

Vulnerability of xylem to embolism in relation to plant hydraulic resistance in *Quercus pubescens* and *Quercus ilex* co-occurring in a Mediterranean coppice stand in central Italy

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SUMMARY

The seasonal patterns of xylem embolism and xylem transport properties in *Quercus pubescens* Willd. and *Quercus ilex* L. trees growing in a natural mixed coppice stand in conditions of severe water stress were investigated. Xylem embolism was evaluated in both dehydrating branches and in apical twigs during a whole year. Measurements of xylem water potential were conducted from predawn to sunset on selected sunny days. On the same days, diurnal courses of leaf conductance were monitored. Measurements of half-hourly sap flow were made by the heat-pulse technique throughout the summer. At the onset of summer, a sharp decrease in water potential was observed in both species. Full recovery of water potentials was observed for both species after the first major rainfall event in September. Both experienced serious embolism throughout the year, ranging between minima of c. 60% (expressed as percentage loss of hydraulic conductivity) after the rains in autumn and after bud burst in spring, and maxima of c. 80% during summer and after freezing-thawing events during the winter season. A significant negative linear relationship was found between water potential and xylem embolism in branches dehydrating in air for *Q. pubescens* and *Q. ilex*. *Q. pubescens* had greater efficiency in hydraulic transport (higher specific conductivity and leaf specific conductivity) by the xylem than *Q. ilex*. In June, leaf conductance was high early in the morning and decreased gradually during the day. Midday depression of leaf conductance, as a result of high evaporative demand combined with water deficit, was observed in both species. In August, leaf conductance of both species was greatly reduced, as water potential dropped to extremely low values, and the stomata were almost completely closed during the afternoon. No hysteresis resulting from plant capacitance was observed in the relationship between shoot water potential and sap flow. *Q. pubescens* exhibited very high values of whole-tree hydraulic resistance between July and September, whereas *Q. ilex* generally showed lower values. The effect of soil moisture depletion on the relationship between sap flow and shoot water potential appears as a lowering of water potential at zero flow. A significant decrease of whole-tree hydraulic resistance in both species was observed with the onset of the autumn, preceding the partial recovery of twig hydraulic conductivity. The results demonstrate that both *Q. pubescens* and *Q. ilex*, although highly tolerant of severe water stress and tissue dehydration, operate at the limits of safety which are surpassed under severe droughts, and prolonged climatic stress might predispose these *Quercus* species to decline.

Key words: cavitation, drought, hydraulic resistance, *Quercus ilex* L., *Quercus pubescens* Willd., water relations, xylem embolism.

INTRODUCTION

In Mediterranean environments, characterized by a long dry season in which rainfall does not meet evapotranspirative demand, xylem embolism may

represent a serious cause of reduced productivity, through catastrophic xylem dysfunction caused by runaway processes (Tyree & Sperry, 1988). The xylem sap of plants is usually under high tension in the growing season and, therefore, cavitation may be nucleated by the entry of air through conduit wall pores disrupting the continuous column of water

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(the so-called 'air-seeding' hypothesis Zimmermann (1983), Sperry & Tyree (1988)). The effects of freeze-thaw cycles during winter also imply xylem embolism by nucleation of gaseous bubbles formed within the xylem conduits during the transition of water from the liquid to the ice phase (Sucoff, 1969; Cochard & Tyree, 1990; Just & Sauter, 1991; Sperry & Sullivan, 1992; Wang, Yves & Lechowicz, 1992). Cavitated conduits, although initially vapour-filled, quickly fill with air as gas diffuses from surrounding air spaces (Yang & Tyree, 1992). These conduits are then non-conductive even at atmospheric pressure, thus increasing hydraulic resistance in the stem. As a result of cavitation-induced embolism in twigs and petioles, hydraulic conductance (and possibly stomatal conductance) is reduced (Tyree *et al.*, 1991, 1993; Yang & Tyree, 1993, 1994). Therefore, it is possible that the flow of water through the plant can be altered by modification of plant hydraulic architecture, including any changes in the hydraulic resistance of the root system (Cohen, Moreshet & Fuchs, 1987; Bréda *et al.*, 1993; Magnani & Borghetti, 1995).

Extensive xylem embolism has been observed in several species during the growing season even under non-stress conditions (Cochard & Tyree, 1990; Tyree, Alexander & Machado, 1992; Sperry *et al.*, 1994; Kolb & Davis, 1994; Tognetti & Borghetti, 1994; Tyree *et al.*, 1994. Magnani & Borghetti, 1995), in particular in older xylem (Sperry, Donnelly & Tyree, 1988*a*). In fact, cavitation in plants has to be considered a normal event (Milburn, 1991).

Trees differ widely in their vulnerability to drought- and freeze-induced embolism (Tyree & Ewers, 1991). Big conduits are considered efficient for hydraulic conduction but highly vulnerable to cavitation compared with small conduits in a stem, but this is not the case as different taxa are compared (Tyree & Sperry, 1989). In general, the physiological relevance of embolism under field conditions is still unclear.

The purpose of this study was to investigate the seasonal patterns of xylem embolism and xylem transport properties in *Quercus pubescens* and *Quercus ilex* trees growing in a natural mixed coppice stand in conditions of severe water stress (summer 1993 and 1994), by evaluating the vulnerability of both species to water stress-induced embolism (Tyree & Cochard, 1996). We also attempted to relate embolism to the time course of total tree hydraulic resistance and tree water status. In the Mediterranean area these two species, with contrasting leaf habit (deciduous vs. evergreen) and xylem conduit size (ring-porous vs. diffuse-porous), co-occur (Salleo & Lo Gullo, 1990), but the ecophysiological responses of *Q. pubescens* and *Q. ilex* in the field are poorly documented (Borghetti *et al.*, 1993; Damesin & Rambal, 1995; Tognetti *et al.*, 1996*a*). Questions arise about how *Q. pubescens* competes with *Q. ilex* and *vice versa*,

and how the growth of these species is affected by restricted water availability, particularly in rain-deficient years that are expected to occur more frequently in coming decades (Wigley, Briffa & Jones, 1984).

MATERIALS AND METHODS

Site description and plant material

Measurements were conducted from May 1993 to December 1994 in an uneven-aged *Quercus pubescens* Willd. and *Quercus ilex* L. Mediterranean coppice stand (mean height and diameter, 6 m and 11 cm, respectively). Tree ages were determined from stem core samples and ranged between 15 and 25 yr. The sampling site (43° 17' N, 11° 35' E) is located c. 40 km south-east of the city of Siena in Tuscany (central Italy) in a south-west facing slope at 350 m above sea level. Climate (summarized in Table 1) is typically sub-Mediterranean with rainfall occurring during autumn and winter. The dry period extends from mid-June to mid-September. Frost is common in winter and particularly in January and February.

Daily minimum temperatures below -6 °C were observed during January 1993 (in the same year minimum temperature was also frequently below zero in February and March). During December 1993 and, particularly, February 1994, the minimum daily temperature dropped below 0 °C frequently (minimum -5 °C), whereas in January 1994 minimum daily temperature was rarely below zero and the maximum was always above zero. The soil layer (pH 6.9) is of the rendzina type, has a depth of only 5–10 cm and rests on travertine bedrock. As usual in such calcareous areas, roots penetrate crevices to much greater depth and this may provide a source of water throughout the long, dry summers. The stand consists of mainly *Q. ilex* and *Q. pubescens* and secondarily of *Arbutus unedo* L., *Calluna vulgaris* (L.) Hull, *Erica arborea* L. and other isolated tree species with a sparse herbaceous layer.

Monitoring seasonal occurrence of embolism

Xylem embolism in apical twigs 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 (Sperry, Donnelly & Tyree, 1988*a*). Ten to twelve 1-yr-old and 2-yr-old apical twigs were collected monthly from the upper crown of each of six trees per species. Twigs were immediately enclosed in polyethylene bags stored in a cooler in the dark and brought to the laboratory within 2 h of abscission. In the laboratory, twigs were soaked for 15–30 min before measurement in order to release tension in the xylem, and to prevent

Table 1. Monthly precipitation and minimum and maximum temperatures at the study site during the experimental period in 1993 and 1994

Month	1993			1994		
	Rainfall (mm)	T_{\min} (°C)	T_{\max} (°C)	Rainfall (mm)	T_{\min} (°C)	T_{\max} (°C)
Jan.	3.4	3.01	8.80	50.0	3.56	9.79
Feb.	2.2	1.07	10.59	26.6	2.35	9.90
Mar.	36.4	2.39	12.20	1.8	6.46	17.14
Apr.	60.2	7.02	16.49	73.0	5.99	14.80
May	45.6	11.87	22.78	84.6	11.25	22.08
June	20.0	15.09	26.51	72.2	14.24	25.37
July	19.0	16.02	28.28	26.4	19.04	31.21
Aug.	16.4	18.36	31.19	0.8	18.90	32.27
Sept.	88.0	13.70	23.75	143.2	14.9	24.20
Oct.	198.6	10.90	18.15	79.6	10.20	17.80
Nov.	78.6	5.18	10.76	70.2	8.37	15.05
Dec.	71.6	4.16	10.52	21.4	4.06	9.87

artefacts in the measurement of hydraulic conductivity resulting from tissue capacitance (Sperry *et al.*, 1988b). From each twig, a segment 150–200-mm long and about 5 mm in diameter was cut under water and then, while still submerged, recut to avoid further embolism. Both ends of the stem section were fitted with rubber gaskets, trimmed with a razor blade in distilled water and mounted on 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, and it 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, passing through a $0.2 \mu\text{m}$ inline filter (POLYCAP-36AS, Arbor Technologies Inc., Ann Arbor, MI, USA), perfused the samples under a constant pressure gradient maintained by a pressure regulator. Stopcocks allowed selective influx for measuring the initial conductivity (K_i) on one sample at a time under a pressure gradient of 9–10 kPa. The 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 joined to a computer that automatically made the calculations. Measurements of hydraulic conductivity were recorded every 30 s and calculated by averaging ten readings after steady-state had been achieved. Embolism causing per cent loss of hydraulic conductivity ($LOSK$) was assessed from: $LOSK = 100 (K_m - K_i)$. Specific hydraulic conductivity (KS), as a measure of the porosity of the xylem on a cross-sectional area basis, was calculated from: $KS = K_i/A_w$, where A_w is the cross-sectional area of xylem tissue. Leaf specific hydraulic conductivity (LSC), as a measure of xylem efficiency, was calculated from: $LSC = K_i/A_L$, where A_L is the area of foliage supplied by the branch.

Field measurements of water potential, leaf conductance and sap flow

Shoot water potential and leaf conductance were measured on the same trees sampled for the determination of xylem embolism. At regular intervals throughout the summer (1993 and 1994), xylem water potential was measured with a pressure chamber (PMS-100, PMS Instrument Co. Corvallis, OR, USA) on three to six apical twigs per tree. Measurements were conducted from predawn (Ψ_{pd}) to sunset on selected sunny days. On the same days, diurnal courses of leaf conductance (g_s) were monitored on four to six fully illuminated apical leaves per tree (reached through a scaffolding or natural rocks) with a steady-state diffusion porometer (LI-1600, Li-Cor, Lincoln, NE, USA). The natural inclination and azimuth of the leaves were maintained during the measurements. For each measurement, the temperature inside the cuvette, the leaf temperature, the r.h. and the incoming photosynthetically active radiation (PAR) were recorded. Precautions were taken to avoid large differences between external environmental conditions and those inside the cuvette. Cuvette overheating was minimized by shading the cuvette between measurements. Leaf conductances were expressed in molar units to account for differences in temperature and atmospheric pressure due to altitude. Changes in leaf conductance were considered to reflect changes in stomatal conductance, assuming that boundary layer conductance inside the cuvette was constant and large.

Sap flow was measured on two trees per species (*Q. ilex* trees were bigger than *Q. pubescens* trees), among those sampled for water potential measurement, throughout the summers of 1993 and 1994. Measurements of half-hourly sap flow were made by the heat-pulse technique (Borghetti *et al.*, 1993, Raschi *et al.*, 1995; Tognetti *et al.*, 1996a, b). Sap

velocity sensor units (Soil Conservation Centre, Palmerston North, New Zealand) were deployed at breast height on each tree. Each sensor unit consisted of a heating probe that penetrated the xylem, and two thermistor probes. Four units were inserted in four directions at right angles to one another on the stem circumference and penetrated into the xylem at different depths beneath the cambium. Thermistor probes were sited 10 mm above and 5 mm below each heating probe. Heating and thermistor probes were vertically aligned. At 30-min intervals, 5-s heat pulses were automatically triggered by the logger. The calculation of sap velocity from heat-pulse velocity was made after Swanson (1962) and included correction for the effect of wounding (Swanson & Whitfield, 1981), Marshall's (1958) equation being used to convert heat pulse velocity to sap flow.

The relationship between values of water potential in apical twigs (Ψ) and sap flow (F) was examined during the measuring period. Tree hydraulic resistance (R_{tree}) was estimated as the slope of the regression of the relationship between water potential and sap flow ($\Psi = bF + a$) (Cohen *et al.*, 1987). Hysteresis effects were small. Only regressions significant at $P < 0.05$ were used for estimation of tree hydraulic resistances.

Wood, water and air in the sapwood were determined gravimetrically from samples collected with an increment corer (5 mm in diameter) in July 1993 and 1994 on the same trees sampled for embolism measurement. At least six samples were taken from each species and, after collection, the cores were sealed in airtight plastic bags. Volumetric fractions of wood and water were calculated assuming the specific gravity of wood to be 1.53 g cm^{-3} .

Xylem embolism in dehydrating branches

The vulnerability of Xylem to embolism was determined by measuring the extent of percentage loss in hydraulic conductivity expressed as a function of the minimum water potential reached by a dehydrating branch. Several branches (1.5–2 m long) were cut in the field during the spring. Since native state loss in hydraulic conductivity was high throughout the year, branches wrapped in plastic bags were allowed to equilibrate xylem water potential (and to promote air diffusion into cavitated conduits) overnight with the basal end under water. Then branches were recut under water and flushed with distilled degassed water at 150 kPa for 1.5 h. Subsequently, they were 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 three other excised shoots from each branch. Values of water potential and embolism

from branches were plotted on the same graph to produce the vulnerability curve.

RESULTS

The summers of 1993 and 1994 were characterized by high degrees of water stress (Table 1, Fig. 1). Predawn and minimum shoot water potential of *Q. pubescens* and *Q. ilex* were correlated with rainfall. At the onset of summer (late June), a sharp decrease in water potential was observed in both species. In midsummer 1993 (the year with less rainfall) values of minimum water potential dropped to *c.* -4.5 MPa and -6 MPa , respectively in *Q. pubescens* and *Q. ilex* (Fig. 1), whereas predawn values were only slightly higher (-3.6 MPa and -5.7 MPa , respectively in *Q. pubescens* and *Q. ilex*). These values were well below the threshold-values inducing cavitation and even below the point of turgor loss in both species (see Tognetti *et al.*, 1996*a*). Full recovery of water potentials was observed for both species after the first major rainfall event in September. At that time predawn water potential approached spring values. *c.* -0.5 MPa .

Branch segments were always severely embolized throughout the year (1993 and 1994) (Fig. 2), which was partly due to the method of determining maximum hydraulic conductivity (many embolized vessels in older growth rings might have not been blocked permanently by tyloses and therefore contributed to hydraulic conductivity after flushing). Lowest values in percentage loss of hydraulic conductivity were observed after the rains in autumn, *c.* 60% and 50%, respectively for *Q. pubescens* and *Q. ilex* (Fig. 2). Coinciding with freezing-thawing events during the winter season 1993–1994 (December 1993 to February 1994), embolism increased to about 80% in both species. After bud burst, at the beginning of May, xylem embolism showed a decrease to *c.* 60%. Throughout summer 1993 and 1994, embolism fluctuated between 60 and 80%. Specific hydraulic conductivity and leaf specific conductivity were significantly ($P < 0.0001$) higher in *Q. pubescens* than in *Q. ilex* (Table 2).

A significant negative relationship was found between water potential and xylem embolism in branches dehydrating in air for *Q. pubescens* and *Q. ilex* (Fig. 3). According to vulnerability curves, *c.* 40% of hydraulic conductivity was lost at a water potential of -3 MPa or -2 MPa and 80% at a water potential of -5 MPa or -3 MPa , respectively, in *Q. ilex* or *Q. pubescens*. The relationship of *Q. pubescens* was steeper than that of *Q. ilex*.

Figure 4 presents representative selected diurnal curves of sap flow, leaf conductance and shoot water potential for the growing season 1993 and 1994. In particular, daily courses relevant to June 1993 (onset of summer) and August 1994 (peak drought) are

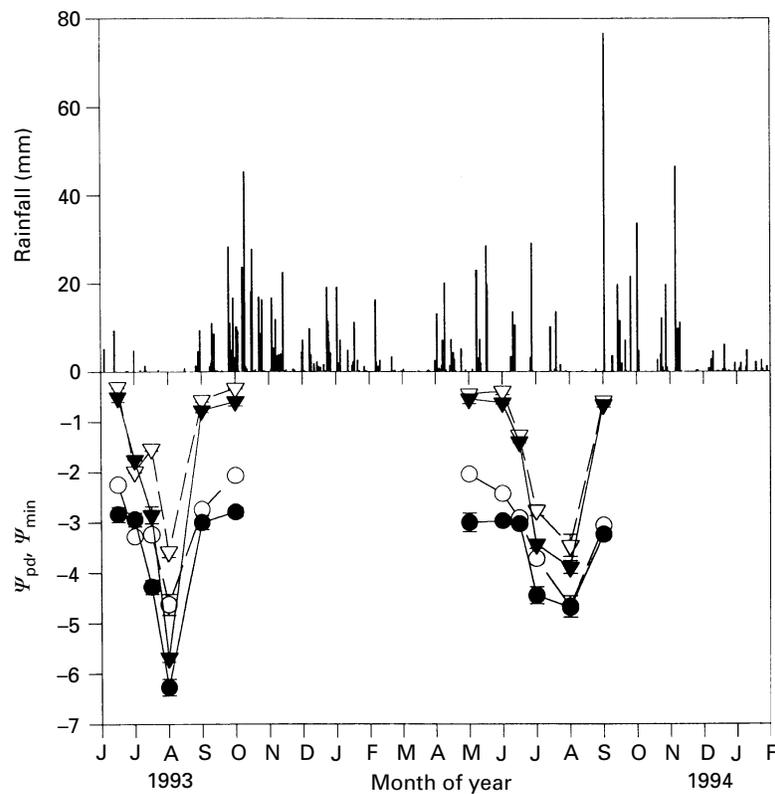


Figure 1. Seasonal changes in predawn and minimum water potential of twigs (Ψ_{pd} and Ψ_{min} , mean \pm SE, error bars not visible indicate SE smaller than the symbol) during summer and daily precipitation (rainfall) at the study site (1993 and 1994). Δ , \blacktriangle , Ψ_{pd} of *Quercus pubescens* and *Q. ilex* respectively; \circ , \bullet , Ψ_{min} of *Q. pubescens* and *Q. ilex* respectively.

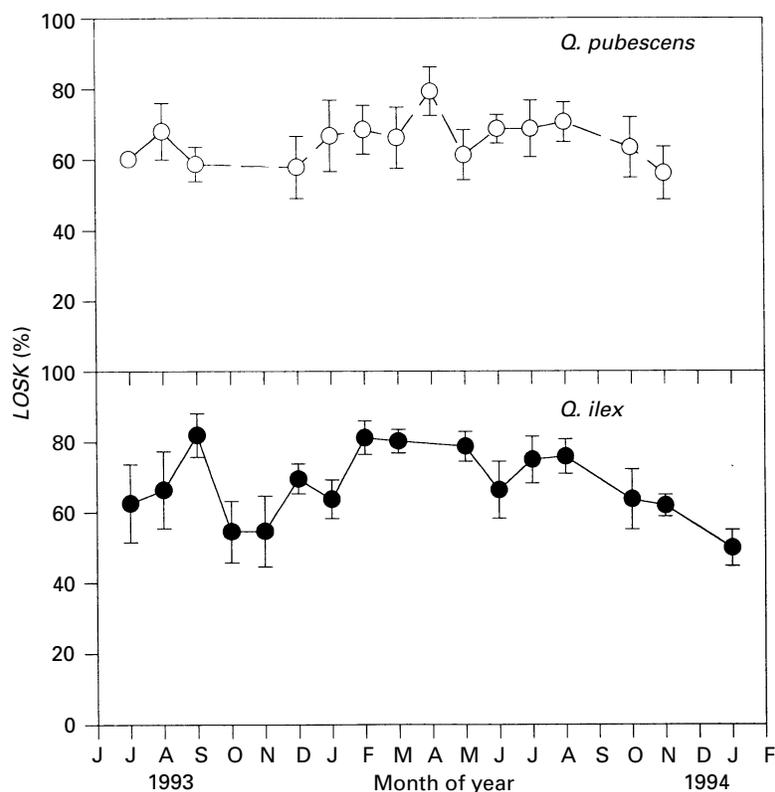


Figure 2. Seasonal changes of xylem embolism in apical twigs, expressed as a percentage loss of hydraulic conductivity ($LOSK$, mean \pm SE, error bars not visible indicate SE smaller than the symbol).

Table 2. Specific hydraulic conductivity (*KS*) and leaf specific hydraulic conductivity (*LSC*) of 1- and/or 2-yr-old branches of *Quercus ilex* and *Q. pubescens*

Species	<i>KS</i> (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	<i>LSC</i> (10 ⁻⁵ kg m ⁻¹ s ⁻¹ MPa ⁻¹)
<i>Q. ilex</i>	0.569 (0.049) a	6.425 (1.025) a
<i>Q. pubescens</i>	1.650 (0.136) b	12.880 (1.526) b

Values are means (\pm SE) of c. 50 samples collected during the growing season. Different letters within a column indicate statistically different values at $P < 0.05$.

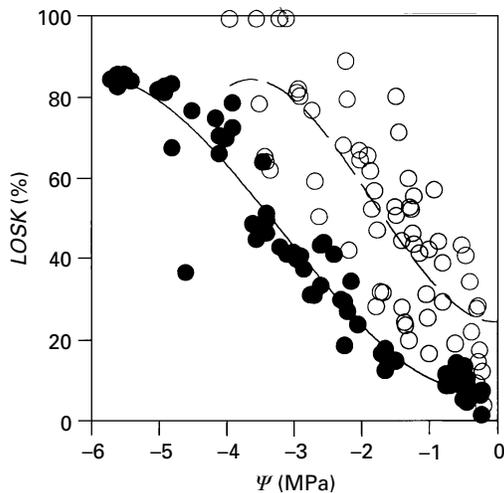


Figure 3. Relationship between percentage loss of hydraulic conductivity (*LOSK*) and xylem water potential of twigs (Ψ) in dehydrating branches. The coefficients of the third order polynomial function for *Quercus pubescens* (●) are: 23.913, 0.850, 14.261, 2.603 ($R^2 = 0.64$, $P < 0.0001$, $n = 66$). The coefficients of the third order polynomial function for *Q. ilex* (○) are: 9.449, 6.933, 8.820, 0.928 ($R^2 = 0.93$, $P < 0.0001$, $n = 66$).

shown. In June, leaf conductance was high early in the morning and decreased gradually during the day (Fig. 4). Midday depression of leaf conductance, as a result of high evaporative demand combined with water deficit (low water potential) was observed in both species. In August, leaf conductance of both species was greatly reduced, as water potential dropped to extremely low values, and stomata were almost completely closed during the afternoon (Fig. 4). After the rain in September, leaf conductance of both species increased to pre-stress values (data not shown). The diurnal changes of sap flow followed normal patterns (Fig. 4), even though absolute values were generally low, in particular for *Q. pubescens*.

No hysteresis resulting from plant capacitance (Jarvis, 1976) was observed in the relationship between shoot water potential and sap flow (Fig. 5). The slope of the regression line represents the tree hydraulic resistance, and the potential at zero flow represents the soil water potential in contact with the roots (Cohen *et al.*, 1987). The slope of the line decreased (more negative, i.e. steeper relationship) by nearly 50%, for *Q. pubescens*, and 40%, for

Q. ilex, during the summer drought, with *Q. pubescens* exhibiting very high values of whole-tree hydraulic resistance between July and September (1993 and 1994) (Fig. 6), whereas *Q. ilex* showed generally lower values. The effect of soil moisture depletion on the relationship between sap flow and shoot water potential appears as a lowering of water potential at zero flow. A significant decrease of whole-tree hydraulic resistance in both species was observed with the onset of the autumn, preceding the partial recovery of twig hydraulic conductivity.

The xylem of the main trunk of *Q. pubescens* was found to be more embolized (by core samples) than that of *Q. ilex* (volume fraction of gas 0.06 vs. 0.11, $P < 0.05$).

DISCUSSION

Q. ilex is a diffuse-porous tree with vessels up to 150 μm in diameter, and *Q. pubescens* a ring-porous tree with vessels up to 500 μm . Zimmermann (1983) proposed that there might be a trade-off between xylem efficiency and xylem safety in woody plants. Those plants having xylem with high specific hydraulic conductivity (high efficiency in water transport because of wide vessels) might inherently be more susceptible to water stress-induced embolism. In any case, comparisons between different tree species in terms of their vulnerabilities to embolism as related to conduit diameter should be carefully interpreted (Lo Gullo *et al.*, 1995). In fact, vulnerability to water-stress-induced cavitation of the xylem conduits has been shown to result from many different factors, including the pore size of pit membranes, and flexibility of the pit membranes (Crombie, Hipkins & Milburn, 1985; Sperry & Tyree, 1990).

During the summer (1993 and 1994), in which the time interval between two subsequent rainy days was several weeks, the percentage loss of hydraulic conductivity in twigs fluctuated between 60 and 80%. Significant amounts of xylem embolism, during the growing season, have also been observed in *Fagus sylvatica* (Magnani & Borghetti, 1995), and *Fraxinus excelsior* and *Populus × canadensis* 'robusta' (Hacke & Sauter, 1996). There were no large

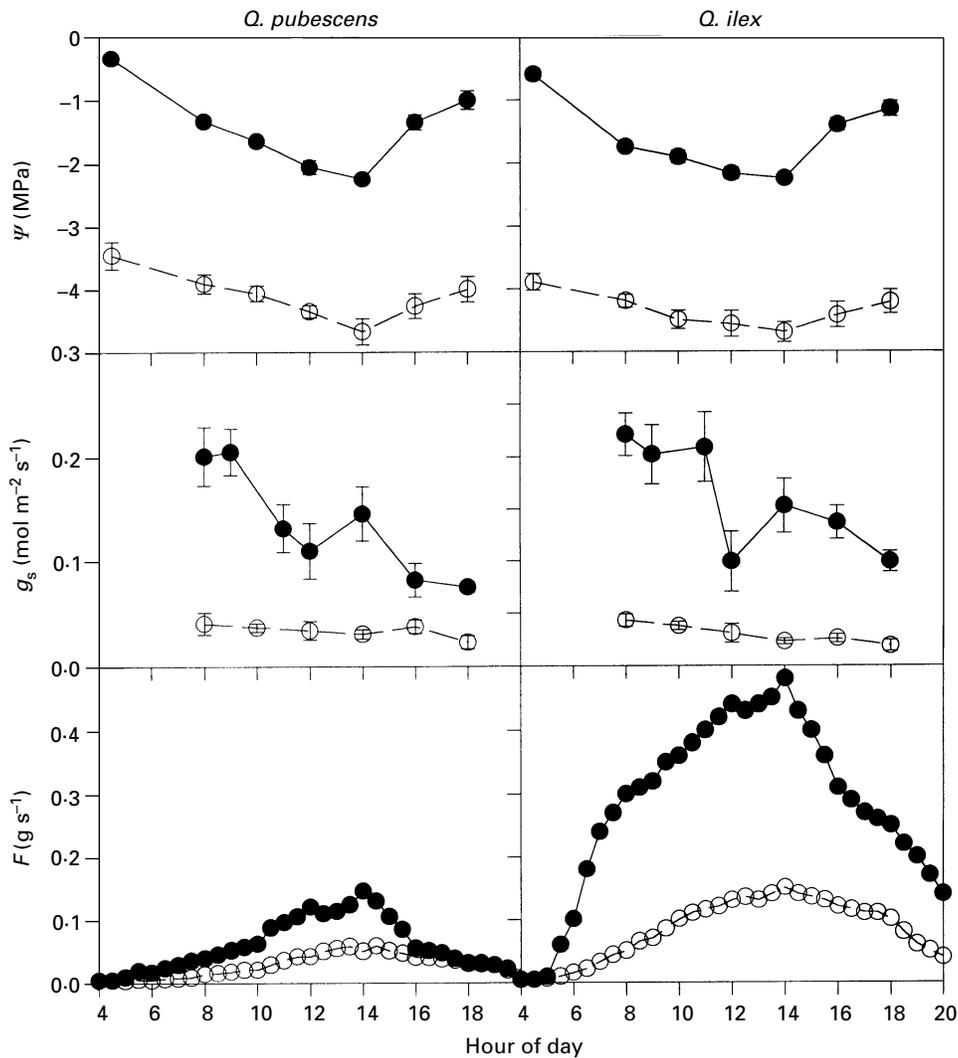


Figure 4. Diurnal patterns of sap flow in trunks (F), stomatal conductance (g_s) and xylem water potential of twigs (Ψ), during the course of two summer days. ●, 7 June 1993; ○, 21 August 1994. Data are means \pm SE, error bars not visible indicate SE smaller than the symbol (SE for sap flow data are not presented).

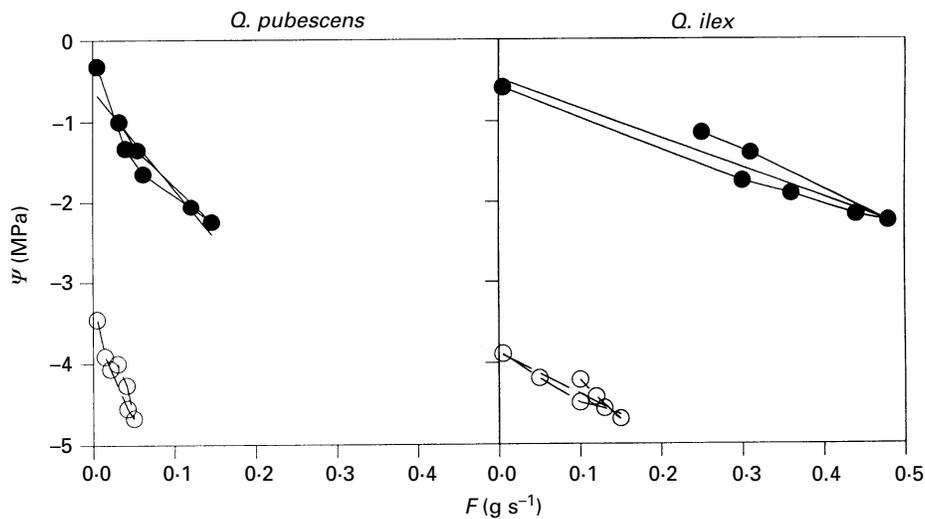


Figure 5. Relationship between the xylem water potential of twigs (Ψ) and sap flow in trunks (F), during the course of two summer days (SE is not presented). ●, 7 June 1993; ○, 21 August 1994. *Q. pubescens* 7 June 1993: $\Psi = -0.62 - 12.23F$; $R^2 = 0.89$, $P < 0.0001$; *Q. pubescens* 21 August 1994: $\Psi = -3.44 - 23.72F$; $R^2 = 0.90$, $P < 0.0001$. *Q. ilex*: 7 June 1993: $\Psi = -0.47 - 3.68F$; $R^2 = 0.92$, $P < 0.0001$; *Q. ilex* 21 August 1994: $\Psi = -3.87 - 5.08F$; $R^2 = 0.88$, $P < 0.0001$.

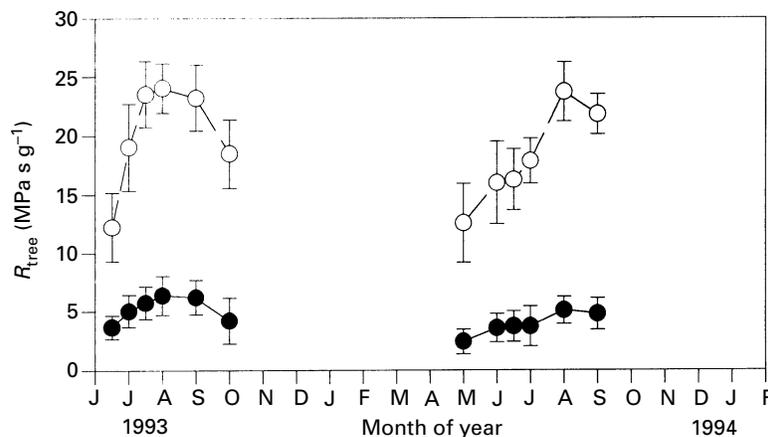


Figure 6. Seasonal changes of whole-tree hydraulic resistance (R_{tree} , mean \pm SE). \circ , *Quercus pubescens*; \bullet , *Q. ilex*. Data are from the slope of the linear relationship between xylem water potential of twigs and sap flow in trunks.

differences in the degree of xylem embolism (branches) between the two *Quercus* species. Both experienced similar degrees of seasonal water stress (i.e. level of water potential of each species down to and below its own osmotic potential at turgor loss point), and the levels of xylem embolism were similar; it was not surprising that artificial dehydration experiments showed a good agreement between the percentage loss of hydraulic conductivity measured under field conditions and those predicted by vulnerability curves (Bréda *et al.*, 1993). Sperry, Perry & Sullivan (1991) suggested, however, that caution is needed when estimating vulnerability of xylem to embolism on refilled material, since the functionality of old xylem with damaged intervessel pit membranes may initially be unnaturally restored. Unlike other Mediterranean species such as *Ceratonia siliqua*, *Olea oleaster* and *Quercus suber* which can tolerate water potential values below the turgor-loss point with minor losses of hydraulic conductivity (Salleo & Lo Gullo, 1993). *Q. pubescens* and *Q. ilex* (which are arcto-boreal species) not only showed losses of hydraulic conductivity of > 60% at the water potential reached but their capability of recovery from embolism also declined consistently (Lo Gullo & Salleo, 1993). On the other hand, xylem dysfunction due to embolism at modest tension might reduce the rates of water extraction and prolong water availability during drought (Sperry & Saliendra, 1994).

Rainfall at the beginning of autumn partially reduced embolism levels. A negative correlation between xylem embolism and precipitation has also been found for *Populus tremuloides* by Sperry (1993) and for *Fagus sylvatica* by Magnani & Borghetti (1995), and thus an active role of precipitation in embolism reversion cannot be ruled out. Sperry *et al.* (1988a), studying *Acer saccharum*, suggested that rainfall could favour the development of root pressure. In our experiment, however, the recovery

was far from being complete in response to rainfall and the high resistance to flow of narrow conduits in apical twigs might make the root pressure, if developed, insufficient to refill them (Lo Gullo & Salleo, 1993). Leaf yellowing (particularly in *Q. pubescens*) observed in mid-summer (at extremely low water potential) might indicate a lack of refilling of cavitated narrow conduits in apical twigs and leaf petioles. Partial reversal of embolisation following cavitation might also be a result of 'Münch water' released from the phloem (Milburn, 1996) that may act, periodically, as a buffer. Recovery from drought-induced embolism is probable if the conduits remain in the cavitated state (i.e. filled with water vapour), but it is much more difficult when vessels are in the embolized state (i.e. filled with air) (Lo Gullo & Salleo, 1993). Because the levels of drought stress reached by trees in this experiment would have caused high pressure differences across the inter-conduit pit membranes, it is likely that transition from the cavitated state to embolized state occurred more frequently. A mechanism for the partial and gradual recovery of xylem function in *Q. pubescens* and *Q. ilex* after early-autumn rainfall could be the construction of new xylem tissue (Kolb & Davis, 1994). In the Mediterranean environment studied, a continued radial growth is common in autumn before leaf abscission (in *Q. pubescens* this occurs in December) and freezing temperature (rarely observed before December).

High levels of xylem embolism in winter have already been reported for other Fagaceae such as *Quercus alba* and *Quercus rubra* (Cochard & Tyree, 1990), and *Fagus sylvatica* (Magnani & Borghetti, 1995) and *Fagus grandifolia* (Sperry, 1993). After exposure to temperatures lower than -3°C (December 1993 and February 1994), xylem embolism increased in correspondence to freezing-thawing cycles. The partial recovery of hydraulic conductivity observed in late spring might be related

to increased cambial activity, producing new functional xylem (Tognetti & Borghetti, 1994). In the early spring, before full leaf expansion, the water pathway provided by the conduits produced in previous years might be sufficient to supply the young growing leaves (Lo Gullo *et al.*, 1995). The large number of embolized conduits and/or the great air volumes developed in them as a result of complete water freezing, might be the cause of the observed lack of full recovery (Lo Gullo & Salleo, 1993). It has been hypothesized 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 many ring-porous trees particularly vulnerable to frost-induced embolism (Cochard & Tyree, 1990). However, both species studied here showed similar values of winter embolism in apical shoots despite differences in xylem anatomy.

The greater efficiency in hydraulic transport by the xylem of *Q. pubescens* shoots (higher specific hydraulic conductivity and leaf specific hydraulic conductivity) might permit higher transpiration rates during spring months, thus facilitating rapid growth and higher rates of photosynthesis. More efficient xylem may be a prerequisite for sustaining the relatively short-lived, metabolically more active leaves of deciduous species (*Q. pubescens*) compared with the long-lived, metabolically more conservative leaves of evergreen, sclerophyllous (*Q. ilex*) species (Larcher, 1995; Kolb & Davis, 1994). The evidence for a lower vulnerability to xylem embolism in *Q. ilex*, would, in theory, allow this species to tolerate higher stem xylem tensions than *Q. pubescens*. It is worth noting that the former species is phytosociologically dominant in the Mediterranean macchia.

Xylem embolism (by core samples) in mid-summer was much lower in trunks than in apical twigs. Zimmermann (1983) stated that embolism should develop first in the terminal part of the trees (i.e. leaves and twigs, which experience higher tensions in water stress conditions), thus preserving the bole and the main branches from embolism damage ('segmentation hypothesis'). In *Quercus cerris*, Lo Gullo *et al.* (1995) found that the embolism induced in the older branches was significantly higher than in 1-yr-old stems suggesting that the greater vulnerability of older stems was a function of the increased length and diameter of conduits with age. It is possible to hypothesize that at least part of the apparent high percentage loss of hydraulic conductivity in older branches results from previously embolized xylem that has not completely refilled (see above) and remains as a residual damage after the stress is relieved. Embolism confined to older xylem, only loosely connected to transpiring leaves, might be of minor functional relevance (Sperry *et al.*, 1991; Magnani & Borghetti, 1995).

Stomatal conductance was greatly reduced by restricted water availability, and as predawn water potential approached values < -3 MPa, corresponding to values of minimum water potential < -5 MPa, stomata were almost completely closed. These values are well below the threshold (which was reached in July) for which increased embolism can be induced, and has previously been observed in Mediterranean *Quercus* species in very dry years or on sites close to desert areas (Rambal & Debussche, 1995). Although stomatal regulation is able to control excess embolism despite decreasing soil water availability (Jones & Sutherland, 1991), with severe drought, as observed in the study period, stomatal control of transpiration cannot prevent the increase of embolism (Bréda *et al.*, 1993). This might trigger irreversible desiccation damage and runaway embolism. At this level of water stress (mid-summer) the sap flow was also strongly reduced. Tognetti *et al.* (1998, unpublished) found, in these species, a strong relationship between leaf conductance, predawn water potential and hydraulic resistance that indicates a control of leaf conductance by root or soil water potential. As reported by Davis & Zhang (1991), soil water stress could trigger root signals stimulating stomatal reactivity. Our results also suggest that plant hydraulic conductance might have an important role in the control of leaf conductance. If stomata close because they sense the reduction in leaf water status, then for a given drop in xylem pressure a loss of hydraulic conductance by cavitation might lead to reduced transpiration. As water stress progresses, cavitation superimposed on stomatal regulation could moderate the soil water extraction more than stomatal regulation alone (Sperry & Saliendra, 1994).

Higher sap flow and lower water potential in *Q. ilex* could be explained by the higher leaf area and trunk diameter of individual trees (Bréda *et al.*, 1993; Tognetti *et al.*, 1988, unpublished) and/or xylem embolism levels. The observed differences might not be an intrinsic species-related feature, rather, they could be due to the favourable competitive status of *Q. ilex* individuals in mixed stands containing *Q. pubescens* (Bréda *et al.*, 1993).

The higher xylem embolism, by core samples, (which reflects a different hydraulic architecture) and the lower sap flow in trunks of *Q. pubescens* might also explain the higher tree hydraulic resistance in this species. A reduction of soil water content during increasing drought stress in the summer could have reduced conductance in the soil compartment (which was shallow), and as a consequence, increased apparent tree hydraulic resistance. The decrease of hydraulic conductance of the soil surrounding the major portion of the root in the course of drought is evidenced by the progressive lowering of zero flow leaf water potential in both *Q. pubescens* and *Q. ilex*. However, *Q. ilex* exhibited

a lower variability in response to drought stress. Soil water stress might have reduced the hydraulic conductance of a fraction of the root system in *Q. ilex*, but an enhanced activity elsewhere within the larger root population of this species could have been responsible for partial compensation. Ramos & Kaufmann (1979) and Levy & Syvertsen (1983), studying citrus seedlings, postulated a reduction in root cell permeability. Bréda *et al.* (1993), suggested that, in *Quercus petraea* trees subjected to water stress, seasonal changes in hydraulic conductance were mainly attributable to modifications in hydraulic properties at the soil–root interface rather than to changes in the embolism of apical twigs. Magnani & Borghetti (1995) found for *Fagus sylvatica* that the embolism of apical twigs did not have a major effect on plant hydraulic resistance observed during the growing season in which soil water potential was estimated to be close to zero. However, a considerable hydraulic resistance at the interface between soil and root is expected when soil dries out (Passioura, 1998), as was in the case of our study. The low hydraulic conductance of the dry soil volume has been found to decrease the hydraulic conductance of the tree by interrupting water transport in a large portion of the root system in citrus trees subjected to partial irrigation and drought, Cohen *et al.* (1987). As water demand in terms of climate and leaf area remains the same, the leaf water potential must decrease to maintain sap flow. Our measurements revealed that water potential recovered in September, after the first major rainfall, but hydraulic resistance decreased only in October when, presumably, root meristems were still active (Magnani & Borghetti, 1995).

Our results demonstrate that both *Q. pubescens* and *Q. ilex* are highly tolerant of severe water stress and tissue dehydration despite the damage by extreme drought, and are adapted to warm and dry climates. Both species, however, operate at the limits of safety which are surpassed under severe droughts (like in summer 1993 and 1994), and prolonged climatic stress might predispose these *Quercus* to decline (Delatour, 1983). On the other hand, they are resprouters and this trait might allow *Q. pubescens* and *Q. ilex* to grow in extremely dry microsites. It is obvious that susceptibility to xylem embolism cannot be used as a sole index of water stress tolerance but must be evaluated in conjunction with other traits that also impart drought resistance and are likely to contribute to the competitive ability of *Q. pubescens* and *Q. ilex* in the Mediterranean basin. In this context, the effect of increasing atmospheric CO₂ concentration on the hydraulic architecture of woody species (Atkinson & Taylor, 1996; Heath, Kerstiens & Tyree, 1997) and the interspecific differences in the response to altered climatic conditions might have consequences on the composition of plant communities.

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