

Is rootstock-induced dwarfing in olive an effect of reduced plant hydraulic efficiency?

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Summary We investigated the hydraulic architecture of young olive trees either self-rooted or grafted on rootstocks with contrasting size-controlling potential. Clones of *Olea europaea* L. (Olive) cv ‘Leccino’ inducing vigorous scion growth (Leccino ‘Minerva’, LM) or scion dwarfing (Leccino ‘Dwarf’, LD) were studied in different scion/rootstock combinations (LD, LM, LD/LD, LM/LM, LD/LM and LM/LD). Shoots growing on LD root systems developed about 50% less leaf surface area than shoots growing on LM root systems. Root systems accounted for 60–70% of plant hydraulic resistance (R), whereas hydraulic resistance of the graft union was negligible. Hydraulic conductance ($K = 1/R$) of LD root systems was up to 2.5 times less than that of LM root systems. Total leaf surface area (A_L) was closely and positively related to root hydraulic conductance so that whole-plant hydraulic conductance scaled by A_L did not differ between experimental groups. Accordingly, maximum transpiration rate and minimum leaf water potential did not differ significantly among experimental groups. We conclude that reduced root hydraulic conductance may explain rootstock-induced dwarfing in olive.

Keywords: grafting, HPFM, Leccino, *Olea europaea*, root hydraulics, transpiration rate, water potential.

Introduction

Trees of perennial fruit crops are mostly propagated by grafting a scion onto a rootstock. Rootstocks are selected on the basis of their performance in different soil types and the scion is selected on the basis of the quality or quantity, or both, of fruit production. Rootstocks inducing reduced vegetative growth and ‘dwarfing’ in scions are increasingly used to develop high-density orchards because they reduce cultural costs associated with harvesting and pruning (Troncoso et al. 1990, Tous et al. 1999). Despite the increasing commercial importance of dwarf orchard trees, little is known about the mechanisms underlying the reduced vegetative growth of scions grafted on low-vigor rootstocks. Early anatomical studies (Beakbane and

Thompson 1939) led to the hypothesis that the phenomenon is related to the influence of rootstocks on tree water relations (Beakbane 1956). In addition, hormonal (Richards et al. 1986, Cutting and Lyne 1993, Kamboj et al. 1999, Sorce et al. 2002), anatomical (Simons 1986, Soumelidou et al. 1994) and nutritional (Jones 1976) mechanisms have been proposed to explain the size-controlling potential of different rootstocks. Lliso et al. (2004) recently suggested that the dwarfing mechanism is related to competition between vegetative and reproductive growth.

Support for the role of tree water relations in the dwarfing effect comes from studies showing that leaf and stem water potentials are lower for scions grafted on dwarfing rootstocks than for scions grafted on vigorous rootstocks (Olien and Lasko 1984, 1986, Basile et al. 2003a, Gonçalves et al. 2006). At a given leaf evaporative flux, the water potential drop from soil to leaves is a function of the hydraulic conductance of the plant (K_{plant}) (Kramer and Boyer 1995). In turn, plant hydraulic conductance is known to influence growth capacity of trees through changes in leaf water status and gas exchange (Willingen and Pammenter 1998, Tyree et al. 1998, Nardini and Tyree 1999, Sperry 2000, Meinzer 2002). Hence, the hypothesis has been advanced that dwarfing rootstocks induce a reduction in K_{plant} and this, in turn, reduces plant vegetative growth. Support for this interpretation comes from studies on grafted apple (*Malus domestica* L.) trees. Cohen and Naor (2002) estimated plant hydraulic properties of apple scions grafted on dwarfing or vigorous rootstocks and found that K_{plant} expressed on a leaf area basis was 63% lower in dwarf plants. Atkinson et al. (2003) investigated the partitioning of hydraulic conductances in apple plants grafted on rootstocks with different degrees of dwarfing and confirmed that the hydraulic conductance of fine roots and stems of dwarf plants was lower than that of vigorous plants. Moreover, the calculated hydraulic conductivity of the graft tissue was lower for trees grafted on dwarfing rootstocks compared with trees grafted on vigorous rootstocks (Atkinson et al. 2003). Clearwater et al. (2004) studied the hydraulic characteristics of kiwifruit vines (*Actin-*

idia sp.) grafted on clonal rootstocks and reported that leaf specific hydraulic conductance was higher in low-vigor rootstocks than in high-vigor rootstocks, which is the opposite of the expected pattern. These authors concluded that changes in plant hydraulics do not provide a mechanistic explanation for changes in the vigor of the scion in *Actinidia* (Clearwater et al. 2004).

Most studies dealing with plant hydraulics of different scion/rootstock combinations are based on estimates of plant hydraulic properties (Cohen and Naor 2002, Clearwater et al. 2004), or on measurements of hydraulic conductivity of root or stem segments (Atkinson et al. 2003). Although these measurements provide an interesting picture of the hydraulic efficiency of vigorous or dwarf grafted trees, direct measurements of the hydraulic conductance of root, graft union, stem and leaf are still largely missing. Only Basile et al. (2003b) have reported direct measurements of the hydraulic conductance of different plant organs. They found that peach (*Prunus persica* (L.) Batsch) trees grafted on dwarfing rootstocks had significantly lower root hydraulic conductance (expressed on a leaf area basis) than trees grafted on vigorous rootstocks, whereas there was no rootstock effect on the hydraulic conductance of the scion or the graft union, suggesting that low root hydraulic conductance plays a central role in the dwarfing mechanism induced by size-controlling peach rootstocks (Basile et al. 2003b).

In this paper, we report measurements of the hydraulic conductance of different organs of olive (*Olea europaea* L.) grafted on rootstocks with contrasting size-controlling potentials. Our study confirms the observations made by Basile et al. (2003b) in peach and provides further evidence that reduced root hydraulic conductance may underlie the root-induced dwarfing phenomenon in grafted fruit tree species.

Materials and methods

Plant material

All experiments were conducted on clones of *O. europaea* cv 'Leccino'. One clone was characterized by vigorous vegetative growth (Leccino 'Minerva', LM) and a second clone showed strongly reduced vegetative growth (Leccino 'Dwarf', LD). Both self-rooted and grafted plants were studied according to the following groups: LD (self-rooted LD clone), LM (self-rooted LM clone), LD/LD (LD scion grafted on LD rootstock), LM/LM (LM scion grafted on LM rootstock), LD/LM (LD scion grafted on LM rootstock) and LM/LD (LM scion grafted on LD rootstock). All plants were propagated in 2002 and grafted at the end of March 2004. Plants were grown in 4-l pots until early March 2005 in a greenhouse of the Department of Arboriculture, University of Palermo, Italy. In March 2005, the plants were transplanted to 3000-l containers filled with a 3:2 (v/v) mixture of peat and fine pumice stone. The soil was fertilized with 2 kg m⁻³ of commercial slow-release N,P,K (13.8,3.5,8) fertilizer and 2 kg m⁻³ of Biotron (40% organic C, 1.3% organic N; Cifo S.p.a.,

S. Giorgio di Piano, Bologna, Italia). The containers were located in an experimental field in Sciacca (Sicily, southern Italy; 37°31' N 13°04' E) at sea level. Plants were kept well irrigated throughout the study.

Hydraulic measurements

Hydraulic measurements were performed in April and July 2005 (360 and 450 days after grafting, respectively) on six plants per experimental group. Measurements were made with a high-pressure flow meter (HPFM) (Tyree et al. 1995). To prevent the potential impact of diurnal periodicity on root hydraulic conductance (Henzler et al. 1999, Tsuda and Tyree 2000), all measurements were made between 0900 and 1200 h during four consecutive days. Trees were excised 5 cm below the graft union. Cuts were made under water by constructing a watertight container around the base of each plant. The HPFM was connected to the root system and five to six transient flow measurements were immediately made. During each measurement, pressure (P) was increased at a rate of 5–8 kPa s⁻¹ up to $P = 0.4$ MPa, while measuring the instantaneous flow every 3 s. The entire sequence of measurements took 5 to 10 min. Measured flow was plotted against the applied pressure, and root hydraulic conductance (K_{root}) was calculated from the slope of the linear regression of the data as described by Tyree et al. (1995). Root hydraulic resistance (R_{root}) was calculated as the inverse of K_{root} . Transient measurements of root hydraulic resistance were preferred to quasi-steady state mode (Raimondo et al. 2005) to avoid potential errors caused by solute concentration at the root tips that might occur during steady-state flow conditions. During the HPFM measurements of root hydraulic properties, the direction of flow is opposite to the physiological direction of transpiration. The reversed flow is expected to push solutes toward the root tips where they would concentrate by reverse osmosis (Tyree et al. 1994). Rapid transient measurements minimize the change in solute concentration and provide good estimates of root hydraulic resistance (Tyree et al. 1995, Bogeat-Triboulot et al. 2002).

During K_{root} measurements, the cut surface of each shoot was kept immersed in water and the entire shoot was enclosed in a plastic bag to prevent evaporation. The base of the stem was connected to the HPFM and the shoot hydraulic resistance (R_{shoot}) was measured in the quasi-steady state mode at a pressure of 0.3 MPa. During measurements, shoots were enclosed in a transparent plastic bag and water was sprayed on the leaves of the bagged shoot to minimize transpiration and facilitate attainment of quasi-steady state conditions. Measurements were made in the open under a light shading net so that photosynthetically active radiation (PAR) at the shoot level was between 500 and 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as measured with a quantum sensor (LI-190S1, Li-Cor, Lincoln, NE) built into the porometer (see below). This procedure was adopted because the hydraulic conductance of leaves of some tree species is strongly down-regulated in dark conditions (Tyree et al. 2005). Leaves were then removed and R was re-measured to obtain R of the leafless stem (R_{residual}). Leaf hydraulic resistance was calculated from $R_{\text{leaf}} = R_{\text{shoot}} - R_{\text{residual}}$. The trunk was then cut immediately above the graft union and the hydraulic resistance

of stems (R_{stems}) was calculated. Finally, a second cut was made below the graft union and the hydraulic resistance of the graft (R_{graft}) was calculated. The hydraulic resistance of the whole plant was computed as $R_{\text{plant}} = R_{\text{root}} + R_{\text{shoot}}$. All hydraulic measurements (including measurements of R_{root}) were corrected to a temperature of 22 °C (calibration temperature for the HPFM). At the end of each experiment, the leaf surface area of the plant (A_L) was measured with a leaf area meter (Li-Cor LI-3000A equipped with an LI-3050 belt conveyer) and all R and K values were scaled by A_L to obtain leaf-specific R and K values (Tyree et al. 1998).

Measurements of transpiration rate and leaf water potential

In July 2005, maximum diurnal leaf transpiration rate (E_L) and minimum water potential (Ψ_L) were measured during two consecutive sunny days before plant excision for the hydraulic measurements. We measured E_L between 1100 and 1400 h on three leaves per plant with a steady-state porometer (Li-Cor LI-1600). Leaves measured for E_L were immediately collected and Ψ_L measured by the pressure chamber method.

Statistics

Data were analyzed with SigmaStat 2.0 (SPSS, Chicago, IL) statistics software. Differences between experimental groups were assessed by one-way analysis of variance (ANOVA). Post-hoc pairwise comparisons between all means were made with Tukey's test. The statistical significance of correlations between parameters was tested by the Pearson product moment correlation.

Results

Whole-plant leaf area did not differ greatly among experimental groups 360 days after grafting (April 2005; Figure 1, filled columns), which was just a few days after plants were transferred to the large containers in the field. Differences were observed in July 2005, however, after plants had been growing in an unrestricted soil volume for a further 90 days. Plants with LD rootstocks had leaf surface areas of 0.3 to 0.5 m², whereas A_L of plants with LM rootstocks was 50–120% greater or about 0.7 m² in LD/LM plants and 1.0 m² in LM plants (Figure 1). The vegetative growth of the different plant types appeared to depend on genetically determined root system characteristics. The LM plants developed large leaf surface areas when self-rooted and when grafted on LM rootstocks, but A_L was strongly reduced when LM scions were grafted on LD rootstocks. In contrast, LD scions had a relatively small leaf surface area both when self-rooted and when grafted on LD rootstocks, but grafting LD scions on LM rootstocks promoted a large and statistically significant increase in leaf area per plant.

Plant hydraulic resistance (R_{plant}) expressed on a leaf area basis (Figure 2, white columns) was about 20×10^3 MPa m² s kg⁻¹ in April and decreased to about 15×10^3 MPa m² s kg⁻¹ in July, with no apparent difference between plant types. The decrease in R_{plant} between April and July probably reflected the effect of plant growth in an unrestricted soil volume. The hy-

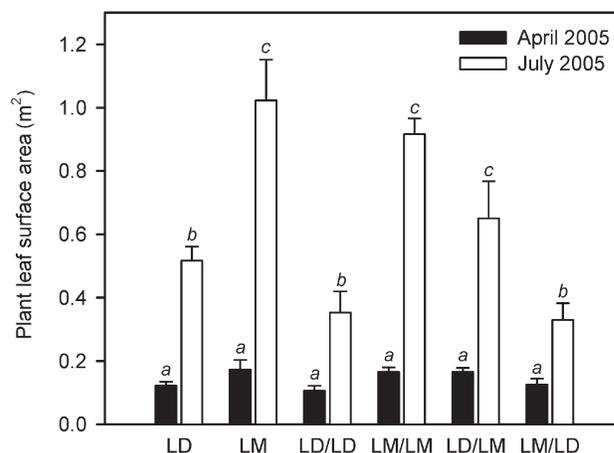


Figure 1. Total leaf surface area of the study plants as measured in April and July 2005. Abbreviations: LD = self-rooted Leccino 'Dwarf' clones; LM = self-rooted Leccino 'Minerva' clones; LD/LD = LD scion grafted on LD rootstock; LM/LM = LM scion grafted on LM rootstock; LD/LM = LD scion grafted on LM rootstock; and LM/LD = LM scion grafted on LD rootstock. Values are means \pm SD. Different letters indicate significant differences according to Tukey's pairwise comparisons ($P < 0.05$).

draulic resistances of individual plant organs (root, shoot, stems and leaves) were not significantly different between experimental groups when expressed on a leaf area basis, indicating that the unit leaf surface area was more or less equally well supplied with water in all plant types. Roots made up the largest fraction of R_{plant} (Figure 3), representing 60–70% of R_{plant} in April and July. By contrast, R_{shoot} was 30–40% of R_{plant} in April, but its contribution decreased to about 30% in July, suggesting that the roots represented the major resistance to water flow in olive plants whether self-rooted or grafted onto different rootstocks. Leaves represented 20–25% of R_{plant} in April and about 10% in July with no difference between groups. The contribution of the graft union to the overall hydraulic resistance was minimal (1–3%; Figure 3) indicating that the functional continuity of the xylem between scion and rootstock was completely restored 360 days after grafting.

Water potential and E_L per unit leaf surface area were similar in the different experimental groups. Minimum diurnal Ψ_L was about -1.60 MPa and maximum E_L was about 10 mmol m⁻² s⁻¹ (Figure 4).

Further insights into the impact of root hydraulic properties on plant vegetative growth were obtained when all hydraulic data of LD versus LM root systems were combined (Figure 5). Root hydraulic conductance not scaled by leaf surface area was significantly lower in LD rootstocks than in LM rootstocks. In particular, LM rootstocks were 1.8 and 2.5 times more conductive than LD rootstocks in April and July, respectively. Scaling root hydraulic conductance by leaf surface area revealed that leaf specific K_{root} did not differ significantly between the two groups in April. In July, however, leaf specific K_{root} was slightly, but significantly, lower in LD rootstocks than in LM rootstocks. When the leaf surface area of single

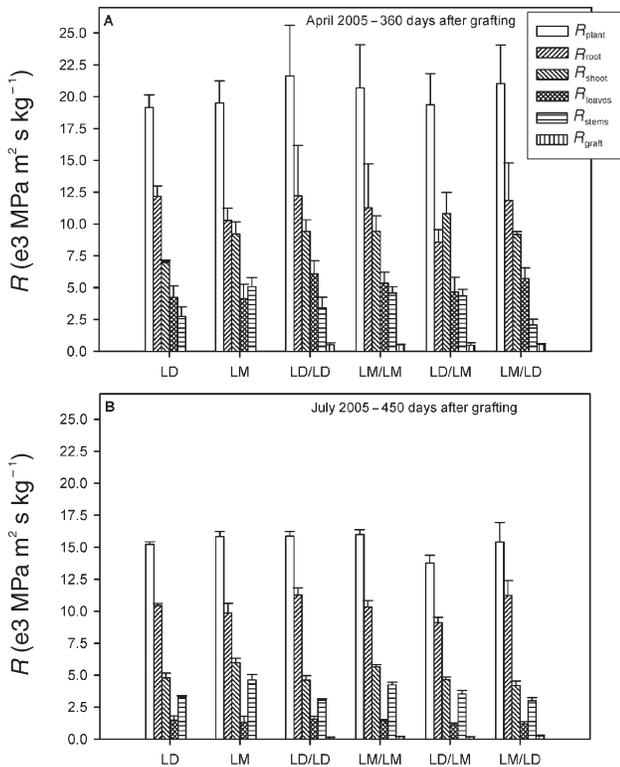


Figure 2. Leaf specific hydraulic resistance (R) of the whole plant and of different plant organs as measured in April (A) and July (B) 2005 in different scion/rootstock combinations. Abbreviations: LD = self-rooted Leccino ‘Dwarf’ clones; LM = self-rooted Leccino ‘Minerva’ clones; LD/LD = LD scion grafted on LD rootstock; LM/LM = LM scion grafted on LM rootstock; LD/LM = LD scion grafted on LM rootstock; and LM/LD = LM scion grafted on LD rootstock. Values are means \pm SD. Differences among experimental groups were not statistically significant (one-way ANOVA).

plants was plotted versus the corresponding root hydraulic conductance (not scaled by A_L), there was a linear positive relationship between the variables (Figure 6) with $r^2 = 0.968$ and $P < 0.0001$.

Discussion

The two rootstocks studied differed markedly in their influence on scion growth. In particular, LD rootstocks effectively reduced scion growth so that plants with root systems of the LD type developed significantly smaller whole-plant leaf areas compared with plants with LM root systems. This effect was rootstock- and not scion-dependent as clearly shown by the different growth patterns of plants having the same scion type, but differing in rootstock type. For example, LM/LM plants developed a total leaf surface area that was about twice that of the LM/LD plants (0.95 versus 0.4 m²; Figure 1). These observations point to a role of roots in determining the dwarfing character observed in LD plants.

Whole-plant hydraulic conductance (K_{plant}) of olive was between 5.0 and 7.0×10^{-5} kg s⁻¹ m⁻² MPa⁻¹, which is in the range previously reported for several woody plants of similar

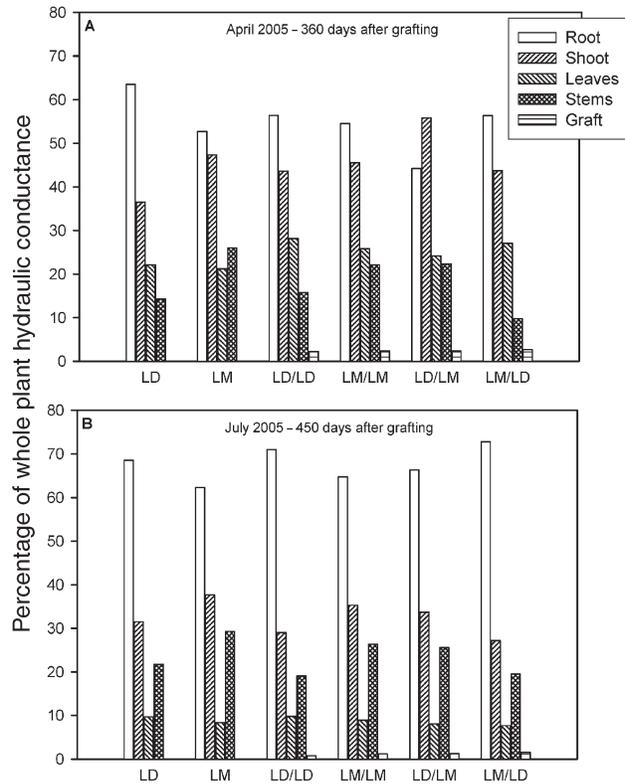


Figure 3. Percentage contribution of different plant organs to the overall plant hydraulic resistance as measured in April (A) and July (B) 2005 in different scion/rootstock combinations. Abbreviations: LD = self-rooted Leccino ‘Dwarf’ clones; LM = self-rooted Leccino ‘Minerva’ clones; LD/LD = LD scion grafted on LD rootstock; LM/LM = LM scion grafted on LM rootstock; LD/LM = LD scion grafted on LM rootstock; and LM/LD = LM scion grafted on LD rootstock.

size. For example, Nardini and Tyree (1999) reported K_{plant} values between 2.5 and 6.0×10^{-5} kg s⁻¹ m⁻² MPa⁻¹ for seven *Quercus* species, and Becker et al. (1999) reported K_{plant} values ranging between 2.5 and about 20×10^{-5} kg s⁻¹ m⁻² MPa⁻¹ for several tropical plants.

Some authors have suggested that rootstock-induced dwarfing results from a perturbation of water transport at the graft union (Simons 1986, Somelidou et al. 1994), possibly caused by graft incompatibility between the two bionts. In some cases, the hydraulic conductivity of the graft tissue has been reported to be lower for trees grafted on dwarfing rootstocks than for trees grafted on vigorous rootstocks (Atkinson et al. 2003). In the plants we studied it is unlikely that reduced vegetative growth of olive scions grafted on LD rootstocks was caused by high hydraulic resistance at the graft level because the contribution of the graft union to the overall hydraulic resistance of the plant was negligible ($< 3\%$) and did not differ significantly among the different scion/rootstock combinations. We conclude, therefore, that the observed twofold variation in leaf surface area observed among experimental groups was not caused by hydraulic constriction at the graft union.

Roots represented between 60 and 70% of whole-plant hydraulic resistance in the spring and summer. A similar parti-

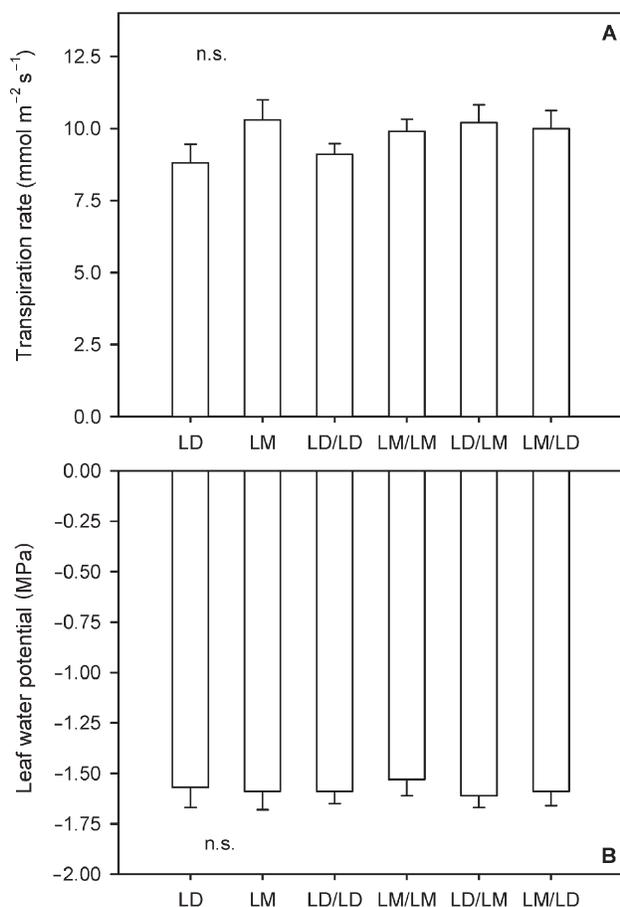


Figure 4. Maximum transpiration rate and minimum leaf water potential measured between 1000 and 1400 h in different scion/rootstock combinations in July 2005. Abbreviations: LD = self-rooted Leccino ‘Dwarf’ clones; LM = self-rooted Leccino ‘Minerva’ clones; LD/LD = LD scion grafted on LD rootstock; LM/LM = LM scion grafted on LM rootstock; LD/LM = LD scion grafted on LM rootstock; and LM/LD = LM scion grafted on LD rootstock. Values are means \pm SD. Differences among experimental groups were not statistically significant (n.s.; one-way ANOVA).

tioning of hydraulic resistances has been reported for several other woody species (Tsuda and Tyree 1997, Nardini and Tyree 1999, Nardini et al. 2000, Trifilò et al. 2004). In a few cases, the magnitudes of root and shoot resistances have been reported to be similar (Moreshet et al. 1990, Nardini et al. 2003). Basile et al. (2003b) computed that the root system accounted for 67–77% of whole-plant hydraulic resistance in peach trees when plants were grafted on a vigorous or a dwarfing rootstock. In the case of young olive trees, root systems apparently represented the major hydraulic bottleneck of the plant, which suggests a major role for root hydraulics in the control of vegetative growth. In contrast with previous reports (Yang and Tyree 1994, Nardini and Salleo 2000), leaves represented a minor fraction of R_{plant} in olive trees, especially in July, when R_{leaves} represented less than 10% of R_{plant} in all experimental groups. Values of leaf hydraulic resistance measured in April were similar to those reported by Nardini (2001)

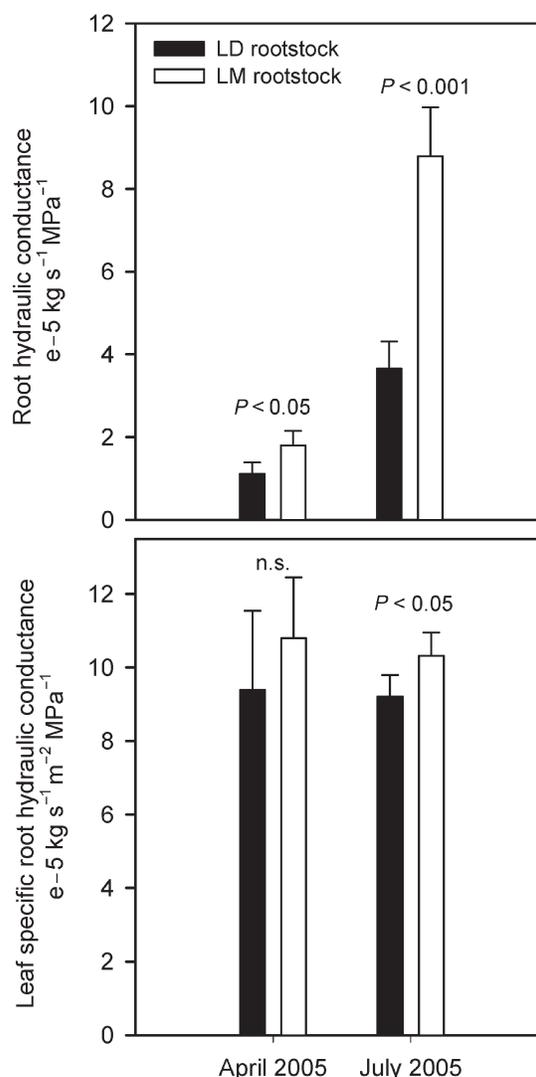


Figure 5. Root hydraulic conductance (not scaled by plant leaf surface area) and leaf specific root hydraulic conductance (scaled by plant leaf surface area) as measured in April and July 2005 in dwarfing (LD, filled columns) and vigorous (LM, open columns) rootstocks. Values are means \pm SD. The P value (one-way ANOVA) is reported where differences are significant. Abbreviation: n.s. = not significant.

for *O. europaea*. In July, however, R_{leaves} was less than 50% of the values recorded in April. Because R_{leaves} values reported in the present study refer to all leaves of the plant, and because plants produced the largest fraction of their A_L between April and July (Figure 1), we conclude that the hydraulic resistance of leaves produced after transplanting to the large containers was much lower than that of leaves produced when the young plants were being raised in small pots. Similar effects were observed for stems and roots so that overall R_{plant} decreased between April and July, suggesting that hydraulic resistance of plants or of their single organs, when measured on plants growing in confined environments, must be interpreted with great caution.

Root hydraulic conductance of dwarfing rootstocks was

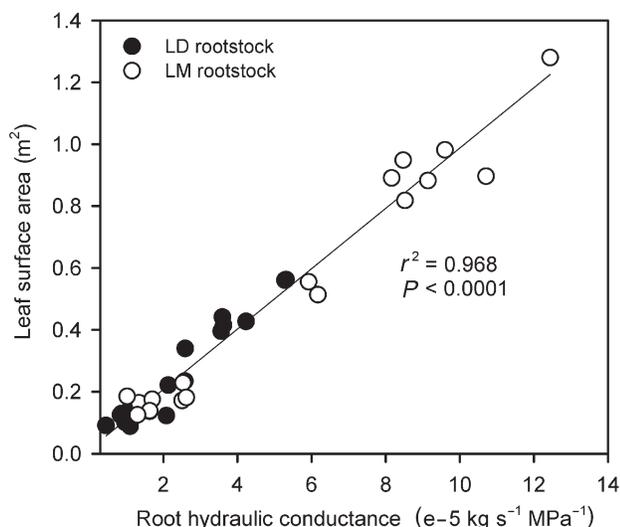


Figure 6. Relationship between plant leaf surface area and the corresponding root hydraulic conductance as measured in dwarfing (LD, ●) and vigorous (LM, ○) rootstocks. The regression line is reported together with the coefficient of determination (r^2) and the P value.

much lower than that of vigorous rootstocks (Figure 5). However, when K_{root} values were scaled by A_L , the difference between LD and LM rootstocks was only about 15%. This suggests that LD root systems with low hydraulic conductance sustained a proportionally smaller whole-plant leaf area compared with the LM rootstocks. This suggestion is further supported by the relationships presented in Figure 6 showing a close linear relationship between the hydraulic efficiency of the root system and the leaf surface area produced by plants. This finding confirms that low root hydraulic conductance is one of the main factors inducing reduced vegetative growth of the scion in plants with LD rootstocks. The relationships between soil water potential, transpiration and leaf water potential are dictated by the hydraulic resistance of the plant (Meinzer 2002) and, in particular, by the plant organ with the largest hydraulic resistance (the root system, in the case of olive). A consequence of this relationship is that root hydraulic conductance of olive governs, to a large degree, the maximum rate of transpiration that can be sustained by the plant because leaves of higher plants tend to operate within a relatively narrow range of water potentials (Nardini and Salleo 2000, Brodribb et al. 2002, Trifilò et al. 2003). A decrease in leaf water potential below a species-specific threshold induces stomatal closure and, eventually, xylem cavitation and embolism (Salleo et al. 2000, Sperry et al. 2003). Because transpiration rate and water potential were both similar among the experimental groups (Figure 4), it follows that reduced transpiration in plants grafted on rootstocks with low hydraulic efficiency could be achieved only by developing a reduced leaf surface area. Larger A_L would lead to imbalanced coordination between liquid- and vapor-phase water transport and, hence, to a consistent drop in leaf and xylem water potentials inducing stomatal closure or catastrophic xylem dysfunction (Tyree and Sperry 1988).

During the evaluation of this manuscript, one reviewer commented that the hypothesis of a hydraulic limitation to vegetative growth imposed by dwarfing rootstocks should be rejected in the case of olive because leaf specific root hydraulic conductance, transpiration rate and leaf water potential did not differ among the experimental groups. We do not agree with this interpretation. Reduction of leaf specific hydraulic conductance is not a pre-requisite for hydraulic limitation of growth. Although hydraulic limitation of plant growth in tall trees has been found to be correlated with reduction of leaf specific hydraulic conductance (Schäfer et al. 2000, Mencuccini 2003), changes in plant hydraulic architecture have also been reported to result in reduced growth in *Pinus palustris* Mill. (longleaf pine) growing in xeric compared with mesic habitats (Addington et al. 2005), even though leaf specific hydraulic conductance, stomatal conductance and leaf water potential did not differ among the experimental groups. Other studies have demonstrated integration among hydraulic architecture, water transport efficiency and leaf growth to maintain homeostasis of leaf water potential and gas exchange (Whitehead et al. 1984, Magnani et al. 2000, Meinzer et al. 2004). Our data are in agreement with these studies and demonstrate that the assumption of “same leaf-specific hydraulic conductance (and, hence, same transpiration rate and leaf water potential) leads to same growth potential” can be misleading.

It is possible that a more direct mechanism is involved in the reduced growth of LD-type plants, causing reduced water supply during leaf expansion to result in smaller leaves (Tyree et al. 1998, Nardini 2002, Wikberg and Ögren 2004, Zwieniecki et al. 2004) and, hence, reduced total leaf surface area. Regardless of whether a direct inhibition of leaf expansion or a fine coordination of liquid water transport and transpiration is involved, the final outcome was that shoots on LD rootstocks developed less biomass compared with scions growing on LM rootstocks.

Although our data demonstrate the existence of a correlation between root hydraulics and plant biomass, they do not provide a full explanation for rootstock-induced dwarfing in olive. Reduced root hydraulic conductance might itself be a consequence of other phenomena such as a hormonal imbalance. Clearly, the basic physiological mechanism(s) involved in the low vigor of the scion as induced by some rootstocks still awaits complete elucidation and that will require detailed analysis of the eventual genetic mutations of dwarfing rootstocks.

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