

# Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil

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## ABSTRACT

Redistribution of water within plants could mitigate drought stress of roots in zones of low soil moisture. Plant internal redistribution of water from regions of high soil moisture to roots in dry soil occurs during periods of low evaporative demand. Using minirhizotrons, we observed similar lifespans of roots in wet and dry soil for the grapevine ‘Merlot’ (*Vitis vinifera*) on the rootstock 101-14 Millardet de Gramanet (*Vitis riparia* × *Vitis rupestris*) in a Napa County, California vineyard. We hypothesized that hydraulic redistribution would prevent an appreciable reduction in root water potential and would contribute to prolonged root survivorship in dry soil zones. In a greenhouse study that tested this hypothesis, grapevine root systems were divided using split pots and were grown for 6 months. With thermocouple psychrometers, we measured water potentials of roots of the same plant in both wet and dry soil under three treatments: control (C), 24 h light + supplemental water (LW) and 24 h light only (L). Similar to the field results, roots in the dry side of split pots had similar survivorship as roots in the wet side of the split pots ( $P = 0.136$ ) in the C treatment. In contrast, reduced root survivorship was directly associated with plants in which hydraulic redistribution was experimentally reduced by 24 h light. Dry-side roots of plants in the LW treatment lived half as long as the roots in the wet soil despite being provided with supplemental water ( $P < 0.0004$ ). Additionally, pre-dawn water potentials of roots in dry soil under 24 h of illumination (L and LW) exhibited values nearly twice as negative as those of C plants ( $P = 0.034$ ). Estimates of root membrane integrity using electrolyte leakage were consistent with patterns of root survivorship. Plants in which nocturnal hydraulic redistribution was reduced exhibited more than twice the amount of electrolyte leakage in dry roots compared to those in wet soil of the same plant. Our study demonstrates that besides a number of ecological advantages to protecting tissues against desiccation, internal hydraulic redistribution of water is a mechanism consistent with extended root survivorship in dry soils.

*Key-words:* electrolyte leakage; root survivorship; root water potential; split pot.

## INTRODUCTION

Roots take up water and serve as a major water conduit in the soil–plant–atmosphere continuum. While transpiration is the major driving force of water movement, water can redistribute within the plant at night or during periods of minimal transpiration, and may exit the roots and hydrate the rhizosphere (Richards & Caldwell 1987). The degree and rate that water redistributes within plant tissues is important, and species vary in resistances along this pathway. In our study, we focus on plant internal redistribution of water and whether or not water flowed out of roots to soil is inconsequential. During extended periods when transpiration is negligible such as night-time, hydraulic redistribution removes gradients in water potential among leaves and roots of different orders of branching (Hinckley, Lassoie & Running 1978; Boyer 1995). However, hydrologic factors that would increase transport to the canopy, including periods of nocturnal transpiration (Donovan *et al.* 1999; Donovan, Richards & Linton 2003), can disrupt internal root-to-root water redistribution. Internal hydraulic redistribution may alleviate plant water stress by maintaining shallow root function (Domec *et al.* 2004), maintaining cell turgor for plant growth (Hsiao & Xu 2000), preventing loss of root hydraulic conductivity (Nobel & Cui 1992), supplying water for night-time increases in leaf turgor (Blum & Johnson 1992) and presumably maintaining leaf water content in plants exposed to drought (Nardini & Pitt 1999). Internal hydraulic redistribution has also been hypothesized to mitigate drought conditions by refilling root xylem embolisms (McCully 1999) and by preserving plant root viability (Huang 1999). Even under severe drought conditions, if a portion of the root system is maintained in wet soil, internal hydraulic redistribution can continue to occur, and fine roots in the dry soil locations can retain their function (Williams, Caldwell & Richards 1993).

We are not aware of any studies that have examined the influence of internal hydraulic redistribution on root water potential status and root tolerance of desiccation, although a number of studies have now investigated water transfer from wet to dry soil through the root system (Baker & van Bavel 1986; Richards & Caldwell 1987; Dawson 1993).

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Espeleta, West & Donovan (2004) demonstrated indirectly that hydraulic lift influences root lifespan by enclosing roots in a hydraulically isolated 'root chamber' and by measuring soil water potential within the chamber. Roots used in the study were relatively large in diameter (0.5–1.0 cm), and although two roots were placed in each chamber at the start of the experiment, there did not appear to be any control over the number or diameter of lateral roots that might have affected final soil water potential. We are not aware of any past examination of the influence of internal water redistribution on root water potential and lifespan of individual roots of the finest laterals of a root system (typically 0.5 mm or less; Eissenstat & Yanai 1997).

Prolonged fine root lifespan can have beneficial implications for the plant as a whole including maintained nutrient uptake capacity (Matzner & Richards 1996; Eissenstat *et al.* 1999) and mineral nutrient retention (Aerts *et al.* 1989). Some reports have indicated that during periods of decreased water availability, the very small movement of water associated with hydraulic redistribution has been adequate enough to prevent root mortality in some species, like oak seedlings (*Quercus agrifolia*; Querejeta, Egerton-Warburton & Allen 2003), four citrus seedlings (*Citrus sinensis* × *Poncirus trifoliata*, *Citrus paridisi* × *Poncirus trifoliata*, *Citrus aurantium* and *Poncirus trifoliata*; Kosola & Eissenstat 1994) and mature grapevines (*Vitis labruscana*; Anderson *et al.* 2003), while others have reported it does not in species such as some bunch grasses (*Schizachyrium scoparium*; Espeleta *et al.* 2004), mesic oaks (*Quercus margaretta*; Espeleta *et al.* 2004) and tomato (*Lycopersicon esculentum* Mill.; Reid *et al.* 1996).

Very fine roots of the ultimate laterals, which may readily lose water as a consequence of higher surface area to volume and/or lack of suberization, may be more susceptible to drought (Huang, Duncan & Carrow 1997). Hydraulic constraints of fine roots such as small xylem vessel diameter and a high incidence of xylem cavitation are believed to contribute to shortened root lifespan (Sperry, Stiller & Hacke 2002). Nonetheless, roots may exhibit plasticity in as much as roots exposed to water stress have been shown to invest in apoplastic barriers such as increased suberization of the exodermis and endodermis, presumably reducing hydraulic flow back into the soil during nocturnal hours where the water potential gradient is in the direction of the soil (North & Nobel 1991). Exodermal secondary cell wall development is hypothesized to provide structural support against radial weakening of the root, thus preventing root collapse and the formation of air gaps between the root and the surrounding soil (Taleisnik *et al.* 1999). Finally, aquaporins within root cells may close and decrease water loss (Stedde 2000). All of the aforementioned factors may serve to increase root lifespan during episodes of drought stress.

We studied the links between internal hydraulic redistribution and root lifespan in grapevines. Researchers have demonstrated previously both the existence of hydraulic redistribution (Smart *et al.* 2005) and similarity in fine root lifespan of grape roots grown in wet and dry soil (Anderson

*et al.* 2003). The objectives of this study were to examine the consequences of internal hydraulic redistribution on root water potential and fine root longevity. We hypothesized that roots in wet and dry soils have similar lifespans when internal hydraulic redistribution occurs. Restricting water movement in the roots by continuous illumination at night should lead to a sustained decline in water potential and a more rapid death of branch roots in dry soil as a consequence of restricting the redistribution of water from branch roots in moist soils.

## MATERIALS AND METHODS

### Field experiment

The *Vitis vinifera* cv. Merlot vineyard was about 8–11 years old during the period of study and was planted in Bale (variant) gravelly loam (fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll). The Mediterranean climate of the Oakville region, Napa County, CA, USA averages 83 cm of annual precipitation and has a mean annual temperature of 14.3 °C [California Irrigation Management and Information System (CIMIS) 2005, <http://www.cimis.water.ca.gov/>]. The vineyard had a north-east to south-west row orientation with vines spaced 2.4 m between rows and 2.2 m within the row, and trained on a bilateral cordon with vertical shoot positioning (VSP) (Winkler *et al.* 1974).

The entire experimental vineyard covered 1 ha and had three irrigation treatments and three rootstock cultivars laid out in a completely randomized block design. Surrounding vine rows served as buffers to separate treatments. Each vine had one emitter, located 50 cm from the trunk on one side of the vine. The irrigation treatments [no irrigation, 40% (deficit irrigation), and 100%  $E_t$ ] were determined using crop evapotranspiration ( $E_{tc}$ ) calculated from the evaporation of a class A pan and the Penman–Monteith equation ( $E_{t0}$ ), and were corrected with crop coefficients ( $K_c$ ) put forward by Pritchard (1992). In this study, only vines in the 100%  $E_{tc}$  treatment on 101-14 Millardet de Gramanet (*Vitis riparia* × *Vitis rupestris*) rootstocks (101-14 Mgt.) were reported.

In April 2002, clear plastic (cellulose acetyl butyrate) root observation tubes (minirhizotrons) were installed. Tubes were 1.3 m long and had 6 cm outside diameter. The tubes were sealed with polyvinyl chloride (PVC) plugs, and the tops were wrapped with black electrical tape and sealed with rubber stoppers to prevent light penetration. When not in use, the tops of the tubes were covered with white metal radiation shields to prevent radiant heating. Each minirhizotron tube was inserted parallel to the vine row at a 30° angle from vertical, and about 60 cm from the trunk. One minirhizotron tube was placed through the dripper zone and the other in the unirrigated zone on the opposite side of the vine. Thus, there were 2 tubes per vine representing irrigated and non-irrigated treatments × 1 irrigation level × 1 rootstock × 6 blocks, for a total of 12 tubes in this study.

Root images were captured using a BTX-100× camera equipped with BTC I-Cap version 4.01 imaging software

(Bartz Technology, Santa Barbara, CA, USA). Images were captured approximately every 2 weeks during the growing season (April–October) and every 4 weeks during the dormant period (November–March). Root diameters were measured with WinRhizo Tron MF software (Regents Inc., Quebec, Canada). Root births were estimated by calculating the date midway between the observation date when a root was first observed and the previous observation date. Similarly, root death was estimated as being midway between the date the root was first observed dead and the previous observation date. Root death was identified by a black and shriveled appearance (Comas, Eissenstat & Lakso 2000) or if the root had disappeared from the window and did not reappear. Roots that transected more than one minirhizotron observation window vertically within the same minirhizotron observation tube, identified by position, were only counted once. Volumetric soil water content was estimated using time domain reflectometry.

The minirhizotrons were used as access tubes for soil moisture determination using a TRIME soil moisture probe (Mesa Systems Co., Medfield, MA, USA) at 20–50, 50–80 and 80–120 cm depths. These depth intervals corresponded with the highest root densities. A soil water retention curve was determined using a pressure plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA, USA) under five levels of pressure to relate volumetric soil moisture (%) estimated by TRIME to soil matric potential (MPa). Bulk density measurements came from a parallel study in the same vineyard and were determined to be  $1.33 \pm 0.10 \text{ Mg m}^{-3}$  at 6–12 cm depth and  $1.45 \pm 0.05 \text{ Mg m}^{-3}$  at 40–46 cm depth (Carlisle, Steenwerth & Smart 2006).

### Greenhouse experiment

To assess mechanisms responsible for the field results, another study was conducted at The Pennsylvania State University greenhouses, University Park, PA, USA. Green cuttings from rootstock 101-14 Mgt. were collected from the Oakville vineyards in August 2005, rooted on a misting bench and then shipped to University Park, PA. The plants were transplanted into a mixture of 50% sand and 50% Hagerstown series soil, which was characterized by a dark brown silt loam layer (20 cm) in 5 L pots. Greenhouse temperatures during the daytime were  $25.0 \pm 3.0 \text{ }^\circ\text{C}$  and during the night-time were  $15.5 \pm 3.0 \text{ }^\circ\text{C}$ .

In November 2005, 6-month-old grapevine root systems were split into two 5 L containers with a 90°, 5 cm PVC elbow with the corner portion removed to bridge the two containers (Eissenstat 1990). The root system was evenly divided between the two outlets of the elbow bridge while the shoot protruded from the central hole. Pots were arranged in a completely randomized design with three replicates per treatment. Treatments were designed to control transpiration at night and to limit hydraulic redistribution by the root system (Caldwell & Richards 1989). Treatments were control (C), supplemental night-time light + supplemental water (LW) and night-time light only

(L). Control plants were kept under natural light conditions (12 h of illumination), while treatments that included supplemental light were kept under 12 h of natural illumination and 12 h of supplemental illumination during the night (minimum  $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$  photosynthetic photon flux density). Sides of the plant that were to be irrigated or left unirrigated were randomly chosen; wet-side pots of the C and L treatments were watered 400 mL once daily with drip irrigation emitters. The LW treatment was watered once daily with 600 mL to maintain leaf water potentials similar to that of the C treatment. Leaf water potentials of C and LW treatment plants were maintained within 0.1 MPa (measured with a Scholander pressure chamber, Soil Moisture Equipment Co.). For each pot, roots were tracked weekly on three 15 cm tall  $\times$  8 cm wide acetate windows located 120° apart on each pot. Root births and deaths were estimated in the same way as in the field study (see previous discussion). If the roots did not die or disappear by the end of the experiment, then the data were treated statistically as censored because the true death date was indeterminable.

Time domain reflectometry was used to measure volumetric soil water daily (TDR 100; Campbell Scientific, Inc., Logan, UT, USA). Probes were constructed from three parallel stainless steel rods, 20 cm in length and 3 mm in diameter. The probes were inserted perpendicular to the soil surface and remained in place for the duration of the experiment. A soil water retention curve was determined in the same way as in the field study (see earlier discussion).

Root water potential was determined using thermocouple psychrometry. Control plants were covered with 100% shade cloth on the evening of measurement to prevent night-time transpiration (Caird, Richards & Donovan 2007). Roots less than 1 week old were collected at pre-dawn (0500 h) from both the wet and dry pots every other day for 2 weeks. Using a razor blade, the acetate window was carefully cut and peeled away, and a first-order root (root with no laterals) about 2 cm in length was severed with the razor blade and removed to a humidified box to prevent water loss. Roots segments were tapped to remove adhering soil particles, loaded into the thermocouple psychrometer chambers (series 74; J.R.D. Merrill Specialty Equipment, Logan, UT, USA) and placed in a cooler until they were brought back to the lab and connected to a computerized data acquisition system (CR-7 data logger, Campbell Scientific, Inc.). Individual root sampling averaged 10 s per root, and total sampling time did not exceed 30 min on any given day. Thermocouple psychrometers were placed in an insulated water bath at 25 °C and measured every 30 min for at least 6 h to allow for temperature and vapor equilibrium. Once equilibrium was reached, three measurements were averaged to estimate water potential. Diurnal patterns of root rehydration were determined by simultaneous sampling of root and leaf water potentials at pre-dawn (0500 h) and midday (1300 h) on two randomly chosen days. Osmotic potential ( $\Psi_s$ ) was determined on the same samples by freezing the sample tissue in liquid N and by equilibrating the psychrometer in the water bath for another 6 h. Thermocouple psychrometers were

calibrated with three salt solutions of known osmolality every 10 measurements. Because of the limited number of available psychrometers and the results of a preliminary study that found a reasonably close relationship of thermocouple psychrometers with the pressure chamber technique ( $R^2 = 0.744$ ,  $P = 0.003$ ), leaf water potentials were measured with a Scholander-type pressure chamber (Soil Moisture Equipment Co.) immediately following root water potential measurements. Leaf osmotic potentials were measured on expressed sap by vapor pressure osmometry (Boyer 1995). Leaves were severed from the plant, placed in plastic syringes and frozen until measurement. The leaves were allowed to thaw for 12 h, and a sample of sap was placed on filter paper for measurement (5500 vapor pressure osmometer; Wescor, Inc., Logan, UT, USA).

Electrolyte leakage was determined on a separate set of new first-order laterals of similar length and weight (Huang, Lakso & Eissenstat 2005). Roots were thoroughly rinsed of all soil particles and immersed in 40 mL of deionized water. Percent electrolyte leakage of the sample was estimated by measuring the electrical conductivity ( $EC$ ) of the water at immersion ( $EC_{initial}$ ), after 30 min ( $EC_{30}$ ) and after disrupting root cell membranes by boiling the sample for 5 min ( $EC_{boil}$ ). Membrane leakage was estimated as a percent of total electrolytes in the roots:

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

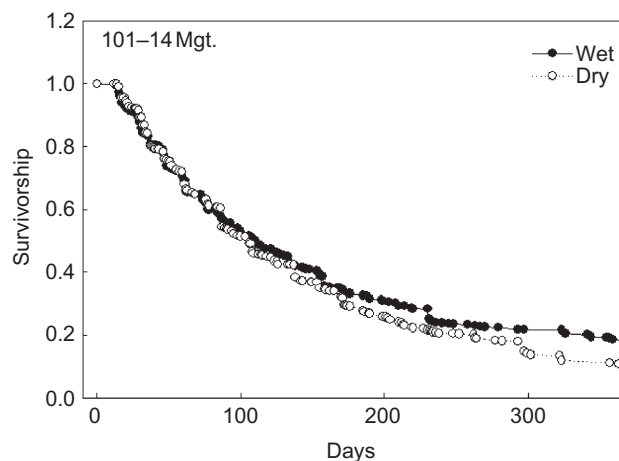
### Statistical methods

Root lifespan data were analysed by Cox proportional hazards regression (PROC PHREG; SAS Institute Inc., Cary, NC, USA). This type of analysis allows the influence of all other covariates to be held constant (Cox 1972). Wilcoxon tests were used to determine significance in root lifespan for field data (SAS Institute Inc.). Components of water potential data were transformed using  $(\log + 1)$  to correct for heteroscedasticity. Soil volumetric moisture and root water potentials over time were analysed using GLM repeated measures (v. 11.0; SPSS Inc., Chicago, IL, USA). Root pre-dawn and midday water potentials were analysed using  $t$ -tests, and root electrolyte leakage was analysed using analysis of variance (ANOVA) (v. 11.0, SPSS Inc.).

## RESULTS

### Field experiment

The years 2003 through 2005 were typical for weather patterns of Napa Valley, CA, USA with cool wet winters and warm summers with no rainfall. Soil moisture in the top 80 cm, the area where the majority of roots are located, averaged 27% (about  $-0.03$  MPa) on the irrigated side of the vine and 22% ( $-0.9$  MPa) on the unirrigated side of the vine during August (a dry month) of all 3 years combined ( $P = 0.0001$ ). Deeper soil ( $>80$  cm) retained more water with an average of 29% water ( $>-0.01$  MPa) in the wet soil



**Figure 1.** Root survivorship for wet and dry sides of *Vitis vinifera* cv. Merlot vines grafted onto 101-14 Millardet de Gramanet (*Vitis riparia* × *Vitis rupestris*) rootstock in Oakville, CA. Vines were watered on one side only and received 100%  $E_t$  through drip emitters located 50 cm from the trunk of the vine. Data include all roots born in years 2003–2005 ( $n = 2187$ ). Differences in root survival in wet and dry soil were not significant ( $P = 0.2055$ ).

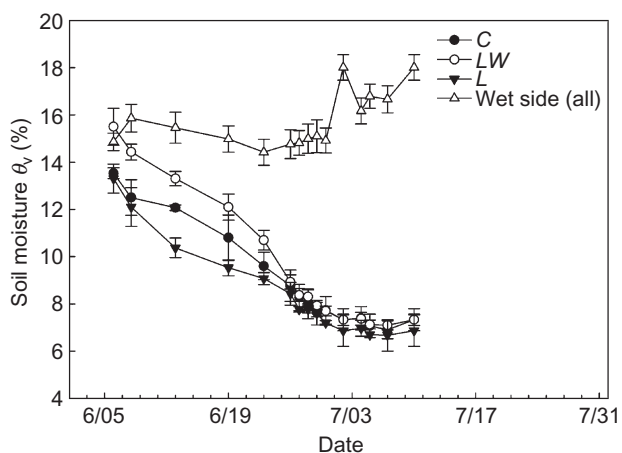
and 28% water ( $-0.02$  MPa) in the dry soil ( $P = 0.150$ ). Fine root first- and second-order diameter averaged 0.42 mm and ranged from 0.11 to 0.98 mm. Fine root median survivorship did not differ between roots in wet soil and roots in dry soil under field conditions (median lifespan: wet = 109 d, dry = 105 d,  $P = 0.2055$ ; Fig. 1). A large portion of the grapevine root system remained apparently unstressed in unirrigated dry soil during the summer months (usually May–October) typical of California summers where precipitation is minimal.

### Greenhouse experiment

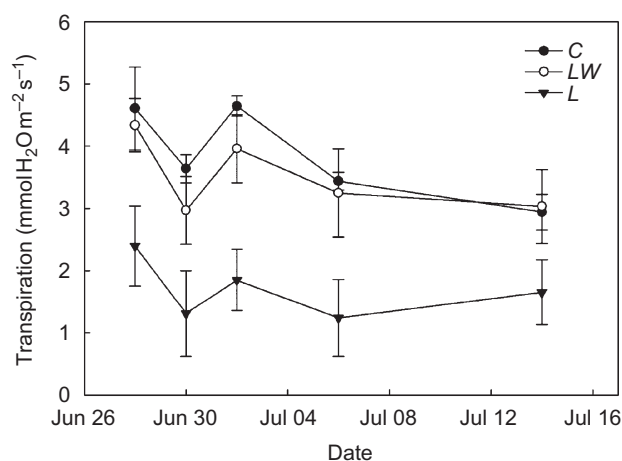
Soil moisture content of the dry-side pots decreased in all treatments compared to the watered pots (Fig. 2). Estimates of soil water potential using the soil moisture release curve indicated that while the soil began at a near saturated condition (day 0 of experiment), the soil in the dry pots reached about  $-3.5$  MPa by day 14,  $-9.0$  MPa by day 21 and reached a minimum of about  $-13$  MPa. Soil moisture in the wet soil was significantly higher than that in the dry side in all treatments ( $P < 0.0001$ ) while dry-side soil moisture was similar among treatments ( $P = 0.512$ ).

Despite similar transpiration rates at day 0 of the experiment (data not shown), after 3 weeks, daytime transpiration of control (C) plants and plants under illumination receiving supplemental water (LW) was almost twice that of the plants subjected to 24 h of illumination without supplemental water (L) ( $P < 0.0001$ ) (Fig. 3). Reduced daytime transpiration in L indicates the increased water use of plants under illumination that did not receive supplemental water to replace water lost.

Despite dry soil conditions, roots in the dry side of the C treatment split pots had similar survivorship to roots grown



**Figure 2.** Volumetric soil moisture content (%) for the wet side of the grapevine, 101-14 Mgt. rootstock cultivar ( $\Delta$ , average of all three treatments), and the dry side by treatment: *C* ( $\bullet$ ), plants with normal irrigation and a 12 h dark nocturnal period; *LW* ( $\circ$ ), plants with supplemental water and 24 h illumination; and *L* ( $\blacktriangledown$ ), plants with normal irrigation but with 24 h illumination ( $\pm 1$  SE). Collectively, 3 h after watering, the watered pots of all vines remained wetter than the un-watered pots ( $P < 0.0001$ ), while dry-side soil moisture was similar among treatments ( $P = 0.512$ ).



**Figure 3.** Midday (1300 h) leaf transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) for the grapevine, 101-14 Mgt. rootstock cultivar, for plants with normal irrigation and a 12 h dark nocturnal period (*C*), for plants with supplemental water and 24 h illumination (*LW*), and for plants with normal irrigation and 24 h illumination (*L*) ( $\pm 1$  SE). Transpiration was lower in *L* plants compared with that of plants in the *C* and *LW* treatments ( $P < 0.0001$ ).

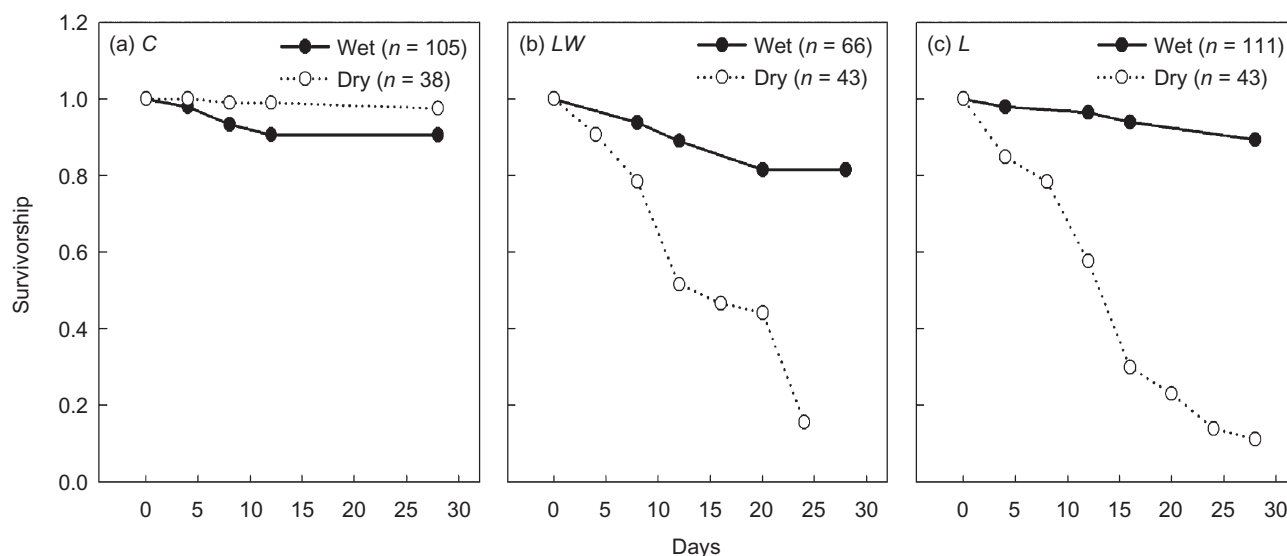
in the wet side of the split pots ( $P = 0.136$ ) (Fig. 4a). In contrast, the treatments with continuous illumination (*LW* and *L*) exhibited shorter lifespans of roots in the dry side of the split pots (Fig. 4b,  $P < 0.0004$  & Fig. 4c,  $P < 0.0001$ ).

Averaged over the entire study, midday leaf water potentials were higher for *C* plants than for *L* plants ( $P = 0.051$ ) but were similar to those of *LW* plants ( $P = 0.865$ ). Pre-dawn water potentials of roots in dry soil under 24 h of

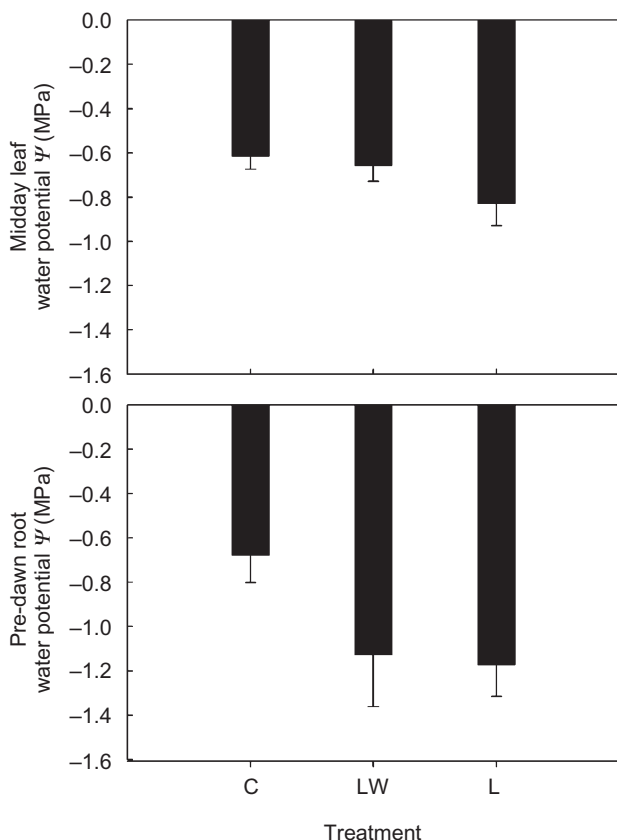
illumination (*L* and *LW*) were nearly twice as negative as for roots of *C* plants ( $P = 0.034$ ) (Fig. 5).

### Diurnal patterns

Changes in plant water potential from midday (1300 h) to pre-dawn (0500 h) gave some indication of the rate of recovery of water potential over night-time. Leaf water potential recovery was considered complete for *C* plants



**Figure 4.** Root survivorship on wet and dry sides of the grapevine, 101-14 Mgt. rootstock cultivar, (a) for plants with normal irrigation and a 12 h dark nocturnal period (*C*), (b) for plants with supplemental water and 24 h illumination (*LW*), and (c) for plants with normal irrigation and 24 h illumination (*L*). Survivorship of roots in the wet side was significantly higher than that in the dry side in (b) and (c) ( $P < 0.0004$ ) but not in (a) ( $P > 0.50$ ).

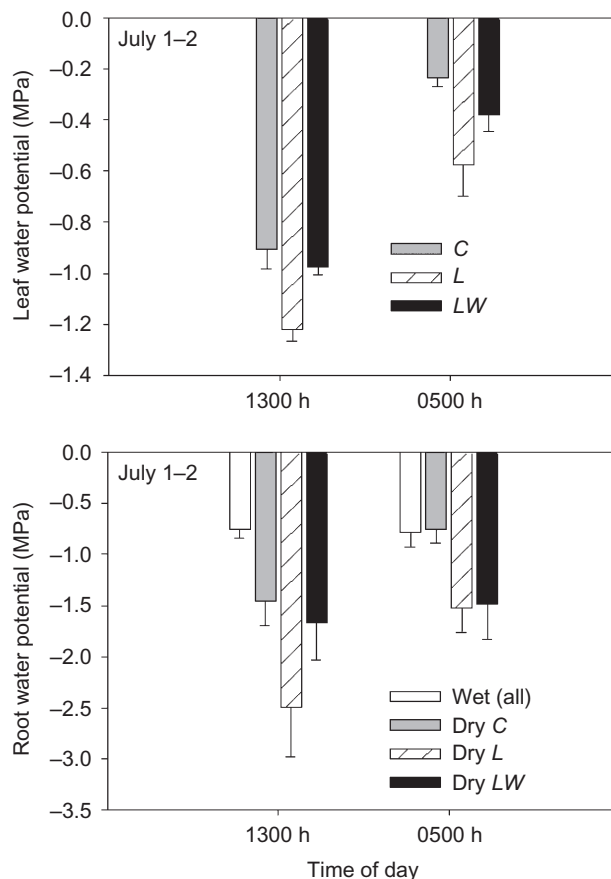


**Figure 5.** Midday (1300 h) leaf water potentials and pre-dawn (0500 h) root water potentials (MPa) averaged over the entire study in the grapevine, 101-14 Mgt. rootstock cultivar, for roots in the dry soil for plants with normal irrigation and a 12 h nocturnal dark period (C), for plants with supplemental water and 24 h illumination (LW), and for plants with normal irrigation and 24 h illumination (L) ( $\pm 1$  SE). Leaf water potentials were lower in L plants ( $P = 0.051$ ) and similar for C and LW treatments ( $P = 0.865$ ), and roots of plants under 24 h of illumination (LW and L) exhibited lower water potentials compared with those of the control (C) ( $P = 0.034$ ).

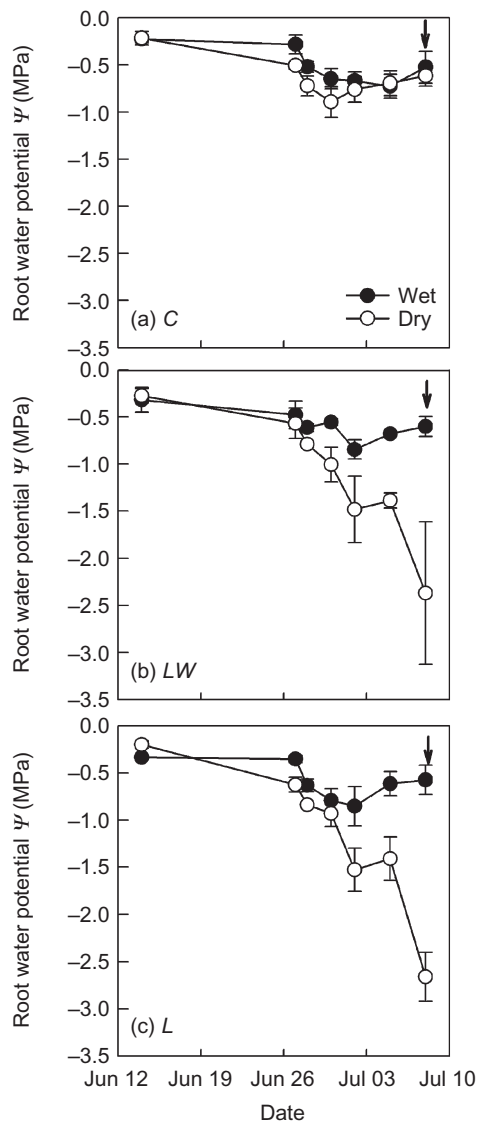
when measured near the end of the experiment on July 1 and 2 (Fig. 6). Pre-dawn leaf water potentials in plants that received supplemental irrigation, LW, were not different from those of the control and were therefore also considered to have complete leaf water potential recovery ( $P = 0.416$ ); however, recovery was incomplete for L plants compared to the control as indicated by lower pre-dawn leaf water potentials in the L plants ( $P = 0.046$ ) (Fig. 6). Roots in dry soil had lower water potentials than roots in wet soil at midday for C, L and LW (all  $P \leq 0.001$ ) plants. Midday and pre-dawn root water potentials in wet soil were similar across light treatments ( $P = 0.848$ ). Light treatments strongly affected the water potential of roots in the dry soil. In C plants, a subsequent rise in root water potentials of dry-side roots from midday (1300 h) to pre-dawn (0500 h) to equal those of roots in wet soil at pre-dawn indicates root water potential recovery is consistent with the movement of water through internal hydraulic redistribution when

nocturnal transpiration is minimized. In contrast, pre-dawn root water potential recovery was incomplete for roots in dry soil of plants that received 24 h illumination plus supplemental water ( $P = 0.040$ ) and for roots in dry soil of plants that only received 24 h illumination ( $P = 0.031$ ) (Fig. 6).

Over the course of the entire experiment, pre-dawn root water potentials of roots in dry soil remained fairly constant and similar to those in wet soil in the C plants ( $P = 0.190$ ), but water potentials of roots in dry soil declined continuously over the experiment in plants exposed to 24 h illumination ( $P = 0.001$ ) (Fig. 7). By the end of the experiment, water potentials of roots in dry soil where plants were illuminated at night had water potentials as low as  $-2.5$  to  $-3.0$  MPa (Fig. 7b,c), whereas plants where normal nocturnal hydraulic redistribution was allowed to occur had water potentials of roots in dry soil of about  $-0.6$  MPa (Fig. 7a).



**Figure 6.** Midday (1300 h) and pre-dawn (0500 h) leaf water potentials (MPa) on July 1 and 2 (upper panel) for the grapevine, 101-14 Mgt. rootstock cultivar, for plants with normal irrigation and a 12 h dark nocturnal period (C, gray bars), for plants with supplemental water and 24 h illumination (LW, black bars), and for plants with normal irrigation but with 24 h illumination (L, hatched bars). Lower panel shows midday and pre-dawn root water potentials (MPa) on July 1 and 2 for roots in wet soil (white bars) and for roots in dry soil in the C (gray bars), L (hatched bars) and LW (black bars) treatments. For statistical significance, see text.



**Figure 7.** Shifts in pre-dawn (0500 h) root water potential over the experiment for the grapevine, 101-14 Mgt. rootstock cultivar on both the watered (●) and dry (○) sides of the plants ( $\pm 1$  SE), (a) for plants with normal irrigation and a 12 h dark nocturnal period (C), (b) for plants with supplemental water and 24 h illumination (LW), and (c) for plants with normal irrigation but with 24 h illumination (L). The treatments, L and LW, had lower water potentials of roots in dry soil than those in wet soil ( $P = 0.001$ ), while the control treatment had similar root water potentials in both wet and dry soil ( $P = 0.190$ ). Arrows indicate sampling time of roots for electrolyte leakage.

Patterns of root electrolyte leakage at the end of the experiment (Fig. 8) were consistent with patterns of root survivorship (Fig. 4) and patterns of pre-dawn root water potential (Fig. 7). Roots of plants in the LW and L treatments exhibited greater electrolyte leakage, an indication of lack of internal water rehydration and subsequent reduced membrane integrity ( $P = 0.046$ ). Overall, illuminated plants had more than a twofold increase in electrolyte leakage in

dry roots compared with roots grown in wet soil ( $P = 0.002$ ) (Fig. 8).

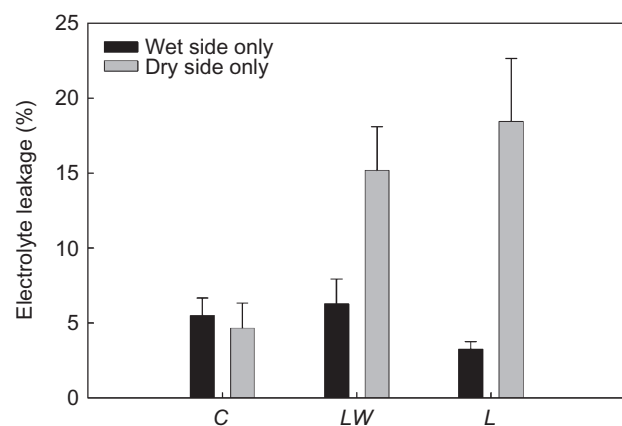
### Components of water potential

Examination of the components of water potential provides insight into mechanisms of how roots cope with dry soil. Roots in dry soil where hydraulic redistribution was disrupted (LW and L treatments) had a pattern, although non-significant, of lower osmotic potentials ( $\Psi_{\pi}$ ) compared to C roots (Fig. 9a,  $P = 0.318$ ). Total water potential ( $\Psi_w$ ) was lower for LW and L treatments compared with C treatment (Fig. 9b,  $P = 0.018$ ). While the C plants retained positive root turgor potential ( $\Psi_p$ ), LW or L roots exhibited less evidence that they were able to retain positive turgor (Fig. 9c,  $P = 0.001$ ).

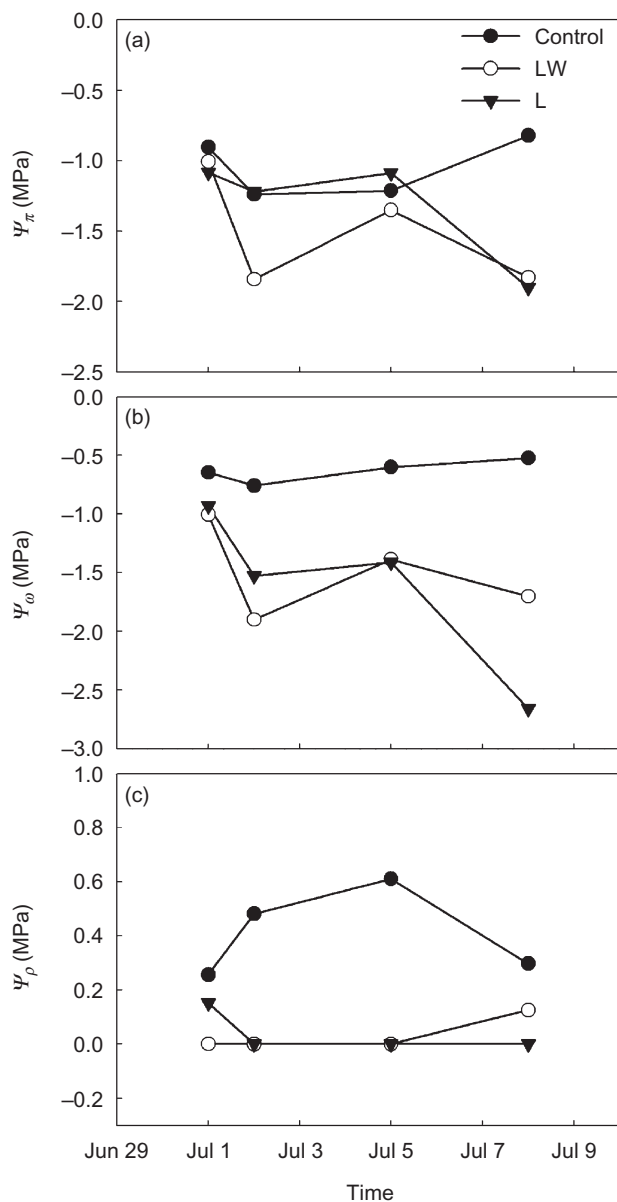
### DISCUSSION

Internal hydraulic redistribution requires minimal night-time transpirational water loss. We successfully devised and implemented a set of treatments using two controls, one for length of illumination and another that controlled for increased transpiration as a result of 24 h of illumination but where root available water was not limiting. This allowed for a direct examination of internal water potential recovery of the finest roots of grape. Internal hydraulic redistribution apparently rehydrated root tissues, when it was allowed to occur under normal night-time conditions, and most likely prevented appreciable first-order root mortality and loss of cell membrane integrity (as estimated by electrolyte leakage).

Inhibiting internal hydraulic redistribution by preventing night-time stomatal closure and maintaining night-time



**Figure 8.** Root electrolyte leakage (%) for roots of the grapevine, 101-14 Mgt. rootstock cultivar at the end of the experiment, for plants with normal irrigation and a 12 h dark nocturnal period (C), for plants with supplemental water and 24 h illumination (LW), and for plants with normal irrigation but with 24 h illumination (L). Black bars represent wet-side roots, and light gray bars represent dry-side roots. Statistical differences were found between treatments ( $P = 0.046$ ) and between wet and dry sides of the plant ( $P = 0.002$ ).



**Figure 9.** Pre-dawn (0500 h) water potential components for roots of grapevine, 101-14 Mgt. rootstock cultivar, in the dry soil only. (a) Osmotic potential ( $\Psi_{\pi}$ ) ( $P=0.318$ ), (b) total water potential ( $\Psi_w$ ) ( $P=0.018$ ), (c) turgor potential ( $\Psi_p$ ) ( $P=0.001$ ). Data are for roots from plants with normal irrigation and a 12 h dark nocturnal period (C) (●), for plants with supplemental water and 24 h illumination (LW) (○) and for plants with normal irrigation but with 24 h illumination (L) (▼).

transpiration with continuous illumination is consistent with the resulting increase in root death, lower root water potentials and more electrolyte leakage by roots in dry soil. Supplemental water to roots in the wet pot did maintain leaf transpiration levels but did not alter water stress as indicated by the lower root  $\Psi$  of roots in the dry soil when nocturnal hydraulic redistribution was presumably disrupted. Moreover, reduced daytime transpiration of plants under continuous illumination without supplemental water

suggests that these plants were unable to replace water lost by closing their stomata at night. Although a number of studies have already established the phenomenon of hydraulic redistribution within many plant species, few have documented direct changes in internal root water potential (but see Burgess *et al.* 1998; Domec *et al.* 2006), an important component for root functioning. Circumstantial evidence of the effects of internal hydraulic redistribution on root lifespan came from a split-pot study that reported a decline in photosynthate allocation to roots in dry soil and a lack of root death in response to drought stress in citrus seedlings (Kosola & Eissenstat 1994).

In grape, we observed a similar lifespan of fine roots in wet and dry soils in the field, presumably because of internal hydraulic redistribution at night. Despite the potential vulnerability of the fine root system to severe decreases in soil moisture (Smucker & Aiken 1992), localized patches of soil moisture in the irrigated zone and deeper soil layers supported root survivorship and even root production in dry soil (see Bauerle 2007). Under extremely heterogeneous field conditions, hydraulic redistribution may aid in maintaining water uptake of the entire root system by rehydrating roots in dry soil (Hultine *et al.* 2003). Our work under greenhouse conditions supported this contention. If plants were exposed to 12 h of darkness, we showed that roots in dry soil in a split-pot system coped with water deprivation by only exhibiting moderate water stress during the day and full recovery of root water potential over the night: there was no decrease in lifespan or increase in membrane leakage. In contrast, plants that were subjected to continuous illumination with or without supplemental water not only exhibited lower midday and pre-dawn root water potentials in dry soil compared with roots in wet soil, but also had decreased root lifespan, probably a result of the loss of cell turgor and increased membrane leakage as the soil dried.

Although it is known that root desiccation can cause a decrease in membrane stability and therefore an increase in electrolyte leakage (Blum & Ebercon 1981), to our knowledge, no studies have evaluated the consequences of decreased root water potential on root vitality. As previously stated, in our study, we were not concerned with the movement of water out of the root system. Instead, the direct measurement of internal rehydration of the finest roots confirmed continuous vitality of roots in dry soil with little apparent ill effects as long as sufficient water was available at night. A more detailed look at the decline of root membrane stability with lowering water potential could provide more insight into the length of time and to what level plant membranes cope with decreasing soil moisture.

Species-specific differences in root xylem vessel diameter can influence water flow within the root. We not only studied the finest and youngest roots of the grape root system but we also used a plant known for its large xylem vessels (Salleo, Lo Gullo & Oliver 1985). Because of the large vessel size of grape, one needs to be cautious about extending our results in grape to species with substantially smaller xylem vessels and greater hydraulic resistance. Nonetheless, we showed



that internal hydraulic redistribution apparently occurred in grape despite the known decrease in vessel size and reduction in hydraulic conductivity associated with roots of the finest orders and plants exposed to moderate drought (Lovisol & Schubert 1998). Understanding variation among species in rates of internal water movement from roots in wet soil to roots in dry soil and its influence on fine root survival may provide additional clues on selection pressures associated with xylem structure.

### Ecophysiological implications

Internal hydraulic redistribution may influence numerous root functions as a direct consequence of increased root persistence in dry soil. The internal transfer of water to portions of the root system in dry soil may allow for enhanced and prolonged nutrient uptake (Matzner & Richards 1996). Moreover, enhanced root growth has been shown to be a consequence of increased photosynthate fixation in plants that perform hydraulic lift (Dawson 1997). Roots of plants that perform hydraulic redistribution may also have a faster recovery response following rain events, thus potentially allowing the plant to better compete for water and nutrients (Burgess *et al.* 1998; Eissenstat *et al.* 1999). Maintaining root function in dry soil by internal hydraulic redistribution has several beneficial implications on the whole plant, such as the reduced carbon costs associated with extended nutrient uptake by the root in relation to building new roots (Eissenstat & Yanai 1997). Extended root function (Domec *et al.* 2004) and lifespan in dry soil may also increase the likelihood of the plant or its mycorrhizal fungi to utilize spatially heterogeneous resources such as water or nutrient patches (Chapin *et al.* 1987).

While hydraulic redistribution effects on plant success under conditions of water limitation are well recognized, lack of hydraulic redistribution caused by root death, shrinkage or xylem embolisms also may occur (North & Nobel 1997; Caldwell, Dawson & Richards 1998). Hydraulic redistribution may also be limited in plants with high axial resistance due to small root xylem vessels or extensive branching (Tsuda & Tyree 1997). Distal roots may show evidence of shorter lifespan during periods of water stress as a result of smaller diameter xylem which is considered more susceptible to cavitation (Tsuda & Tyree 1997). Root survivorship of high-latitude plants with short periods of darkness or plants that transpire at night (Hultine *et al.* 2003) may also have decreased root survival in dry soil as a result of limited hydraulic redistribution.

In summary, we found that despite differences in soil moisture, internal hydraulic redistribution of water is the most obvious link to prolonged root lifespan in the finest laterals of the root system. Circumventing hydraulic redistribution by maintaining transpiration during nocturnal hours prevented roots in dry soil from rehydrating at night and resulted in reduced root turgor, increased electrolyte leakage and decreased root survivorship.

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