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Research paper

Growth and physiology of olive pioneer and fibrous roots exposed to soil moisture deficits

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In woody plants, pioneer roots are the main roots used to expand the root system horizontally and vertically whereas fibrous 'feeder' roots are chiefly used in the absorption of water and nutrients. Because of their different roles, we expected newly emerged pioneer and fibrous roots to respond differently to restrictions in soil moisture. We hypothesized that fibrous roots would exhibit greater growth plasticity and greater physiological impairment from soil moisture deficits, especially under heterogeneous conditions. We compared the responses of fibrous and pioneer roots of olive seedlings (*Olea europaea*) to localized and uniform soil moisture deficits in transparent containers in the greenhouse. In comparison with uniformly wet conditions, uniformly dry conditions caused reduced shoot photosynthesis and reduced shoot growth, but no significant effect on root morphology, root respiration (measured in aerated buffer solution using excised roots) or electrolyte leakage as a function of root age. Under heterogeneous soil moisture conditions, root growth tended to preferentially occur in the moist sector, especially in the pioneer roots. In comparison with pioneer roots in the moist sector, pioneer roots in the dry sector had higher tissue density and higher suberin content, but no shift in root respiration, non-structural carbohydrates or electrolyte leakage. In contrast, fibrous roots in the dry sector exhibited evidence of impaired physiology in older (>38 days) roots compared with similar age fibrous roots in the moist sector. While we anticipated that, compared with pioneer roots, fibrous roots would be more sensitive to soil moisture deficits as expressed by higher electrolyte leakage, we did not expect the strong growth plasticity of pioneer roots under heterogeneous soil moisture conditions. Differentiating the responses of these two very different root types can improve our understanding of how different portions of the root system of woody plants cope with soil moisture deficits.

Keywords: carbohydrate allocation, ^{13}C NMR spectroscopy, drought stress, electrolyte leakage, *Olea europaea*, root partitioning.

Introduction

One of the most pervasive stresses plants must cope with is limited water. Over the course of evolution, plants have colonized increasingly arid environments and developed many mechanisms for exploiting limited soil moisture that is both spatially and temporally heterogeneous (Grubb 1998). One way plants respond to this heterogeneity is by producing roots of different functions. In woody plants, new roots are often categorized into two classes: fibrous (=absorptive, short or feeder) and pioneer (=framework, long or skeletal) (Lyford and Wilson 1964, Wilcox 1964, Horsley and Wilson 1971, Kolesnikov 1971, Lyford 1980, Sutton and Tinus 1983). The two root types are typically distinguished primarily based on diameter, but also may include mycorrhizal colonization, rate of growth and length before branching. Pioneer roots are of relatively coarse diameter, fast growth and extended length at birth, and primarily serve the roles of vertical and horizontal soil exploration for an expanding root system, as the progenitors of fibrous root branches, as the conduits for water and nutrient transport to the stem, and as key storage organs for non-structural carbohydrates and mineral nutrients. Pioneer roots have limited mycorrhizal colonization and often have relatively long average life expectancies, as these

are the roots that undergo secondary growth (Wells and Eissenstat 2003, Zadworny and Eissenstat 2011). In contrast, fibrous roots are ephemeral, typically living no more than 1–2 years (Wells and Eissenstat 2001, Anderson et al. 2003, Xia et al. 2010), do not typically undergo secondary growth, commonly are colonized by mycorrhizal fungi, and thus are associated primarily with water and nutrient absorption (Rubio et al. 2004, Xia et al. 2010, Zadworny and Eissenstat 2011). Despite the very different functions of these two root types, there is very limited research that contrasts the morphological and physiological responses of pioneer and fibrous roots to environmental stresses including soil moisture deficits (Guo et al. 2004).

One challenge in contrasting fibrous with pioneer roots is incorporating the influence of root age. Fibrous roots, for example, can show dramatic declines in nitrate (Volder et al. 2005, 2009) and phosphate uptake (Bouma et al. 2001) and respiratory activity (Comas et al. 2000, Volder et al. 2005) from the first days of life to ages of only 1 or 2 weeks. Although the effects of age on pioneer roots have not been studied in the same manner, particularly in woody plants, we would not necessarily expect that pioneer roots respond in the same way as fibrous roots. In comparison with fibrous roots, pioneer roots have high construction costs per unit length (Guo et al. 2004, Huang et al. 2010) with several hypodermal layers that likely would lead to high growth respiration while the roots are still growing and developing, which might take two or more weeks (Zadworny and Eissenstat 2011). Beyond this growth-development phase, we might expect the declines in metabolic activity with age to be slower in the sturdier pioneer roots than the more ephemeral fibrous roots (cf., Bouma et al. 2001), but this has never been quantified.

In interpreting research on root responses to soil moisture deficits, responses where the whole root system is exposed to relatively uniform soil moisture deficit conditions may depart markedly from a situation where only a portion of the root system is exposed to dry soil (Eissenstat and Yanai 1997). Under natural soil conditions, uniform soil moisture deficits across the rooting zone would be a rarity. In contrast to uniform soil moisture conditions, under heterogeneous moisture conditions roots should preferentially grow in favorably moist locations and may discard roots in unfavorably dry locations (Richards and Cockroft 1975, Coutts 1982, Fort et al. 1998, Green and Clothier 1999). Under heterogeneous conditions, therefore, competition for carbohydrates is occurring as much between different branches of the roots system as between roots and shoots, as found among treatments in uniform drought experiments (Kosola and Eissenstat 1994).

We expect the physiology of pioneer and fibrous roots to respond differently to soil moisture deficits. Most studies on root responses of woody plants to soil moisture deficits have focused primarily on the fibrous roots or entire root branches that are dominated by fibrous roots, especially with regard to physiology (Kosola and Eissenstat 1994, Green and Clothier 1999, Bryla et al. 2001, Huang et al. 2005). Although we are not aware of comparisons of non-woody pioneer and fibrous root responses to soil moisture deficits, responses of axial roots of annuals, which share some structural and architectural similarities to non-woody pioneer roots, and lateral roots arising from axial roots, have been compared. In sorghum, axial roots exposed to soil moisture deficits exhibited lower hydraulic conductance than axial roots in moist soil, which was partially attributed to accelerated deposition of lignin and suberin in the hypodermis and endodermis (Cruz et al. 1992).

Responses of different kinds of cactus roots to soil moisture deficits have been studied by Nobel and colleagues. *Opuntia ficus-indica* main roots, which are functionally similar to non-woody pioneer roots, when exposed to soil moisture deficits show an increase in cross-sectional areas of the epidermis/exodermis and the sclerenchyma/endodermis. The increases were due to increases in cell layers in the exodermis and sclerenchyma. The same cells also showed an increase in extractable lipids of up to 50% and reduced radial conductivity (L_r) (North and Nobel 1995). Lipids in suberized cell walls generally decrease water permeability (Vogt et al. 1983). Similar to main roots, fibrous laterals exposed to drought have shown a decrease in L_r (Lopez and Nobel 1991) but reduction for young lateral roots is typically smaller than for main roots (North and Nobel 1992). After rewetting, radial conductivity was never fully restored in main roots while in lateral roots it was restored to nearly the same value estimated before drought stress (North and Nobel 1992).

We expect that because pioneer roots are formed to develop the framework of the tree whereas the main role of fibrous roots is water and nutrient absorption, there would be fundamental differences in how these two types of roots respond to both uniform and heterogeneous soil moisture deficits. To study this question, we chose the tree crop olive (*Olea europaea*), a very drought-tolerant species. In comparison with fibrous roots, we hypothesized that pioneer roots would show higher tolerance to soil moisture deficits, as indicated by less reduction in respiration in older roots, less cellular damage and greater suberization. Moreover, we expected that plants under uniform soil moisture deficits would allocate biomass primarily to fibrous roots to increase absorptive surface area for water acquisition, whereas under heterogeneous soil moisture conditions there would be very limited fibrous root growth in dry soil regions and most root growth would occur in moist soil regions. We expected pioneer roots, in contrast, to show less growth plasticity in response to different soil moisture conditions.

Materials and methods

The study was conducted from May to August 2009 in State College, PA, USA. Plants were grown in the greenhouse with a

temperature range of 20–30 °C. Artificial lighting was supplied to ensure a photoperiod of 14 h. Self-rooted, 1-year-old, 12-cm-tall, olive plants (cv. Arbequina) were grown in 'rhizoboxes' with dimensions of 30 cm (length) × 60 cm (height) × 3 cm (thick). One side of the rhizobox was solid while the other side was covered by a clear Plexiglas cover to allow visual tracking of roots over time and easy access to roots at the end of the harvest. During the experiment the Plexiglas was covered with a second, opaque cover to prevent light intrusion into the soil environment. Soil media was a well-drained substrate (pH 6.7) composed of 60% sand and 40% Hagerstown silt loam (15 cm depth) soil from the Pennsylvania State University, Russell E. Larson Agricultural Research Center, near State College, PA, USA. Each box was divided into two hydraulically isolated chambers. Fertilizer was provided uniformly to all plants. Over the period from transplanting to initiation of the irrigation treatments, a total of 225 mg of N (ammonium-N 86 mg, nitrate-N 67 mg, urea-N 72 mg), 198 mg of P (as P_2O_5) and 187 mg of K (as K_2O) was provided.

Plants were randomly assigned to three treatments with seven replicates. After an establishment period of 34 days during which all systems were well watered, the irrigation treatments were initiated and maintained for 75 days. The restricted irrigation treatment was designed to impose substantial drought stress on the treated plants, resulting in reduced plant growth without killing the plants. Plants were exposed either to continuously *uniformly* irrigated equally in both halves of the container (WW), continuously deficit irrigated *uniformly* in both halves of the container with one-third the quantity of water as above (DD), and a third set of plants that were *heterogeneously* irrigated with full watering in half the container (Wh) and only partial (one-third) watering in the other side of the container (Dh). Water was supplied daily to reduce moisture fluctuations. The whole soil volume was irrigated equally through small pierced pipes running along the length of the boxes that enabled uniform wetting of the soil volume without creating persistent wet and dry areas within a sector. Over the course of the entire experiment each W sector was irrigated with a cumulative total of 2190 ml of water and each D sector irrigated with 740 ml. This resulted in a total of 4380 ml of water irrigated for each WW plant, which was sufficient to create a well-watered treatment given the small number of leaves (<20 leaves per plant) and minimal evaporation at the soil surface. Soil water content at the end of the experiment averaged 6.36 ± 0.28 (SE) in W and 2.65 ± 0.17% in D sectors. Soil water content was defined on a gravimetric basis and calculated as (weight of fresh soil sample – oven-dried soil sample)/(oven dried soil sample). The oven-dried samples were dried until weight stabilized (~72 h).

Shoot measurements

Shoot growth was evaluated weekly by monitoring stem length. Every 3 weeks, photosynthetic rate, transpiration rate and stomatal conductance were measured at 15.00 h on recent, fully expanded leaves at a light intensity of 800 μmol m^{-2} s^{-1} using a LiCor 6400 photosynthesis measurement system (Li-Cor Inc. Lincoln, NE, USA). Leaf water potential (pressure chamber), leaf turgor potential and leaf osmotic potential were measured at peak stress at 15.00 h and at predawn at the end of the experiment. A VAPRO 5520 osmometer (Wescor Inc. ELITech Group Co., Logan, UT, USA) was used to measure osmotic potential. Leaf turgor potential was estimated by the difference between total leaf osmotic potential and extracellular osmotic potential, measured respectively after and before freezing tissues two times in liquid N over 5 min, to cause cell membrane disruption. Total non-structural carbohydrates (TNCs) were analyzed colorimetrically on freeze-dried tissues and separated into storage pools (starch, by enzymatic digestion) and metabolically active pools (soluble sugars) on leaves at the end of the experiment (Nelson 1944, Somogyi 1952).

Root measurements

All roots analyzed in the experiment were first or second order (where first-order roots are roots with a tip and no other branch roots) to minimize the influence due to root position within the branching hierarchy. Roots were traced approximately every 2 weeks using colored markers to track roots of different ages from birth until the end of the experiment, when all roots were harvested (Days 75–76). Thus, harvested roots that were of young age were born later in the experiment. Roots on first observance were divided into two classes based on diameter—coarse-diameter pioneer roots (range 0.65–1.43 mm) and fine-diameter fibrous roots (range 0.32–0.58 mm). At the end of the experiment (Day = 75) the Plexiglas plates were removed leaving a sheet of acetate over the soil surface with each traced root of known age shown. Each root was harvested individually by cutting the acetate window with a razor blade and removing the roots with forceps. Roots sampled for physiological evaluation were divided into five different age classes: 1–4, 5–20, 21–38, 39–56 and 57–75 days old. All root samples evaluated in this experiment arose only from roots born during the experiment. In contrast, stem and leaf samples used to determine biomass partitioning included tissue that was grown both prior and during the experiment.

Root respiration was considered an index for root metabolic activity. Specific root respiration rate was determined on excised roots by measuring O_2 consumption using a Clark-type oxygen electrode (Hansatech Oxygraph, King's Lynn, UK). Roots of known age were collected, rinsed, and immediately immersed in a 2-(*N*-morpholino) ethanesulfonic acid buffer solution and allowed to stabilize at 22 °C for ~20 min. Roots were then inserted in the Oxygraph chamber at constant temperature (22 °C). Oxygen depletion inside the sealed chamber was measured until a steady slope displaying the O_2 consumption rate

could be identified (~15 min). Following respiration measurements, roots were dried for 48 h at 70 °C and weighed.

As an indicator of stress (e.g., Huang et al. 2005), root electrolyte leakage was used to assess cell membrane stability and integrity as described in Martin et al. (1987). Roots were immersed in deionized water and electrical conductivity (EC) of the water was measured before roots were inserted ($EC_{initial}$), after 30 min of immersion (EC_{30}) and again after boiling the sample for 5 min (EC_{total}). Electrolyte leakage was calculated using the following relationship:

$$\text{Electrolyte leakage}(\%) = 100 \times \frac{EC_{30} - EC_{initial}}{EC_{total} - EC_{initial}}.$$

Total non-structural carbohydrate concentration and nuclear magnetic resonance analysis were performed on freeze-dried tissues of roots of mixed age (maximum age: 75 days) produced after plant installation into split-boxes and collected at the end of the experiment. Total non-structural carbohydrate concentration was measured with the same procedure indicated for leaves.

Nuclear magnetic resonance spectroscopy (^{13}C-CPMAS NMR) was used to assess the influence of soil moisture deficits on the chemical composition of the different roots. Samples were filled into zirconium dioxide rotors and spun in a magic angle spinning probe at a rotation speed of 6.8 kHz to minimize chemical anisotropy using a Bruker DSX 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). A ramped 1H pulse was used during a contact time of 1 ms to prevent Hartmann–Hahn mismatches. The delay time was set to 1 s for the root samples. Chemical shifts (driven by electron shielding of C moieties) were referenced to tetramethylsilane, whereas the TMS signal was set at 0 ppm. The chemical shift of –10 to 45 ppm covers paraffinic structures, whereas the signal at 30 ppm indicates long-chain aliphatic structures (e.g., lipids, suberin) (Kögel-Knabner, 2002). Changes in the relative intensities of the alkyl-C shift region were assigned to changes in suberin content, the predominant aliphatic compound in roots. Alcohol and ether structures as found in carbohydrates have a resonance between 45 and 110 ppm, as given here by relative intensities of the O/N alkyl C region (Kögel-Knabner, 2002). The aromatic compounds are recorded with resonances between 110 and 160 ppm with predominating aromatic and phenolic C peaks at 130 and 150 ppm, which essentially derive from lignin (Kögel-Knabner 1997). For integration, chemical shift regions were used as given: alkyl C (–10 to 45 ppm), O/N-alkyl C (45–110 ppm), aryl/olefine C (110–160 ppm) and carbonyl/carboxyl/amide C (160–220 ppm).

Statistics

For shoot traits, we compared the three irrigation treatments (WW, DD, WD) by one-way analysis of variance (ANOVA). For root traits, the uniformly irrigated plants were analyzed separately from the heterogeneously irrigated plants. A two-way ANOVA was used to compare moisture regime and root type (pioneer versus fibrous). Thus, for the uniform irrigation treatment the moisture regimes were DD or WW and for the heterogeneous irrigation treatment, the moisture regimes were Dh and Wh. Note for the heterogeneous treatment, the effects of different moisture regimes were compared on roots of the same plant, thus eliminating potential shoot differences on root physiology. For a particular irrigation treatment (uniform or heterogeneous), the interaction term in a two-way ANOVA (moisture regime × root type) would test whether fibrous roots were more affected than pioneer roots by soil moisture deficits. Because of potential ontogenetic effects for root mass partitioning, whole-plant biomass was used as a covariate in the two-way analysis of covariance. We also used multiple regression to examine the influence of age on physiology.

Results

Shoot growth, photosynthesis and water relations

The three irrigation treatments successfully created a broad range in shoot growth. Differences in shoot extension and gas exchange among treatments increased with time over the experiment (data not shown). Averaged over the entire experiment, shoot extension rate was 0.37 mm/day in DD and 1.6 mm/day in WW with the heterogeneously irrigated plants (DhWh) intermediate (Figure 1a). Shoot relative growth rate (RGR) for the DD treatment was 23% less than that of WW plants (Figure 1b). Water shortage in DD induced a gradual reduction in photosynthetic activity compared with DhWh and WW (data not shown). After 75 days, plants in the WW treatment exhibited significantly faster photosynthetic rates than those in the DD treatment (9.2 and 2.8 μmol CO_2 m^{-2} s^{-1} respectively, Figure 1c). The reductions in photosynthetic activity were also linked to reductions in leaf TNC, with leaves in the DD treatment ~15% (relative terms) lower than those in the WW and DhWh treatments ($P < 0.034$; Figure S2 available as Supplementary Data at *Tree Physiology* Online).

Leaf water potential at the end of the experiment in DD plants averaged –2.8 MPa at 15.00 h (Figure 1d). Osmotic potential in leaves was also significantly decreased by soil moisture deficits, having values of –2.24 MPa for WW plants and –4.57 MPa for DD plants at peak stress at 15.00 h (Figure 1e) with a leaf turgor of 1.90 MPa for WW and 0.65 MPa for DD (data not shown). Similarly, stomatal conductance was also strongly reduced by soil moisture deficits (Figure 1f).

Biomass partitioning

Biomass partitioning examined in this study represents total shoot growth over the life of the plant but estimated fibrous

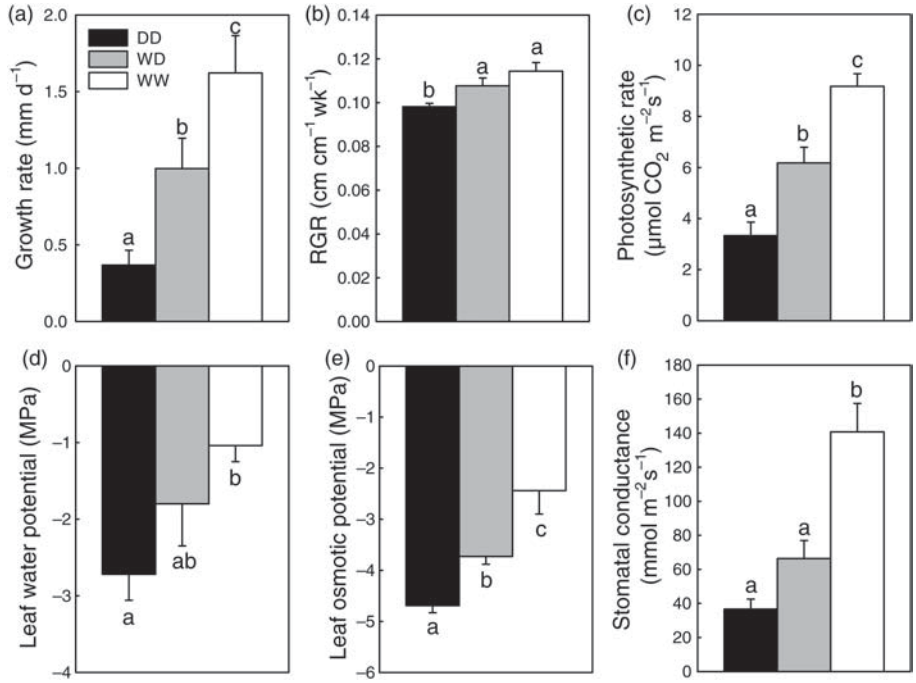

Figure 1. Shoot growth and leaf physiological responses to three irrigation treatments. Each growth container was divided into two hydraulically isolated chambers. Plants were continuously well watered equally in both halves of the container (WW), subjected to a continuously deficitirrigated uniformly in both halves of the container with one-third the quantity of water as above (DD) or heterogeneously irrigated with full watering in half the container and only partial (one-third) watering in the other side of the container (DhWh). Means followed by a different letter were significantly different ($P < 0.05$).

and pioneer root biomass represents that only associated with growth during the experimental period. There was no correlation between fibrous root mass and total plant mass that might cause ontogenetic effects (plant size) to confound comparisons among the drought treatments (Figures S1A and S1C available as Supplementary Data at *Tree Physiology* Online). Pioneer root mass was correlated with total plant mass, so ontogenetic effects (plant size) were included as a covariate in the analysis of moisture regimes. In comparing the moisture regimes WW and DD in the uniform irrigation treatment, no effect of soil moisture on fibrous or pioneer root biomass independent of plant size effects was observed, although moisture deficits (DD) substantially reduced shoot biomass (Table 1). Moreover, we did not observe significant shifts in the proportion of total root biomass that was partitioned to fibrous roots in DD compared with WW treatment. In addition, there was no evidence that fibrous root biomass was more responsive to soil moisture deficits than pioneer root biomass in the uniform irrigation treatment (moisture regime (WW, DD) × root-type interaction, $P = 0.89$). In the heterogeneously irrigated treatment, while we did not observe a significant root type by moisture regime (Wh,Dh) interaction ($P = 0.21$), the percentage of total root dry weight that was fibrous in the Dh sector was more than twice that of roots in the Wh sector ($P = 0.01$ by *t*-test; Table 1).

Table 1. Biomass (dry weight) and biomass partitioning of shoot mass, fibrous root mass, and pioneer root mass in uniformly or heterogeneously irrigated seedlings that were either well watered (WW, Wh) or deficit irrigated (DD, Dh, see Figure 1). Shoot biomass represents all shoot biomass including tissue growth during and prior to the start of the experiment. For fibrous and pioneer roots only roots that were produced during the experiment were collected. Comparisons of root mass were only made between WW and DD and between Dh and Wh. Means followed by a different letter were significant at $P < 0.05$. For root organs (fibrous and pioneer), comparisons were made between both moisture regime and root type. The interaction of root type and moisture regime was not significant in either the uniform or heterogeneous treatment ($P > 0.21$)

Organ or trait	Uniform		Heterogeneous	
	WW	DD	Wh	Dh
Shoot (g)	3.85a	2.26b	3.30a	
Roots				
Pioneer (g)[2]	0.724[1]a	0.660a	0.425q	0.123p
Fibrous (g)[2]	0.129[1]b	0.141b	0.072r	0.047s
Root mass that was fibrous (%)	10.1a	15.0a	10.9q	25.8p

[1]When total plant mass was accounted for (ontogenetic effects), there were no significant differences between WW and DD pioneer or fibrous roots (see Figure S1 available as Supplementary Data at *Tree Physiology* Online).

[2]Retransformed means of log data; statistical comparisons were on log-transformed data.

Root morphology

Pioneer and fibrous roots in the uniform soil moisture deficit (DD) treatment had similar morphology as those in the WW treatment (Tables 2 and 3). In the heterogeneous irrigation treatment, pioneer and fibrous roots had higher tissue density in the Dh sector than the Wh sector, but diameter was unaffected by soil moisture deficits. Drought treatment generally did not influence pioneer roots differently from fibrous roots. The only evidence of a differential response was where pioneer roots tended to exhibit a greater increase than fibrous roots in tissue density in the Dh sector compared with that in the Wh sector ($P = 0.10$; Table 2).

Root chemistry

Because of limited differential responses of pioneer and fibrous roots to soil moisture deficits as well as the expense associated with analysis, only roots in the heterogeneous treatment were examined by ^{13}C-NMR. We consistently found shifts in root chemistry, as assessed by solid-state ^{13}C-NMR, as a result of soil moisture deficits (Figure 2, Table 3). Compared with fibrous roots across moisture regimes, pioneer roots had significantly higher intensities of O/N-alkyl C structures, which are predominantly derived from structural and non-structural carbohydrates (e.g., cellulose and starch). Fibrous roots overall showed a higher level of alkyl C (suberin-like compounds) than pioneer roots. Localized soil moisture deficits led to increases in alkyl C for both pioneer roots and fibrous roots with no evidence of a significant differential response to soil moisture deficits in the two root types. No clear differences among pioneer and fibrous roots were recorded for the aromatic-C region, predominantly derived from lignin phenolic structures.

Total non-structural carbohydrate concentrations differed by root class with pioneer roots generally containing higher TNC concentration than fibrous roots ($P < 0.0001$, Figure 3, Table 3). In the uniform treatments, water limitation (DD) resulted in TNC slightly lower than those in the WW treatment. In the heterogeneous treatment differences due to moisture regime were not significant. For TNC of roots in the uniform and heterogeneous treatments, there was no significant interaction of moisture regime with root type. In addition, pioneer roots from the heterogeneous treatment had a lower fraction of TNC in the form of starch than fibrous roots ($P = 0.003$, Table 3, Figure 3). In the uniform treatment, soil moisture deficits increased the fraction of TNC that was starch in fibrous roots but not in pioneer roots (moisture regime × root-type interaction, $P = 0.026$).

Metabolism and stress indicators

Respiration was significantly influenced by root class and age. The highest metabolic activity was observed in young roots >4 days old and decreased sharply with root age during the first 20 days of life, after which metabolism exhibited a slow and gradual decline with age (data not shown). In general, fibrous

Table 2. Morphological characteristics of first-order pioneer and first-order fibrous roots under uniform and heterogeneous irrigation regimes (see Figure 1 for treatment description). Means followed by a different letter differ significantly at $P < 0.05$. For all parameters the main effect of moisture regime was not significant in the uniformly irrigated plants ($P > 0.28$). For the heterogeneously irrigated plants, tissue density was affected by moisture regime at $P = 0.005$ and the interaction of moisture regime x root type was significant at $P = 0.10$.

Trait	Uniform		Heterogeneous	
	WW	DD	Wh	Dh
Diameter (mm)				
Pioneer	0.97a	0.90a	1.03p	0.95p
Fibrous	0.44b	0.48b	0.45q	0.43q
Specific root length (m/g)				
Pioneer	4.71a	3.87a	4.35p	3.54p
Fibrous	16.5b	15.8b	16.6q	16.9q
Tissue density (g/cm^3)				
Pioneer	0.34a	0.45a	0.32p	0.50q
Fibrous	0.41a	0.40a	0.39p	0.44q

Table 3. Summary of *P* values from analysis of variance for physiological and structural traits of fibrous and pioneer roots in uniform and heterogeneous water regimes. Numbers in bold significant at $P < 0.05$.

	Tissue density	Alkyl-C	TNC	Starch fraction of TNC	Respiration (roots > 38 days old)	Electrolyte leakage
Uniform irrigation regime						
Treatment (WW vs. DD)	0.297	–	**0.036**	0.282	0.094	0.558
Root type (Pioneer vs. Fibrous)	0.130	–	> **0.0001**	0.257	0.1089	0.749
Treatment × Root type	0.146	–	0.387	**0.026**	0.087	0.523
Heterogeneous irrigation regime						
Treatment (Wh vs. Dh)	**0.005**	**0.021**	0.434	0.227	0.055	**0.010**
Root type (Pioneer vs. Fibrous)	0.582	**0.001**	> **.0001**	**0.0025**	0.054	**0.035**
Treatment × Root type	0.089	0.986	0.585	0.525	0.063	**0.024**

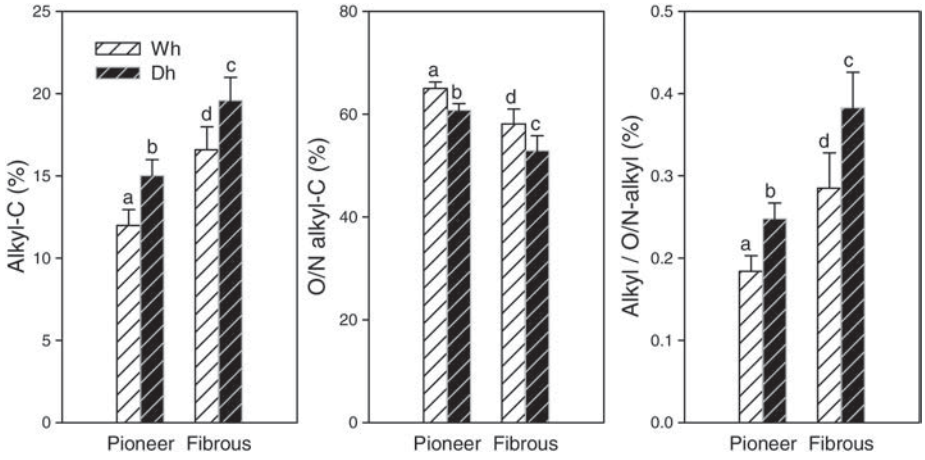

Figure 2. Relative intensities of the NMR spectrum in the alkyl C region (mainly suberin, the predominant aliphatic compound in roots), the O/N-alkyl C region (alcohol and ether structures as found in carbohydrates) and the ratio of aliphatic to carbohydrate structures (alkyl/ON-alkyl) for pioneer and fibrous roots in wet sector (Wh) and dry sector (Dh) of the heterogeneous treatment. Roots of mixed age (maximum 75 days) produced after plant installation on split-boxes and collected at the end of the experiment were used in the analysis. Different letters indicate significant ($P < 0.05$) differences due to root type and moisture regime. The interaction of root type by moisture regime was not significant for any spectral region ($P > 0.59$). For the aromatic (e.g., lignin) region of the spectrum, neither moisture regime nor root type was significant ($P > 0.20$; data not shown).

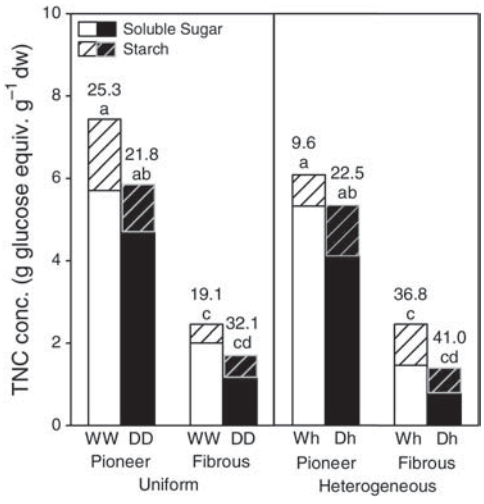

Figure 3. Total non-structural carbohydrate concentration in pioneer and fibrous roots, where TNC was partitioned into two components: starch (hatched) and soluble sugars (unhatched). Roots of mixed age (maximum 75 days) produced after plant installation on split-boxes and collected at the end of the experiment were used in the analysis. Different letters indicate significant ($P < 0.05$) differences due to root type and moisture regime, respectively. Moisture regimes in the uniformly irrigated treatment were either fully irrigated (WW) or irrigated at only one-third of full irrigation (DD). Moisture regimes in the heterogeneously irrigated plants were split into two sectors, with one sector irrigated fully (Wh) and the other irrigated one-third of full irrigation (Dh). Moisture regime was significant in the uniform irrigation treatment ($P = 0.036$). The interaction of root type by moisture regime was not significant in either the uniform or heterogeneous irrigation treatments ($P > 0.39$). Numbers above bar indicate the percent starch relative to the total non-structural carbohydrates (starch/TNC). For the uniform irrigation treatment, the moisture by root type interaction in starch/TNC was significant ($P = 0.026$). Neither moisture regime nor the interaction significantly affected starch/TNC in the heterogeneous irrigation treatment (Table 3).

roots had faster respiration than pioneer roots. Over all root ages the influence of water availability on pioneer root respiration was minimal in the uniform irrigation treatment. In fibrous roots older than 38 days, respiration tended to be lower in the roots exposed to soil moisture deficit than the roots in moist soil, but this interaction was not significant (Table 3: homogeneous, $P = 0.087$; heterogeneous, $P = 0.063$; Figure 4a).

Electrolyte leakage increased with root age, being low in roots younger than 38 days and increasing in older roots ($P < 0.0001$; data not shown). For roots older than 38 days, a significant interaction of root type with soil moisture regime was found in the heterogeneous irrigation treatments (Figure 4b, Table 3). In particular, localized soil moisture deficit dramatically increased electrolyte leakage in the fibrous roots, while pioneer roots were largely unresponsive.

Discussion

We found that pioneer and fibrous root systems responded differently to soil moisture limitation, especially under heterogeneous soil moisture conditions. Localized soil moisture deficits caused older fibrous roots to be more compromised than pioneer roots in terms of membrane integrity (electrolyte leakage). Pioneer roots, however, responded more strongly than fibrous roots, in terms of biomass allocation, in contrast to our original hypothesis. Not all responses, however, differed between pioneer and fibrous roots. Both pioneer and fibrous roots accumulated aliphatic compounds (suberin) and exhibited decreases in total non-structural carbohydrates in response to soil moisture deficits.

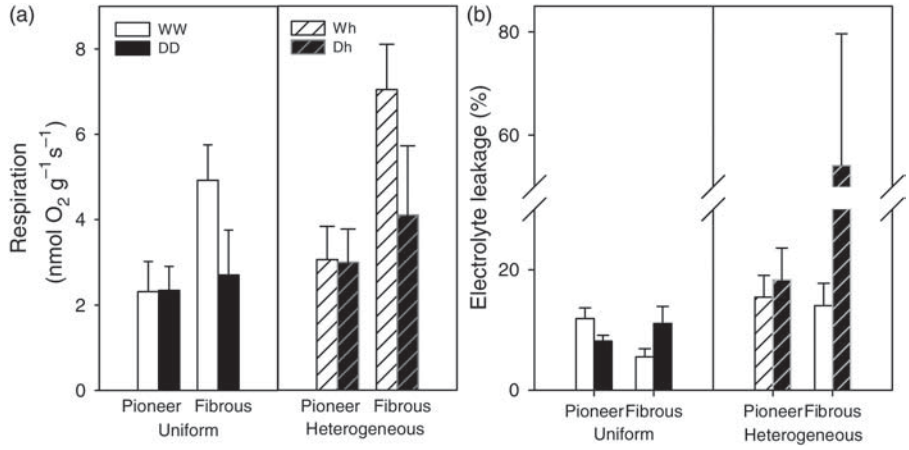

Figure 4. Respiration (a) and electrolyte leakage (b) in pioneer and fibrous roots older than 38 days in different soil moisture regimes (+SE). Moisture regimes in the uniformly irrigated treatment were either fully irrigated (WW) or irrigated at only one-third of full irrigation (DD). Moisture regimes in the heterogeneously irrigated plants were split into two sectors, with one sector irrigated fully (Wh) and the other irrigated at one-third of full irrigation (Dh). See Table 3 for significance of various factors.

Pioneer olive roots had more than twice the total non-structural carbohydrate concentration compared with fibrous roots and higher O/N-alkyl C signal intensities, an indicator of both structural and non-structural carbohydrates. This is commonly associated with pioneer root development into the perennial root system, which has an important carbohydrate storage function (Guo et al. 2004, Zadworny and Eissenstat 2011). While soil moisture regimes did not affect TNC concentration in the two root types, the roots in the Dh treatment generally exhibited reduced signal intensity of O/N-alkyl C.

Patterns of non-structural carbohydrate storage were also influenced by soil moisture deficit. We found lower TNC concentration in leaves and roots in the DD treatment compared with those in the WW treatment. One likely reason was that the much lower C assimilation in the DD treatment led to reduced non-structural carbohydrate storage. We also observed drought-induced increases in the percentage of TNC that was starch, especially in fibrous roots in the DD moisture regime. This may be partly caused by the low absolute concentration of TNC in fibrous roots in dry soil and partly because of the low metabolism observed in these roots, leading to a higher fraction of starch compared with soluble sugars.

While we had hypothesized that pioneer roots might exhibit greater suberization in response to soil moisture deficits than fibrous roots, this was not observed. The drought-induced increase in alkyl C signals, an indicator of aliphatic compounds associated with suberin, were similar in pioneer and fibrous roots. Moreover, the drought-induced shift in the alkyl/O/N-alkyl C ratios, indicating a shift from carbohydrate (both structural and non-structural) accumulation to suberin accumulation, was also similar in fibrous and pioneer roots. Enhanced suberization of cell walls under drought stress has been reported for roots of a variety of plant species (Noldt et al. 2001, Enstone et al. 2002). For instance, the drought-resistant Amazonian tree species *Carapa guianenis* Aubl. has heavily modified exodermal cells under dry conditions, including substantial lignification and suberin lamellae. However, unlike *Carapa guianenis*, we did not observe evidence of drought-induced enhanced lignification (relative intensities of aromatic C) in the roots of olive.

Respiratory declines with age found in this study are in agreement with previous studies (Bouma et al. 2001, Volder et al. 2005). Soil moisture deficits had variable effects on root respiration and no significant reductions were observed, in contrast to previous studies (Bryla et al. 2001, Huang et al. 2005). The variability observed here may indicate that some roots were being maintained by active osmotic adjustment while others experienced disrupted metabolic activity due loss of cellular integrity as suggested by their increased electrolyte leakage.

Uniform and heterogeneous soil moisture deficits elicited markedly different root biomass and physiological responses. Under uniform soil moisture conditions, the fraction of total root biomass that was fibrous was similar (10–15%) between DD and WW treatments but under localized soil moisture deficits (Dh), root biomass partitioning to pioneer roots was strongly inhibited and fibrous root fraction increased to 26% compared with 11% in the moist sector (Wh). Similarly, older fibrous roots showed more stress in terms of increased electrolyte leakage under localized soil moisture deficits (Dh) compared with those exposed to uniform soil moisture deficits (DD). When a root system is exposed to uniform soil moisture deficits, the whole plant is stressed and there are few options in shifting allocation among the different root branches in the root system to improve soil moisture acquisition. In contrast, when soil moisture deficits are localized, the plant can respond by preferentially allocating to roots in the moist soil regions and reducing allocation to those in the dry regions, which might lead to shifts in growth and likely leads to abandoning the maintenance of roots in the

unfavorable soil, as indicated by the high electrolyte leakage. This might be accomplished by reduced production of osmoticants or reduced activity of free-radical scavenging enzymes as well as induction of programmed cell death.

Conclusions

Olive successfully copes with soil moisture deficits by a wide range of responses. Aboveground, they tolerate very low shoot water potentials and still maintain positive photosynthesis, partly by strong osmotic adjustment. Belowground we found that biomass partitioning to pioneer roots was more strongly influenced by soil moisture deficits compared with fibrous roots. In contrast, older fibrous roots were more sensitive than similar age pioneer roots to prolonged exposure to soil moisture deficits, as indicated by increased electrolyte leakage in fibrous roots. This may enable olive plants to maintain the main framework of the root system with a high capability for exploration in moist soil both vertically and horizontally while shedding fibrous roots in soil regions where soil moisture is least available.

Supplementary Data

Supplementary data for this article are available at *Tree Physiology* online.

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