

Response of a mature *Pinus laricio* plantation to a three-year restriction of water supply: structural and functional acclimation to drought

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Summary The response of mature forest stands to a reduction in water availability has received little attention. In particular, the extent to which a short-term reduction in gas exchange can be alleviated in the long-term by acclimation processes is not well understood. We studied the impact of a severe reduction in water availability on the water relations and growth of 35-year-old *Pinus laricio* Poiret. trees in a replicated experiment. Sapwood and needle increments, soil and tree water status, stand transpiration, xylem embolism and plant hydraulic architecture were monitored over a 3-year period in control and drought-treated plots. Needle length was reduced in drought-treated trees by 30, 19 and 29%, and sapwood increments by 50, 27 and 24% over the 3 years. Drought did not result in tree mortality or in extensive xylem embolism or foliage dieback. On the contrary, a conservative water-use strategy was observed, because minimum leaf water potentials did not differ between treatments or over the season. Plant hydraulic resistance was also unaffected by restricted water availability. Stand transpiration was strongly reduced by drought treatment over the summer, but not during the winter, despite significant differences in leaf area between control and drought-treated trees, implying higher transpiration rates per unit leaf area in the droughted plants. This suggests that water transport capacity, rather than the amount of leaf area, controlled stand transpiration, which is at variance with expectations based on experiments with seedlings and short-term experiments with mature trees.

Keywords: growth, homeostasis, hydraulic resistance, long-term effects, transpiration.

Introduction

Water availability is the main factor limiting plant and forest growth in dry climates (Waring and Running 1998). Despite great uncertainty about the impact of global warming on the

amount and distribution of precipitation over the next century, most general circulation models predict a marked reduction in soil water availability during the summer in the Mediterranean area (Intergovernmental Panel on Climate Change 1990, Wetherald and Manabe 1995). Together with the increase in evaporative demand brought about by higher temperatures, such a decline in water availability is likely to result in more severe water stress, which could offset the positive effects of elevated CO₂ concentration ([CO₂]) on forest growth (Intergovernmental Panel on Climate Change 1996, 1997).

Process-based models used to assess the impact of climate change on forests should therefore include a better representation of the response of mature trees to long-term drought. However, although forest response to elevated [CO₂] is increasingly well understood (DeLucia et al. 1999, Idso 1999), we know little about the mechanisms involved in tree response to drought.

Over the short term, drought has a substantial impact on gas exchange (Schulze 1993). It has been suggested that, over the long term, structural changes could alleviate the direct effects of water stress on stomatal conductance and photosynthesis (Geiger and Servaites 1991). Leaf area index, in particular, is known to be sensitive to soil water availability (Gholz 1982). Xylem embolism may also be of great significance in the response of woody plants to soil dryness (Grace 1993). Xylem is known to be a vulnerable pipeline, often at the brink of dysfunction (Tyree and Sperry 1988), and extreme water potentials can trigger extensive xylem cavitation resulting in foliage dieback or plant death (Tyree et al. 1993, Saliendra et al. 1995). There is substantial evidence that a certain amount of drought-induced cavitation of xylem conduits is a regular occurrence in tree species under field conditions (Borghetti et al. 1993, 1998, Sperry et al. 1994, Tognetti and Borghetti 1994, Jackson et al. 1995, Magnani and Borghetti 1995, Irvine et al. 1998). However, it is possible that either stomatal closure (Saliendra et al. 1995) or changes in allocation and functional

allometry (Magnani 2000) maintain minimum water potentials above the threshold for extensive cavitation, thereby preventing substantial loss of water transport capacity and foliage damage. To explore these possibilities we need to understand how stomatal mechanisms, structural acclimation and foliage mortality combine in the overall response to drought.

Although the effects of water availability on small seedlings are well documented (Seiler 1985, Cregg 1994), our knowledge of the response of mature trees to drought under field conditions is incomplete. Only a handful of field experiments have looked at the long-term effects of drought on both function and growth of mature trees. Many studies have focused on the long-term effects of irrigation or artificial drought on soil processes and stand growth (Axelsson and Axelsson 1986, Snowdon and Benson 1992, Gower et al. 1992, Bredemeier et al. 1998), and a few studies have looked at the impact on physiology as well, but with a perspective generally limited to a single year (Borghetti et al. 1998, Irvine et al. 1998). Because of the slow dynamics of tree acclimation, the results obtained from short-term experiments could prove misleading, and very few studies have combined the two approaches with a long-term perspective (Ewers et al. 1999, 2000).

We carried out a field experiment in which the effects of an artificial reduction in water availability on the function and growth of mature *Pinus laricio* Poiret. trees were followed over a 3-year period at a site in southern Italy where tree growth is limited by drought. Although widely planted in Mediterranean Europe (Debazac 1964, 1965), *P. laricio* appears to be sensitive to extended periods of drought, which can trigger a long-term decline in growth (Lebourgeois and Becker 1996). We monitored changes in gas exchange and growth and their combined effects on plant water relations and xylem cavitation, to provide a better understanding of the mechanisms involved in the drought response of mature forest trees.

Materials and methods

Study site

The research was carried out in an even-aged *P. laricio* stand growing in Calabria, Italy. Environmental conditions at the study site are detailed in Table 1, together with key stand variables at the beginning of the experiment. Six 10-m² experimental plots were located randomly in the stand. Although the plots differed in tree density (ranging between 1100 and 2300 trees ha⁻¹), they had similar sapwood basal areas (32.7 ± 1.5 m² ha⁻¹) with no significant differences among treatments ($P = 0.56$). Lateral water movement at the plot perimeter was prevented by a 50-cm-deep ditch, extending down to the unconsolidated material (C horizon). The ditch was lined with a heavy polythene sheet. In December 1994, the water supply to the soil was restricted in three plots (RW treatment) by a transparent plastic roof suspended 1.5 m above ground. Precipitation was not interrupted in the other three plots (unrestricted water supply; UW treatment). Scaffolds provided access to the forest canopy in one RW and one UW plot.

Table 1. Site and stand characteristics (Dimase and Iovino 1996).

Location	Sila, Calabria, Italy
Latitude	39°28'
Longitude	16°30'
Altitude (m a.s.l.)	1080
Slope (°)	0–3
Soil type	Lithic Xerumbrepts ¹
Soil depth (cm)	40
Soil texture (%)	Sand 73; silt 21; clay 6
Underlying rock	Granite
Annual rainfall 1921–1987 (mm)	1155
Annual temperature (°C)	8.9
Age of trees (years)	35
Tree density (trees ha ⁻¹)	1500
Diameter at 1.3 m aboveground (cm)	18.9
Height (m)	15.3
Leaf area index	2.5
Overbark basal area (m ² ha ⁻¹)	42.0
Sapwood basal area (m ² ha ⁻¹)	29.2

¹ According to Soil Taxonomy (Soil Survey Staff 1975).

Measurements of environmental, ecophysiological and growth variables

Tree growth and water relations were measured throughout 1995, 1996 and 1997, with the most intensive sampling during the growing periods. Measurements were restricted to trees close to the center of the plots (i.e., 3–5 m from the nearest trench), to reduce artifacts arising from root damage.

Incoming global radiation, air temperature and vapor pressure deficit were continuously measured by an automated weather station 3 m above the canopy. Daily precipitation was measured at a meteorological station nearby. Soil water content was periodically measured by time domain reflectometry (TDR) (Topp et al. 1980). The apparent dielectric constant of the soil was measured at three locations per plot with a Trase reflectometer (Soilmoisture, Santa Barbara, CA) connected to the ends of two 45 cm × 5 mm stainless steel cylindrical probes fully inserted in the soil 5 cm apart. Readings of soil dielectric constant were translated to a measure of soil volumetric water content, based on laboratory calibration with dehydrating soil samples from the experimental site.

Sapwood water content was monitored by TDR (Constantz and Murphy 1990, Irvine and Grace 1997) on five trees per plot. The apparent dielectric constant of the sapwood at breast height was measured by the Trase reflectometer connected to the ends of two 5 cm × 3 mm probes inserted radially into the stem 5 cm apart. A disk of bark had been removed around the point of insertion of the probes and the exposed area coated with vaseline to prevent loss of water from the xylem. The apparent dielectric constant of the sapwood was related to the water content of the sapwood by laboratory calibration with dehydrating wood samples (Cinnirella and Saracino 1998).

Stem circumference at a height of 1.3 m was monitored throughout the experiment on five trees per plot with custom-built aluminum girth bands (Liming 1957). Four trees per treatment adjacent to the scaffolding towers were selected for

measurement of predawn needle water potential, water content of shoot xylem, needle and shoot length and sap flow density. Needle length was periodically measured with a caliper on 10–16 current-year needles sampled on three twigs from the sunlit crown of each tree. The length of the same shoots was also measured.

Predawn water potential was measured with a Scholander pressure chamber (PMS Instrument Co., Corvallis, OR) on 1-year-old needles sampled from six twigs located in the upper sunlit part of the tree crown. Measurements were made immediately after twigs had been detached.

The xylem water content of 1-year-old twigs was also measured on three shoots excised from the upper part of the tree crown. Twig segments were debarked, wrapped in sealing film and taken to the laboratory. The volumetric water fraction (V_f) was calculated on 2–3-cm-long segments as $V_f = (W_f - W_d)/(\rho_w V_f)$, where W_f , W_d and V_f are fresh weight, dry weight and fresh volume of debarked twig segments, respectively, and ρ_w is density of water (Borghetti et al. 1991).

Starting in autumn 1996, sap flow density was measured on four trees per treatment, by the continuous heating method developed by Granier (1985, 1987). On each tree, two probes, 2 cm × 2 mm, were inserted in the xylem at the base of the live crown. Probes were vertically aligned at a distance of 10 cm from each other. The upper probe was continuously heated, whereas the lower probe was used as a reference. The temperature difference between the probes was recorded with an M93 data logger (Micros, Conegliano Veneto, Italy) every 15 min and converted to sapflow density using the equations reported by Granier (1985). Stand transpiration was computed by multiplying mean sapflow density (i.e., transpiration per unit area of sapwood) of measured trees in the UW and RW plots by stand sapwood basal area (Granier and Loustau 1994), after correcting for sapwood tapering between the base of the live crown and breast height. Sapwood area was inferred from inspection of wood cores extracted with a Pressler borer from stems of measured trees at the end of the experiment.

On 2 days in 1996 and 3 days in 1997, the diurnal course of needle water potential was also determined by the pressure chamber technique on the same plants sampled for sap flow density. Soil-to-needle stand hydraulic resistance was computed as the slope of the relationship between needle water potential and stand transpiration (Jarvis 1976, Irvine et al. 1998). Based on a visual analysis of data, sapflow measured at the base of the crown was assumed to lag transpiration by 45 min (Granier and Loustau 1994).

Results

Soil water content and predawn water potential

During the study, precipitation showed a bimodal pattern, with a maximum in autumn and a peak in late winter with little rainfall over the summer (Figure 1). As a result, soil water deficits developed in the control (UW) treatment during the summer. Soil water content in the UW plots, integrated over the top 45 cm, ranged from 0.27 to 0.10 m³ H₂O m⁻³ of soil, with a

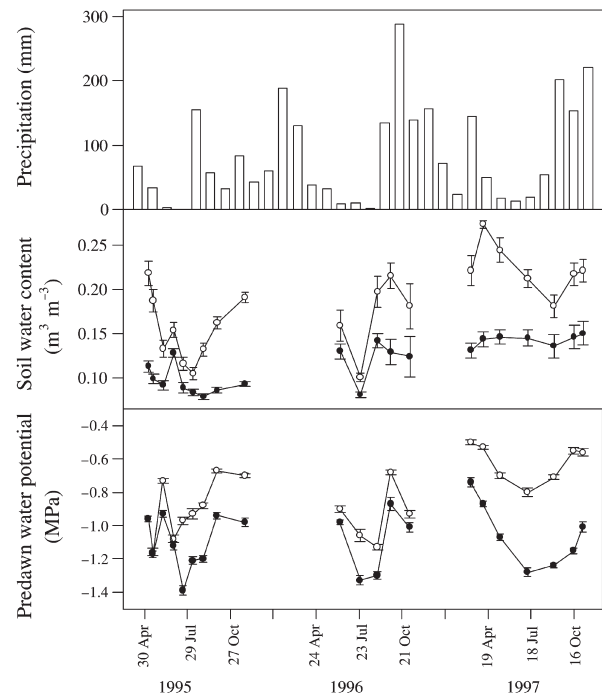


Figure 1. Seasonal course of monthly precipitation, volumetric soil water content in the top 50 cm of soil and predawn needle water potential in RW (●) and UW (○) plots during the study period. Vertical bars indicate ± 1 standard error.

marked seasonal pattern throughout the experiment (Figure 1). The RW treatment effectively restricted soil water recharge, resulting in water contents ranging between 0.15 and 0.08 m³ m⁻³, with little variation over the year. The sudden rise in soil water content observed in June 1995 and August 1996 was probably caused by precipitation circumventing the plastic roof. Minimum soil water contents occurred in the second half of July 1996, in both the UW and RW treatments. The above-average precipitation in 1997 resulted in a marked increase in soil water contents.

Predawn water potentials of UW and RW trees showed distinct and parallel fluctuations over the three growing seasons (Figure 1). Maximum values of predawn water potential in UW and RW trees were -0.5 and -0.74 MPa, respectively. The peak drought always occurred in July, with minimum predawn water potentials of -1.16 and -1.39 MPa in UW and RW trees, respectively. When grouped over all dates, predawn water potential was significantly ($P < 0.01$) lower in RW trees than in UW trees. There was a significant ($P < 0.01$) treatment effect on predawn water potential on 17 out of 21 measurement dates. The effect of the treatment increased over the experiment. The largest differences between treatments were observed in summer 1997, mainly as a result of the strong soil water recharge in the UW plots that resulted in predawn water potentials consistently above -0.9 MPa.

Tree growth

The imposed restriction in water supply had a considerable impact on tree growth, although the effects differed depending

Table 2. Tissue growth in drought-treated (RW) and control (UW) *P. laricio* trees. Values of relative basal area (BA) increment, final needle and shoot length are compared for the two treatments (mean \pm SE; probability levels based on a two-tailed *t*-test).

		RW	UW	<i>P</i>
Relative BA growth (% year ⁻¹)	1995	0.93 \pm 0.13	1.86 \pm 0.13	< 0.001
	1996	2.30 \pm 0.29	3.15 \pm 0.25	0.046
	1997	1.85 \pm 0.20	2.45 \pm 0.20	0.044
Needle length (cm)	1995	7.1 \pm 0.1	10.3 \pm 0.1	< 0.001
	1996	9.3 \pm 0.4	11.5 \pm 0.4	0.002
	1997	7.4 \pm 0.3	10.5 \pm 0.4	< 0.001
Shoot length (cm)	1995	9.4 \pm 0.3	13.4 \pm 0.1	< 0.001
	1996	11.8 \pm 0.7	14.3 \pm 0.5	0.012
	1997	9.0 \pm 0.6	14.2 \pm 0.6	< 0.001

on the tissue (Table 2). The influence of tree water status on growth was explored by plotting needle length, shoot length and mean sapwood increment of individual trees against their mean predawn water potential over the year, computed for each tree as the integral of predawn water potential divided by time (Figure 2). Growth traits were well differentiated by tree water status in 1995, showing a strong negative correlation with mean predawn water potential. In 1996, a weaker correlation was found for shoot length and almost no correlation for needle length. In 1997, shoot and needle length appeared to be strongly correlated with predawn water potential, whereas no correlation was observed for sapwood increments.

Needle and shoot growth in RW trees over the 3 years were only 70, 81 and 71% and 70, 82 and 63% of control values, respectively (Figure 3). Sapwood increments, on the contrary, were most severely affected by the RW treatment at the begin-

ning of the experiment, with a 50% reduction in 1995, but reductions of only 27 and 24% below the control in the following years. Differences in basal area increments over the second and third years were just statistically significant ($P = 0.05$) (Table 2). Because of the difference in longevity between needles and sapwood, leaf and sapwood areas were reduced by different extents in the RW trees by the end of the study. *Pinus laricio* leaves typically last 3 years, so by the end of 1997 all of the needles on RW trees were on average 30% shorter than those on UW trees. Based on the conservative assumption that the reduced water supply had no additional effects on the number of needles formed and shed each year, we can assume a similar 30% reduction in tree and stand leaf area. Active sapwood of *P. laricio* trees consisted of 31 annual rings, accounting for almost 99% of plant basal area. As a result, the radial growth reduction over the 3 years of the study translated into a 2% decline in sapwood area.

Transpiration and canopy stomatal conductance

The diurnal courses of meteorological variables and transpiration during selected days in November 1996 and July 1997 are shown in Figure 4. Predawn water potential did not differ sig-

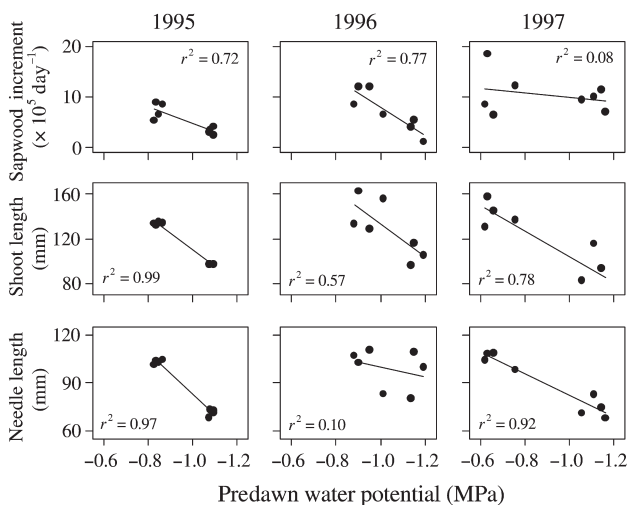


Figure 2. Changes in growth sensitivity to soil water availability in different *P. laricio* tissues during the study. The weighted mean predawn water potential was computed for each growing season as the predawn water potential integral divided by time. Sapwood increment is the relative sapwood area increment per unit of time over the year. All data refer to individual trees for which growth and water potential values were available ($n = 8$).

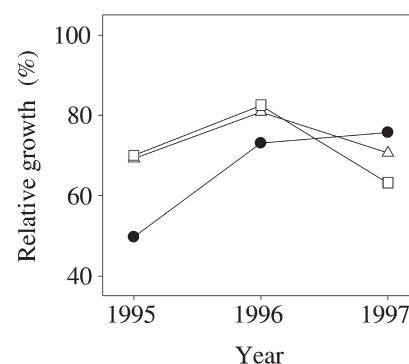


Figure 3. Variable response of growth to a long-term restriction in soil water availability in different *P. laricio* tissues. Symbols: mean needle length, Δ ; mean shoot length, \square ; and sapwood area increment, \bullet . Needle and shoot growth were measured on four plants per treatment and sapwood increments were measured on 15 trees per treatment.

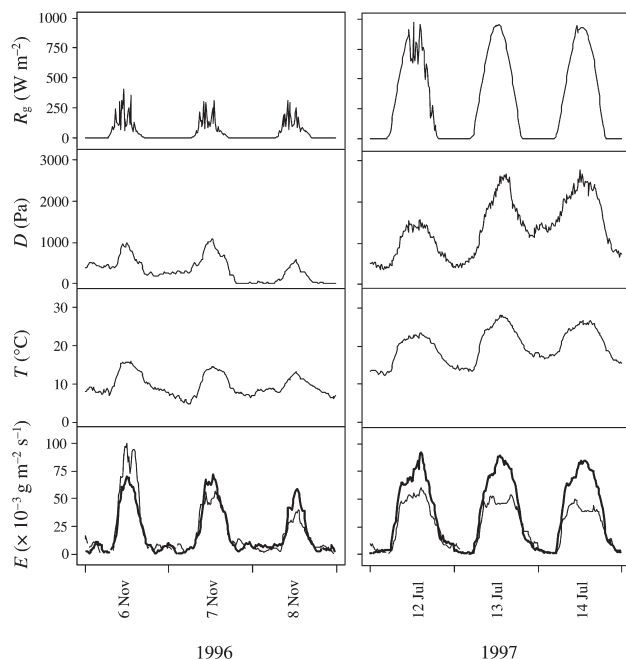


Figure 4. Daily course of transpiration in *P. laricio* under contrasting conditions. Mean transpiration rate (E) in RW (thin line) and UW trees (thick line) over the course of 3 days during the winter and at the height of the summer drought is compared with the development of global radiation (R_g), vapor pressure deficit (D) and air temperature (T), as measured above the canopy. Transpiration is expressed on a ground area basis.

nificantly between UW and RW trees in November 1996 (-0.9 ± 0.02 versus -1.0 ± 0.03 MPa), but there was a considerable treatment difference by the following July, corresponding to the peak of summer drought (-0.8 ± 0.02 versus -1.3 ± 0.02 MPa). As a result, the transpiration rate of RW trees did not differ from that of UW trees in the first period, but was reduced up to 40% at the peak of summer drought. In particular, there was a marked midday plateau in transpiration rates under drought conditions in RW trees but not in UW trees.

To examine the combined effects of air and soil humidity on transpiration, data were grouped into nine homogeneous periods distributed over the 2 years, centered around individual measurements of predawn water potential. Data for six of these intervals are presented in Figure 5, plotted against air vapor pressure deficit (D) above the canopy. Transpiration showed a curvilinear response to D , reaching a plateau at about 1 kPa. The effect of the treatment, however, changed over the year (Figure 6): transpiration of RW trees fell to 57% of that of UW trees over the summer, as a result of the more negative soil water potentials, but recovered to rates similar to those of UW trees during the autumn. Because of the differences in leaf area, we infer that, under well-watered conditions, transpiration per unit leaf area was higher in RW trees than in UW trees.

Stand hydraulic resistance and minimum water potential

Plant soil-to-leaf hydraulic resistance (on a ground area basis)

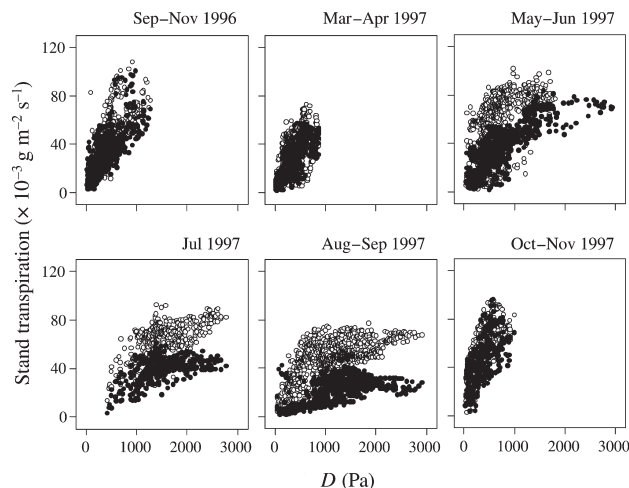


Figure 5. Environmental effects on transpiration rate in control and drought-treated *P. laricio* plots. The response of transpiration rate to air vapor pressure deficit (D) in RW (●) and UW (○) trees is presented for six homogeneous periods over the season.

was determined on four occasions from the slope of the relationship between needle water potential and stand transpiration (Figure 7). Mean values for the RW and UW trees were 7.7 and 11.7 MPa s m^{-1} , respectively. Although values were always less for RW trees than for UW trees, covariance analysis demonstrated that the slopes were not significantly different on any occasion.

Under conditions of natural and artificial drought, stomatal closure effectively counterbalanced the effects of soil dryness, maintaining minimum daily leaf water potentials at around -1.6 MPa, with minimum values of around -1.7 MPa over the summer. There were no significant differences between treatments in leaf water potential ($P = 0.11$), which was largely decoupled from predawn water potentials (Figure 8). The sensitivity of needle water potential to predawn water potential was only 0.15, i.e., a drop of 1 MPa in predawn water potential

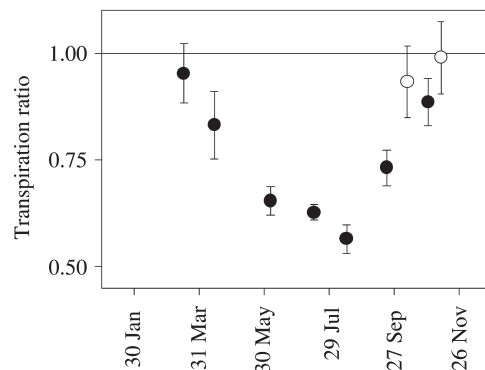


Figure 6. Effects of a long-term restriction in water supply on *P. laricio* transpiration over the course of the season. The ratio of instantaneous transpiration rate in RW trees to UW trees was averaged over homogeneous periods in 1996 (○) and 1997 (●). Values are means \pm LSD.

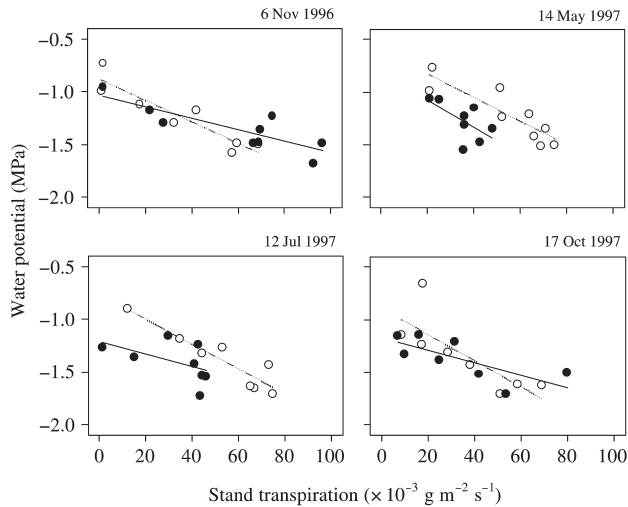


Figure 7. Effects of a long-term restriction in water supply on the hydraulic resistance of *P. laricio* trees. Whole-plant resistance per unit sapwood area was computed as the slope of the relationship between needle water potential and stand transpiration rate in RW (●) and UW (○) stands. Resistances were evaluated under a range of environmental conditions over the course of the season.

translated into a minimum water potential change of no more than 0.15 MPa.

Seasonal changes in xylem water content

The seasonal courses of xylem volumetric water content in terminal shoots and in stem sapwood are reported in Figure 9. Because measurements were made on 1-year-old shoots, i.e., shoots formed during the previous year, samples from RW trees produced only one xylem ring under a restricted water supply in 1995, but both xylem rings in 1996 and 1997.

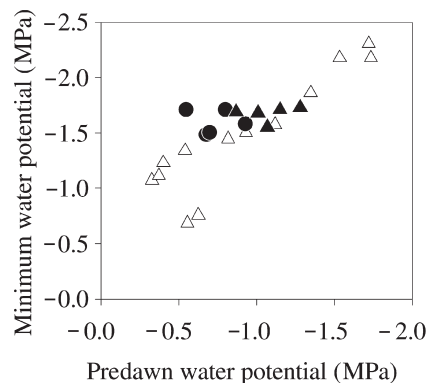


Figure 8. Isohydic and anisohydic behavior in mature trees and seedlings of *P. laricio*. In mature trees, despite differences in predawn water potential over the course of the season and between RW (●) and UW (▲) trees, minimum needle water potential over the day was conserved. In an experiment on potted seedlings, on the contrary, minimum leaf water potential over the day (△) was sensitive to changes in predawn water potential (data from Lebourgeois et al. 1998).

In both UW and RW trees, clear seasonal fluctuations in terminal shoot water content were observed in 1995 and 1997, with a considerable decline over the spring and early summer and a recovery in the autumn to the same values attained at the beginning of the year. A different pattern was observed in 1996: no marked seasonal fluctuation was apparent and the shoot xylem water content was at its highest value in both treatments, despite rather negative predawn water potentials. When pooled over all measurement dates, the water volume fraction of shoot xylem showed a significant ($P < 0.01$) treatment effect between UW trees ($0.57 \pm 0.04 \text{ m}^3 \text{ m}^{-3}$, mean \pm standard error) and RW trees ($0.50 \pm 0.01 \text{ m}^3 \text{ m}^{-3}$; Figure 9A). However, a significant ($P < 0.01$) treatment effect was found on four dates only (marked with an asterisk in Figure 9A), and terminal shoot water contents in RW trees did not differ significantly from control values at the end of the study.

Treatment differences in stem water content at breast height were greater than treatment differences in terminal shoot water content. The volumetric water fraction of the stem xylem, as assessed by the TDR, averaged 0.48 ± 0.04 and $0.39 \pm 0.03 \text{ m}^3 \text{ m}^{-3}$ for UW and RW trees, respectively, over the whole experiment (Figure 9B). Stem xylem water content was about $0.5 \text{ m}^3 \text{ m}^{-3}$ at the beginning of the experiment, with no significant differences between RW and UW trees. It later declined in both RW and UW trees, as seen also in apical shoots. Recovery later in the season was detected only in UW trees. As a result, by the end of 1995 the volumetric water fraction of stem xylem differed significantly between UW and RW trees (0.53 versus $0.38 \text{ m}^3 \text{ m}^{-3}$, $P < 0.01$). However, at the beginning of 1996, the volumetric water fractions of stem xylem of both UW and RW trees were similar to those measured in the corresponding period in 1995 (0.37 and $0.42 \text{ m}^3 \text{ m}^{-3}$ in RW and UW trees, respectively). From the middle of 1996 until the end of the experiment xylem water contents usually differed between treatments. At the beginning of 1997, low values were recorded in trees in both treatments; values in UW trees later fluctuated between $0.53 \text{ m}^3 \text{ m}^{-3}$ in May and $0.43 \text{ m}^3 \text{ m}^{-3}$ in September, whereas only small fluctuations around $0.4 \text{ m}^3 \text{ m}^{-3}$ were observed in RW trees.

Overall, only a slight positive correlation was observed between xylem water content of stems and apical twigs and predawn water potentials ($r^2 = 0.33$ and 0.28 , respectively; data not shown).

Discussion

The RW treatment resulted in a realistic simulation of the reduction in water availability predicted by climate change scenarios for the Mediterranean region (Intergovernmental Panel on Climate Change 1990). Given the characteristics of the soil and underlying rock at the study site (Dimase and Iovino 1996), it is likely that some tree roots explored soil pockets between rock crevices and extracted water from deep in the soil profile. This is confirmed by the observation that minimum predawn water potentials did not differ among years in trees in the RW plots, despite large differences in the volumetric water

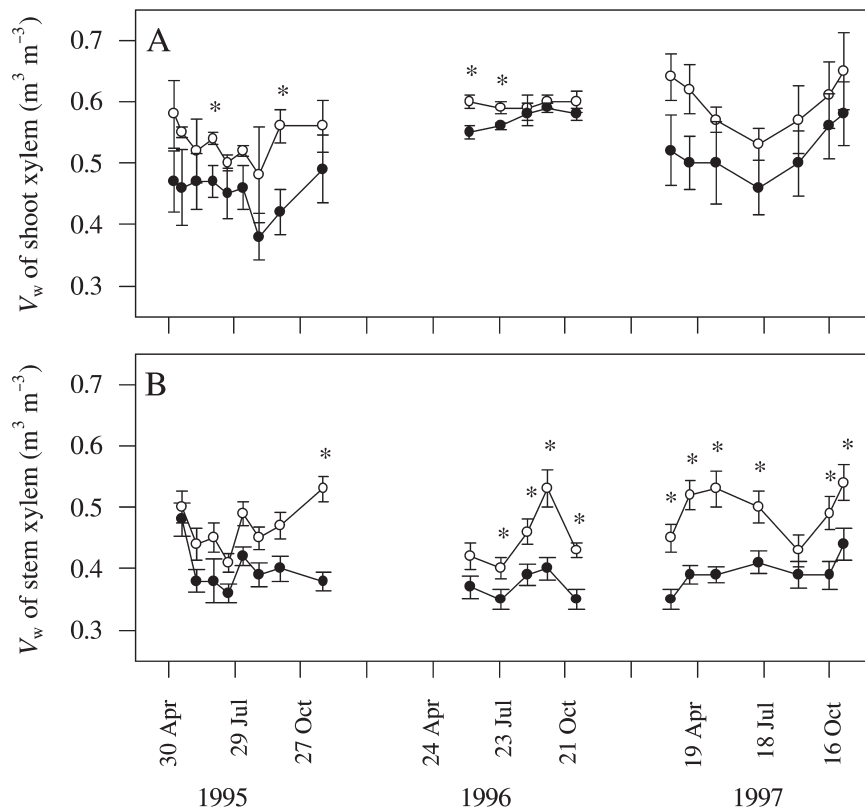


Figure 9. Effects of a long-term restriction in water supply on xylem volumetric water content (V_w) in *P. laricio*. Seasonal changes in sapwood water content in the stem (A) and in terminal shoots (B) of RW (●) and UW (○) trees during the study period. Values are means \pm SE. Significant differences ($P < 0.01$) between treatments are marked by an asterisk.

content of the top soil layers (Figure 1). As a result, the RW treatment did not cause forest dieback, but resulted in a growth decline and in the slow acclimation of tree structure and function to the new environment. Thus, even under Mediterranean conditions, it appears that forests will be able to adapt to the predicted climate change (Intergovernmental Panel on Climate Change 1996).

Growth was strongly reduced by the artificial drought. Based on local growth and yield tables for the species (Castellani 1970), current annual increments over the study period for the UW and RW stands were estimated at 5.5 and 3.6 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$, respectively, i.e., a 35% reduction in response to drought. Nutrient dynamics could have played a role in this reduction, because water availability is known to affect the decomposition rate of soil organic matter, and the roofs constructed to interrupt rainfall likely excluded atmospheric depositions from the RW plots.

Changes in carbon allocation between transpiring leaves and conducting sapwood in response to water availability are well documented (Gower et al. 1995): drought-treated trees tend to allocate less carbon to new foliage production than irrigated trees of similar diameter (Axelsson and Axelsson 1986, Gower et al. 1992). A more pronounced effect of drought on needle production than on stemwood increments has also been observed over a single season in Scots pine (Irvine et al. 1998), but not in Aleppo pine (*Pinus halepensis* Mill.) (Borghetti et al. 1998). In *P. laricio*, a shift in resource allocation from leaves to sapwood production was apparent only after 3 years, suggesting that similar dynamics could account for the lack of

a treatment response in Aleppo pine over a single season. If so, caution is needed when drawing conclusions about the response of forest growth to drought and other disturbances on the basis of results obtained from short-term experiments (Pothier and Margolis 1991).

Tree hydraulic resistance did not differ significantly between treatments (Figure 7), and resistance per unit leaf area was therefore lower in drought-treated trees than in control trees because of the smaller leaf area of the drought-treated trees. This finding corroborates recent results for *P. taeda* L. trees subjected to a combination of irrigation and fertilization (Ewers et al. 1999, 2000): both leaf area-to-sapwood area ratio and leaf-specific hydraulic resistance were increased by irrigation, although the effect was reversed by fertilization. In contrast, short-term precipitation interception experiments have shown a significant increase in leaf-specific hydraulic resistance in response to drought in *P. sylvestris* L. (Irvine et al. 1998) and *Quercus petraea* L. ex Liebl. (Breda et al. 1993). The discrepancy between the results of the long- and short-term studies probably reflects the slow dynamics of tree acclimation.

In *P. laricio*, homeostasis in midday leaf water potential can be maintained under extreme conditions of water restriction. Similar isohydric behavior has also been reported for *P. resinosa* Ait. (Sucoff 1972), *P. pinaster* Ait. (Loustau et al. 1990) and *P. edulis* Engelm. (Linton et al. 1998) under natural drought conditions. However, homeostasis in midday leaf water potential is not characteristic of all *Pinus* species. For example, it has not been observed in *P. sylvestris* (Irvine et al.

1998) or *P. halepensis* (Borghetti et al. 1998).

Isohydic behavior was not observed in potted seedlings of *P. laricio* subjected to an artificial drought (Figure 8), suggesting that it is a characteristic of mature trees. In this context, we note two differences between mature trees and potted seedlings. First, in mature trees under natural conditions, the balance between leaf area index and site water availability, together with stomatal closure in response to drought, effectively maintained predawn water potentials within a narrower range than observed in potted seedlings, despite natural and artificially induced drought. Second, homeostatic mechanisms kept midday water potentials fairly constant in mature trees, but not in potted seedlings, where minimum leaf water potentials displayed high sensitivity to soil water availability.

Minimum water potentials under field conditions have often been reported to match closely the threshold value triggering extensive xylem cavitation (Sperry and Pockman 1993, Sperry 2000). Tyree and Sperry (1988) concluded that homeostatic mechanisms maintain minimum leaf water potentials within a safety range, preventing the onset of a positive feedback cycle of decreasing water potentials and xylem conductivities that eventually result in foliage dieback. The absence of runaway cavitation in either apical twigs or stem xylem as a result of leaf water potential homeostasis supports this conclusion.

A large part of total plant hydraulic resistance is known to reside in terminal twigs (Mencuccini and Grace 1995), whose water content (a good proxy for extent of embolism in conifers) did not differ significantly between treatments. The observed difference between treatments was small and was already apparent at the beginning of the experiment, suggesting that it could be the result of the limited sample size. The main effect of the restriction in water availability was to hinder the ability of the trees to recover from summer embolism. Xylem refilling was observed during the autumn in stems of UW trees but not in stems of RW trees (Figure 9B). We did not examine mechanisms involved in xylem refilling under natural conditions.

The functional basis of the observed isohydic behavior has not been elucidated. Tardieu and Simonneau (1998) suggested that it could arise from the interaction of an ABA signal originating in the roots and leaf water status, which would modulate stomatal response to the chemical messenger of soil dryness. In the long term, however, the maintenance of leaf water potentials will also involve a structural acclimation, because both tree hydraulic architecture and leaf gas exchange potential could acclimate to drought. The contribution of stomatal and structural responses can be explored by means of a simple model of plant water relations. The water potential gradient across the soil–plant continuum can be expressed as:

$$\Psi_1 = \Psi_s - E^{\max} f_s R, \quad (1)$$

where Ψ_1 and Ψ_s are leaf and soil water potential, respectively, E^{\max} is maximum stand transpiration, f_s is a reduction factor accounting for the effects of soil water availability ($f_s = 1$ under well-watered conditions) and R is plant hydraulic resis-

tance. Under conditions of decreasing soil water potential over the season, stomatal closure is mainly responsible for the maintenance of leaf water potential. It is interesting, however, to compare the two treatments under the well-watered conditions that occurred over the winter months. Assuming for both treatments $f_s = 1$ and a constant tree hydraulic resistance, the maintenance of a fixed minimum Ψ_1 requires that maximum stand transpiration be similarly constant:

$$E^{\max} = -\frac{\Psi_1}{R} = \text{constant}, \quad (2)$$

which is in good agreement with the experimental data (Figure 6). Despite the difference in leaf area index between the two treatments, stand transpiration under well-watered conditions during the winter did not differ between the two treatments. This implies an increase in maximum transpiration per unit leaf area in the drought-treated trees that exactly counterbalanced the reduction in stand leaf area index. Thus, under well-watered conditions, transpiration appears to be controlled by water transport capacity, rather than by the amount of transpiring foliage.

Although the results of short-term experiments on potted seedlings (Seiler 1985, Cregg 1994) are not in agreement with the suggestion that transpiration is controlled by water transport capacity, supporting evidence comes from studies on mature trees under field conditions. Thus, transpiration of irrigated and control, naturally drought-exposed *P. taeda* trees did not differ under well-watered conditions, either over the winter as in the present study (Ewers et al. 1999) or during the summer after thorough rewatering (Ewers et al. 2000), despite a 14% greater leaf area index in irrigated plots. Similarly, Maherali and DeLucia (2000) reported significantly higher maximum stomatal conductances in *P. ponderosa* Dougl. ex P. Laws. & C. Laws trees at a dry site than at a wet site, which largely counterbalanced the lower plant and stand leaf area at the dry site (Carey et al. 1998). Additional evidence is provided by defoliation experiments. For example, Reich et al. (1993), who studied the long-term effects of artificial defoliation in *P. resinosa* Ait., reported that a 33% reduction in leaf area enhanced stomatal conductance of the remaining foliage during the following year, with the result that minimum leaf water potentials of defoliated and control plants were the same. A similar response was observed by Pataki et al. (1998) in a short-term study in which a stepwise reduction in plant leaf area of up to 55% resulted in stimulation of transpiration by the remaining needles, and defoliation had no effect on whole-plant transpiration.

In conclusion, the behavior of forest stands in response to drought differs from that expected based on experiments with seedlings or short-term experiments on mature trees. Mature trees, in particular, appear to have an array of acclimation mechanisms, involving changes in both growth allocation and tissue functional characteristics, that help maintain a high degree of functional homeostasis in water relations and reduce forest vulnerability to even extreme events.

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