. In *P. tremula* and *A. unedo*, percent loss of hydraulic conductivity was similar at both sites in

Table 1. Percent loss of hydraulic conductivity (Loss K) of branches of *Q. ilex*, *Q. pubescens*, *P. tremula*, *F. ornus* and *A. unedo* growing at the CO₂ spring and at the control site. Values are means (\pm SE, $n = 13$ – 32) of samples collected about monthly during the course of the experiment from June 1993 to January 1995. Data are grouped per season. The probability (P) is shown in the lower part of the table.

Season	<i>Q. pubescens</i>		<i>Q. ilex</i>		<i>F. ornus</i>		<i>P. tremula</i>		<i>A. unedo</i>	
	Control	CO ₂ spring	Control	CO ₂ spring	Control	CO ₂ spring	Control	CO ₂ spring	Control	CO ₂ spring
Winter	72 \pm 4.0	63 \pm 3.3	66 \pm 3.9	64 \pm 2.5	65 \pm 5.5	64 \pm 5.2	47 \pm 3.6	34 \pm 6.5	67 \pm 5.1	63 \pm 5.5
Spring	65 \pm 2.9	48 \pm 4.7	57 \pm 4.1	59 \pm 2.1	62 \pm 2.6	53 \pm 2.4	51 \pm 3.8	40 \pm 2.7	55 \pm 3.9	50 \pm 3.9
Summer	70 \pm 4.1	59 \pm 4.2	66 \pm 3.5	63 \pm 2.7	72 \pm 3.9	65 \pm 4.6	74 \pm 4.4	75 \pm 6.3	55 \pm 6.0	57 \pm 5.5
Fall	46 \pm 4.2	50 \pm 2.1	53 \pm 4.0	55 \pm 2.7	50 \pm 4.0	48 \pm 2.3	50 \pm 3.9	36 \pm 3.9	58 \pm 3.8	39 \pm 3.1
Site	0.0051		0.9328		0.0801		0.0057		0.0409	
Season	0.0000		0.0017		0.0000		0.0000		0.0050	
Site \times Season	0.0752		0.7573		0.6409		0.3599		0.0971	

summer. In *F. ornus*, but not *Q. ilex*, there was a tendency for a lower percent loss of hydraulic conductivity in trees at the CO₂ spring site than in trees at the control site in summer. In autumn, *A. unedo* and *P. tremula* showed more consistent recovery of percent loss of hydraulic conductivity at the CO₂ spring site than at the control site, whereas recovery of percent hydraulic conductivity loss was delayed in *F. ornus* trees at the CO₂ spring site compared with trees at the control site (data not shown). In contrast, recovery from winter embolism was earlier for *F. ornus* trees at the CO₂ spring site than for trees at the control site (data not shown). After bud burst, in late spring, xylem embolism decreased to 40–60% in all species. *Quercus pubescens*, *F. ornus* and *A. unedo* trees growing at the CO₂ spring showed a larger decrease in xylem embolism in spring than trees growing at the control site, whereas *Q. ilex* displayed the opposite trend.

The relationship between initial hydraulic conductivity and stem segment diameter, after log-transformation to linearize the data (Figure 2), showed a general increase ($P < 0.01$) in hydraulic conductivity as stem diameter increased for all species except *F. ornus*. For *F. ornus*, *P. tremula* and *A. unedo* the relationship differed ($P < 0.05$, ANCOVA) between sites.

Overall, initial specific hydraulic conductivity was significantly different between species ($P < 0.0001$, ANOVA) but not between sites (Table 2); however, the interaction between species and site was significant ($P < 0.05$). *Quercus pubescens*, *Q. ilex* and *A. unedo* trees growing at elevated CO₂ concentration had an increased hydraulic efficiency (higher initial specific hydraulic conductivity), whereas the other two species showed the opposite tendency. However, the differences in initial specific hydraulic conductivity (with the exception of *Q. pubescens*) between diffuse-porous and ring- or semiring-porous species were small (Wang et al. 1992). Considering all species, those with high initial specific hydraulic conductivity were prone to greater loss of conducting capacity during winter months.

In all species studied, there was a significant negative relationship ($P < 0.001$) between water potential and xylem embolism in branches dehydrating in air (Figure 3). About 40% of hydraulic conductivity was lost at water potentials ranging from -1 to -3 MPa depending on the species, with *Q. ilex* being the least vulnerable and *P. tremula* the most vulnerable. An 80% loss of hydraulic conductivity was reached at water potentials varying from -2 MPa (*P. tremula*) to -5 MPa (*Q. ilex*), and at intermediate values (-3 to -4 MPa) for the other species. Differences in xylem vulnerability between control trees and trees from the CO₂ spring were small and followed no particular trend.

Discussion

Zimmermann (1983) postulated that there was a trade-off between xylem efficiency and xylem safety in woody plants. Plants with high specific hydraulic conductivity (high efficiency in water transport because of wide vessels) may be inherently more susceptible to drought-induced embolism than plants with low specific hydraulic conductivity. However, comparisons between tree species on the basis of the relation-

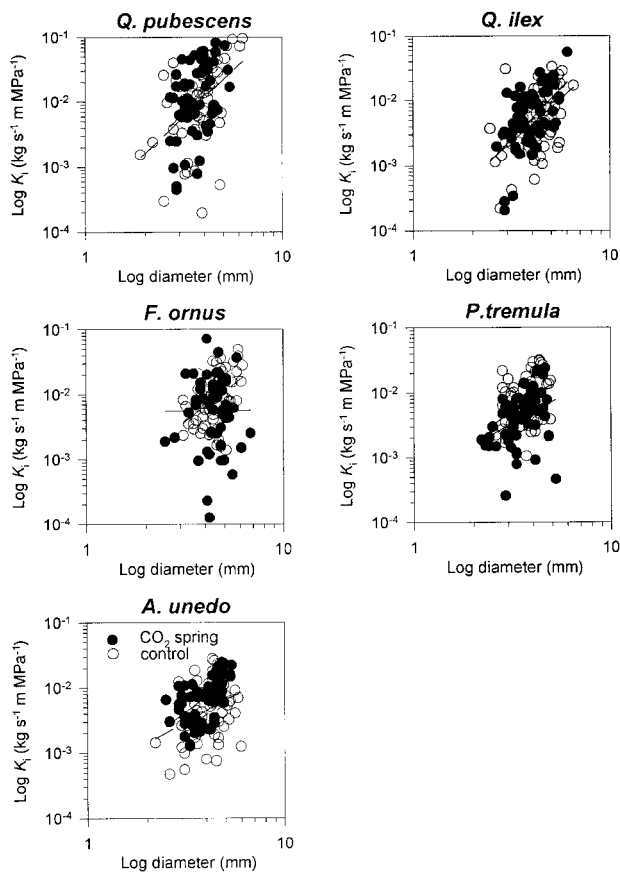


Figure 2. Relationship between initial specific hydraulic conductivity and branch diameter for *Q. pubescens*, *Q. ilex*, *F. ornus*, *P. tremula* and *A. unedo* trees at the control (○) and CO₂ spring (●) sites. Data were analyzed by least squares regression, following log-transformation of both variables to linearize the data. Regression values (a = intercept and b = slope): *Q. pubescens*, $a = -3.654$ (control) and -3.763 (CO₂ spring), $b = 2.856$ (control) and 3.220 (CO₂ spring); *Q. ilex*, $a = -3.963$ (control) and -4.143 (CO₂ spring), $b = 2.666$ (control) and 3.112 (CO₂ spring); *F. ornus*, $a = -3.587$ (control) and -2.267 (CO₂ spring), $b = 2.267$ (control) and 0.021 (CO₂ spring); *P. tremula*, $a = -3.354$ (control) and -3.307 (CO₂ spring), $b = 2.142$ (control) and 1.673 (CO₂ spring); *A. unedo*, $a = -3.348$ (control) and -3.495 (CO₂ spring), $b = 1.654$ (control) and 2.253 (CO₂ spring). Values of R^2 ranged between 0.11 and 0.32 (with the exception of *F. ornus* CO₂ spring trees that showed values even lower than 0.01).

ship between vulnerability to embolism and conduit diameters can be misleading (Lo Gullo et al. 1995), because vulnerability to drought-induced cavitation of xylem conduits can result from many factors, including pit membrane pore size and the flexibility of the pit membranes (Crombie et al. 1985, Sperry and Tyree 1990). In addition, conduit structure and functionality can be influenced by atmospheric water vapor deficit (Whitehead et al. 1983). Because elevated CO₂ concentration may affect the sensitivity of stomatal conductance to vapor pressure deficit (Tognetti et al. 1998, 1999), we postulated that environmentally driven changes in xylem differentiation might be mediated by the growth atmospheric CO₂ concentration.

A more efficient xylem may be a prerequisite for sustaining the relatively short-lived, metabolically more active leaves of

Table 2. Specific hydraulic conductivity (K_S) of branches of *Q. ilex*, *Q. pubescens*, *P. tremula*, *F. ormus*, and *A. unedo* growing at the CO₂ spring and at the control site. Values are means (\pm SE, $n = 56-65$) of samples collected about monthly during the course of the experiment from June 1993 to January 1995. The probability (P) is shown in the lower part of the table.

Species	K_S (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	
	CO ₂ spring	Control
<i>Q. pubescens</i>	1.798 \pm 0.206	1.500 \pm 0.178
<i>Q. ilex</i>	0.590 \pm 0.059	0.549 \pm 0.078
<i>F. ormus</i>	0.692 \pm 0.117	0.710 \pm 0.066
<i>P. tremula</i>	0.548 \pm 0.047	0.920 \pm 0.091
<i>A. unedo</i>	0.688 \pm 0.048	0.531 \pm 0.063
Species	0.0000	
Site	0.7585	
Species \times Site	0.0312	

deciduous species (*Q. pubescens* and secondarily *F. ormus* and *P. tremula*) compared with that required for long-lived, metabolically less active leaves of evergreen (*Q. ilex* and *A. unedo*), sclerophyllous species (Kolb and Davis 1994, Larcher 1995). Enhanced efficiency of hydraulic transport (high initial specific hydraulic conductivity) may permit increased transpiration rates during the spring, thus facilitating rapid growth and high rates of photosynthesis. Atkinson and Taylor (1996) found an increase in hydraulic efficiency in seedlings of *Quercus robur* L. when grown at elevated CO₂ concentration and associated this response with the observed increase in mean vessel size; however, this relationship was not observed in seedlings of *Prunus avium* (L.) L. \times *Prunus pseudocerasus* Lindl.

The generally high percent embolism measured in this study may in part be an experimental artifact caused by the method of determining maximum hydraulic conductivity. Many embolized vessels in older growth rings, particularly of ring-porous trees, have been temporarily blocked by tyloses and could, therefore have contributed to hydraulic conductivity after the experimental flushing procedure. However, the oldest branch segments measured were only 1–2 years old. In *Quercus cerris* L., Lo Gullo et al. (1995) found that the embolism induced in older stem parts was significantly higher than in 1-year-old stems, suggesting that the greater vulnerability of older stems was a function of the increased length and diameter of conduits with age. It has been suggested that embolism of older xylem that is only indirectly associated with transpiring leaves may be of minor functional relevance (Sperry et al. 1991, Magnani and Borghetti 1995).

Significant amounts of xylem embolism during the growing period have been observed in other hardwoods including *Fagus sylvatica* L., *Fraxinus excelsior* L. and *Populus \times canadensis* "robusta" (Magnani and Borghetti 1995, Hacke and Sauter 1996). In our study, species differed with respect to the degree of xylem embolism exhibited with *A. unedo* and *Q. ilex*

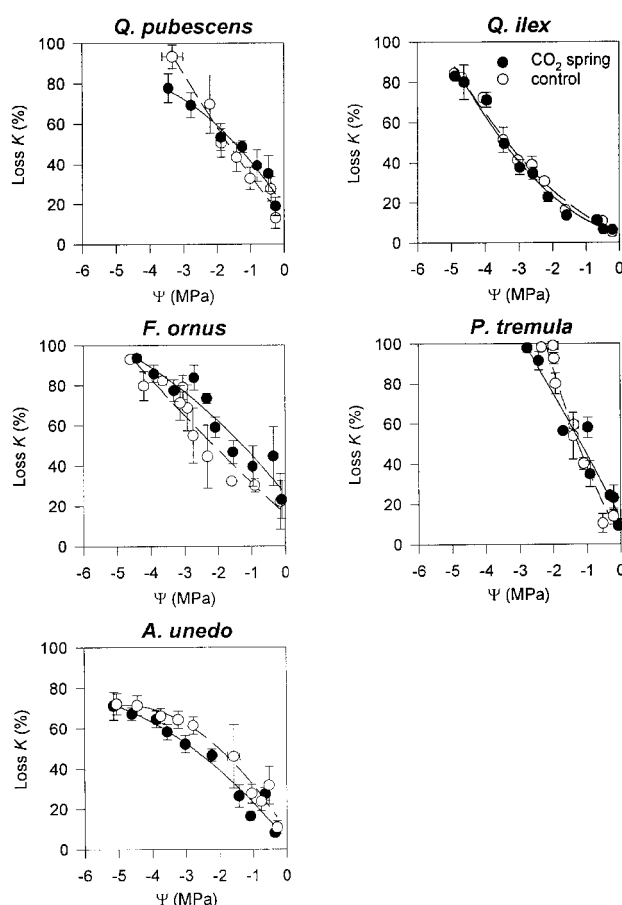


Figure 3. Relationship between percent loss of hydraulic conductivity (Loss K) and xylem water potential of branches (Ψ) in dehydrating branches of *Q. pubescens*, *Q. ilex*, *F. ormus*, *P. tremula* and *A. unedo* trees at the control (○) and CO₂ spring (●) sites. Each line represents a second-order polynomial function fitted to the data. Coefficient values are: 12.457, -20.853, 1.126 (control, $R^2 = 0.97$) and 18.943, -25.081, -2.415 (CO₂ spring, $R^2 = 0.96$) for *Q. pubescens*; 4.684, -6.625, 2.109 (control, $R^2 = 0.99$) and 4.554, -4.136, 2.643 (CO₂ spring, $R^2 = 0.98$) for *Q. ilex*; 16.006, -15.058, -0.447 (control, $R^2 = 0.91$) and 25.933, -20.583, -1.190 (CO₂ spring, $R^2 = 0.91$) for *F. ormus*; 4.796, -25.764, 7.734 (control, $R^2 = 0.96$) and 12.950, -31.962, -0.523 (CO₂ spring, $R^2 = 0.94$) for *P. tremula*; 8.541, -26.123, -2.706 (control, $R^2 = 0.96$) and 4.070, -20.536, -1.436 (CO₂ spring, $R^2 = 0.95$) for *A. unedo*.

being the least embolized species in summer. The finding of a lower vulnerability to xylem embolism in *A. unedo* and *Q. ilex* compared with the other species examined would, in theory, allow these species to survive higher stem xylem tensions than the other species. It is worth noting that these species are phytosociologically dominant in Mediterranean-type forests.

An active role of precipitation in embolism reversion cannot be ruled out. Sperry et al. (1988a) suggested that rainfall could favor the development of root pressure in *Acer saccharum* Marsh. In our experiment, however, there was only partial recovery of percent loss of hydraulic conductivity in response to rainfall, perhaps indicating that the high resistance to flow of narrow conduits in apical branches makes root pressure

insufficient to refill them (Lo Gullo and Salleo 1993). A mechanism for the partial and gradual recovery of xylem function after early autumn rainfall (particularly evident in *A. unedo* and *P. tremula* trees at the CO₂ spring site compared with control trees of the same species) could involve the formation of new xylem tissue (Kolb and Davis 1994). In the Mediterranean environment, continued radial growth is common in many species in autumn before leaf abscission. Moreover, a reduction in transpiration in response to elevated CO₂ concentration may result in quicker recovery of xylem conductivity after the end of drought because of reduced water consumption.

Substantial winter xylem embolism has been reported for many species including *Quercus alba* L. and *Quercus rubra* L. (Cochard and Tyree 1990), *Fagus sylvatica* (Magnani and Borghetti 1995) and *Fagus grandifolia* J.F.Ehrh. (Sperry 1993), and *Alnus cordata* (Loisel.) Duby (Tognetti and Borghetti 1994). It has been shown that the size of bubbles formed during freezing depends on the diameter of the conduit and that large bubbles take a long time to dissolve (Ewers 1985), making ring-porous trees particularly vulnerable to freeze-thaw-induced embolism (Cochard and Tyree 1990). However, in our experiment, all of the species (except *P. tremula*), regardless of species-specific vessel size, showed a similar percent loss of hydraulic conductivity during the winter. During the winter months, *P. tremula* and *A. unedo* trees at the CO₂ spring site showed a consistently lower percent embolism compared to control trees of the same species, which may be associated with modifications in xylem structure in trees grown in elevated CO₂ concentration. The partial recovery of hydraulic conductivity observed in late spring might be related to the production of new functional xylem as a result of increased cambial activity (Tognetti and Borghetti 1994). In early spring, before full leaf expansion, the water pathway provided by the conduits produced in previous years may be sufficient to supply the young growing leaves (Lo Gullo et al. 1995).

Differences in the timing and degree of spring recovery among the study species may be related to differences in the effects of elevated CO₂ concentration on phenological state (e.g., cambial activity). In particular, *Q. pubescens*, *F. ornus* and *A. unedo* trees growing at the CO₂ spring show reduced stomatal conductance compared with control trees of the same species during springtime (Jones et al. 1995, Bettarini et al. 1998, Tognetti et al. 1999) that was reflected in a more consistent recovery of percent embolism. In *Q. ilex*, the difference in percent embolism between trees growing at the CO₂ spring site and trees growing at the control site was small and sometimes in the opposite direction to that observed in *Q. pubescens*. Tognetti et al. (1998) observed that the stomatal response to elevated CO₂ concentration was also limited in *Q. ilex*. Despite the observed species-dependent differences between trees growing at the CO₂ spring sites and trees growing at the control site, there was no clear indication of an alteration in the hydraulic properties of the xylem (hydraulic conductivity and vulnerability to embolism) associated with xylem structure (ring-porous, semiring-porous or diffuse-porous wood types), or leaf habit (evergreen or deciduous) in response to elevated

CO₂ concentration. Because the species-dependent differences between trees growing at the control and CO₂ spring sites were often correlated with stomatal response (see also Heath et al. 1997), we postulate that these differences and the interaction with seasonal stress events might strongly influence the competitive ability of these species in a global change scenario.

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