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## Root morphology and water transport of *Pistacia lentiscus* seedlings under contrasting water supply: A test of the pipe stem theory

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### Abstract

Restoration of degraded Mediterranean areas often requires the reintroduction of key-stone woody species but the establishment of seedlings of native species is frequently poor. This is partly due to insufficient knowledge of the ecology of these species at the seedling stage. Fast rooting and efficient water supply under water limiting conditions may be crucial to withstand summer drought and ensure establishment. However, knowledge of the relationship between root morphology and the water transport capacity of Mediterranean woody species in response to drought is still scarce. We evaluated the effect of low water availability on biomass allocation, root morphology and transpiration of a common Mediterranean shrub species, *Pistacia lentiscus* L. Seedlings of this species were grown in pots filled with soil under glasshouse conditions for 6 months, and irrigated either weekly (W+) or monthly (W−). Low water availability strongly reduced all fractions of biomass, and decreased relative biomass allocation belowground. Average diameter of fine roots colonising the soil was higher in W+ plants, but this resulted in only marginal effects on specific root length. Water limitation did not affect the topology of secondary roots colonising the soil. Surprisingly, the ratio of leaf area to coloniser roots surface area was higher in W− seedlings. Sapwood area was strongly correlated with leaf area, secondary roots cross-sectional area, and surface area of fine roots colonising the soil when all seedlings were pooled. In agreement with the pipe stem theory, the ratio of sapwood area to leaf area was not affected by watering regime. Plant water loss when soils were taken to field capacity was significantly correlated with leaf area, sapwood area, secondary roots cross-section area and coloniser roots surface area. Water loss at high water availability was greatly reduced in W− plants, as leaf area decreased and transpiration rates on a leaf area basis were similar in W+ and W− seedlings. *P. lentiscus* showed limited capacity to acclimate to low water availability by modifying biomass allocation and root morphology. Thus, parallel to what has been observed aboveground, this species can benefit from periods of high water availability by showing relatively high root growth rates, but may respond poorly to water scarcity.

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**Keywords:** *Pistacia lentiscus*; Root architecture; Root topology; Drought; Transpiration

### 1. Introduction

In semiarid areas, long-term land use has frequently resulted in the disappearance of woody sprouters (Grove and Rackham, 2001). These species are important for ecosystem functioning because they protect the soil from erosion, recover quickly after disturbance, and promote biodiversity by facilitating other vascular plants, thus providing shelter and food for fauna (Herrera, 1982; Maestre and Cortina, 2005). In degraded semiarid areas, a decrease in anthropic pressure may not be enough to ensure

spontaneous colonisation, and species reintroduction may be an essential step in the restoration of these habitats (Whisenant, 1999; Vallejo et al., 2005).

Seedling survival in Mediterranean semiarid lands is usually low, and short-term aboveground growth of seedlings is often negligible due to drought during the first summer after transplanting into the field (Vilagrosa et al., 1997; Maestre et al., 2003; Cortina et al., 2004). The rapid development of a deep root system that can access water stored lower in the soil profile may be essential for successful seedling establishment (Joffre et al., 2002; Otieno et al., 2006). Drought may not only increase the proportion of biomass allocated belowground (Nicotra et al., 2002), but also the geometry of the root system (Fitter, 1986; Taub and Goldberg, 1996; Ryser, 2006). Species native to lower rainfall environments tend to produce roots with

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longer links (Nicotra et al., 2002), and higher specific root length (Poot and Lambers, 2003; Tjolkner et al., 2005). Drought may increase the length of root links within a species (Jupp and Newmann, 1987; Fitter and Stickland, 1992; Berntson, 1994). Many plants respond to water limitation by inhibiting lateral branching (Malamy, 2005), and thus forming more herringbone-like root systems (Fitter, 1986; Berntson, 1994). Herringbone systems are considered more efficient in capturing high mobility resources such as water (Fitter, 1991). However, there are exceptions to this general pattern. For example, Taub and Goldberg (1996) found that dicots but not grasses showed this type of response. Furthermore, there was no relationship between rainfall level and root branching pattern in a range of Australian perennial plants (Nicotra et al., 2002). These studies suggest that both species and functional group play a part in determining root responses to water availability.

One way to understand these complex relationships is to study how changes in root system morphology affect plant water transport capacity using the pipe stem theory. This has been used to compare the diameter of stems and roots of different developmental order (Bouma et al., 2001; Oppelt et al., 2001), and to relate the cross-sectional area of conductive tissues to the surface area of transpiring tissues (Shinozaki et al., 1964a,b). For adult individuals of woody species, the ratio of sapwood area to leaf area may increase as water availability is reduced (Callaway et al., 1994; Mencuccini and Grace, 1995; Jackson et al., 1999; Mencuccini and Bonosi, 2001).

*Pistacia lentiscus* L. (mastic tree) is one of the most important woody species in the Mediterranean Basin, where it is a common part of the climax vegetation in semiarid coastal areas (Tomaselli, 1981). *P. lentiscus* has been proposed as a suitable species for the restoration of semiarid areas due to its adaptations to drought and resistance to disturbance (Vallejo et al., 2000). It also has high root growth capacity (Fonseca, 1999; Trubat et al., 2004) and plasticity to contrasted nutrient availability (Trubat et al., 2006), and responds readily to low water availability by modifying aboveground morpho-functional traits (Vilagrosa et al., 2003). However, little information is available on belowground morpho-functional strategies of this species to withstand drought.

We studied the belowground responses of *P. lentiscus* to contrasting water regimes to test the hypothesis that low water availability would promote biomass allocation belowground, increase the ratio of sapwood area to leaf area, and modify root morphology, thus improving its capacity to supply water aboveground.

## 2. Material and methods

In spring 2000, *P. lentiscus* seeds collected in the Region of Valencia, Spain were planted into 150 cm<sup>3</sup> plugs filled with a mixture of limed sphagnum peat and cocopeat (1:1, v:v). In January 2001 in the UK, seedlings were planted into 8 L pots filled with a 3:1 (v:v) mixture of quartz sand and soil. As a surrogate for Mediterranean soil we used a flinty silty clay loam topsoil (Panhole series) of pH 7.9 (0.01 M CaCl<sub>2</sub>) collected from the plough layer (23 cm) of an arable field over-

lying chalk on the Rothamsted Estate, Hertfordshire, UK. Total nitrogen was 0.061%, loss on ignition 9.1%, available phosphorus 36.4 mg L<sup>-1</sup> and exchangeable potassium 214 mg L<sup>-1</sup> (modified Morgan method).

Pots were watered to saturation in mid January 2001 and kept in a glasshouse throughout the period of study. Average temperature was 24 °C. Plants were either watered weekly (W+ seedlings) or monthly (W- seedlings) with tap water. By July 12 (175 days after the onset of the experiment) W- seedlings had received 4.1 L of water, and W+ seedlings had received 9.0 L of water. Each treatment was replicated 16 times.

On July 13 we estimated transpiration rate from the weight loss of 10 seedlings per treatment (9:00–17:00 GMT; average PPFD 460 μmol m<sup>-2</sup> s<sup>-1</sup>; average air temperature range: 25–31 °C). Containers had been previously sealed to avoid evaporation losses. In mid July 2001 we harvested the 16 month-old seedlings and carefully washed out the root system. We separated three root fractions: the first order tap root, higher order roots remaining within the original root plug and higher order roots colonising the pot (hereafter *colonisers*), and measured the diameter of secondary roots at their proximal end. The cortex was separated at the root collar level, and under the assumption that all xylem was sapwood, we measured the sapwood area. Cross-sectional area of the main secondary roots was also measured. Total root length, total surface area and average root diameter were obtained by using WinRhizo software (Régent Instruments, Québec, Canada) to analyse scanned root images (A3 Epson 836XL flatbed scanner with a transparency adapter operating at 300 dpi. Images were 8-bit greyscale and saved in uncompressed TIFF format).

The topology of root systems can be described by three variables, magnitude, total exterior pathlength and altitude (Fitter, 1985). Magnitude is the number of root tips in the whole root system. Total exterior pathlength is calculated as the sum of all links (internodes) counted from each of the root tips back to the base of the root system. Altitude is the single longest individual path from a root tip back to the base link. We counted the number of root tips and calculated the topological indices of root system altitude and root system total external pathlength for a randomly chosen secondary colonising root from each pot, according to the method of Werner and Smart (1973) as modified by Berntson (1995).

Leaves were scanned and analysed as for roots to obtain leaf area. All biomass fractions were dried at 65 °C for 3 days to determine the dry weight. We estimated the root weight ratio (RWR) of coloniser roots as the ratio between coloniser roots and that of the whole root system. Both the ratio between total belowground and total biomass (RWR for the whole root system), and the RWR of coloniser roots were analysed by using the natural logarithms of both terms of the ratio. Specific root length (SRL) was estimated as the ratio between root length and biomass.

Treatment effects were evaluated by using one-way ANOVA with one fixed factor (water). For topological analyses, the magnitude of the root system (number of tips) was included in the analysis as a covariate. We used Pearson correlation coefficients to explore the relationship between pairs of variables. Variables

Table 1

Leaf area, biomass, RWR and main morphological traits of the secondary and higher order roots of *Pistacia lentiscus* seedlings colonising the soil as affected by contrasted water availability

	Low Water (W–)	High Water (W+)	F	p
Leaf area (cm <sup>2</sup> )	147 ± 12	352 ± 19	9.9	0.06
Foliar D.W. (mg)	3048 ± 284	8273 ± 485	86.5	<0.001
Stem D.W. (mg)	1099 ± 122	3322 ± 208	86.8	<0.001
Main root D.W. (mg)	1422 ± 144	3957 ± 189	113.4	<0.001
Plug Roots D.W. (mg)	398 ± 24	747 ± 71	26.0	<0.001
Coloniser Roots D.W. (mg)	656 ± 67	2560 ± 204	0.0 <sup>a</sup>	<0.001
RWR	0.380 ± 0.011	0.387 ± 0.007	15.3	<0.001
Coloniser RWR	0.102 ± 0.008	0.134 ± 0.006	32.5	<0.001
Coloniser Length (cm)	1842 ± 136	6820 ± 571	121.0	<0.001
Coloniser Surface Area (cm <sup>2</sup> )	316 ± 26	1250 ± 99	133.7	<0.001
Coloniser Diameter (mm)	0.541 ± 0.009	0.588 ± 0.009	7.0	0.013
Coloniser SRL (cm mg <sup>-1</sup> )	2.96 ± 0.14	2.66 ± 0.09	3.1	0.088
Coloniser Tissue Density (g cm <sup>-3</sup> )	0.146 ± 0.009	0.140 ± 0.004	0.3	0.586

F-values and associated probabilities from the analysis of variance are given; all data are mean ± S.E. (n = 16). See text for definitions of terms.

<sup>a</sup> Mann–Whitney U-test.

were transformed when required to ensure homoscedasticity and normality. All statistical analyses were performed by using the SPSS 10.0.6 statistical package (SPSS Inc., Chicago, USA).

### 3. Results

Drought substantially reduced all fractions of biomass (Table 1). Biomass allocation belowground decreased with drought as shown by a decreased RWR. The biomass of colonising roots was well correlated with that of the main root and the plug roots ( $r = 0.886$ ,  $n = 32$ ,  $p < 0.001$ ;  $r = 0.708$ ,  $n = 32$ ,  $p < 0.001$ , respectively). Coloniser roots in W+ seedlings had greater diameter and had relatively greater biomass allocation than in W– seedlings. Specific root length showed a trend towards lower values in coloniser roots of W+ seedlings, but differences were not statistically significant.

Magnitudes of secondary roots chosen for topological analysis ranged from 34 to 239, and were not significantly different for W– and W+ seedlings (Table 2). This, together with the use of magnitude as a covariate, ensures that this variable was not a confounding factor in architectural analysis. Average link length was not affected by the watering regime. Water availability had no effect on the topological indices we measured (Table 2; Fig. 1).

Table 2

Link lengths and topological indices of secondary roots of *Pistacia lentiscus* seedlings as affected by contrasted water availability

	Low Water (W–)	High Water (W+)	F	p
Magnitude	97 ± 12	100 ± 11	0.02	0.888
Link length (cm)	0.32 ± 0.02	0.40 ± 0.04	1.39	0.246
log(a)	1.53 ± 0.04	1.54 ± 0.03	–	–
log(Pe)	3.20 ± 0.08	3.19 ± 0.08	–	–
a/E (a)	1.40 ± 0.08	1.41 ± 0.08	0.01	0.915
Pe/E (Pe)	1.36 ± 0.09	1.27 ± 0.11	0.34	0.564

F-values and associated probabilities from the analysis of variance are given; all data are mean ± S.E. (n = 16). a: root altitude, Pe: total external pathlength, E: expected values for a randomly branched root system.

Decreased water availability resulted in lower sapwood area and cross-sectional area of secondary roots (Table 3). W+ plants showed higher coloniser roots surface area to leaf area than W– plants. Watering had no effect on any other relationship between leaf area, sapwood area, secondary roots cross section area and coloniser root surface area (data not shown). When both treatments were pooled, leaf area was strongly correlated with sapwood area, secondary roots cross-sectional area, and surface area of fine roots colonising the soil (Table 4; Fig. 2). The surface area of fine roots inside the root plug was not related to any of these variables.

Water losses were correlated with leaf area, sapwood area, secondary roots cross-sectional area and coloniser roots surface area when all seedlings were pooled (Table 4). Water loss was more than two-fold higher in well-watered seedlings than in W– seedlings. We found no differences in transpiration rate on a leaf area or root surface area basis between W– and W+ seedlings (Table 3). Results agreed with early morning and midday IRGA gas exchange measurements (data not shown) made on the same

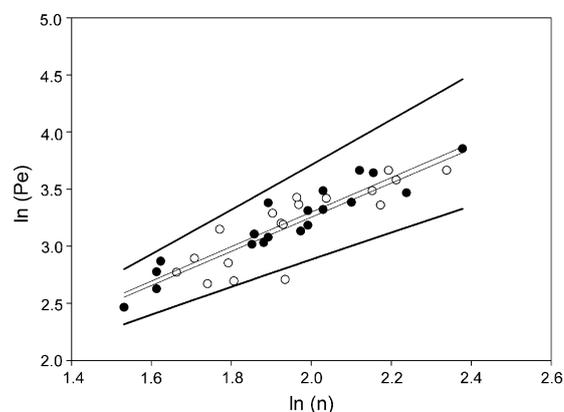


Fig. 1. Relationship between magnitude ( $n$ ) and total external pathlength (Pe) of secondary roots of *Pistacia lentiscus* seedlings grown at low (W–, ●) or high (W+, ○) water availability. Regression lines correspond to W– seedlings (upper) and W+ seedlings (lower). Upper and lower bold lines correspond to estimations of perfect herringbone and dichotomous systems, respectively.

Table 3  
Morphological attributes of the main root, secondary roots (second), and transpiration of 1-year old *Pistacia lentiscus* seedlings grown for 6 months at contrasted water regimes and then irrigated to field capacity

	Low water (W–)	High water (W+)	F	p
Sapwood area (mm <sup>2</sup> )	5.6 ± 0.8	9.4 ± 1.0	9.8	0.006
Secondary roots cross-section (mm <sup>2</sup> )	15.9 ± 1.7	30.1 ± 5.7	4.6	0.045
Sapwood area: Leaf area (×10 <sup>2</sup> )	4.37 ± 0.39	4.71 ± 0.20	0.035	0.853
Secondary roots cross-section: Leaf area (×10 <sup>2</sup> )	13.03 ± 0.74	14.26 ± 1.73	0.00	0.997
Secondary roots cross-section: Sapwood area	3.23 ± 0.36	3.08 ± 0.38	1.73	0.683
Coloniser roots surface area: leaf area	2.58 ± 0.43	6.52 ± 0.65	16.914	<0.001
Water losses (ml 8 h <sup>-1</sup> )	11.3 ± 1.2	26.7 ± 2.2	38.1	<0.001
Water losses: Leaf Area (ml h <sup>-1</sup> cm <sup>-2</sup> )	0.010 ± 0.003	0.009 ± 0.002	111 <sup>a</sup>	0.534
Water losses: Root surface area (ml h <sup>-1</sup> cm <sup>-2</sup> )	0.003 ± 0.001	0.002 ± 0.001	0.126	0.725

F values are from one-way ANOVA. All data are mean ± S.E. (n = 10).

<sup>a</sup> Mann–Whitney U-test.

Table 4  
Pearson correlation coefficients for leaf area, morphological attributes of the root system and transpiration of 1-year old *Pistacia lentiscus* seedlings grown for 6 months at contrasted water availability regimes and then irrigated

	Leaf area	Sapwood area	Secondary roots cross section area	Fine roots plug surface area	Coloniser roots surface area
Sapwood area	0.912***				
Second roots cross-section area	0.850***	0.758***			
Fine roots plug surface area	0.409	0.438	0.414		
Coloniser roots surface area	0.641***	0.589***	0.633***	0.410	
Water loss	0.657***	0.650***	0.595***	0.541	0.879*

n = 20 in all cases. Asterisks correspond to significant differences at \*p < 0.05 and \*\*\*p < 0.001.

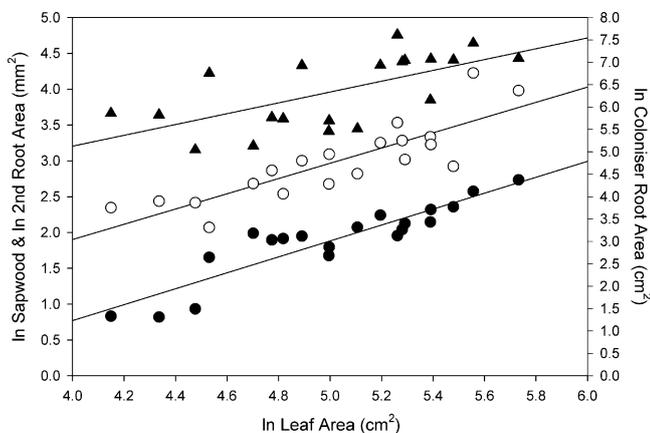


Fig. 2. Relationship between leaf area and either sapwood area (●), secondary roots cross-section (○) or coloniser roots surface area (▲). Both irrigation treatments are pooled. Regression lines are drawn for comparison.

day that whole plant water use was measured ( $r = 0.826$ ,  $n = 32$ ,  $p < 0.001$ , and  $r = 0.825$ ,  $n = 32$ ,  $p < 0.001$ , for an 8 h integration of IRGA measurements made at early morning and midday, respectively).

#### 4. Discussion

Root proliferation in *P. lentiscus* seedlings was highly restricted in dry soils, together with the decline of other biomass fractions. Reductions in the growth of water stressed plants have been widely described in the literature, and may be caused by

stomatal closure, photoinhibition, loss of cell turgor, and direct effects of dehydration on photosynthetic processes (Kayser, 1987; Dubey, 1997; Escalona et al., 1999). Furthermore, the proportion of coloniser roots was lower in water-stressed seedlings, indicating that relatively less biomass was allocated to exploring the soil volume. The root weight ratio increased in seedlings with higher water supply. This was unexpected as plants commonly respond to reduced water availability by increasing biomass allocation belowground (Bloom et al., 1985). Some studies have found an increase in the root-to-shoot ratio of Mediterranean plants growing at low water potentials (Squire et al., 1987), but others not (Fonseca, 1999; Rodriguez-Cousinho, 2000; Bacelar et al., 2007). Further evidence that increased water availability did not inhibit biomass allocation belowground in this species is provided by the reduction in the amount of leaf area that was supported by a given surface area of coloniser roots in W+ seedlings. The magnitude of the changes in biomass allocation belowground in response to drought although significant, is relatively modest (Poorter and Nagel, 2000), and may differ even in phylogenetically related plants (Bacelar et al., 2007; Chambel et al., 2007). It has been suggested that the absence of changes to biomass allocation patterns in droughted plants may be a consequence of similar levels of osmotic adjustment in leaves and roots (Sobrado and Turner, 1986), and carbohydrate storage in both aboveground and belowground tissues (Rodrigues et al., 1995; Bacelar et al., 2007). Rooting in deep soil horizons may be an essential component of a plant's strategy to withstand drought at the seedling stage in environments with seasonal drought (Joffre et al., 2002; Otieno et al., 2006; David et al.,

2007). Thus, fast root growth at the early stages of development, when water is available, rather than a resource-balance driven response, may represent an evolutive advantage in these environments.

Biomass of roots colonising new areas of the soil was well correlated with that of other fractions of the root system. This result has been observed in field plantations (Vilagrosa et al., 1997; Fonseca, 1999), and may be because the main root, which grew very little in length after being air-pruned in the nursery at ca. 12 cm, continued to increase in diameter and weight. Thus it is likely that, despite air-pruning, the main root maintained its role as storage organ. These results indicate that the biomass of the main root at this stage can be used as a reliable estimator of the biomass of the entire root system.

Average diameter of coloniser roots of drought stressed seedlings was lower than that of seedlings receiving frequent watering. We found no differences in average root tissue density, and consequently the decrease in average diameter in water stressed plants resulted in a trend towards higher SRL in W-seedlings (a trend that was marginally significant). The observed effect of low water availability on fine root diameter has been documented in the literature (Fitter, 1985), although exceptions also exist (Fitter, 1985; Hipps et al., 1995). Roots of droughted plant may show lower vessel diameter, and increased lignification and suberization (Cruz et al., 1992; Steudle, 2000a). These changes would result in increased root density, a response that was not observed in *P. lentiscus* plants. An increase in SRL has also been related to a decrease in secondary wall thickening in the exodermis (Eissenstat and Achor, 1999). The role of changes in SRL and root diameter in droughted plants is complex (Ryser, 2006). Increased deposition of lignin and suberin, and decreased vessel diameter, would increase root longevity, reduce water loss, and decrease the risk of cavitation (Eissenstat et al., 2000; Hacke et al., 2000; Steudle, 2000b). Conversely, it would reduce hydraulic conductance and increase construction cost (Cruz et al., 1992; Eissenstat et al., 2000; Hacke et al., 2000). Increased number of terminal meristems under high water availability may have favoured the increase in average diameter, as root thickness in *P. lentiscus* increases at distal root links. Increased SRL has been related to an increase in exploitation efficiency (Fitter, 1991), hydraulic conductivity (Eissenstat, 1997) or uptake efficiency (Comas et al., 2002; but see the discussion on this topic in Eissenstat and Yanai, 1997). Specific root length in the entire root system tends to be lower in low rainfall habitats (Poot and Lambers, 2003; Tjolkner et al., 2005). This may partly reflect the increase in main root biomass, as SRL of secondary roots can show the opposite trend (Nicotra et al., 2002). Other Mediterranean woody species have shown null or negative responses of average root diameter to water limitation (Fonseca, 1999; Rodriguez-Cousinho, 2000). Under natural conditions it is difficult to separate the effects of water limitation from those of other factors affecting root growth such as resistance to penetration and nutrient limitation. Resistance to penetration frequently results in increased average root diameter (Misra and Gibbons, 1996). However, drought preconditioned *P. lentiscus* seedlings produce new roots with lower SRL than untreated seedlings when planted in fertirrigated vermiculite with low bulk density

(Fonseca, 1999), suggesting that the response observed in the present study may be directly related to water availability.

Average link length was rather small as compared to other herbaceous and woody species (Fitter et al., 1988; Hipps et al., 1995; Nicotra et al., 2002), probably because of the small link lengths of high order roots. We found no significant effect of drought on link length, in contrast with the reduction in average link length in *P. lentiscus* seedlings growing in microcosms reported by Green et al. (2005). Rodriguez-Cousinho (2000) found a reduction in distal link (EE) length in *Ceratonia siliqua* and *Quercus suber* after drought preconditioning in the nursery. Hipps et al. (1995) observed no changes in the average distance on the first order axis between second-order roots in peach trees subjected to contrasted irrigation regimes. The increase in link length with reduced water supply has been observed in herbaceous plants (Fitter and Stickland, 1992; Berntson and Woodward, 1992), and may be related to the exploration of a higher soil volume, but there are exceptions to this (Jupp and Newmann, 1987). There is evidence that external and internal links may behave differently (Fitter and Stickland, 1992; Berntson, 1994; Bouma et al., 2001; Williamson et al., 2001). Unfortunately, we did not measure average link length in different topological classes.

Secondary roots were not clearly herringbone or dichotomous. Taub and Goldberg (1996) found no significant departure from random branching in four dicots from a relatively rich Mediterranean site. Containerised *Q. suber*, *C. siliqua*, *Juniperus oxycedrus* and *Quercus coccifera* cannot be ascribed to either herringbone or dichotomous topologies (Fonseca, 1999; Rodriguez-Cousinho, 2000). Decreased water availability often favours the dominance of the root axis, leading to more herringbone-like root systems (Fitter, 1986; Taub and Goldberg, 1996), although at intense water deficits root tip death may promote branching (Jupp and Newmann, 1987).

We found no evidence of changes in root topology in *P. lentiscus* seedlings resulting from the treatments. Topological response to water in *P. lentiscus* may have been offset by other factors, particularly the availability of suitable sites for root expansion. Alternatively, seedlings may not be responsive to soil resources at this level. Our results support the suggestion that topology may be less sensitive to resource availability than other morphological variables, as has been observed elsewhere (Glimskär, 2000). In containerised seedlings, *P. lentiscus* and *Q. coccifera* roots appear to be less sensitive to changes in the irrigation regime than *J. oxycedrus* (Fonseca, 1999). Rodriguez-Cousinho (2000) found little changes in root topology of *C. siliqua* and *Q. suber* seedlings subjected to drought preconditioning. A particular root topology does not seem to be associated with species from low or high rainfall environments, at least at the seedling stage (Nicotra et al., 2002). A herringbone pattern may be associated with more efficient root systems in terms of the volume of soil explored per volume of root produced (Fitter, 1987; Fitter et al., 1991), or to a higher exploration potential (Berntson, 1994). The model used by Fitter (1987), however, assumed a gradual decrease in root diameter as development order increased. This relationship was not confirmed for *P. lentiscus* in the present study (data not shown), nor

was it for a variety of other species (McCrary and Comerford, 1998; Glimskär, 2000; Bouma et al., 2001). This topic clearly deserves more attention because of the implications it has for estimations of construction costs and nutrient uptake efficiency.

Leaf area was well correlated with sapwood area, secondary roots cross-sectional area and coloniser roots surface area, and the ratio of sapwood area to leaf area did not differ between W– and W+ seedlings, suggesting that the pipe model (Shinozaki et al., 1964a,b) is valid at the level of proximal and distal ends of the root system in *P. lentiscus* seedlings. Moreover, the square of sapwood diameter was well correlated with the sum of the squares of the diameters of secondary roots ( $r=0.658$ ;  $n=22$ ;  $p<0.001$ ), and the slope of the regression between these two variables was 2.6, similar to the values found in adult tropical woody species, and not far from the theoretical value of 2 (Oppelt et al., 2001). Similarly, the slope of the relationship between sapwood diameter and average diameter of secondary roots ( $2^\circ$  root diameter =  $0.0915 + 0.3772$  Sapwood diameter,  $n=21$ ,  $R^2=0.5514$ ) was very close to the value found by Bouma et al. (2001) for seven halophytic species. The strength of these relationships is remarkable, and may be related to the minimisation of the energy associated with water transport and mechanical stability (West et al., 1999; McCulloh and Sperry, 2005). Furthermore, they suggest that reasonable estimates of leaf area or fine root surface area can be obtained from measures of sapwood area or root collar diameter in *P. lentiscus* seedlings, provided that drought has not been too strong (see below). The lack of response of the ratio of sapwood area to leaf area to contrasted water availability is in disagreement with observations of increased sapwood to leaf area with drought for a given species (Callaway et al., 1994; Mencuccini and Grace, 1995; Jackson et al., 1999; Mencuccini and Bonosi, 2001). The opposite trend – decreased proportional biomass allocation to sapwood at low water availability – has been associated with development stage (canopy closure) (White et al., 1998), which is clearly not the case in the present experiment. Shumway et al. (1993) found no changes in sapwood to leaf area ratio in well watered vs. drought stressed *Quercus rubra* and *Liriodendron tulipifera* seedlings. In this case the proportion of potentially functional xylem increased in stressed plants, which probably affected specific hydraulic conductivity. We did not measure the amount of sapwood area corresponding to functional xylem. Cavitation may have occurred in W– seedlings, and thus the ratio of functional xylem area to leaf area in water stressed seedlings may have been indeed lower than in W+ seedlings. Villar-Salvador et al. (1997) suggested that the absence of a clear relation between the ratio of sapwood area to leaf area and rainfall in *Q. coccifera* and *Quercus faginea*, as compared to *Quercus ilex*, resulted from leaf area losses that were unmatched by reductions in sapwood area. Relevant losses of hydraulic conductivity from cavitation in *P. lentiscus* seedlings occur at xylem potentials below  $-3$  MPa, whereas leaf shedding begins at below  $-6$  MPa (Vilagrosa et al., 2003). Water stress was probably milder in the present experiment (indeed leaf abscission was very low in W– seedlings), and W– seedlings may have retained the structure inherited from the nursery phase, with little changes in leaf area. Considering the leaf shedding and hydraulic con-

ductivity dynamics of *P. lentiscus* seedlings (Vilagrosa et al., 2003), an increase in the leaf area to functional xylem area ratio between  $-3$  and  $-6$  MPa, followed by a sudden decrease as drying progresses would be expected.

Transpiration rate on a leaf area and fine root surface area basis was not affected by previous irrigation regime after watering to field capacity, suggesting that despite the contrast in size, plants grown under low water supply had similar leaf specific hydraulic conductivity to frequently watered plants. The need to account for hydraulic conductivity when studying changes in leaf area to sapwood area ratios have been emphasised by Mokany et al. (2002). The lack of effects of watering on the ratio between leaf area and conducting surface area, or on leaf specific conductivity, and the higher leaf area to absorbing surface area in W– plants may reflect insensitivity of *P. lentiscus* or, as mentioned above, the maintenance of a nursery-acclimated hydraulic structure in stressed plants.

In conclusion, drought after planting does not promote the allocation of biomass to belowground parts and coloniser roots in *P. lentiscus* seedlings, nor does it affect the topology of secondary roots. It does, however, promote small changes in the morphology of coloniser roots. Drought seems to affect whole seedling morphology and function by hampering growth rather than changing allocation patterns or modifying water transport efficiency. These changes may not be enough to balance the limited colonisation of new areas of the soil volume, and thus seedlings established on drying soils may be unable to withstand summer drought. Poor rooting is thus likely to be responsible for high mortality rates at the seedling stage in this species.

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