

Water stress responses of seedlings of four Mediterranean oak species

M. N. FOTELLI,¹ K. M. RADOGLU^{1,3} and H.-I. A. CONSTANTINIDOU²

¹ Forest Research Institute, National Agricultural Research Foundation (NAGREF), Vassilika, 57006 Thessaloniki, Greece

² Department of Agriculture, Aristotle University of Thessaloniki, Greece

³ Author to whom correspondence should be addressed

Received October 6, 1999

Summary Effects of water stress on phenology, growth, stomatal activity and water status were assessed from April to November 1996 in 2-year-old seedlings of *Quercus frainetto* Ten. (*Quercus conferta* Kit.), *Quercus pubescens* Willd., *Quercus macrolepis* Kotschy (*Quercus aegilops* auct.) and *Quercus ilex* L. growing in containers in northern Greece. All four species developed more than 50% of their total leaf area before the beginning of June—an adaptation to arid climates. Well-irrigated plants tended to develop greater individual leaf area, number of leaves per plant, total plant leaf area, height and root:shoot ratios than water-stressed plants, but the difference between treatments was not significant for any parameter in any species. *Quercus macrolepis* appeared to be the most drought-tolerant of the four species. It maintained the highest number of leaves of the smallest size and increased the proportion of fine roots during drought. In all species, drought caused significant decreases in stomatal conductance and predawn and midday water potentials from mid-July until the end of August, when the lowest soil water content and highest mean daily air temperatures and midday leaf temperatures occurred; however, the responses were species-specific. Among the four species, *Quercus macrolepis* sustained the highest stomatal conductance despite very low water potentials, thus overcoming drought by means of desiccation tolerance. *Quercus ilex* decreased stomatal conductance even before severe water stress occurred, thereby avoiding desiccation during drought. *Quercus pubescens* had the highest water potential despite a high stomatal conductance, indicating that its leaf water status was independent of stomatal activity. *Quercus frainetto* was the least drought-resistant of the four species. During drought it developed very low water potentials despite markedly reduced stomatal aperture.

Keywords: growth, phenology, *Quercus*, stomatal conductance, water potential.

Introduction

Structural and physiological adaptations to drought determine the growth and survival of forest tree species in dry climates (Tenhunen et al. 1987). For example, highly efficient water use can provide a substantial adaptive advantage on xeric sites (Ni

and Pallardy 1991). The variety of ecosystem types in the Mediterranean region reflects the adaptability of native woody plants to drought, because water availability is the main factor regulating the productivity of these ecosystems (Dafis 1982). The considerable variation in ecophysiology of Mediterranean oaks is comparable with that observed in the Californian chaparral and tropical dry forests (Gallego et al. 1994).

Oak forests account for 23% of the total forest area in Greece and are adapted to the extremely dry summers that characterize this Mediterranean region (Radoglou 1996). About 75% of the oak forests are degraded, low productivity, coppice forests (Dafis 1982). Eleven oak species occur in Greece, growing in environments that differ widely in water availability. Among these oak species, four are important for reforesting degraded soils of the dry Mediterranean ecosystem. *Quercus pubescens* Willd., a deciduous species native to Greece, predominates on poor soil that is prone to drought. It is a codominant species in many mature communities over large areas of the Mediterranean basin. *Quercus ilex* L. is an evergreen species native to Greece that is able to utilize mainly rainwater and thus modulate its growth throughout the dry season according to the rainfall. Both species are highly plastic and resistant to environmental stresses. The deciduous *Q. macrolepis* Kotschy (*Quercus aegilops* auct.) is also considered a drought-resistant species, capable of growing on shallow and poor soils. It is a dominant Mediterranean species. *Quercus frainetto* Ten. (*Quercus conferta* Kit.) is a deciduous species that shows low tolerance to drought and high temperatures and has a high demand for soil water. It occurs in the forests of southeast Europe and is common in Greece (Athanasiadis 1986, Romane and Terradas 1992, Valentini et al. 1992, Damesin et al. 1997). Based on the distribution of these species in Greece, the following ranking in drought tolerance has been suggested (Athanasiadis 1986): *Quercus frainetto* (least tolerant) < *Q. ilex* < *Q. macrolepis* < *Q. pubescens* (most tolerant). However, differences in the ability of these species to withstand drought are reported in the literature (e.g., Valentini et al. 1992).

The water relations and responses of oak and other species to water stress have been studied (Dreyer et al. 1992, Valentini et al. 1992, Tognetti et al. 1999), but there are few comparative studies on Mediterranean oaks. A detailed knowledge of how

environmental conditions affect the growth of native oak species could contribute to better management of natural Mediterranean ecosystems during the current period of decreasing precipitation at Mediterranean latitudes.

Water deficits have both long- and short-term effects on plants. Processes such as stomatal opening and closure can be affected within minutes, whereas processes such as leaf expansion may be affected over a period of months (Myers 1988). Therefore, we measured both short-term physiological responses and long-term morphological and phenological responses to drought to obtain a comprehensive picture of oak seedling responses to water stress.

We hypothesized that (1) water stress would promote significant changes in the ecophysiological and growth responses of different oak species at the seedling growth stage, and (2) differences in ecophysiological and growth responses should reveal drought adaptation attributes among species. To test these hypotheses we compared water-stress effects on growth, leaf water potential and stomatal conductance of *Q. frainetto*, *Q. pubescens*, *Q. macrolepis* and *Q. ilex*. The seedling stage of growth was chosen to evaluate short and long-term responses to water stress, because seedlings are more susceptible to drought than saplings and adult trees (Margolis and Brand 1990).

Materials and methods

Experimental design

The experiment was conducted during the 1996 growing period at the experimental garden of the Thessaloniki Forest Research Institute (40°35' N, 22°58' E, 10 m a.s.l.). Two-year-old seedlings of *Quercus frainetto*, *Q. pubescens*, *Q. macrolepis* and *Q. ilex* were transplanted from paper pots to experimental containers (four seedlings per species per container, eight containers per species). Each container had a volume of 0.225 m³ (diameter 0.93 m and depth 0.33 m) and a hole at the bottom for drainage. The mixture in the containers consisted of peat and soil (1:3). The soil comprised silt (5–8%), clay (16–39%) and sand (56–75%), pH 7.2–7.5.

The control (well-watered) plants were watered regularly to field capacity, whereas the drought-treated plants received no irrigation other than natural rainfall. There were eight seedlings per species per treatment and measurements were conducted on four seedlings per species per treatment (one seedling per container).

A weather station at the experimental site recorded air temperature and air relative humidity. Mean annual precipitation over a 30-year span is 409 mm (range 211–704 mm), and the area experiences a 5-month dry season from May to September. According to the mean monthly precipitation of the last 30 years, August and September are the driest months, with 22 and 21 mm of precipitation, respectively.

Volumetric determination of soil water content

Soil water content (SWC, %) was measured weekly from April 30 to November 4, 1996 with a moisture analyzer

(Brabender OHG, Duisburg, Germany) on four 10-g samples per treatment. The SWC was used as an index of drought intensity. Field capacity and wilting point were measured with a pressure membrane extractor. Pressures of 0.35 MPa and 1.50 MPa were applied to measure field capacity and wilting point, respectively.

Leaf area

Total leaf area per plant was estimated weekly on four randomly selected seedlings per treatment. The leaves of each seedling were subdivided into three size categories and the leaves of each category were counted. The area of a representative leaf per category was measured with an LI-3000 portable leaf area meter (Li-Cor, Inc., Lincoln, NE). Total leaf area per plant was estimated as the sum of the leaf areas of the three categories.

Growth

Height growth was measured monthly from the soil surface to the highest active bud, and biomass was determined after the experiment ended on November 16, 1996. Roots, branches and main stems (without leaves) were oven-dried (48 h, 75 °C) and dry weights determined for four seedlings per treatment. Roots were divided into three size categories: R1 (diameter < 2 mm), R2 (2–5 mm), and R3 (> 5 mm). Total root:shoot ratio (R:S; shoot = branches + main stem) and R1, R2 and R3 as a percentage of total root per seedling were estimated.

Water potential

Predawn and midday leaf (xylem) water potentials (Ψ) were measured approximately weekly on two fully expanded leaves from each of four seedlings per treatment with a pressure chamber (Wescor, Inc., Logan, UT) (Turner 1988). One leaf was harvested between 0630 and 0730 h for the determination of predawn (April, August through November) or dawn (May through July) water potential, and the other leaf was harvested between 1130 and 1230 h for the determination of midday water potential. Leaves were put in plastic bags and refrigerated during transport to the laboratory. Water potential measurements were performed within 1–2 h of collection. A set of pilot measurements performed at the beginning of the experiment verified that there was no significant change in leaf water potential between harvesting and transport to the laboratory. Because each species initiated leaf expansion at a different time, the time of the first measurement was adjusted accordingly.

Stomatal conductance

Stomatal conductance was measured weekly on one fully expanded leaf of four plants per treatment with an LI-1600 portable porometer (Li-Cor, Inc.). Measurements were taken from April 24 until November 4, 1996 between 1100 and 1300 h, when incoming photosynthetically active radiation (PAR) was > 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Changes in leaf conductance were considered to reflect changes in stomatal conductance, assuming that the boundary

layer conductance inside the cuvette was constant and large. Conductance measurements began on different dates for each species studied, depending on the onset of leaf expansion. In the evergreen *Q. ilex*, the first measurements were made on leaves of the previous growing season. Leaf temperature, relative humidity (RH) inside the cuvette, and PAR were recorded during each measurement (data not shown).

Data analysis

Analysis of variance and non-parametric methods for multiple comparison were applied on a 95% significance level. For the non-parametric methods, the Kruskal-Wallis and Nemeyi tests were used, and for distribution checking, the Kolmogorov-Smirnov and Chi-Square tests were used (Sokal and Rohlf 1995).

Results

Weather and soil conditions during the experiment

Seasonal changes in soil water content of the containers, daily precipitation, field capacity and wilting point during the growing season are shown in Figure 1. Throughout the experimental period, soil water content was significantly lower in the drought treatment than in the well-watered treatment. Soil water content was always lower than field capacity in the drought treatment. Until June 19, soil water content in the drought treatment was above wilting point; it then decreased abruptly and returned to above wilting point only when rain fell on consecutive days. The extreme stress period for drought-treated plants extended from June 19 to September 19, and the lowest soil water content in the drought treatment was recorded on August 14 (1.8%).

There was a large difference between mean monthly precipitation during the growing season (April to November) over the last 30 years (range 21–64 mm) and that during the 1996

growing season (4–22 mm), with the 1996 growing season being much drier. Relative humidity varied from 30 to 90%. Mean daily air temperature fluctuated from 3 to 25 °C, with the highest values observed from early July until mid-August, which is typical for Greece (e.g., Radoglou 1996). High leaf temperatures (measured with a porometer daily between 1100 and 1300 h) were noted, with a maximum of 41 °C in mid-July.

Plant growth

Seasonal patterns of leaf size, leaf number per plant and total plant leaf area are shown in Figures 2, 3 and 4, respectively. In all four species, new leaves appeared from mid-April until the beginning of May. Leaf size, number of leaves per plant and consequently total leaf area tended to be higher in well-watered seedlings than in drought-treated seedlings, for all four species. However, the effects of drought on these parameters were largely masked by the high variation among measurements. Drought-treated seedlings lost their leaves at the beginning of September, whereas well-watered plants kept their leaves until the beginning of November.

In early June, before weather conditions became stressful, well-watered plants of *Q. frainetto*, *Q. pubescens* and *Q. macrolepis* had developed 58, 50 and 61% of their final leaf area, respectively. *Quercus ilex* carried 100% of its final leaf area in early June, because it retained leaves of the previous growing season; however, this initial total leaf area decreased later in the season when the old leaves abscised.

Under both well-watered and drought conditions, *Q. macrolepis* had significantly smaller but more numerous leaves compared with the other species. Under drought conditions, *Q. frainetto* exhibited significantly higher values of total leaf area up to July than the other species, but during the period with the highest soil water deficit, it suffered an abrupt decrease in leaf area, resulting in the lowest leaf area after *Q. ilex*.

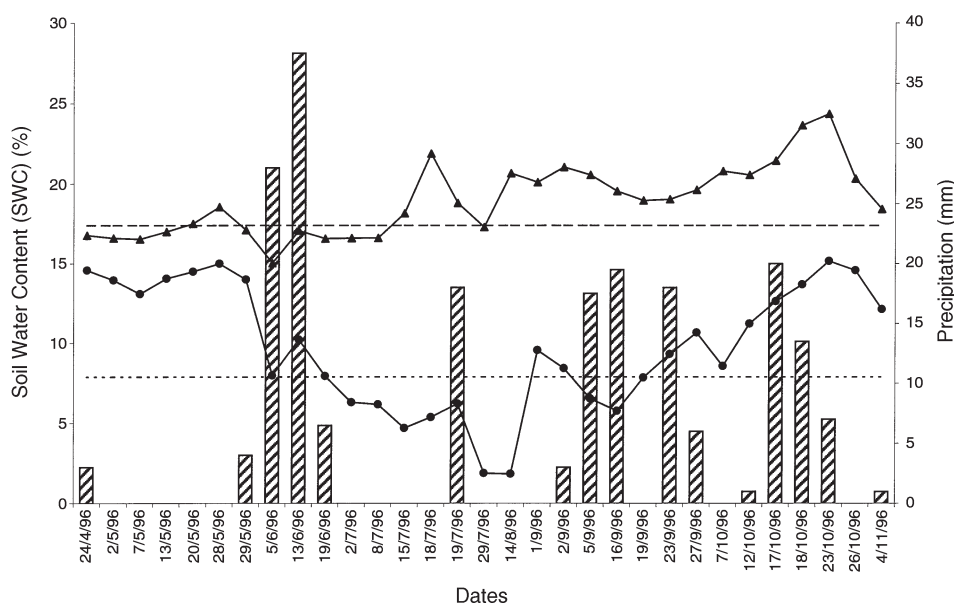


Figure 1. Seasonal variation in soil water content (%) of well-watered (▲) and drought-treated (●) seedlings during the 1996 growing season (April to November). Daily precipitation, represented by hatched bars, was recorded at a meteorological station located 50 m away. Field capacity (---) and wilting point (----) (%) are represented by the two parallel lines. There was a statistically significant difference between drought-treated and well-watered seedlings at the 95% level for all dates shown.

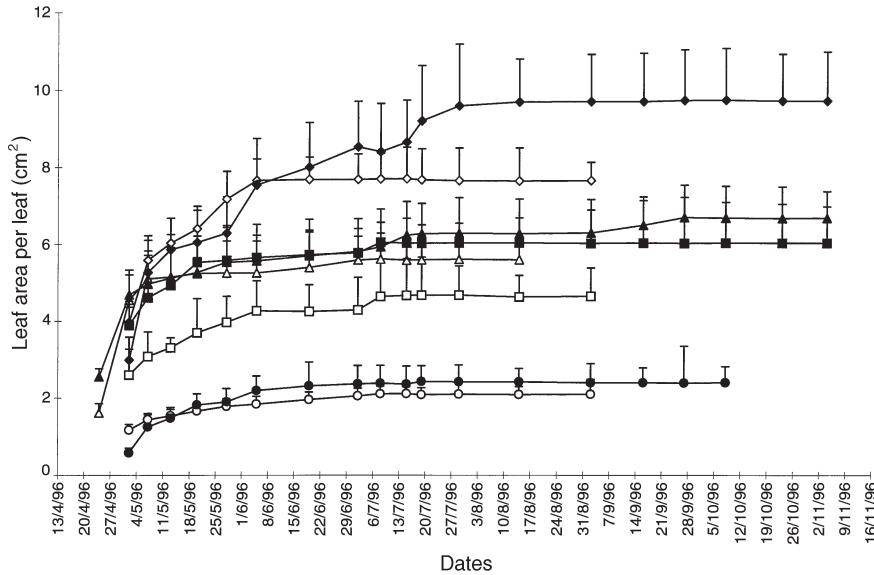


Figure 2. Seasonal variation in leaf area per leaf of well-watered (closed symbols) and drought-treated (open symbols) seedlings of *Q. frainetto* (◆, ◇), *Q. pubescens* (▲, △), *Q. macrolepis* (●, ○) and *Q. ilex* (■, □). Vertical bars indicate + SEM. No statistical differences at the 95% level were detected.

There were no significant differences between well-watered and drought-treated seedlings in seasonal height increment (Figure 5). Among the species studied, *Q. macrolepis* exhibited the greatest height growth and *Q. pubescens* the least

height growth; this difference was maintained throughout most of the growing season in both treatments.

Dry weights of main stems, branches and roots of the four species are presented in Figure 6, and the R:S ratio and R1, R2

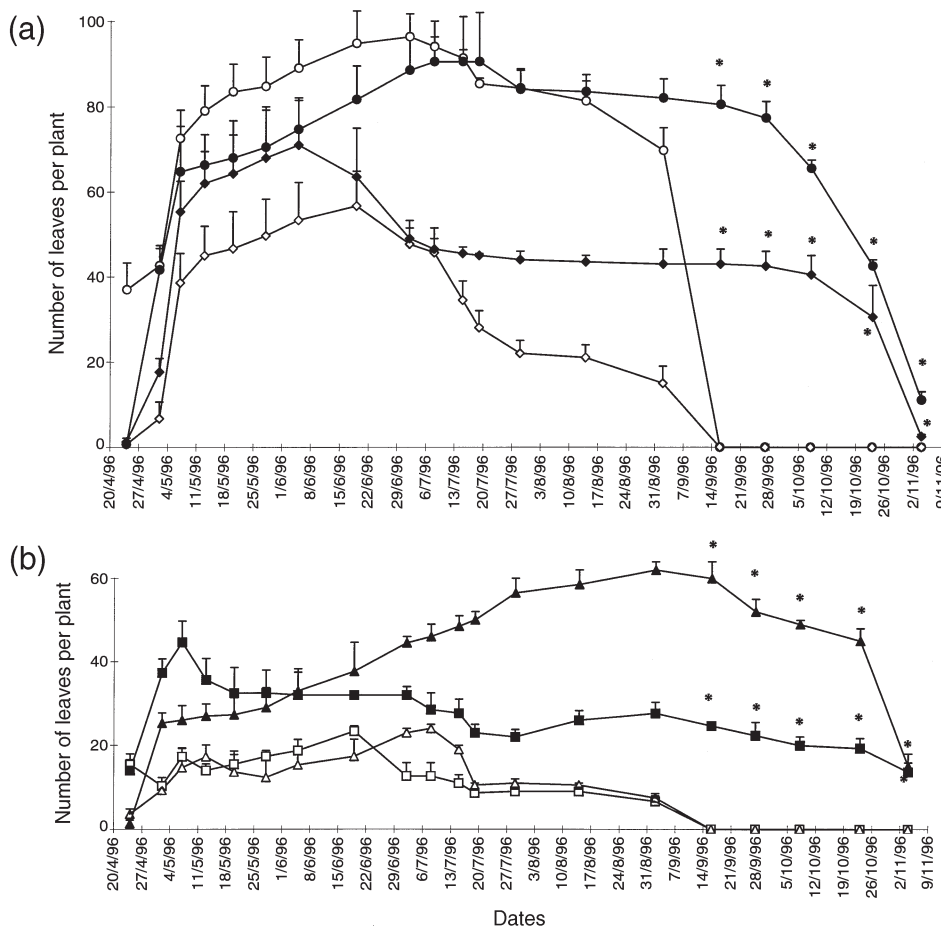


Figure 3. Seasonal variation in number of leaves per plant of well-watered (closed symbols) and drought-treated (open symbols) seedlings of (a) *Q. frainetto* (◆, ◇) and *Q. macrolepis* (●, ○), and (b) *Q. pubescens* (▲, △) and *Q. ilex* (■, □). Vertical bars indicate + SEM. An asterisk (*) signifies a statistically significant difference between drought-treated and well-watered seedlings at the 95% level.

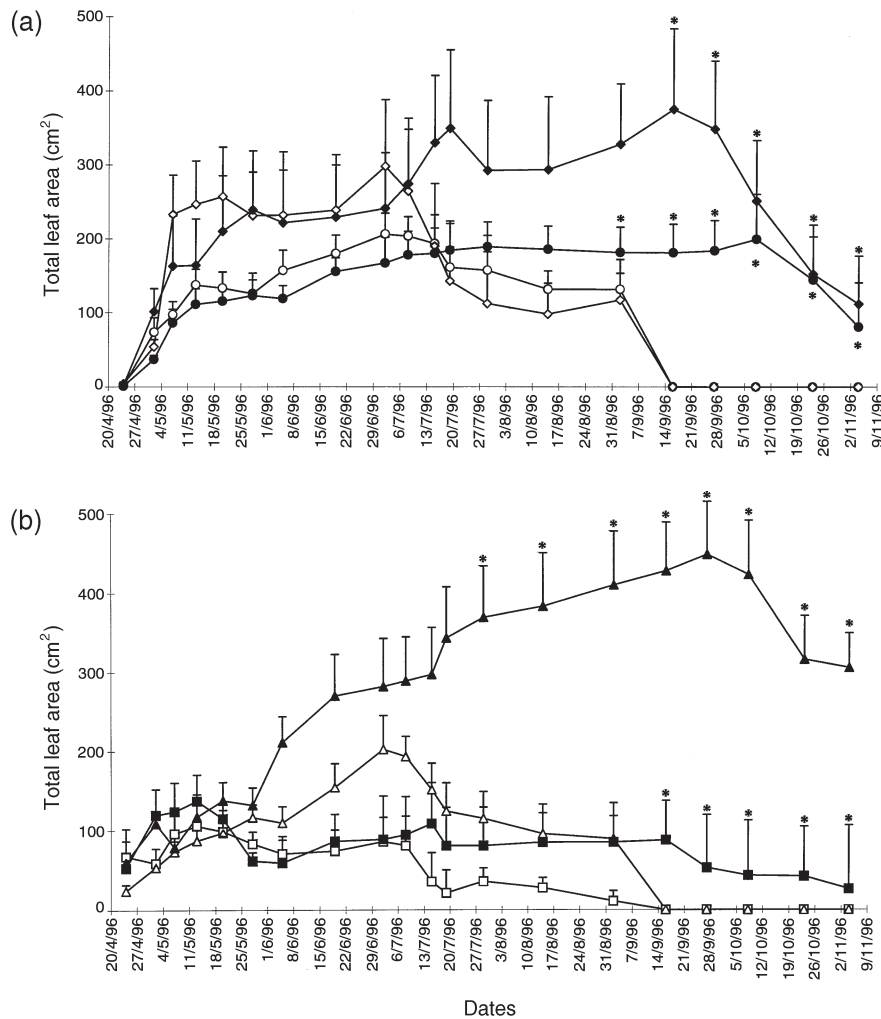


Figure 4. Seasonal variation in total plant leaf area of well-watered (closed symbols) and drought-treated (open symbols) seedlings of (a) *Q. frainetto* (◆, ◇) and *Q. macrolepis* (●, ○), and (b) *Q. pubescens* (▲, △) and *Q. ilex* (■, □). Vertical bars indicate + SEM. An asterisk (*) signifies a statistically significant difference between drought-treated and well-watered seedlings at the 95% level.

and R3 percentages are shown in Table 1. In *Q. pubescens*, *Q. macrolepis* and *Q. ilex*, whole-plant biomass tended to be higher in well-watered plants than in drought-treated plants,

whereas *Q. frainetto* seedlings had similar dry weights under both well-watered and drought conditions (Figure 6). *Quercus macrolepis* was the only species that produced a higher per-

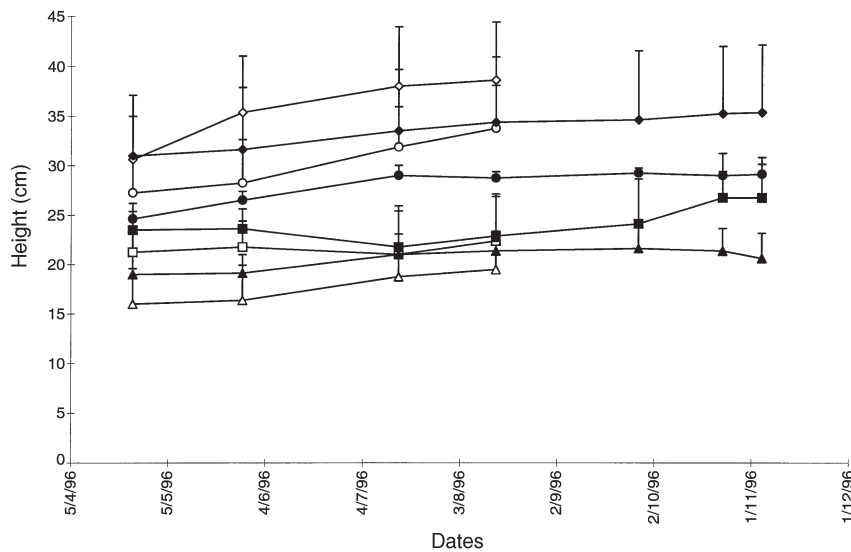


Figure 5. Seasonal variation in height of well-watered (closed symbols) and drought-treated (open symbols) seedlings of *Q. frainetto* (◆, ◇), *Q. pubescens* (▲, △), *Q. macrolepis* (●, ○) and *Q. ilex* (■, □). Vertical bars indicate + SEM. Statistical differences at the 95% level were not detected.

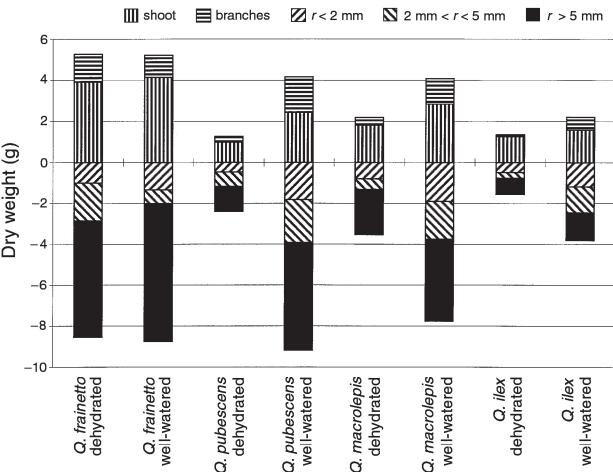


Figure 6. Dry weights of main stems, branches and roots of well-watered and drought-treated seedlings of *Q. frainetto*, *Q. pubescens*, *Q. macrolepis* and *Q. ilex*.

centage (31%) of fine roots (diameter < 2 mm) when subjected to drought than when well-watered (25%; Table 1). Drought had no significant effect on the R:S ratio in any species.

Plant water status

Seasonal curves of predawn (Ψ_{Pd}) and midday (Ψ_{Mid}) water potentials are shown in Figure 7. In general, leaf water potential followed the seasonal course of soil water content (SWC), with low values occurring when SWC reached its minimum value (approximately 1.8%) at the end of August (Figure 1). From the beginning of July, a major reduction in water potential was observed in all drought-treated seedlings, leading to leaf loss by mid-September (Figure 4).

On average, Ψ_{Pd} was 1.5 MPa higher than Ψ_{Mid} , and this difference remained constant throughout the experimental period for both well-watered and drought-treated seedlings. Although water potential recovered diurnally, a gradual seasonal decline in Ψ_{Pd} was observed in drought-treated seedlings.

In all species, Ψ_{Pd} was significantly lower for drought-

treated seedlings than for well-watered seedlings from mid-August until early September, when Ψ_{Pd} values of drought-treated plants were at a minimum (Figure 7). Values of Ψ_{Mid} became significantly lower for drought-treated seedlings than for well-watered seedlings around the end of July or mid-August for *Q. frainetto*, *Q. ilex* and *Q. macrolepis*, and around the beginning of September for *Q. pubescens*.

Values of Ψ_{Pd} and Ψ_{Mid} differed among species. Values of Ψ_{Pd} and Ψ_{Mid} were lower under both growing conditions in *Q. macrolepis* and *Q. frainetto* than in *Q. pubescens* and *Q. ilex*. Under drought conditions, the lowest Ψ_{Pd} values of the four oak species, measured on September 2, were -4.9, -4.7, -3.7 and -2.4 MPa for *Q. frainetto*, *Q. macrolepis*, *Q. ilex* and *Q. pubescens*, respectively, with the Ψ_{Pd} value of *Q. pubescens* being significantly higher than that of the other species. On the same date, the corresponding Ψ_{Mid} values were -5.9, -6.0, -4.8 and -5.0 MPa, and the difference was significant only between *Q. macrolepis* and *Q. pubescens* (data not shown). In general, *Q. pubescens* and *Q. ilex* sustained the highest water potentials among the four species, with *Q. ilex* having a slightly lower water potential than *Q. pubescens*. Minimum annual Ψ_{Pd} was highly correlated with maximum seasonal differences in Ψ_{Pd} . *Quercus macrolepis* and *Q. frainetto* had the most negative Ψ_{Pd} and also exhibited the greatest seasonal differences in Ψ_{Pd} (Figure 8).

The seasonal pattern of stomatal conductance (g_s) is shown in Figure 9. At the end of July, when SWC was 1.8% (Figure 1) and midday water potential ranged between -2.8 and -3.5 MPa (Figure 7), g_s abruptly declined in all species under both well-watered and drought conditions. Stomatal closure started as Ψ_{Pd} reached -1 to -2 MPa, and was close to zero at Ψ_{Pd} values of -2.5 MPa in *Q. pubescens*, -3.5 MPa in *Q. ilex* and -4.5 MPa in *Q. frainetto* and *Q. macrolepis*.

Drought-treated seedlings had significantly lower g_s than well-watered seedlings from July until early September, and the period of significant differences varied with the species (Figure 9). Among the four species, *Q. macrolepis* had significantly higher g_s than the other species when weather and soil water conditions were not stressful (early May until mid-June). By mid-July, both *Q. macrolepis* and *Q. pubescens* had

Table 1. Root to shoot ratios (R:S) and root categories (R1 = roots with diameter < 2 mm; R2 = roots with diameter 2–5 mm; R3 = roots with diameter > 5 mm) presented as percentages of total root dry weight of the studied species under well-watered and drought conditions. Measurements were made at the end of the experiment (November 1996). Each value is the mean of four measurements.

Species	Treatment	R1 (%)	R2 (%)	R3 (%)	R:S
<i>Q. frainetto</i>	Drought	21	22	57	1.6 ± 0.2
	Well-watered	30	18	52	1.7 ± 0.2
<i>Q. pubescens</i>	Drought	19	30	51	1.9 ± 0.2
	Well-watered	20	23	57	2.2 ± 0.2
<i>Q. macrolepis</i>	Drought	31	12	58	1.6 ± 0.1
	Well-watered	25	24	52	1.9 ± 0.2
<i>Q. ilex</i>	Drought	31	17	51	1.2 ± 0.1
	Well-watered	31	33	36	1.7 ± 0.2

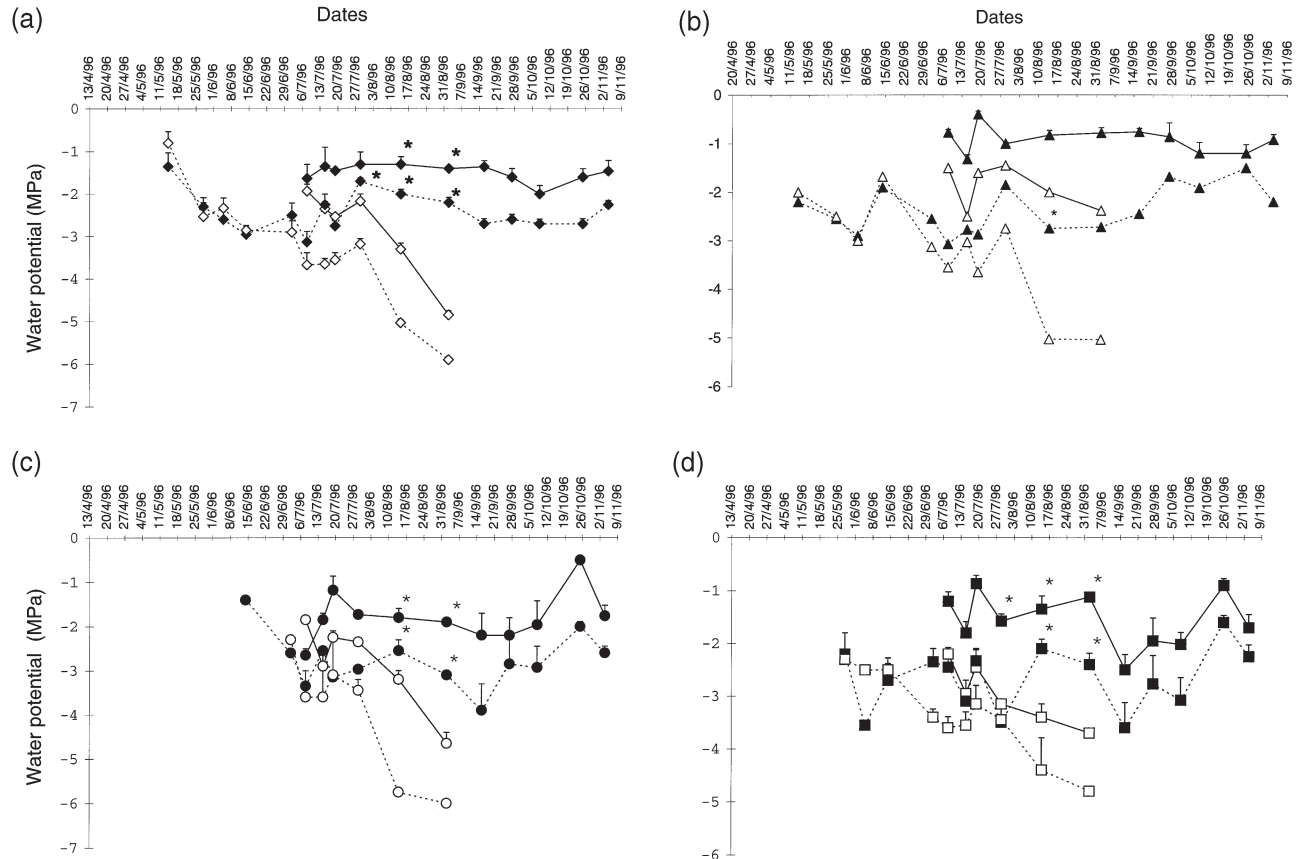


Figure 7. Seasonal variation in predawn (solid lines) and midday (dashed lines) leaf water potentials of well-watered (closed symbols) and drought-treated (open symbols) seedlings of (a) *Q. frainetto*, (b) *Q. pubescens*, (c) *Q. macrolepis* and (d) *Q. ilex*. Vertical bars indicate + SEM. An asterisk (*) signifies a statistically significant difference between drought-treated and well-watered seedlings at the 95% level.

significantly higher g_s than the other species. The highest recorded stomatal conductance values were 0.5, 0.8, 0.8 and 0.3 mol m⁻² s⁻¹ in *Q. frainetto*, *Q. pubescens*, *Q. macrolepis* and *Q. ilex*, respectively.

There was a direct relationship between Ψ_{Mid} and g_s under drought conditions in *Q. frainetto*, *Q. pubescens* and *Q. macrolepis*, but not in *Q. ilex* (Figure 10). This indicates that as Ψ_{Mid} decreased below -2.0 MPa, g_s declined in parallel in *Q. frainetto*, *Q. pubescens* and *Q. macrolepis*. *Quercus macrolepis* presented the strongest relationship between declining stomatal conductance and declining water potential.

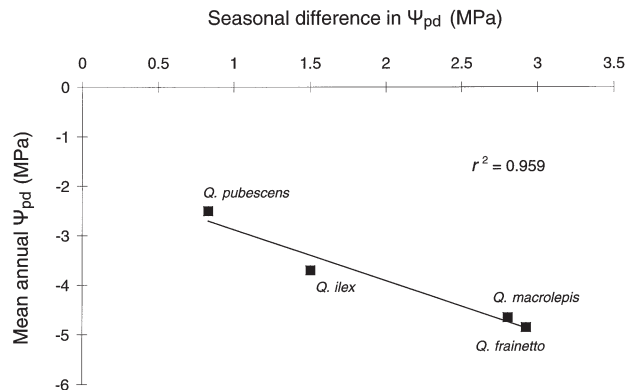


Figure 8. Correlation between minimum annual predawn water potential (Ψ_{pd}) and seasonal differences in predawn water potential in drought-treated seedlings of *Q. frainetto*, *Q. pubescens*, *Q. macrolepis* and *Q. ilex*.

Discussion

Plant growth

Severe water stress during July and August reduced soil water content to 1.8% and led to leaf loss in all species by mid-September. Early leaf loss under conditions of water deficit indicates a severe effect of drought (Sala and Tenhunen 1994). The four oak species made rapid leaf growth (more than 50% of final leaf area) early in the growing season before the driest period occurred. This pattern of leaf growth is an adaptation to arid environments (e.g., Radoglou 1996). Water stress had no effect on height increment of the four oak species.

Tognetti et al. (1999) suggested that structural changes occur in oak that influence seasonal water relations and determine the operational range for water potential. Among the oak

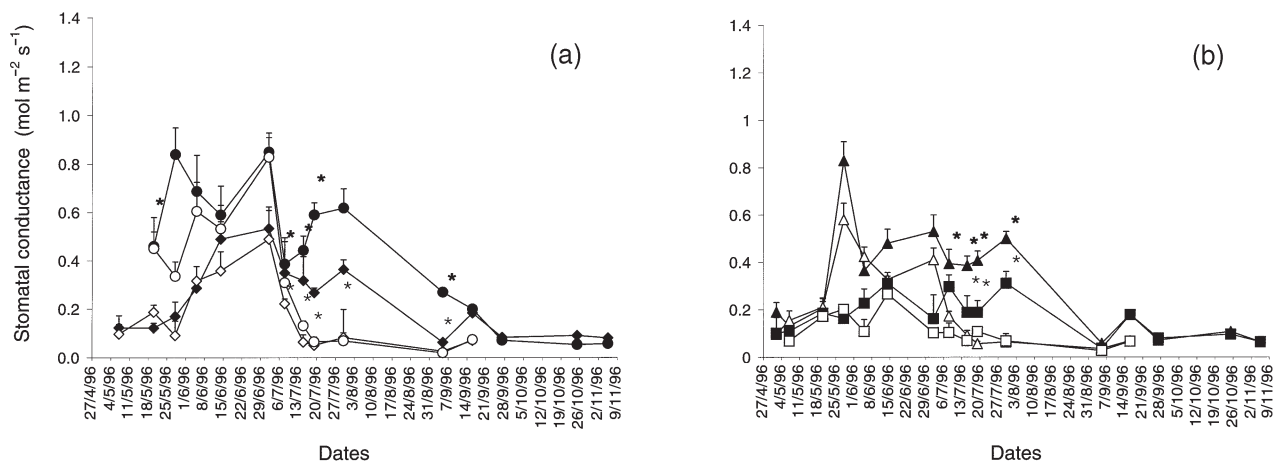


Figure 9. Seasonal variation in stomatal conductance of well-watered (solid symbols) and drought-treated (open symbols) seedlings of (a) *Q. frainetto* (◆, ◇) and *Q. macrolepis* (●, ○), and (b) *Q. pubescens* (▲, △) and *Q. ilex* (■, □). Vertical bars indicate + SEM. An asterisk (*) signifies a statistically significant difference between drought-treated and well-watered seedlings at the 95% level.

species, *Q. macrolepis* was the only one that responded to water depletion by increasing the proportion of fine roots, thus enhancing its water absorbing area. Several studies (Teskey and Hinckley 1981, Dreyer et al. 1992, Callaway and Mahall 1996) have shown that soil water depletion regulates new root formation in some oak species. In addition, both well-watered and drought-treated *Q. macrolepis* seedlings had leaves that

were smaller and more numerous than those of the other oak species examined. Small leaves, in combination with other adaptive mechanisms, is a response that allows oak species to survive dry conditions (e.g., Hamerlynck and Knapp 1996). Furthermore, the relatively small total leaf area possessed by *Q. ilex* under drought conditions is a growth adaptation to xeric sites, and has also been observed in *Q. rubra* L. growing

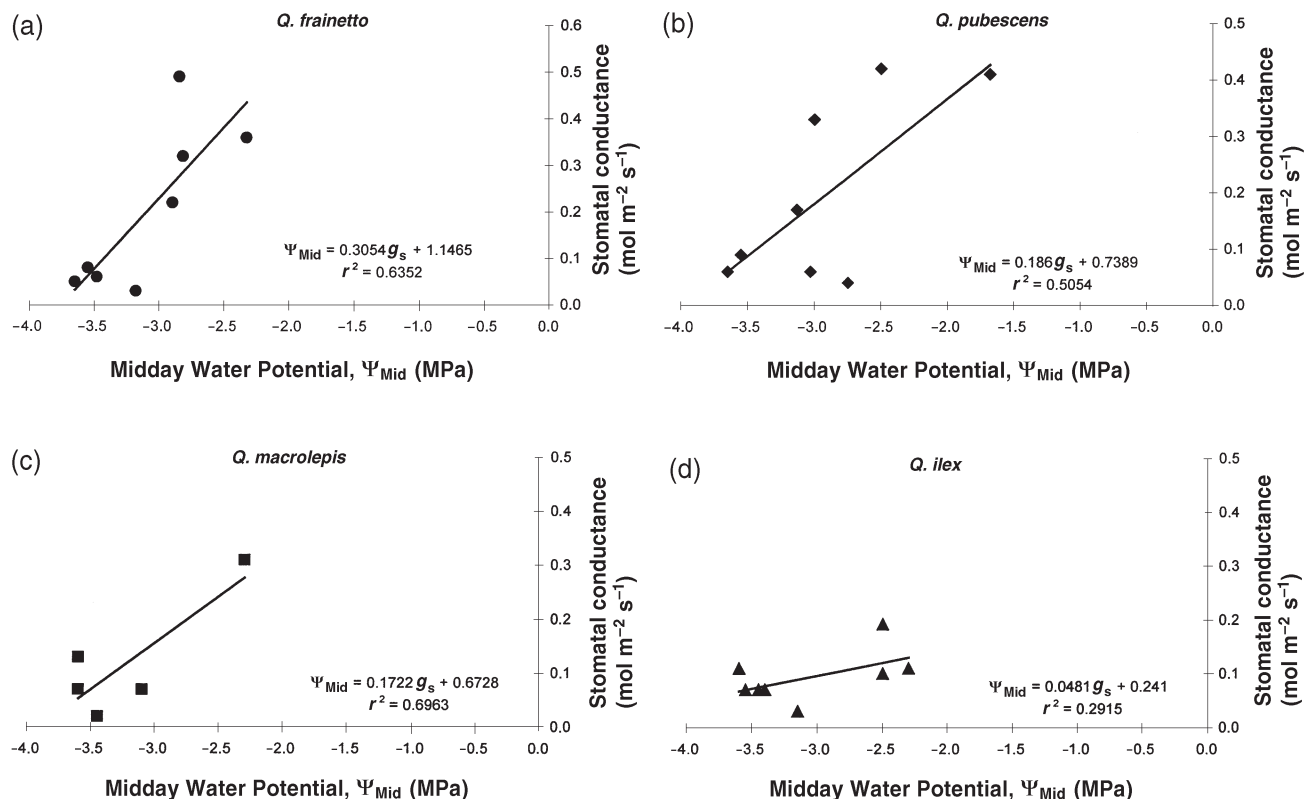


Figure 10. Relationship between stomatal conductance (g_s) and midday leaf water potential (Ψ_{Mid}) under drought conditions during the 1996 growing season for (a) *Q. frainetto*, (b) *Q. pubescens*, (c) *Q. macrolepis* and (d) *Q. ilex*.

under drought conditions (Kubiske and Abrams 1992). In contrast, *Q. agrifolia* Née has a high leaf area under drought conditions, perhaps enabling this evergreen oak species to maximize winter and spring carbon uptake (Goulden 1996). In our study, the deciduous *Q. frainetto* had the highest leaf area but the lowest drought resistance among the species studied. There were no significant differences in R:S ratio between drought-treated and well-watered seedlings in any of the species studied. A similar finding has been reported for *Q. rubra* (Kubiske and Abrams 1992).

Plant water status

In all four species, severe drought significantly reduced water potentials from early July onward. A similar drought response has been reported for *Q. pubescens* growing in the Mediterranean (Tognetti et al. 1999). The simultaneous reduction in stomatal conductance with decreasing water potentials is a characteristic response of Mediterranean species to diurnal and seasonal deficits and is an indicator of conservative water use (Aussenac and Valette 1982, Turner 1986, Archer and Rambal 1992, Castell et al. 1994).

On average, Ψ_{pd} was 1.5 MPa higher than Ψ_{mid} , and this difference remained constant throughout the experimental period for both drought-treated and well-watered seedlings, indicating that water potential recovered diurnally from the extreme stress experienced between 1200 and 1600 h. Tognetti et al. (1998) reported midsummer minimum midday water potentials of -4.5 MPa and -6 MPa in *Q. pubescens* and *Q. ilex*, respectively, and corresponding predawn values of -3.6 and -5.7 MPa, respectively.

A gradual seasonal decline in Ψ_{pd} was observed in all drought-treated seedlings, and the seedlings did not recover to pre-drought values. Rising Ψ_{pd} eventually caused irreversible damage to leaf tissues (beginning of September), evidenced by leaf yellowing and abscission, as reported for *Q. ilex* by Kyriakopoulos and Larcher (1976). Stomatal conductance did not recover to pre-drought values either, a finding that also points to irreversible cell damage. However, g_s never fell to zero, indicating that the oak seedlings maintained stomatal conductance at low water potentials. Tognetti et al. (1999) also reported no recovery of stomatal control of transpiration; however, they observed recovery of water potential to pre-drought values in *Q. pubescens* trees. The differential stomatal response between the studies may be attributable to differences between mature trees and 2-year-old seedlings.

Ranked in increasing order of water potentials, *Q. macrolepis* had the lowest, followed by *Q. frainetto*, *Q. ilex*, and then *Q. pubescens* (the highest). Similarly, Damesin et al. (1997) reported slightly lower Ψ_{pd} in *Q. ilex* than in *Q. pubescens* growing in Mediterranean habitats under various levels of water availability. The strong correlation between the most negative Ψ_{pd} and the greatest seasonal differences in predawn water potential, which is attributed to differences in water relations among *Quercus* species, emphasizes the different drought responses of the species examined.

Generally, our water potential values are lower than those reported for oaks in similar experiments. For example, Ψ_{mid} values in the range of -2.5 to -4.0 MPa have been reported for *Q. macrolepis*, *Q. ilex* and *Q. pubescens* (Comin et al. 1987, Guyon 1987, Dreyer et al. 1990). Minimum Ψ_{pd} values for *Q. rubra* ranged from -1.8 to -2.4 MPa (Kubiske and Abrams 1992, Abrams 1994, Fort et al. 1997) and for *Q. alba* L. values ranged from -2.2 to -2.9 MPa (Teskey and Hinckley 1981) and for *Q. pyrenaica* Willd. a value of -0.3 MPa was reported (Gallego et al. 1994). During the summer, a Ψ_{pd} of -0.3 MPa and a Ψ_{mid} of -2.4 MPa were measured in *Q. suber* L. (Faria et al. 1996).

It has been suggested that *Quercus* seedlings have the ability to sustain favorable water conditions during a drought by maintaining a relatively high water potential (desiccation avoidance response) (e.g., Abrams 1990, Ni and Pallardy 1991, Valentini et al. 1992). However, Rambal (1993) concluded that *Quercus* seedlings decrease their water potential during drought (desiccation tolerance response). The significant differences in stomatal conductance between species can be explained by correlating stomatal activity with the progress of water stress. Initially, *Q. ilex* had a high g_s ; however, g_s of *Q. ilex* became the lowest of all species early in the growing season, whereas water potentials remained relatively high throughout the season, indicating that this species copes with diurnal and seasonal water deficits by avoiding desiccation (cf. Huc et al. 1994). Epron and Dreyer (1990) concluded that *Q. ilex* exhibits increased instantaneous water-use efficiency with increasing drought severity, suggesting that this species is well-adapted to drought conditions. Among the species studied, highest water potentials were measured in *Q. pubescens*, implying a drought avoidance response. However, stomatal conductance was significantly higher than that of *Q. ilex*, suggesting that the stomatal regulation exhibited by *Q. pubescens* was probably independent of actual leaf water status. Our observations for *Q. ilex* and *Q. pubescens* are consistent with the results of other studies (Valentini et al. 1992, Sala and Tenhunen 1994, Davies et al. 1994). *Quercus macrolepis*, on the other hand, appears to cope with summer water stress through mechanisms of dehydration tolerance, reflected in the highest stomatal conductance and the lowest water potentials among the species examined. *Quercus macrolepis* showed the strongest relationship between the decline in stomatal conductance and the decline in water potential, supporting the hypothesis that it is a drought-tolerant species (cf. Guehl et al. 1991). Different behavior was exhibited by *Q. frainetto*. We observed a large decline in leaf water potential and a concomitant reduction in stomatal conductance. Such responses are indicative of moderate drought resistance, because stomatal closure was not sufficient to maintain adequate leaf water potentials. The drought response of *Q. frainetto* was similar to that of *Q. robur* (Fort et al. 1997).

Conclusions

Severe water stress during July and August 1996 significantly reduced soil water content (down to 1.8%) and led to leaf loss

in *Q. frainetto*, *Q. pubescens*, *Q. macrolepis* and *Q. ilex* seedlings by mid-September. However, the drought-induced decreases in individual leaf area, number of leaves per plant, total plant leaf area, height and root:shoot ratio were not significant. All species exhibited early leaf area development (more than 50% of the final) before growing conditions became stressful. Compared with the other species, *Q. macrolepis* maintained the largest number of leaves of the smallest size, a response that enabled this species to sustain its population in dry conditions. In addition, it was the only species that responded to water depletion by increasing fine root production, thus enhancing its water absorbing root area.

Extreme drought conditions during July and August significantly reduced stomatal conductance and predawn and midday leaf water potentials in all four oak species. However, the four species possessed different mechanisms for coping with water stress. *Quercus macrolepis* showed dehydration tolerance characteristics including high stomatal conductance and relatively low water potential. In contrast, *Q. ilex* maintained relatively higher water potentials and initiated early stomatal closure before serious alteration of water status, thus demonstrating a drought avoidance response. By sustaining both high water potential and stomatal activity, *Q. pubescens* exhibited stomatal regulation independent of its actual water status. The least drought-resistant species was *Q. frainetto*. This species developed low water potentials despite having a very low stomatal conductance. We conclude that seedlings of *Q. macrolepis*, *Q. ilex* and *Q. pubescens*, but not *Q. frainetto*, possess responses to water stress that confer the capacity to maintain biological activity during arid summers in the Mediterranean zone.

Acknowledgments

The authors thank Dr. Y. Raftoyiannis for advising and helping in the statistical analysis of the data, and Dr. G. Jackson, University of Edinburgh, for helpful criticisms of the first draft of this manuscript.

References

- Abrams, M.D. 1990. Adaptations and responses to drought in *Quercus* species in North America. *Tree Physiol.* 7:227–238.
- Abrams, M.D. 1994. Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. *Tree Physiol.* 14:833–842.
- Archer, G. and S. Rambal. 1992. Comparative water relations of four Mediterranean oak species. *Vegetatio* 99/100:177–184.
- Athanasiadis, N.H. 1986. Forest botany. (Trees and bushes of the forests in Greece), Giahoudi Giapoulis, 1st Edn. Thessaloniki, Greece, 309 p. In Greek.
- Aussenac, G. and J.C. Valette. 1982. Comportement hydrique estival de *Cedrus atlantica* Manetti, *Quercus ilex* L. et *Quercus pubescens* Willd. et de divers pins dans le Mont Ventoux. *Ann. For. Sci.* 39:41–62.
- Callaway, R.M. and B.E. Mahall. 1996. Variation in leaf structure in *Quercus douglasii* trees differing in root architecture and drought history. *Int. J. Plant Sci.* 157:129–135.
- Castell, C., J. Terradas and J.D. Tenhunen. 1994. Water relations, gas exchange, and growth of resprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L. *Oecologia* 98:201–211.
- Comin, M.P., A. Escarre, C.A. Gracia, M.J. Lledo, R. Rabella, R. Save and J. Terradas. 1987. Water uptake by *Q. ilex* L. in forests near Barcelona, Spain. In *Plant Responses to Stress. Functional Analysis in Mediterranean Ecosystems*. NATO ASI Series, Series G, Ecological Sciences, Vol. 15. Eds. J.D. Tenhunen, F.M. Catarino, O.L. Lange and W.C. Oechel. Springer-Verlag, Berlin, pp 59–266.
- Dafis, S. 1982. The present state of the forests in our country. *Scientific Bulletin Geotechnica*, pp 18–22. In Greek.
- Damesin, C., S. Rambal and R. Joffre. 1997. Between-tree variations in leaf $\delta^{13}\text{C}$ of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability. *Oecologia* 111:26–35.
- Davies, W.J., F. Tardieu and C.L. Trejo. 1994. How do chemical signals work on plants that grow on dry soil? *Plant Physiol.* 104:309–314.
- Dreyer, E., F. Bousquet and M. Ducrey. 1990. Use of pressure volume curves in water relation analysis of woody shoots: influence of dehydration and of four European oak species. *Ann. For. Sci.* 47:285–297.
- Dreyer, E., A. Granier, N. Breda, H. Cochard, D. Epron and G. Aussenac. 1992. Oak trees under drought constraints: eco-physiological aspects. In *Proc. Int. Congr.: Recent Advances in Studies on Oak Decline*. Selva di Fasano, Bridisi, pp 293–322.
- Epron, D. and E. Dreyer. 1990. Stomatal and non-stomatal limitation of photosynthesis by leaf water deficits in three oak species: a comparison of gas exchange and chlorophyll a fluorescence data. *Ann. Sci. For.* 47:435–450.
- Faria, T., J.I. Garcia-Plazaola, A. Abadia, S. Cerasoli, J.S. Pereira and M.M. Chaves. 1996. Diurnal changes in photoprotective mechanisms in leaves of cork oak (*Quercus suber*) during summer. *Tree Physiol.* 16:15–123.
- Fort, C., M. Fauveau, F. Muller, P. Label, A. Granier and A. Dreyer. 1997. Stomatal conductance, growth and root signaling in young oak seedlings subjected to partial soil drying. *Tree Physiol.* 17:281–289.
- Gallego, H.A., M. Rico, G. Moreno and I. Santa Regina. 1994. Leaf water potential and stomatal conductance in *Quercus pyrenaica* Willd. forests: vertical gradients and response to environmental factors. *Tree Physiol.* 14:1039–1047.
- Goulden, M.L. 1996. Carbon assimilation and water-use efficiency by neighboring Mediterranean-climate oaks that differ in water access. *Tree Physiol.* 16:417–424.
- Guehl, J.M., G. Aussenac, J. Bouachrine, R. Zimmermann, J.M. Pennes, A. Ferhi and P. Grieu. 1991. Sensitivity of leaf gas exchange to atmospheric drought, soil drought and water-use efficiency in some Mediterranean *Abies* species. *Can. J. For. Res.* 21:1507–1515.
- Guyon, J.P. 1987. Étude des courbes pression-volume de rameaux de trois espèces forestières. *Acta Oecol. Oecol. Appl.* 8:363–370.
- Hamerlynck, E. and A.K. Knapp. 1996. Photosynthetic and stomatal responses to high temperature and light in two oaks at the western limit of their range. *Tree Physiol.* 16:557–565.
- Huc, R., A. Ferhi and J.M. Guehl. 1994. Pioneer and late stage tropical rainforest tree species (French Guiana) growing under common conditions differ in leaf gas exchange regulation, carbon isotope discrimination and leaf water potential. *Oecologia* 99:297–305.
- Kubiske, M.E. and M.C. Abrams. 1992. Photosynthesis, water relations and leaf morphology of xeric versus mesic *Quercus rubra* seedlings ecotypes in central Pennsylvania in relation to moisture stress. *Can. J. For. Res.* 22:1402–1407.

- Kyriakopoulos, E. and W. Larcher. 1976. Changes in water potential in leaves of *Quercus ilex* L. during desiccation. *Z. Pflanzenphysiol.* 77:268–271.
- Margolis, H.A. and D.G. Brand. 1990. An ecophysiological basis for understanding plantation establishment. *Can. J. For. Res.* 20: 375–390.
- Myers, B.J. 1988. Water stress integral—a link between short-term stress and long-term growth. *Tree Physiol.* 4:315–323.
- Ni, B.-R. and S.G. Pallardy. 1991. Response of gas exchange to water stress in seedlings of woody angiosperms. *Tree Physiol.* 8:1–9.
- Oliveira, Q., O.A. Correia, M.A. Martins-Loucao and F.M. Catarino. 1992. Water relations on cork-oak (*Quercus suber* L.) under natural conditions. *Vegetatio* 99/100:199–208.
- Radoglou, K.M. 1996. Environmental control of CO₂ assimilation rates and stomatal conductance in five oak species growing in field conditions in Greece. *Ann. Sci. For.* 53:269–278.
- Rambal, S. 1993. The differential role of mechanisms for drought resistance in a Mediterranean evergreen shrub: a simulation approach. *Plant Cell Environ.* 16:35–44.
- Romane, F. and J. Terradas. 1992. *Quercus ilex* L. ecosystems: functions, dynamics and management. *Advances in Vegetation Science* 13, Kluwer Academic Publishers, Boston, MA, 376 p.
- Sala, A. and J.D. Tenhunen. 1994. Site-specific water relations and stomatal conductance of *Q. ilex* in a Mediterranean watershed. *Tree Physiol.* 14:601–617.
- Sokal, R.R. and J.F. Rohlf. 1995. *Biometry*. 3rd Edn. W.H. Freeman and Company, New York, 850 p.
- Tenhunen, J.P., O.L. Lange and R.W. Pearcy. 1987. Diurnal variations in leaf conductance and gas exchange in natural environments. *In* *Stomatal Function*. Eds. E. Zeiger, G.D. Farquar and I.R. Cowan. Stanford University Press, Stanford, pp 323–351.
- Teskey, R.O. and T.M. Hinckley. 1981. Influence of temperature and water potential on root growth of white oak. *Physiol. Plant.* 52: 363–369.
- Tognetti, R., A. Longgobucco and A. Raschi. 1998. 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. *New Phytol.* 139:437–447.
- Tognetti, R., A. Longgobucco, F. Miglietta and A. Raschi. 1999. Water relations, stomatal response and transpiration of *Quercus pubescens* trees during summer in a Mediterranean carbon dioxide spring. *Tree Physiol.* 19:261–270.
- Turner, N.C. 1986. Adaptation to water deficits: a changing perspective. *Aust. J. Plant Physiol.* 13:175–190.
- Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. *Irrig. Sci.* 9:289–308.
- Valentini, R., G. Scarascia Mugnozza, E. Giordano and A. Vannini. 1992. Water relations of Mediterranean oaks. Possible influences on their dieback. *Proc. Int. Congr. Recent Advances in Studies on Decline. Selva di Fasano, Brindisi*, pp 439–446.