RESEARCH PAPER

Differential responses of grapevine rootstocks to water stress are associated with adjustments in fine root hydraulic physiology and suberization

F.H. Barrios-Masias¹, T. Knipfer¹ and A.J. McElrone¹,², *

¹ Department of Viticulture and Enology, University of California, Davis, CA 95616, USA
² United States Department of Agriculture-Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA

* To whom correspondence should be addressed. E-mail: ajmcelrone@ucdavis.edu

Received 2 December 2014; Revised 16 May 2015; Accepted 28 May 2015

Editor: Jeremy Pritchard

Abstract

Water deficits are known to alter fine root structure and function, but little is known about how these responses contribute to differences in drought resistance across grapevine rootstocks. The ways in which water deficit affects root anatomical and physiological characteristics were studied in two grapevine rootstocks considered as low–medium (101-14Mgt) and highly (110R) drought resistant. Rootstocks were grown under prolonged and repeated drying cycles or frequent watering (‘dry’ and ‘wet’ treatments, respectively), and the following parameters were evaluated: root osmotic and hydrostatic hydraulic conductivity (\(Lp_{os}\) and \(Lp_{hyd}\), respectively), suberization, steady-state root pressure (\(P_{rs}\)), sap exudation rates, sap osmotic potential, and exosmotic relaxation curves. For both rootstocks, the ‘dry’ treatment reduced fine root \(Lp\), elicited earlier root suberization and higher sap osmotic potential, and generated greater \(P_{rs}\) after rewatering, but the rootstocks responded differently under these conditions. \(Lp_{os}\), \(Lp_{hyd}\), and sap exudation rates were significantly higher in 110R than in 101-14Mgt, regardless of moisture treatment. Under ‘dry’ conditions, 110R maintained a similar \(Lp_{os}\) and decreased the \(Lp_{hyd}\) by 36% compared with ‘wet’ treatments, while both parameters were decreased by at least 50% for 101-14Mgt under ‘dry’ conditions. Interestingly, build-up of \(P_{rs}\) in 110R was 34% lower on average than in 101-14Mgt, suggesting differences in the development of suberized apoplastic barriers between the rootstocks as visualized by analysis of suberization from fluorescence microscopy. Consistent with this pattern, 110R exhibited the greatest exosmotic \(Lp_{os}\) (i.e. \(Lp_{os}\) of water flowing from roots to the soil) as determined from relaxation curves under wet conditions, where backflow may have limited its capacity to generate positive xylem pressure. The traits studied here can be used in combination to provide new insights needed for screening drought resistance across grapevine rootstocks.

Key words: Drought resistance, fine root conductivity, \(Lp\), root hydraulics, traits, \textit{Vitis}.

Introduction

Based on field observations, commercially available grapevine rootstocks are commonly categorized as ranging from low to high drought resistance. However, the mechanisms by which root hydraulic traits contribute to differential drought resistance among grapevine rootstocks are not well understood. Rootstock performance is better defined for other biotic stressors, soil conditions, and vigour induction, and has contributed to the expansion of grapevine cultivation across diverse environmental conditions (Christensen, 2003). Root systems that maintain water uptake capacity under...
high transpirational demands and low water availability may increase the drought resistance of a rootstock, and a greater understanding of how root hydraulic traits affect plant performance under these conditions can improve germplasm selection and improve irrigation management under diminishing water supply (Passioura, 2002; Comas et al., 2013; Barrios-Masias and Jackson, 2014; Seversike et al., 2014).

One fundamental function of a root system is to match canopy water demands needed to sustain carbon assimilation and plant growth (Saliendra and Meinzer, 1992; Kramer and Boyer, 1995). In woody plants, the root system consists of both mature, well-suberized roots (i.e. secondary growth) and young, elongating, not fully mature roots (Comas et al., 2013). The relative contribution to water uptake of each portion of the root system depends on their length, developmental stage, and anatomical and physiological characteristics (Queen, 1967; Kramer and Boyer, 1995; Gambetta et al., 2013). In grapevine, the mature, well-suberized part of a woody root system possesses a low root hydraulic conductivity ($L_p$) relative to young elongating roots (Queen, 1967; Kramer and Boyer, 1995). To date, there is a lack of understanding of how, when, and under what conditions fine roots transition to a mature suberized state, and if their development varies among rootstocks and with water availability. One recent study has suggested that a sustained proliferation of young roots late in the growing season was associated with a higher $L_p$ of a drought-resistant grapevine rootstock (Alsina et al., 2011). A decrease in $L_p$ under drought could also be beneficial by reducing water loss from roots to the drying soil (Oosterhuis and Wiebe, 1986; North and Nobel, 1991; Steudle, 2000; Aroca et al., 2012). Roots can be conditioned to regulate flow and direction of water movement (Molz and Peterson, 1976; Richards and Caldwell, 1987), and understanding how soil moisture regulates these properties could be used to improve genotype-specific management for grapevine rootstocks.

Water deficits affect how water moves from the soil to the root xylem by changing the relative contribution of the apoplastic and cell-to-cell pathways (Steudle and Frensch, 1989; Schreiber, 2010; Aroca et al., 2012). The contribution of each pathway depends on the existing driving force (i.e. water potential gradient) and the hydraulic properties of the path (Azaizeh and Steudle, 1991; Steudle, 2000; Lopez et al., 2003; Hachez et al., 2012; Gambetta et al., 2013). Under drought, the matric potential of the soil decreases (i.e. water is held more tightly in porous surfaces) and a higher driving force is needed for root water uptake; as long as the soil–root contact is maintained (Comas et al., 2013). A hydrostatic force (i.e. negative pressure gradient) resulting from shoot transpiration is the main driver for water movement into and across roots, and flow under these conditions is dominated by the apoplastic pathway. Flow in the cell-to-cell pathway is driven mainly by an osmotic gradient from the soil to the root, and under water deficit this pathway can contribute more to the movement of water across fine roots (Steudle and Meshcheryakov, 1996; Knipfer and Fricke, 2011). This switch in pathway contribution to water uptake has been associated with the development of apoplastic barriers (i.e. exodermis and endodermis) and the abundance and activity of aquaporins embedded in cell membranes that alter root $L_p$ (Saliendra and Meinzer, 1992; Steudle, 2000; Hachez et al., 2012; Gambetta et al., 2013). Osmotic adjustment by the active accumulation of solutes plays a role in how grapevines respond to drought (Düring and Dry, 1995; Patakas et al., 2002). This mechanism can help to maintain water movement into roots, and allows continued root growth under decreasing soil moisture (Chaves et al., 2003). Accumulation of solutes in grapevine root tissues could increase the contribution of the cell-to-cell pathway especially under low flow conditions imposed by drought stress (e.g. Vandeleur et al., 2009). Little is known of how changes in osmotic gradients and fine root conductivity contribute to differential responses of grapevine rootstocks to drought stress, and whether they play an important role in limiting root leakiness to drying soil conditions.

Generation of root pressure ($P_r$) results from an osmotically driven water flow usually considered to be induced by the accumulation of solutes in the xylem sap (Kramer and Boyer, 1995). Although details of the phenomenon are still under debate (e.g. Enns et al., 2000; Pickard, 2003; Wegner, 2014), it is generally thought that the presence of a suberized barrier (i.e. Casparian band) can act as a differentially permeable membrane for the accumulation of solutes in the stele (Steudle, 2000). In this case, the timing and extent (i.e. how completely these barriers form) of suberized barriers in fine roots may affect the ability of a rootstock to develop $P_r$. While $P_r$ has been linked to the process of refilling embolized xylem vessels (Sperry et al., 1987; Brodersen et al., 2010; Brodersen and McElrone, 2013; Knipfer et al., 2015) and to rootstock vigour (in kiwifruit; Clearwater et al., 2007), this hydraulic trait could be used to inform us about the formation of suberized barriers. Greater $P_r$ generation would suggest that suberized barriers have developed earlier and more completely, helping to prevent back flow of water and solutes; $P_r$ could provide an integrated measurement to compare differences in suberization across rootstock materials.

Using widely adopted commercial rootstocks, the idea was tested that drought resistance in grapevine rootstocks is associated with maintaining fine root conductivity under water deficits, limiting leakiness back to drying soils, and adjusting osmotically to maintain water uptake. Two commercial grapevine rootstocks, characterized as low–medium (101-14Mgt) and highly (110R) drought resistant, were studied under two soil moisture regimes to determine how root anatomical and physiological hydraulic traits respond to soil water deficits and affect water uptake. Root pressure–flow relationships, fine root conductivity under both osmotic and hydrostatic pressure gradients and into and out of the roots, sap exudation, root pressure generation, sap osmotic potential, and osmotic relaxation curves were measured to determine the half-time of water exchange between root xylem and the medium ($T_{1/2}$). These measurements were combined with analysis of suberization in young roots.

**Materials and methods**

**Plant material and experimental set-up**

A series of experiments were conducted in greenhouse facilities during the summers of 2013 and 2014 at the University of California,
All experiments were conducted under the same growing conditions. Two grapevine rootstocks were grown from green cuttings: 110 R (V. berlandieri × V. rupestris parentage) is considered as high vigour and drought resistant; and 101-14Mgt (V. riparia × V. rupestris parentage) is considered as low to moderate vigour and low–medium drought resistant (Christensen, 2003). Plant material was obtained from actively growing green shoots from mother plants maintained in greenhouses or in the field. For each experiment, the material was obtained from the same location (i.e. either the greenhouse or the field). The green cuttings were segments of stems that included two nodes on either end, and of similar size (i.e. stem dry weight was similar between rootstocks by the end of the experiments). The top node had a bud for shoot growth, and the bud in the bottom node was cut off to promote root growth. The bottom node of the cutting was dipped into a 2.5% rooting solution (Earth Die & Mfg. Inc., Portland, OR, USA) and watered to pot capacity once a day for at least four consecutive days to help establishment. No further water was used. Relative humidity was estimated to be between 40% and 80%, from data available for other greenhouses in the same growing period as the present studies. The average solar radiation was 303 W m⁻² (California Department of Water Resources, 2015).

After the establishment period, plants were randomly assigned to two moisture treatments: (i) plants with prolonged and repeated drying cycles or (ii) plants with frequent watering (‘dry’ and ‘wet’ treatments, respectively). Plants were kept under the ‘dry’ and ‘wet’ treatments for a period of 15–20 d, with the ‘dry’ treatment receiving water every 5 d on average. This watering regime ensured that roots in the ‘dry’ treatment grew most of the time under lower soil moisture availability than the ‘wet’ treatment. All pots were weighed daily to estimate the soil moisture content from water loss and total evapotranspiration. The ‘wet’ treatment received water every day to replace water lost. Irrigation was always done with 10% Hoagland solution for both moisture treatments. The experiments were terminated at the end of a drying cycle, and when the first root tips reached the base of the pots. This approach was used to prevent root tip damage and negative effects on root development (e.g. root bound). For all the experiments conducted in this study (details below), the average minimum moisture content that the ‘dry’ and ‘wet’ treatments reached was 7–9% and 15–17%, respectively.

Plant biomass

On the day that measurements were conducted, the shoot was severed 3 cm above the soil surface, leaving a stump for conducting whole-root system measurements (details below). The leaf area of the excised shoot was measured by taking an image of all the leaves and then processing with ImageJ software. After each experiment, the sand was washed off from the root system, and a digital image taken to evaluate root diameter at the three root segments of interest (see staining procedure) in a subset of roots (n=16). The short 3 cm stem, and the shoot and the root fresh weights were recorded. All biomass was dried at 60 °C and weighed after 24 h.

Staining procedure

A berberine–aniline blue fluorescent staining procedure was used to stain hand-cut root sections as described by Brundrett et al. (1988). Root cross-sections were taken at three different locations along the maturation zone, where the endodermis and the primary xylem and phloem differentiate (see fig. 2 in Gambetta et al., 2013): (i) before any lateral root had emerged (2–3 cm from tip); (ii) where lateral roots were growing (4–6 cm from tip); and (iii) before the rupture and loss of the cortex cell layers (8–10 cm from the tip). After staining, root sections were mounted on a slide and observed within a 4 h period using an Olympus VanoxAH-2 microscope (Olympus Optical Co., Ltd, Shibuya-Ku, Tokyo, Japan) or a Zeiss Axio microscope (Carl Zeiss, Oberkochen, Germany). A total of eight plants were used, with several cross-sections from each root segment.

Osmotic experiment

A root exudation study was conducted at the end of the third drying cycle, and all plants (i.e. ‘dry’ and ‘wet’ treatments) received enough water within 2 h prior to measurement to ensure that all pots reached their maximum water-holding capacity. It was assumed that soil matric potential was close to zero and that osmotic potential dominated the overall soil water potential. The experiment was done on intact, whole-root systems after the shoot was severed leaving a 3 cm stump where a 2.0 mm internal diameter Tygon tubing (R-3603) was tightly fitted. Sap was sampled in a pre-weighed pipette tip at time intervals of 30 min to be able to collect at least 15 µl of sap exudate. A total of four collections were made over a period of 120 min from when the shoot was cut. At each collection time, the pipette tip containing sap was weighed, the sap transferred to a microcentrifuge tube, and immediately placed on ice. For each root system, the collected exudate was saved into two tubes (i.e. tube 1, 30 and 60 min; and tube 2, 90 and 120 min collection times). This was done to test if the osmolarity of the sap changed during the first and second half of the entire collection time (i.e. 120 min total).

Within 24 h of the root exudation experiment (i.e. sap collection), the roots were harvested for biomass (see details above), and sand samples were collected from five different locations along the length of the pot to measure the osmotic potential of the root medium. The osmotic potential of the soil solution in the sand was estimated according to a dilution technique described by Sands and Reid (1980). About 18 g of bulk, wet sand were collected in a 50 ml Falcon tube and kept at 4 °C. Deionized water was added to increase the moisture to 0.3 g g⁻¹, and the sand and water were mixed thoroughly and allowed to settle for 1 h. The supernatant was transferred to a 2.0 ml Eppendorf tube, centrifuged at 7500 rpm for 10 min, and the osmotic potential determined. The osmotic pressure of the sap and soil extract was measured using the VAPRO 5600 osmometer (Wescor Inc., South Logan, UT, USA).

Hydrostatic experiments

Similar to the previous experiment, an intact root system was used to measure Lₚₒₒₑ. The shoot was severed and the root system was carefully removed from the pot and submerged in water to wash off the sand. The root system was immediately placed in a plastic container with deionized water inside a custom-made pressure chamber (PMS Instrument Company, Albany, OR, USA). A 1 ml graduated pipette was connected to a 1 cm stem protruding from the chamber lid. Pressure–flow relationships were measured by pressure increments of 0.05 MPa until a final pressure of 0.25 MPa. At each step, a period of at least 5 min was taken to obtain a steady-state flow and take a measurement (i.e. volume per time). After the pressure–flow measurements, the root system was removed, rinsed, padded dry, and fresh weight recorded.

Root pressure experiments

A system composed of several pressure transducers (PX26-005GV, Omega Engineering, Inc., Stamford, CT, USA) connected to a datalogger (model CR7, Campbell Scientific, Logan, UT, USA) was assembled for continuous data collection to facilitate

Downloaded from http://jxb.oxfordjournals.org/ by guest on July 9, 2015
numerous simultaneous root pressure measurements. The \( P \) measurements of the intact root systems were determined by connecting a pressure transducer on the 3 cm stump left after the shoot was severed (Supplementary Fig. S1 available at JXB online). An hour prior to this (i.e. ~08.00 h), all plants were fully watered to saturation to ensure similar soil water availability on both moisture treatments. The shoot was covered by a Mylar-wrapped plastic bag for at least 15 min, excised with the stem under water, and then measured for stem water potential with a Scholander-style pressure chamber (#3005; SoilMoisture Equipment Corp., Goleta, CA, USA). A 20 mM potassium chloride (KCl) solution was used to wet the area of the cut. A 1 cm segment of the remaining stem was inserted into semi-rigid 2 cm PVC tubing filled with a 1 cm non-toxic, dental impression polymer (Pentron Clinical Technologies, LLC, Wallingford, CT, USA). The pressure transducer and the connection to the stem were filled with 20 mM KCl and extra care was taken to remove any air bubbles. The pressure transducer and the stem connection were connected through a polypropylene male–female luer fitting (Value Plastics, Fort Collins, CO, USA). Plastic repair epoxy was used to seal all joints of the connection (Part #: 17394, Ace Hardware Corp., Oak Brook, IL, USA). Once attached, data were recorded continuously every 10 s throughout the duration of the studies. A 3 h period after the root systems were connected to the pressure transducers was taken to reach a steady-state root pressure (\( P_{rs} \); see Fig. 1) and to ensure that no leaks were present. At this point, the \( P \) changed at a 2.2 ± 1.9% rate over a period of 1 h and the system was considered to be at a steady state under well-watered conditions. Subsequently, 150 ml of sucrose solution (40 mM) were added to each pot, which resulted in water flow out of the root driven by an osmotic pressure gradient between the root and soil to obtain an exosmotic relaxation curve (time: 0 min; Fig. 1). After a new steady-state was reached after ~190 min, a similar amount of deionized water was applied to decrease the osmotic potential of the soil medium and induce water to move from the soil to the root. The \( P \) was expected to increase to a similar level to the initial \( P_{rs} \) and confirm that the system had no leaks. Pots were free to drain excess sucrose solution or water applied at 0 and 190 min, respectively. After completing the experiments, pressure transducers were detached from the root system, and the pots were kept at 4 °C until further processing within the next 24 h, as described above.

Calculations of hydraulic conductivity

Root hydraulic conductivity (\( L_p \)) was determined by volume flow rate divided by the driving force for the flow of water between root xylem and medium and the whole-root system fresh weight. In young grapevine rootstocks with no lignified roots, the total fine root fresh weight strongly correlates with root surface area (Gambetta et al., 2012).

From the root exudation experiment, the sap exudate rate (units: \( \text{m}^3 \text{g-} \text{FW}^{-1} \text{s}^{-1} \)) was calculated from the slope of the linear regression (\( R^2 \geq 0.97 \)) of exudate amount over time (\( \text{m}^3 \text{s}^{-1} \)), and the fresh weight of the entire root system (g FW). The osmotic potential was expressed in MPa, where 0.75 mM Osmol kg\(^{-1}\) corresponds to 0.1 MPa (Fricke et al., 2014). The root osmotic conductivity (\( L_{pw} \); units: \( \text{m}^3 \text{g-} \text{FW}^{-1} \text{s}^{-1} \text{MPa}^{-1} \)) was calculated by relating the sap exudate rate and the difference in osmotic potential between the xylem sap and the root medium. From the hydrostatic experiments, the root hydrostatic conductivity (\( L_{hpw} \); units: \( \text{m}^3 \text{g-} \text{FW}^{-1} \text{s}^{-1} \text{MPa}^{-1} \)) was calculated by the slope of the linear regression (\( R^2 \geq 0.98 \)) from the pressure–flow relationship (units: \( \text{m}^3 \text{s}^{-1} \text{MPa}^{-1} \)) and the fresh weight of the entire root system (g FW).

\( P \) was obtained immediately before the osmotic relaxation experiments were started (Fig. 1). The osmotic relaxation curves were used to estimate the half-time of water exchange between root xylem and soil medium (\( T_{1/2} \)). The raw data (i.e. ~130 data points for each curve) were fitted using the NLIN procedure of SAS (SAS Institute, Cary, NC, USA) into the exponential function: 

\[
y(t) = a + b \times e^{-c(t-r)}
\]

where \( a \) is the final stationary root pressure, \( b \) is the difference between the initial and final root pressures, \( c \) is the corresponding rate constant of the relaxation in pressure \([k = \ln(2)/T_{1/2}]\), and \( t \) is time (Steudle and Fresnch, 1989; Knipfer et al., 2007). The fit of the osmotic curves was tested, and the \( R^2 \) was on average >0.96. Based on the determination of \( L_p \) from root pressure probe experiments (e.g. Azaizeh and Steudle, 1991), the osmotic Lp (\( L_{os} \)) was estimated by \( L_{os} = \ln(2)/(T_{1/2} \times \text{FW} \times \beta) \), where \( \text{FW} \) is the total root fresh weight and \( \beta \) is the elastic coefficient of the root and measuring system. \( \beta \) was considered to be constant across rootstock and moisture treatment, and a value of unity was used; thus, these data are reported as arbitrary units. Typical values for \( \beta \) have been reported to be between \( 0.5 \times 10^9 \) and \( 2 \times 10^9 \text{m}^3 \text{g}^{-1} \text{MPa}^{-1} \) (Azaizeh and Steudle, 1991; Knipfer et al., 2007).

Statistical analysis

For all experiments, the statistical design was a randomized complete block design (RCBD). The main factors were ‘rootstock’ and ‘moisture’ treatments. Each of the exudation and root pressure experiments had two blocks, and during intense data collection (e.g. root pressure or sap exudate), each experiment was evaluated in 2 d (i.e. one block per day) with all measurements conducted at similar times among blocks and experiments. The exudation experiments had a total of 32 plants and the two root pressure experiments had a total of 16 plants each. The hydrostatic experiments had a total of 28 plants measured across three experiments. For the root pressure experiments, the data from both experiments were pulled together. Thus, ‘experiment’ was included as a third factor, and data analyses were conducted using the MIXED procedure of SAS, Version 9.3 (SAS Institute). For the exudation and hydrostatic experiments, the analysis of variance (ANOVA) was performed using the GLM procedure of SAS. The Shapiro–Wilk W test for normal distribution and Levine’s test for homogeneity of variance were used to test that data fulfilled the ANOVA assumptions. Data were transformed as necessary when assumptions were not met. Tukey–Kramer HSD test was used to determine significant differences among treatments.

Results

Suberized barriers developed closer to the root tips in both rootstocks with prolonged drying cycles compared with frequent watering (Fig. 2). Plants in the ‘dry’ treatment showed
a more advanced development of the exodermis around the root from sections at the beginning of the maturation zone and before any lateral roots had emerged (i.e. 2–3 cm away from the root tip; Fig. 2). In this zone, plants in the ‘wet’ treatment had minimal or no signs of suberization in the exodermis. Within the maturation zone, where lateral roots were growing (4–6 cm from tip), the exodermis of the ‘dry’ treatment was almost completely suberized and the endodermis showed a more advanced degree of suberization than the ‘wet’ treatment (Fig. 3). The exodermis and endodermis of the ‘wet’ treatment showed areas with no or minimal suberization although the degree of suberization was more advanced compared with sections closer to the root tip (i.e. Fig. 2). Differences in the degree of suberization between the moisture treatments were less obvious further from the root tip and closer to the initiation of the secondary growth (data not shown). While the fluorescence microscopy method may not be conclusive about subtle differences, it appears that the pattern of suberization differs across the rootstocks in response to the moisture treatment. Suberization at both the exo- and endodermis develops earlier and more completely for 101-14Mgt compared with 110R even under frequent watering (Figs 2, 3).

Root sap exudation rates were similar between the two moisture treatments, but it was on average 40% higher in 110R than in 101-14Mgt (9.6E-12 ± 5.8E-13 m³ g-FW⁻¹ s⁻¹ and 6.8E-12 ± 3.7E-13 m³ g-FW⁻¹ s⁻¹, respectively; P < 0.01; Fig. 4A). Interestingly, under ‘dry’ conditions, 110R maintained a >50% higher exudation rate than 101-14Mgt despite its lower root pressure (i.e. Pₑ). The sap osmotic potential was 13% higher in the ‘dry’ than in the ‘wet’ treatment (moisture P < 0.001; Fig. 4B). The osmotic potential of the sap did not change with the time of collection (i.e. first versus second hour of collection; data not shown). The soil solution osmotic potential was not different across moisture treatments and rootstocks (0.052 ± 0.001 MPa).

Lₚ varied by moisture conditions and across the rootstocks (Figs 4C, 5, 6). Under osmotic driving forces, the Lₚₒₛ decreased under prolonged drying cycles compared with frequent watering (moisture P < 0.01), and 110R had higher Lₚₒₛ than 101-14Mgt (rootstock P < 0.01) (Fig. 4C). The ‘dry’ treatment reduced the Lₚₒₛ of 101-14Mgt by 50% compared with the ‘wet’ treatment, but no differences were observed within 110R. In the ‘dry’ treatment, the 110R Lₚₒₛ was twice as high as the Lₚₒₛ of 101-14Mgt, but in the ‘wet’ treatment the Lₚₒₛ was similar between the rootstocks (Fig. 4C). Under hydrostatic driving forces, the Lₚₕᵧd decreased in the ‘dry’ treatment by 45% compared with the ‘wet’ treatment (moisture P < 0.001; Fig. 5), and 110R had on average a 1.3-fold greater Lₚₕᵧd than 101-14Mgt (rootstock P < 0.05; Fig. 5). The reduction in Lₚₕᵧd under ‘dry’ conditions was 55% in 101-14Mgt and 36% in 110R compared with their respective ‘wet’ treatments. The Lₚₕᵧd was 2-fold greater than the Lₚₒₛ under well-watered conditions (i.e. ‘wet’ treatment), and under ‘dry’ conditions the Lₚₕᵧd was 1.8- and 1.5-fold greater than the Lₚₒₛ of 101-14Mgt and 110R, respectively.

The T₁/₂ was higher in the ‘dry’ than in the ‘wet’ treatment of 110R, but all other comparisons between rootstock × moisture interactions were similar (Fig. 6). When Lₚₒₛ was derived from the measured T₁/₂, the rootstocks were affected differently by the moisture treatment (Fig. 6). For 110R, the Lₚₒₛ was 65% lower in the ‘dry’ than in the ‘wet’ treatment, decreasing water outflow for low-flow rates driven by an osmotic gradient. In contrast, the Lₚₒₛ was similar between the moisture treatments of 101-14Mgt.

The Pₑ increased on average by 44% in plants with prolonged drying cycles (‘dry’, 0.10 ± 0.01 MPa; ‘wet’, 0.14 ± 0.01 MPa).
0.07 ± 0.01 MPa; Fig. 7). The $P_{rs}$ increased by 67% for 110R growing under ‘dry’ conditions compared with the ‘wet’ treatment. The $P_{rs}$ in 101-14Mgt was not different between the ‘dry’ and ‘wet’ treatments. Overall, 110R had ~35% lower $P_{rs}$ than 101-14Mgt, regardless of moisture treatment.

Total evapotranspiration was >40% lower in plants under prolonged drying cycles than under frequent watering, with no differences between rootstocks (Table 1). Most of the evapotranspiration was due to soil evaporation (data not shown) because of the relatively small leaf area of the plants. The total fresh root biomass increased 30% in plants under ‘dry’ conditions compared with ‘wet’ conditions (2.52 ± 0.27 g and 1.94 ± 0.27 g, respectively; Table 1). The root dry matter content was 11% higher in plants under ‘dry’ than under ‘wet’ conditions (‘dry’, 9.08 ± 0.28 g; ‘wet’, 8.18 ± 0.28 g), and it was 20% higher in roots of 110R than in roots of 101-14Mgt, regardless of moisture treatment (data not shown). On average, plants had 3–5 main roots actively growing with intact tips, and the rate of growth is estimated as 0.8 cm d$^{-1}$ for the fast growing roots (i.e. roots that reached the bottom of the pot), regardless of rootstock. Root diameter varied among roots at the same developmental stage even from the same plant, but root thickness decreased consistently from the tip (1.60 mm on average; not including the 1 cm closest to the tip) towards the more mature root segments. The root segment between 4 cm and 6 cm from the tip had on average a 9% smaller diameter (1.46 mm in average), while the 8–10 cm segment from the root tip had a 27% smaller diameter (1.16 mm in average). The thicker roots were ~1.94 mm and the thinner roots were 0.87 mm in diameter. The root:shoot ratio was not affected by moisture availability, and it was consistently higher in 110R than in 101-14Mgt by at least 35% (Table 1).

![Fig. 3. Patterns of suberization in the maturation zone for two grapevine rootstocks (101-14Mgt and 110R) grown under ‘dry’ and ‘wet’ conditions. Root sections were taken 4–6 cm from the root tip, where lateral roots had emerged. Suberization of the exodermis (arrows; A–D) is more advanced on both rootstocks grown under ‘dry’ conditions (A and C), while under ‘wet’ conditions (B and D) areas not yet suberized are easily observed (arrowheads). Suberization of the endodermis (arrows; E–H) is more developed under ‘dry’ conditions (E and G), while under ‘wet’ conditions (F and H) areas less suberized are observed (arrowheads). Scale bars=100 μm.](http://jxb.oxfordjournals.org/)

Discussion

This study found that two grapevine rootstocks exhibited differential responses of hydraulic physiology when subjected to water stress, which could help to explain their known differences in drought resistance. The drought-resistant rootstock (110R) maintained water uptake capacity (i.e. unchanged $L_{p_{os}}$ and smaller decrease in $L_{p_{hyd}}$) and reduced root leakiness (i.e. larger decrease in $L_{p_{os_{ex}}}$) under drought conditions (summarized in Fig. 8). Both rootstocks exhibited increased suberization under water deficit, but the low drought-resistant rootstock (101-14Mgt) appeared to develop these layers more rapidly even under well-watered conditions. This qualitative assessment of suberization was consistent with greater $P_{rs}$ in 101-14Mgt under both watering treatments. Grapevine rootstocks that maintain higher root $Lp$ would be able to maintain a greater water supply
Fine root hydraulic physiology in grapevine rootstocks subjected to drought

The findings demonstrate the utility of studying drought resistance as a suite of inter-related traits as found in other study systems (Comas et al., 2013; Tramontini et al., 2013; Barrios-Masias and Jackson, 2014).

The formation of apoplastic barriers reduces root $L_P$ and forces a greater proportion of water to pass through the cell-to-cell pathway (Steudle, 2000). For both rootstocks studied here, suberized apoplastic barriers formed more rapidly and closer to the growing root tip in response to water deficit (i.e. the roots matured more rapidly). Suberization of the exodermis is a common response to water deficit and other stressors for many woody and annual species (Zimmermann and Steudle, 1998; Schreiber, 2010; Hachez et al., 2012; Comas et al., 2013). In grapevine, suberization has been shown to start in the maturation zone, and was linked to a reduced fine-root $L_P$ (Gambetta et al., 2013). Similar reductions in root $L_P$ with suberization have been documented for other crops (Saliendra and Meinzer, 1992; Zimmermann and Steudle, 1998). It was found, however, that the water deficit treatment caused a greater reduction in $L_{P\text{os}}$ and $L_{P\text{hyd}}$ in the drought-sensitive 101-14Mgt compared with the drought-resistant 110R. This could result from incomplete or slightly delayed formation of apoplastic barriers or differences in the chemical composition of the suberin in 110R under water deficit compared with 101-14Mgt (Schreiber et al., 2010). Previous
work has documented greater inherent aquaporin expression and activity in 110R compared with 101-14Mgt (see Lovisolo et al., 2008; Gambetta et al., 2012). Higher aquaporin activity could maintain water uptake capacity (i.e. less hydraulic resistance of the tissue) through a greater contribution of the cell-to-cell pathway (e.g. similar \( L_p \) osm under ‘dry’ and ‘wet’ conditions).

Under conditions of high transpirational demand, the rate at which water uptake is taken up through the apoplastic pathway can result in transient water stress in rootstocks with lower \( L_p \) hyd (e.g. 101-14Mgt) and hamper growth and yield. On the other hand, the lower \( L_p \) of the drought-sensitive rootstock (i.e. 101-14Mgt) may conserve water in the soil profile for longer periods of time (Oosterhuis and Wiebe, 1986; Aroca et al., 2012; Hachez et al., 2012; Tramontini et al., 2013). Some viticulturalists advocate for the use of rootstocks like 101-14Mgt in order to control vigour and utilize soil water resources more judiciously in growing regions with little to no summer precipitation (Greenspan, 2006, 2008). The current findings may help to explain mechanisms behind differences in drought resistance for which growers have already been accounting. The drought-sensitive rootstock (101-14Mgt) is known to recover slowly from water deficit; earlier suberization and decreased \( L_p \) would limit uptake potential upon rewatering and the rootstock may need to resume growth of root tips that have lower hydraulic resistance in order to take full advantage of the available water. These hydraulic characteristics may also contribute to the inherently lower vigour of 101-14Mgt.

The osmotic potential of the xylem sap increased with prolonged soil drying cycles in both rootstocks studied here. Changes in osmotic potential of leaf and root extracts have been observed as a response to water deficit for many plant species including grapevine (Patakas et al., 2002; Lian et al., 2004; Dichio et al., 2006), and are thought to help maintain favourable water potential gradients under drying conditions. By saturating the soil with water before the sap exudate measurements, soil water potentials were close to zero, allowing any changes in the osmotic driving gradient to be accounted for. Sap exudate rates were significantly lower in 101-14Mgt under dry conditions despite an increase in the sap osmotic potential (i.e. larger driving gradient), which is consistent with the measurements of increased hydraulic resistance along the uptake pathway. When accounting for sap osmotic potential, Salindra and Meinzer (1992) found that sugarcane cultivars differed in sap osmolality. This is consistent with the idea that mechanisms other than an osmotic process affect the development of a \( P_r \) rs of whole-root systems of two grapevine rootstocks (110R and 101-14Mgt) grown under ‘dry’ and ‘wet’ conditions. Data shown is the composite of two experiments. Values are mean ±standard error (n=8).

### Table 1. Water evapotranspired, total fresh root biomass, and root:shoot ratio of two grapevine rootstocks (110R and 101-14Mgt) grown under ‘dry’ and ‘wet’ conditions. Data shown is the composite of two experiments. Values are mean ±standard error (n=8). Means followed by different letters are significantly different at P<0.05.

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Moisture</th>
<th>Water evapotranspired (ml)</th>
<th>Fresh root biomass (g)</th>
<th>Root:shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>101-14</td>
<td>Dry</td>
<td>400 ±27 b</td>
<td>2.6 ±0.3 a</td>
<td>0.27 ±0.06 b</td>
</tr>
<tr>
<td>101-14</td>
<td>Wet</td>
<td>736 ±27 a</td>
<td>2.0 ±0.3 a,b</td>
<td>0.31 ±0.06 a,b</td>
</tr>
<tr>
<td>110R</td>
<td>Dry</td>
<td>392 ±32 b</td>
<td>2.5 ±0.3 a</td>
<td>0.53 ±0.06 a</td>
</tr>
<tr>
<td>110R</td>
<td>Wet</td>
<td>656 ±32 a</td>
<td>1.9 ±0.3 b</td>
<td>0.42 ±0.06 a,b</td>
</tr>
<tr>
<td>Main factor: Rootstock</td>
<td>NS</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>P&lt;0.0001</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. Steady-state root pressure (\( P_r \)) of whole-root systems of two grapevine rootstocks (110R and 101-14Mgt) grown under ‘dry’ and ‘wet’ conditions. Data shown are the composite of the two root pressure experiments. Values are the mean ±standard error (n=8). Means followed by different letters are significantly different at P<0.05.
back to the drying soil as root pressure increased. Conversely, the lower values of $P_{rs}$ for frequently watered 110R suggests less fully developed suberin layers that would result in backflow of accumulated solutes and water. Although root pressure is an effect of root osmotic potential (Kramer and Boyer, 1995), the $P_{rs}$ as measured in this study integrates several traits (e.g. formation of suberized barriers, osmotic adjustment, and $L_p$) that affect the root system capacity to generate a positive xylem pressure. Regardless of the exact role that $P_t$ plays in the response of grapevines to drought stress, the present results suggest that this parameter can provide useful information about differential development and functioning of suberized barriers across rootstocks that are extremely difficult to glean from microscopic or chemical analysis of the tissues.

The results show that grapevine rootstock responses to water deficit can be influenced by management (as suggested by Chaves et al., 2003; Comas et al., 2013). Irrigation strategies that account for rootstock characteristics could maintain water uptake capacity of young roots by managing soil moisture content to capitalize on genotype by management interactions. The moisture treatments simulated cycles of wetting and drying that rootstocks may encounter under real field management conditions (i.e. weekly or large pulse irrigation events). The focus of this study was on young fine roots because: (i) they contribute disproportionately more to root system water uptake (Gambetta et al., 2012, 2013); and (ii) the rate of maturation and suberization of this tissue would affect the water uptake capacity of the root system and differ among rootstocks. In this study, infrequent watering stimulated root growth, which is considered important to maintaining stomatal conductance under declining soil moisture (Alsina et al., 2011). Given that grapevine fine root functioning can change dramatically when subjected to water deficit over a short time period, this suggests that irrigation management could be used to modify the uptake capacity of a root system throughout the season. For example, 101-14Mgt could benefit from smaller but more frequent irrigation events to maintain a more conductive fine root tissue for a longer period into the growing season. Under persistent drought and limited water resources, rootstocks like 110R that maintain high water uptake capacity while limiting water loss to the soil may be ideal, and this suggests that drought resistance involves more than just deep rooting characteristics as suggested by Padgett-Johnson et al. (2003). In grapevine, breeding and selection of rootstocks for drought resistance may include screening protocols based on exudation experiments. The advantage is that they are straightforward to conduct and provide useful information that indicates changes in $L_p$ as affected by environmental conditions.

Supplementary data
Supplementary data are available at *JXB* online.

**Figure S1.** A pressure transducer connected to an intact grapevine root system after the shoot was severed 3 cm above the surface.

**Acknowledgements**
This work was supported by a National Institute of Food and Agriculture-Specialty Crops Research Initiative grant and funding from the American Vineyard Foundation to AJM, and by the US Department of Agriculture-Agricultural Research Service Current Research Information System (research project no. 5306-21220-004-00).

**References**


