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Original Article

Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure

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ABSTRACT

Drought induces xylem embolism formation, but grapevines can refill non-functional vessels to restore transport capacity. It is unknown whether vulnerability to embolism formation and ability to repair differ among grapevine species. We analysed *in vivo* embolism formation and repair using x-ray computed microtomography in three wild grapevine species from varied native habitats (*Vitis riparia*, *V. arizonica*, *V. champinii*) and related responses to measurements of leaf gas exchange and root pressure. Vulnerability to embolism formation was greatest in *V. riparia*, intermediate in *V. arizonica* and lowest in *V. champinii*. After re-watering, embolism repair was rapid and pronounced in *V. riparia* and *V. arizonica*, but limited or negligible in *V. champinii* even after numerous days. Similarly, root pressure measured after re-watering was positively correlated with drought stress severity for *V. riparia* and *V. arizonica* (species exhibiting embolism repair) but not for *V. champinii*. Drought-induced reductions in transpiration were greatest for *V. riparia* and least in *V. champinii*. Recovery of transpiration after re-watering was delayed for all species, but was greatest for *V. champinii* and most rapid in *V. arizonica*. These species exhibit varied responses to drought stress that involve maintenance/recovery of xylem transport capacity coordinated with root pressure and gas exchange responses.

Key-words: cavitation; drought; recovery; *Vitis*; vulnerability; water stress; xylem.

INTRODUCTION

Cultivars of grapevines and perennial fruit crops are often grafted onto rootstocks to control plant growth and provide resistance to both soil-borne pests (e.g. phylloxera, nematodes and fungal pathogens) and abiotic conditions (e.g. drought, salinity and soil type) (Pongrácz 1983; Christensen 2003; DeJong *et al*. 2004). Different rootstocks allow flexibility in growing a particular fruiting cultivar across diverse growing conditions and soil types and have enabled the geographic expansion of vineyards and orchards while maintaining the desirable qualities of the harvested fruit. Grapevine rootstocks are known to affect scion gas exchange, water-use efficiency and vigour (Candolfi-Vasconcelos *et al*. 1994; Padgett-Johnson *et al*. 2000; Marguerit *et al*. 2011; Gambetta *et al*. 2012; Tramontini *et al*. 2013) suggesting xylem hydraulic function may contribute to these known differences, but this is not well studied.

Long-distance water transport from roots to leaves is necessarily dependent on maintenance of xylem functionality. Xylem water flow is driven predominantly by a negative pressure (i.e. tension) generated at the transpiring leaf surface that propagates to the roots through continuous columns of water in xylem conduits ('cohesion-tension mechanism', Dixon & Joly 1894). Under conditions of drought and/or high transpiration, xylem tensions may exceed a threshold beyond which gas emboli form and hydraulic transport capacity is impaired. Prolonged xylem dysfunction can lead to leaf wilt, decreased productivity and ultimately plant death. Xylem vulnerability to drought-induced embolism has been associated with the aridity of a species' native habitat in both gymnosperms and angiosperms (Pockman & Sperry 2000; Maherali *et al*. 2004; Willson & Jackson 2006; Choat *et al*. 2007). For example, *Juniperus* species native to mesic habitats of eastern North America were shown to exhibit higher xylem vulnerability than species native to the arid-southwestern United States (Willson & Jackson 2006). We speculate that a similar trend in xylem vulnerability to embolism may also apply for grapevine species native to different habitats. Moreover, grapevines are known to repair embolized vessels to restore xylem functionality (Sperry *et al*. 1987; Holbrook *et al*. 2001). Recent work has utilized x-ray computed microtomography (microCT) to visualize embolism spread and repair *in vivo* for *Vitis vinifera* and data suggest that living cells embedded in the xylem matrix play an active role in this repair process (e.g. Brodersen *et al*. 2010, 2013a; Brodersen & McElrone 2013; McElrone *et al*. 2013). However, it is not known whether the ability to repair differs between closely related species. For example, species native to mesic habitats when subjected to drought may require enhanced ability to repair

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embolized vessels because they are more susceptible to embolism formation. This interplay between embolism formation and repair is likely linked to varying strategies that species use to tolerate and recover from drought stress.

In grapevine, embolized vessels can be refilled after re-watering when transpiration is minimized (Holbrook *et al*. 2001) and/or when isolated from tensions of functional vessels (Brodersen *et al*. 2010). Delayed or incomplete recovery of stomatal conductance and transpiration after re-watering may positively contribute to embolism repair (Miyashita *et al*. 2005; Lovisolo *et al*. 2008). At minimized transpiration, it has long been speculated that root pressure contributes to embolism repair in some species by mitigating xylem pressures (Tyree *et al*. 1986; Ewers *et al*. 1997; Holbrook & Zwieniecki 1999; Cao *et al*. 2012; Wegner 2013). Root pressures of 0.02 to 0.5 MPa have been reported for herbaceous (e.g. Bramley *et al*. 2009; Knipfer & Fricke 2010) and woody species (e.g. Ewers *et al*. 2001) including grapevine (Scholander *et al*. 1955; Sperry *et al*. 1987; Tibbetts & Ewers 2000). For grapevine, root pressure apparently plays an important role in the spring refilling of freeze-induced embolism to restore hydraulic transport capacity of the xylem (Sperry *et al*. 1987). Hence, it is possible that differences in embolism repair and recovery from drought stress after re-watering among grapevine species may be related to species-dependent difference in gas exchange recovery and root pressure, but this has yet to be investigated.

In the present study, we utilized synchrotron-based microCT technology to investigate *in vivo* xylem vulnerability to drought-induced embolism and its subsequent repair after re-watering in three grapevine species (*V. riparia*, *V. arizonica* and *V. champinii*). Rootstocks of *V. riparia* and *V. champinii* are commercially available and all three species represent germplasm currently being evaluated for future commercial use. *V. riparia* is native to more mesic habitats of eastern North America and encompasses a large latitudinal range, while *V. arizonica* and *V. champinii* are native to arid habitats of southwestern United States and northern Mexico (for details see http://plants.usda.gov/java/). A previous water relations study conducted on these species in a common vineyard experiment in California resulted in *V. arizonica* and *V. champinii* being ranked as drought-tolerant and *V. riparia* as drought-sensitive (Padgett-Johnson *et al*. 2003). Here, we follow-up on those observations with the objective to provide a more detailed understanding of the physiological traits and processes involved in resistance to and recovery from drought stress of these grapevines species. Given the role of xylem tensions on both embolism formation and repair, microCT data were complemented with measurements of leaf gas exchange and root pressure in order to investigate a possible coordination of those three physiological parameters.

MATERIAL AND METHODS

Plant material and growth conditions

Plants of *V. riparia* ('Riparia Gloire'), *V. arizonica* ('b43-17') and *V. champinii* ('Ramsey') were grown from 5 to 10 cm long herbaceous cuttings obtained from established parent plants at the University of California, Davis, experimental vineyards. The bottom end of the cutting was dipped in 2.5% rooting solution (Earth Science Products, Wilsonville, OR, USA), placed into plastic trays filled with perlite and transferred to a mist room at 25 °C bottom heat. After approximately 4 weeks, when there was evidence for root growth, cuttings were transplanted into 0.7 L plastic pots filled with a soil mix of 40% washed sand, 20% sphagnum peat moss, 20% redwood compost and 20% pumice rock. Subsequently, plant growth was maintained for 4 to 12 weeks under greenhouse conditions (approximated day/night temperature of 8/25 °C, photoperiod of 15/9 h, relative humidity of 35% and photosynthetic active radiation of 500 μE m^{-2} s^{-1} during the day). In the greenhouse, plants were drip irrigated twice daily with water supplemented with calcium (90 μg mL^{-1}), magnesium (24 μg mL^{-1}), potassium (124 μg mL^{-1}), nitrogen as NH_4^+ (6 μg mL^{-1}), nitrogen as NO_3^- (96 μg mL^{-1}), phosphorus (26 μg mL^{-1}), sulfur (16 μg mL^{-1}), iron (1.6 μg mL^{-1}), manganese (0.27 μg mL^{-1}), copper (0.16 μg mL^{-1}), zinc (0.12 μg mL^{-1}), boron (0.26 μg mL^{-1}) and molybdenum (0.016 μg mL^{-1}) at pH 5.5 to 6.0. Plants used in experiments were approximately 0.7 to 1.0 m tall with an average leaf area of 1463 cm^2 (*V. riparia*), 621 cm^2 (*V. arizonica*) and 1378 cm^2 (*V. champinii*) and root fresh weight of 51 g (*V. riparia*), 32 g (*V. arizonica*) and 47 g (*V. champinii*). To induce drought stress, irrigation of continuously well-watered plants was removed up to 3 d prior to analysis. The time period of drought stress depended on the targeted plant water status as measured by stem water potential (Ψ_{stem}). The drought period was followed by a re-watering period of up to 5 d during which the soil was maintained fully hydrated.

Water status

Plant water status was determined by measuring Ψ_{stem} with a Scholander pressure chamber (Soil Moisture Equipment Corp 3005, Goleta, CA, USA). Leaves were covered with a sealed and foiled plastic bag for more than 20 min, subsequently excised at the base of the petiole with a sharp razor blade and placed immediately into the chamber with the excised petiole end protruding out of the chamber. The chamber was slowly pressurized (~0.03 MPa s^{-1}) while the cut end of the petiole was monitored. When water started to emerge from the excised petiole surface, the corresponding pressure was recorded and defined as Ψ_{stem}.

X-ray microCT

Plants were scanned at the microCT facility (Beamline 8.3.2) at the Lawrence Berkley National Laboratory Advanced Light Source (ALS) (for details, Brodersen *et al*. 2010, 2013a; McElrone *et al*. 2013). The day of scanning, plants were transported from the greenhouse to the ALS around 3 h before the start of analysis. At the ALS, Ψ_{stem} was monitored frequently. Groups of replicate plants (n = 4–8), which had been exposed to different water treatments ('well-watered' with $\Psi_{stem} > -0.6$ MPa, 'drought-stressed' with $\Psi_{stem} < -1.0$ MPa

and 're-watered' with $\Psi_{stem} < -1.0$ MPa under stress and $\Psi_{stem} > -0.6$ MPa after 5 d of watering) in the greenhouse, were subjected to a single scan within 24 h after being at the ALS and were analysed randomly within experimental blocks, where a replicate from each treatment by species combination was scanned within a 2–3 h block of time. Single scans eliminate possible artefacts of tissue damage by x-ray exposure. In addition, previously drought-stressed plants ($n = 3$ of each species) were subjected to multiple scans over time after re-watering to study the time course and dynamics of embolism repair. For visualization of plant tissue, the pot of an intact plant was placed in an aluminum cage and fixed on an air-bearing stage. To reduce vibrations and stem movement during the scan, a plastic cylinder was mounted on top of the aluminum cage. During sample preparation, great care was taken that branches and petioles were not damaged. After the plant was set-up properly, a 1 to 5 mm section of the stem just above the soil was scanned in the 15 to 19 keV synchrotron x-ray beam, while the plant was rotating in 0.25° increments yielding 720 to 1032 two-dimensional images with a ~4.5 μM pixel resolution captured on a 4008×2672 pixel CCD camera (#PCO and PCO.edge, PCO AG, Kehlheim, Germany). The acquired two-dimensional projection images were reconstructed into a stack of transverse images with a custom software plug-in for Fiji imaging-processing software (www.fiji.sc, ImageJ) that used Octopus 8.3 software (Institute for Nuclear Sciences, Ghent University, Belgium) in the background. The number of embolized vessels and vessel diameter were determined from representative transverse images taken out of the three-dimensional (3D) image stack using a semi-automated routine within Fiji (for details, Brodersen *et al*. 2013a). The total number of embolized and water-filled vessels was counted on the same transverse image manually using the 'point selection' tool in Fiji software. The percentage of embolized vessels was determined by [(number of embolized vessels/total number of vessels) × 100%]. In some cases, when the resolution of the transverse microCT image was insufficient, total vessel number was obtained from light micrographs as taken from the scanned stem. The scanned stem portion was harvested, stored in 70% ethanol, sectioned with a sliding microtome (American Optical Company SI Division AO-860, Buffalo, NY, USA), stained with 1% (aq.) Toluidine Blue O for 1 min and rinsed with water and photographed with a digital camera mounted on a microscope (#DFC425, Leica Microsystems Limited, Buffalo Grove, IL, USA). Using Avizo 6.2 software (VSG, FEI Company, Hillsboro, OR, USA), embolized vessels of scanned plant tissue were screened for water droplet formation by panning through the 3D image in longitudinal direction.

Leaf gas exchange

Transpiration and stomatal conductance of plants ($n = 5$ of each species) was measured midday between 1100 and 1300 h in the greenhouse using a portable gas exchange system (Li-1600, Li-Cor, Inc., Lincoln, NE, USA). Three mature and healthy leaves were selected on each plant, which were treated as subsamples, and were measured repeatedly when plants were 'well-watered' ($\Psi_{stem} \geq -0.6$ MPa), under drought-stress ($\Psi_{stem} < -1.0$ MPa), and 1 d and 2 d after re-watering ($\Psi_{stem} > -0.6$ MPa). When one of the selected leaves showed severe signs of necrosis or leaf dieback in response to drought stress, it was discarded from the analysis. Ψ_{stem} was determined on other mature leaves within 15 to 30 min after completion of gas exchange measurements.

Root pressure

Plants that were well-watered or subjected to different levels of drought stress were transported from the greenhouse to the laboratory 1 to 3 h before analysis. In the laboratory, Ψ_{stem} was measured and subsequently the stem was excised under water with sharp pruning scissors (XP-3-330, Ronan Tools, San Jacinto, CA, USA) 2 to 3 cm above the soil. The portion of the excised stem segment that was still connected to the root system was attached to a 2 cm piece of semi-rigid Polyvinyl Chloride tubing using epoxy glue (20445 Flow-Mix 5-Minute Epoxy, Devcon, Danvers, MA, USA). Subsequently, the tubing was entirely back-filled with water containing 20 mM KCl and connected via polypropylene male–female luer fittings (FTLL013-6 and MTLL013-6005, Value Plastics, Fort Collins, CO, USA) to a pressure transducer (PX26-005GV, Omega Engineering, Inc. Stamford, CT, USA). Pressure transducers were connected to a data logger (CR7, Campbell Scientific Inc., Logan, UT, USA). Root pressure was recorded continuously every 5 min for up to 3 d while the soil was fully hydrated. In drought-stressed plants, we often observed gas bubbles rising from the excised stem surface during the first hour following rehydration of the soil. If this was the case, the connection of stem and pressure transducer was opened to release the gas and subsequently the tubing was entirely refilled with solution before the pressure transducer was reconnected. A steady-state root pressure, which was equivalent to the maximum pressure, was reached after 4 to 30 h. When signs of water leakage were detected during the time period of pressure recordings, samples were discarded. In theory, a steady-state root pressure is reached in a 'closed system' (e.g. a root-system sealed off by a pressure transducer) when it equals root xylem osmotic pressure (Knipfer & Fricke 2010; Wegner *et al*. 2013).

Data analysis

Statistical and regression analysis were performed with SAS (version 9.2, SAS Institute, Cary, NC, USA) and SigmaPlot (version 8.0, Systat Software Inc., San Jose, CA, USA). The 'PROC REG' procedure in SAS was used for linear regression analyses. Non-linear regression analyses were performed using SigmaPlot. Mean values and standard errors were determined using the 'PROC MEANS' procedure in SAS. Statistical differences between means and between slopes of linear regression lines were determined by analysis of variance using the 'PROC GLM' procedure in SAS; when

Table 1. Stem water potential (Ψ_{stem}) and corresponding percentage of embolized vessels of three grapevine species under well-watered (n = 4 plants each), drought-stressed (n = 7–8 plants each) and re-watered for 5 d (n = 7 plants each) conditions

	Vitis riparia		*Vitis arizonica*		*Vitis champinii*	
Treatment	Ψ_{stem} (MPa)	Embolized vessels (%)	Ψ_{stem} (MPa)	Embolized vessels (%)	Ψ_{stem} (MPa)	Embolized vessels (%)
Well-watered	$-0.23 \pm 0.01^{a,x}$ (−0.2 to −0.3)	$9.9 \pm 5.1^{a,x}$ (1.3 to 22)	$-0.27 \pm 0.02^{a,x}$ (−0.2 to −0.3)	$5.0 \pm 1.4^{a,x}$ (1.1 to 8.0)	$-0.37 \pm 0.05^{a,y}$ (−0.2 to −0.5)	$1.3 \pm 0.2^{a,x}$ (0.7 to 1.8)
Drought-stressed	$-1.90 \pm 0.20^{b,x}$ (−1.2 to −2.6)	$40.8 \pm 8.9^{b,x}$ (12.0 to 89.6)	$-1.59 \pm 0.12^{b,x}$ (−1.0 to −1.9)	$22.9 \pm 4.1^{b,x,y}$ (8.5 to 36.1)	$-2.02 \pm 0.21^{b,x}$ (−1.3 to −2.8)	$14.2 \pm 3.9^{a,y}$ (0.7 to 28.2)
Re-watered	$-0.29 \pm 0.03^{a,x}$ (−1.4 to −2.8) to (−0.2 to −0.4)	$16.7 \pm 3.4^{a,x}$ (6.4 to 29.1)	$-0.31 \pm 0.03^{a,x}$ (−1.0 to −2.1) to (−0.2 to −0.6)	$10.2 \pm 3.5^{a,x}$ (3.3 to 29.3)	$-0.36 \pm 0.03^{a,x}$ (−1.3 to −2.8) to (−0.2 to −0.5)	$9.3 \pm 3.1^{a,x}$ (0.4 to 21.2)

Percentages of embolized vessels were determined from transverse microCT images (see Fig. 1) through the grapevine stem (total vessel number and diameter were 495 ± 63 and 53 ± 2 μM in *Vitis riparia*, 458 ± 79 and 57 ± 3 μM in *V. arizonica* and 547 ± 43 and 51 ± 3 μM in *V. champinii*, respectively). Data are given as mean ± SE (range from minimum to maximum). Different letters 'a' and 'b' indicate significant differences between treatments within each species and different letters 'x' and 'y' indicate significant differences between species within each treatment ($P < 0.05$).

differences between means were significant, a multiple comparison of means *post hoc* Tukey ($P < 0.05$) was applied.

RESULTS

Embolism formation and repair

Under well-watered conditions, plants of *V. riparia*, *V. arizonica* and *V. champinii* exhibited a comparable Ψ_{stem} of −0.2 to −0.5 MPa and low percentages of embolized vessels (<10%) (Table 1). On average more vessels were embolized in plants of *V. riparia* (9.9%) than in *V. arizonica* (5.0%) and *V. champinii* (1.3%), but those differences were not significant. For all three species, embolized vessels seen under well-watered conditions were most often found in the oldest xylem adjacent to the pith (Fig. 1, top row).

In drought-stressed plants of all three species, Ψ_{stem} was < −1.0 MPa and average values of Ψ_{stem} were comparable among species (−1.9 MPa in *V. riparia*, −1.6 MPa in *V. arizonica*, −2.0 MPa in *V. champinii*) and significantly lower than under well-watered conditions (Table 1). In comparison with well-watered conditions, drought-stress resulted in a significant increase in average percentage of embolism of around fourfold for *V. riparia* (40.8%) and *V. arizonica* (22.9%) but not for *V. champinii* (14.2%); *V. champinii* did exhibit the largest increase in average percentage of embolism (i.e. ~ 10-fold increase), but the absolute percentage of embolism was still the lowest of the three species. Across species, drought stress induced significantly more embolism in *V. riparia* as compared with *V. champinii*. In plants of *V. riparia* and *V. arizonica*, many more embolized vessels formed further away from the pith in a radial pattern, whereas for *V. champinii* the few vessels that embolized were distributed more randomly (Fig. 1, middle row).

In plants re-watered for 5 d after experiencing drought stress, Ψ_{stem} recovered to a value of −0.2 to −0.6 MPa in all three species and was comparable with well-watered conditions (Table 1). In comparison with drought-stressed plants, average percentage of embolism was significantly lower in re-watered plants of *V. riparia* (16.7%) and *V. arizonica* (10.2%) but not for *V. champinii* (9.3%). In re-watered plants of all three species, the remaining embolized vessels were again concentrated around the pith (Fig. 1, bottom row).

Similar to results from single-scan experiment (see Table 1; Fig. 1), re-watered plants of the three species exhibited differences in the time course of embolism repair when subjected to multiple microCT scans over 40 h (Fig. 2).

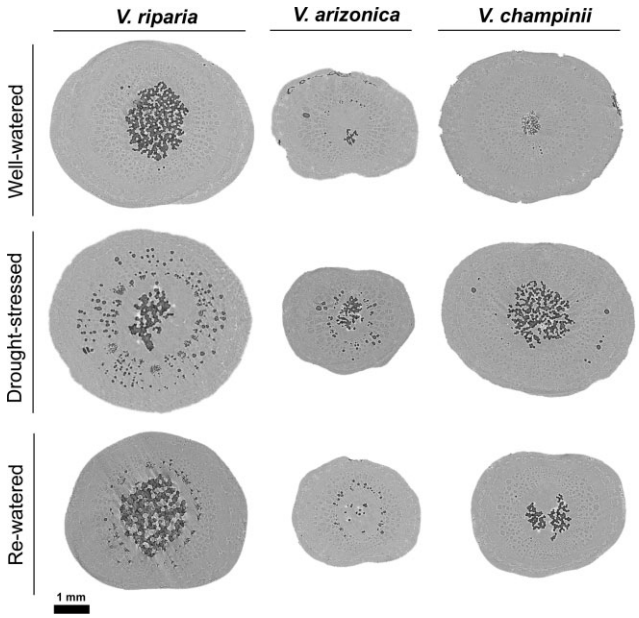

Figure 1. Representative transverse microCT images through grapevine stems (*Vitis riparia*, *V. arizonica* and *V. champinii*) showing embolism in well-watered (Ψ_{stem} > −0.5 MPa), drought-stressed (Ψ_{stem} < −1.5 MPa) and re-watered plants (Ψ_{stem} > −0.5 MPa). Embolized vessels appear dark gray and water-filled vessels as light gray. The variably hydrated pith is visible in the centre of each cross-section.

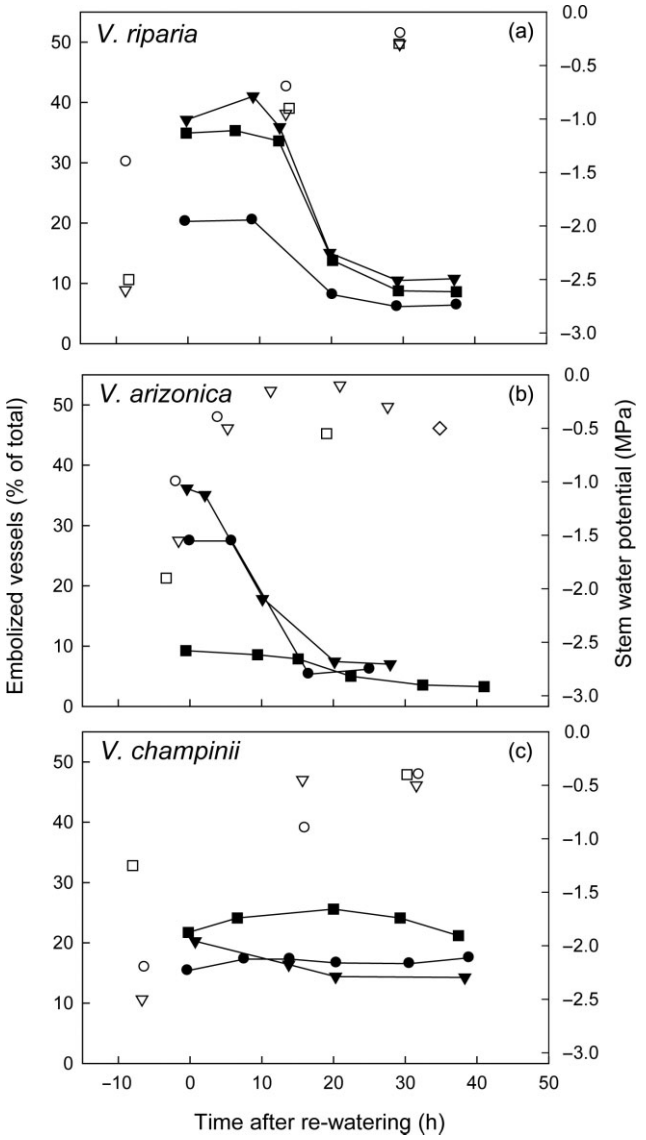

Figure 2. Representative time courses of the change in percentage of embolized vessels (closed symbols) and stem water potential (open symbols) after re-watering following drought stress in plants ($n = 3$ each) from the three grapevine species: (a) *Vitis riparia*, (b) *V. arizonica* and (c) *V. champinii*. Each line shows the recovery of a single plant scanned multiple times after re-watering.

Substantial embolism repair was found in *V. riparia* and *V. arizonica*, but not in *V. champinii*. Individual *V. riparia* and *V. arizonica* plants showed an increase in Ψ_{stem} to >−1.0 MPa and a decline in embolism after only 10 h (Fig. 2a,b); recovery in Ψ_{stem} and percentage of embolism appeared to be most rapid in *V. arizonica*. After 30 h, *V. riparia* and *V. arizonica* plants recovered to $\Psi_{stem} \geq -0.5$ MPa and percentage of embolism was <15% for both species. In contrast, the level of embolism remained similar even 40 h after re-watering for *V. champinii* plants even though Ψ_{stem} recovered similarly to the other two species (Fig. 2c).

Longitudinal microCT images revealed water droplets emerging into the lumen of embolized vessels for all three species (Fig. 3), including *V. champinii* despite its apparent lack of refilling. In line with the time course of embolism repair, we found that in *V. riparia* (Fig. 3a) and *V. arizonica* (Fig. 3b) water droplets started to emerge much earlier than in *V. champinii* (Fig. 4c) and completed the refilling process. After 28 h of recovery, all three vessels in *V. riparia* and *V. arizonica* had almost entirely refilled, whereas embolized vessels in *V. champinii* had just initiated refilling.

Leaf gas exchange

Among species, transpiration responded differentially to drought stress and after re-watering even though average values of Ψ_{stem} were comparable (Fig. 4a); responses in stomatal conductance exhibited an identical pattern (Supporting Information Fig. S1). Under well-watered conditions, transpiration was highest in *V. champinii* (14.7 mmol m^{-2} s^{-1}), followed by *V. arizonica* (9.8 mmol m^{-2} s^{-1}) and *V. riparia* (8.2 mmol m^{-2} s^{-1}). Drought stress caused a significant decrease in transpiration and stomatal conductance, which was most dramatic in *V. riparia* (by ~95%), followed by *V. arizonica* (by ~80%) and *V. champinii* (by ~75%) (Fig. 4b). *V. riparia* had an average transpiration rate of 0.4 mmol m^{-2} s^{-1} under drought stress, which was significantly lower than in *V. champinii* (3.3 mmol m^{-2} s^{-1}); in *V. arizonica*, average transpiration (1.9 mmol m^{-2} s^{-1}) was intermediate. Re-watering of plants of all three species resulted in a recovery of Ψ_{stem} to ≥ −0.6 MPa within 1 d, comparable with well-watered conditions, but the recovery of leaf gas exchange was delayed in all three species. Among species, average transpiration rates of *V. riparia* plants remained significantly lower during the re-watering period than of *V. champinii* plants (3.7 versus 7.8 mmol m^{-2} s^{-1} after 1 d, 5.6 versus 10.1 mmol m^{-2} s^{-1} after 2 d, respectively). Transpiration recovered most rapidly in *V. arizonica* and was similar to well-watered conditions on day 2, whereas *V. champinii* and *V. riparia* plants only recovered to ~50% on day 1 and ~70% on day 2.

Combining data from Table 1 (i.e. percentage of embolism) and Fig. 4b (i.e. transpiration) revealed that transpiration rates decreased with increasing percentages of embolized vessels across all three species (Fig. 4c). Low values of transpiration of *V. riparia* were associated with the highest percentages of embolized vessels under well-watered, drought-stressed and re-watered conditions. In contrast, *V. champinii* maintained the highest transpiration rates and yet the lowest percentage of embolism (Fig. 4c).

Root pressure

Root pressure measurements were taken on plants subjected to drought stress and re-watered immediately before data collection. Steady-state root pressures increased linearly for *V. riparia* and *V. arizonica* with increasing drought severity (i.e. more negative Ψ_{stem}) (Fig. 5a,b). Such a response in root pressure was not found for *V. champinii* (Fig. 5c). Root pressure in *V. riparia* was ~0.03 MPa in well-watered plants and increased to ~0.15 MPa in previously drought-stressed plants (Fig. 5a). A similar trend was observed for *V. arizonica*, but

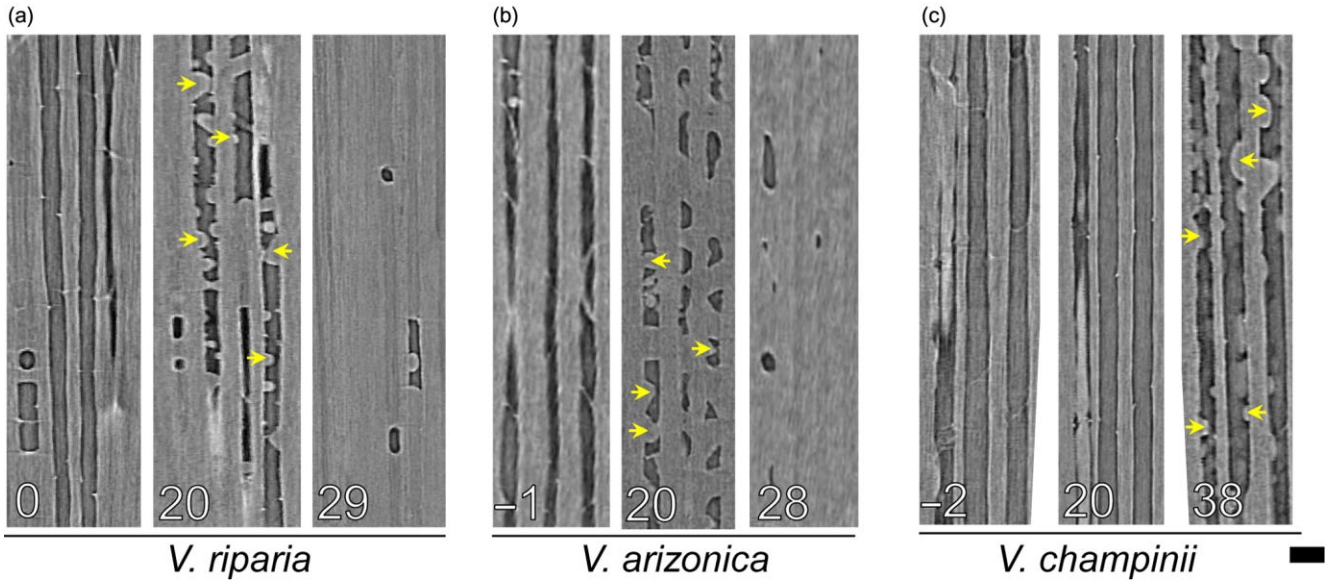

Figure 3. Longitudinal microCT images through the grapevine stem of representative (a) *Vitis riparia* (square symbols in Fig. 2a), (b) *V. arizonica* (triangle symbols in Fig. 2b) and (c) *V. champinii* (square symbols in Fig. 2c) plants. Numbers in images are the time in hours after re-watering. For each species, images show the same three embolized vessels over the time course of recovery. Examples of water droplets emerging into the lumen of these vessels are indicated by yellow arrows. Scale bar ≈ 150 μM.

root pressures were generally higher than those of *V. riparia* (Fig. 5b). In contrast, root pressure for *V. champinii* was always ~0.15 MPa for both well-watered and previously drought-stressed plants (Fig. 5c).

DISCUSSION

This study provides strong evidence that grapevine species differ in xylem embolism formation/repair, leaf gas exchange and root pressure in response to drought stress and re-watering. According to microCT analysis, xylem vessels of *V. riparia* were more vulnerable to drought-induced cavitation as compared with *V. champinii*, while *V. arizonica* was intermediate in susceptibility to cavitation. Embolism repair, as a component of drought recovery following re-watering, was initiated by water droplet formation in all three species, similar to our previous data collected for *V. vinifera* (Brodersen *et al*. 2010, 2013a). Vessel refilling was most pronounced for *V. riparia* and negligible or non-existent for *V. champinii*. In line with these findings, contrasting behaviours between *V. riparia* and *V. champinii* were also found in terms of leaf gas exchange and root pressure. Drought stress caused the greatest reduction in transpiration for *V. riparia* and after re-watering remained significantly lower as compared with *V. champinii*. Also after re-watering, root pressures generated by *V. riparia* and *V. arizonica* were higher for plants that experienced more severe drought stress, but such a response was not observed for *V. champinii*. Our data suggest a tight linkage between embolism formation/repair, leaf gas exchange and root pressure for grapevine species and highlight that species from varied native habits have different strategies to cope with and recover from drought stress.

Drought stress

We observed differences in embolism susceptibility under drought stress (i.e. $\Psi_{stem} < -1.0$ MPa) among grapevine species, with *V. riparia* being the most vulnerable (41%) and *V. champinii* (14%) being the least vulnerable species. *V. arizonica* exhibited an intermediate susceptibility to drought-induced cavitation with on average 23% embolism at a Ψ_{stem} of ~−1.5 MPa. Previous research demonstrated that *V. vinifera* has ~50% embolism under a similar Ψ_{stem} of ~−1.5 MPa and embolism spread radially via xylem vessel relays that provide connections between conduits (Choat *et al*. 2010; Brodersen *et al*. 2013a). In comparison with *V. arizonica*, Brodersen *et al*. (2013b) found that *V. vinifera* stems have higher xylem network connectivity because of the presence of ~25% more xylem vessel relays connecting large diameter vessels; also, xylem vessel relays of *V. vinifera* were less resistant to the spread of air and pathogens because they lacked pit membranes between small relay conduits. Hence, the presence/absence of structures such as xylem vessel relays can play a significant role in embolism spread and most likely account for differences in embolism susceptibility among closely related species, such as the ones studied here. The low susceptibility of *V. champinii* to drought-induced embolism and the randomly distributed nature of embolized vessels may have been the result of a less integrated xylem network with fewer or structurally different xylem vessel relays. However, obtaining reliable estimates of xylem network connectivity and vessel relay structure among grapevine species requires a comprehensive anatomical study.

Data collected with non-invasive imaging techniques (such as microCT) suggest that the xylem of grapevines is less

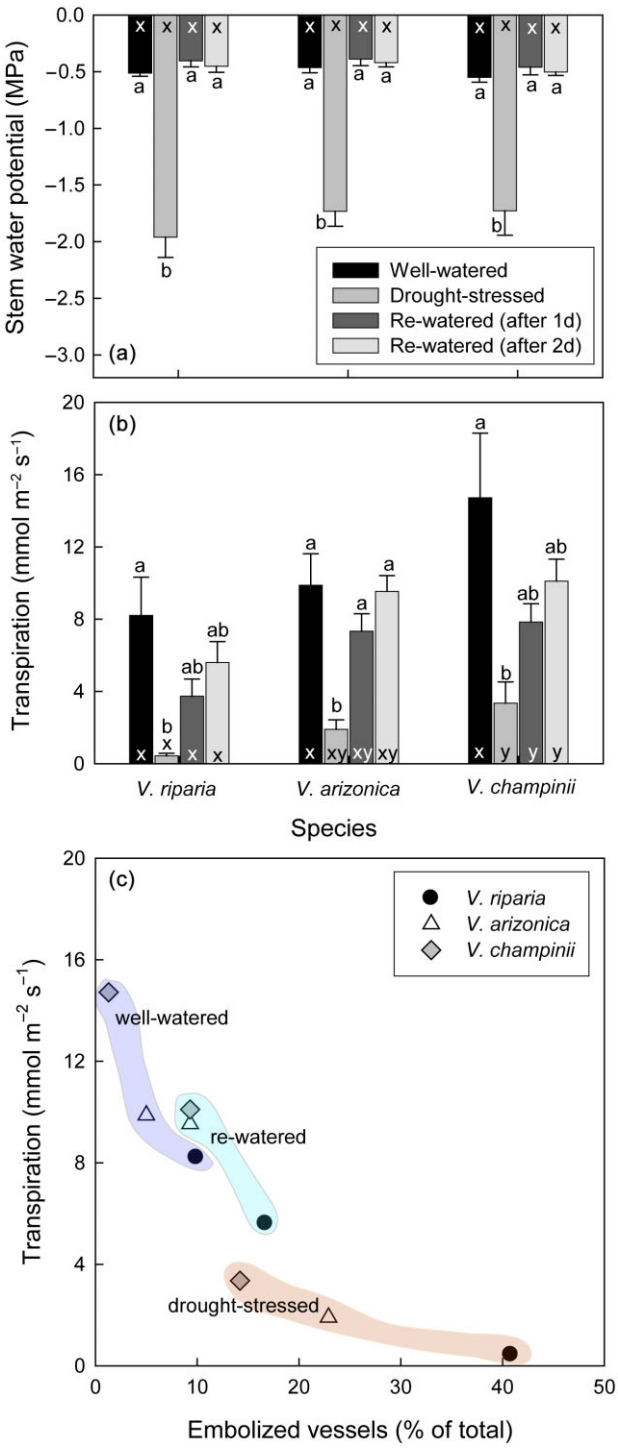

Figure 4. Mid-day measurements of (a) stem water potential and (b) transpiration of three grapevine species when well-watered, under drought-stress and after re-watering. Bars are means ± SE of the same five plants. Different letters 'a' and 'b' indicate significant differences between treatments within each species and letters 'x' and 'y' indicate significant differences between species within each treatment ($P < 0.05$). (c) For each species, mean values of percentage of embolized vessels from Table 1 were related to corresponding transpiration rates from Fig. 4b.

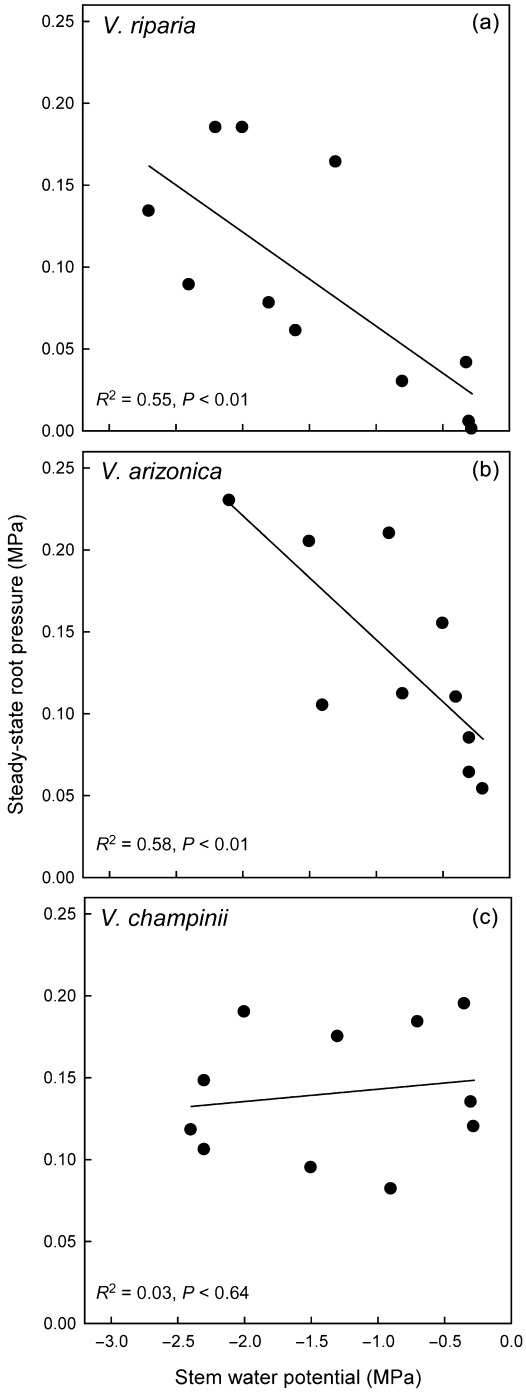

Figure 5. Relationship of stem water potential and steady-state root pressure for (a) *Vitis riparia*, (b) *V. arizonica* and (c) *V. champinii* plants. Each symbol represents an individual plant. For each plant, stem water potential was measured before cutting the stem and rehydrating the soil (x-axis) and steady-state root pressure was measured on the excised stem after re-watering at full soil moisture (y-axis). Linear regression lines across data points were modelled using the function of $y = a + b \times$ ($y = 0.07 - 0.06x$ for *V. riparia*, $y = 0.69 - 0.08x$ for *V. arizonica*, $y = 1.51 + 0.01x$ for *V. champinii*). The slopes of the linear regression lines were significantly different between *V. riparia* and *V. champinii* ($P < 0.02$) and *V. arizonica* and *V. champinii* ($P < 0.01$).

susceptible to cavitation than previously reported (Choat *et al*. 2010; McElrone *et al*. 2012; Brodersen *et al*. 2013a). Using invasive hydraulic measurements, Tibbetts & Ewers (2000) reported that *V. riparia* exhibits already ~50% embolism at Ψ_{stem} of as high as –0.2 MPa (similarly for *V. vinifera*; Jacobsen & Pratt 2012). Our microCT data highlight that percentage of embolism in *V. riparia* is <10% for $\Psi_{stem} > -0.5$ MPa; in *V. arizonica* and *V. champinii*, the maximum percentage of embolism was only 36% for Ψ_{stem} of up to –1.9 MPa. There is evidence in the literature that the relatively high embolism susceptibility reported for grapevines is an artefact associated with destructive hydraulic measurement techniques on long-vesseled species (Choat *et al*. 2010; McElrone *et al*. 2012, 2013; Cochard *et al*. 2014). Current literature also suggests that other plant species are more resistant to cavitation than previously thought questioning the reliability of hydraulic techniques for analysis of embolism formation/repair in general (Cochard & Delzon 2013; Rockwell *et al*. 2014; Wang *et al*. 2014). Hence, in order to obtain reliable estimates of embolism susceptibility and repair, we recommend using microCT technology on intact grapevine plants or to verify the reliability of alternative methods used to estimate embolism formation (e.g. Vergeynst *et al*. 2014).

A connection between a loss in hydraulic transport capacity by embolism formation and reduced transpiration by stomatal closure has been reported for a wide range of species (Sparks & Black 1999; Salleo *et al*. 2001; Brodribb *et al*. 2003; Choat *et al*. 2007; Martorell *et al*. 2013). The data of our study highlight that this relationship extends to the *Vitis* genus as well and suggest that there is a link between the amount of xylem embolism formation and stomatal closure, although we do not know the exact timing of both processes. In line with our data, field measurements of Padgett-Johnson *et al*. 2003) also indicated that drought stress caused the most significant decrease in stomatal conductance in *V. riparia*, followed by in *V. arizonica* and then in *V. champinii*.

Re-watering and recovery

Using microCT technology, we revealed for the first time a difference in the time course of embolism repair between grapevine species. Data on embolism repair of *V. vinifera* collected by Brodersen *et al*. (2010) showed a substantial reduction in embolized vessels within 24 h of re-watering. A similar time course of embolism repair following a similar stress event was found here for *V. riparia* and *V. arizonica*, where the majority of embolized vessels were refilled in a matter of hours following rehydration. For plants stressed to a Ψ_{stem} of ~–1.5 MPa, *V. arizonica* initiated refilling in less than 5 h, while *V. vinifera* (data from Brodersen *et al*. 2010) and *V. riparia* initiated this process in 7 and 10 h, respectively. In contrast, even when embolism repair was initiated in *V. champinii* (as evidenced by the presence of water droplets in some samples) and Ψ_{stem} had recovered to pre-drought levels, embolism repair was limited or incomplete in this species. This differential response in embolism repair among the three species was similar regardless of whether samples were scanned multiple times within 40 h of re-watering (Fig. 2) or scanned once 5 d after re-watering when the plants have had substantial time to repair (Table 1). Results from the single-scan experiment also helped to confirm that the lack of refilling in *V. champinii* was not due to altered cell metabolism or death associated with radiation exposure.

The limited amount of embolism in *V. champinii* could be below a threshold that triggers signalling mechanisms needed to initiate embolism repair. Secchi & Zwieniecki (2013) recently suggested that a chemical signal (i.e. oxygen) associated with embolism formation is responsible for triggering refilling in *Populus*. Alternatively, vessel-associated paratracheal parenchyma cells in *V. riparia* and *V. arizonica* may possess a different metabolic capacity that favours refilling of stored carbohydrates needed to repair stem embolism. Salleo *et al*. (2008) reported that starch depolymerization in vessel-associated parenchyma cells of *Laurus nobilis* is associated with embolism repair. Finally, the greater number of remaining functional vessels in *V. champinii* following drought stress most likely provided sufficient hydraulic transport capacity to the leaves, which resulted in a less extensive reduction in transpiration.

Similar to differences in embolism repair, the three grapevine species tested here also exhibited differences in the generation of root pressure after re-watering. Root pressure has long been suspected to play a significant role in embolism repair in grapevines (e.g. Sperry *et al*. 1987; Ewers *et al*. 1997) and root pressures of up to 0.1 MPa have been measured on intact grapevine stems (Scholander *et al*. 1955; Tibbetts & Ewers 2000) and cut side branches (Sperry *et al*. 1987; Ewers *et al*. 1997) with bubble manometers. Tibbetts & Ewers (2000) measured root pressures of 0.02 to 0.08 MPa for *V. riparia* before springtime bud break (i.e. no transpiration); their values were similar to the ones reported here for well-watered plants. However, we found that plants of *V. riparia* and *V. arizonica* generated increasingly higher root pressures with increasing severity of drought experienced prior to re-watering. This response suggests that root pressure is directly or indirectly associated with xylem refilling, which occurs at night and when xylem tensions are reduced (Holbrook *et al*. 2001; Brodersen *et al*. 2010). The generation of elevated root pressure after re-watering may aid a more rapid delivery of water through the remaining functional vessels to sites of embolism repair (Brodersen & McElrone 2013) and may reflect greater metabolic activity of solute transport by vessel-associated parenchyma both in stems and roots. Vessel-associated parenchyma are found in most plant species in which embolism repair has been documented (Martinez-Cabrera *et al*. 2009; Brodersen & McElrone 2013) and similar cells presumably exist along the entire length of the xylem transport pathway (i.e. from roots to shoots). Hence, we believe that active solute loading and associated water movement into conduits regulated by vessel-associated parenchyma cells is involved in both the generation of root pressure and refilling of embolized vessels in grapevines (see Pickard 2003; Wegner 2013).

In line with data on *V. vinifera* (Holbrook *et al*. 2001; Brodersen *et al*. 2010), we found that refilling of embolized

vessels is initiated by water droplets which exuded into the lumen of embolized vessels. Cryo-SEM data already suggested a similar refilling mechanism for sunflower (Canny 1997) and potentially for *Passiflora caerulea* (Canny *et al*. 2007). Based on our data, we have to reject the possibility of a 'bottom up' refilling mechanism in grapevines, as reported for *Arabidopsis* (Lee *et al*. 2013) and as intuitively expected from the presence of root pressure (Isnard & Silk 2009). A 'bottom up' refilling mechanism may be observed in those plant species that have less active paratracheal parenchyma cells, but based on our microCT that show emerging water droplets and no rising water column, it is unlikely to occur in young grapevine.

Slow recovery of stomatal opening after re-watering is attributed to abscisic acid accumulation and persistence in leaves following a water-deficit event (Buckley 2005; Lovisolo *et al*. 2008). In all three grapevine species studied here, transpiration was slow (>2 d) to recover after re-watering while Ψ_{stem} was comparable with well-watered condition within 1 d. However, transpiration remained lowest for *V. riparia* after re-watering, which could contribute to its ability to repair embolism by minimizing xylem tensions. In contrast, gas exchange remained highest in *V. champinii* after re-watering, which may have prevented embolism repair as it is thought to be most effective when transpiration and thus xylem tensions are minimized (Tyree *et al*. 1986; Holbrook *et al*. 2001), although it has been documented in xylem under tension (Brodersen *et al*. 2010).

There is evidence in the literature that embolism repair after re-watering can differ in timing and degree among plant species (Brodersen & McElrone 2013). For example, Hacke & Sperry (2003) found that hydraulic transport recovered by ~40% in *Laurus nobilis* and only by ~15% in *Acer negundo* within 1 d even though both species recovered in Ψ_{stem} from –2.5 to –1.0 MPa. The authors speculated that this difference is associated with varied damage under drought (e.g. leaf dieback). Drought stress in combination with supraoptimal temperatures can cause leaf dieback and ultimately leaf shedding in grapevines (Chaves *et al*. 2010). For the three grapevine species studied here, we did not observe leaf dieback when Ψ_{stem} was >–1.5 MPa. For more negative Ψ_{stem}, *V. champinii* and *V. arizonica* showed signs of leaf dieback, whereas these symptoms were rarely observed for *V. riparia*. Leaf dieback and less embolized vessels in the stem of *V. champinii* and *V. arizonica* may be a result of hydraulic segmentation, which potentially allows vessels in leaf petioles to cavitate first and subsequently limit the propagation of embolisms to the stem (Zufferey *et al*. 2011).

CONCLUSION

Our results collected on three grapevine species (*V. riparia*, *V. arizonica*, *V. champinii*) from varied native habitats demonstrate that a coordination of several plant hydraulic parameters contribute to strategies for dealing with drought stress and recovery. The relative performance of the species can be summarized as follows: (1) embolism susceptibility, *V. riparia* > *V. arizonica* > *V. champinii*; (2) embolism repair, *V. riparia* > *V. arizonica* > *V. champinii*; (3) reduction in transpiration under drought, *V. riparia* > *V. arizonica* > *V. champinii*; (4) recovery in transpiration after re-watering, *V. arizonica* > *V. riparia* ≈ *V. champinii*; (5) response of root pressure after re-watering, *V. arizonica* ≈ *V. riparia* > *V. champinii*. Through a single drought and recovery cycle, *V. champinii* maintains high levels of gas exchange by limiting embolism formation. *V. riparia* on the other hand faces the threat of significant embolism formation under drought, which may require elevated metabolic activity for embolism repair and generation of elevated root pressures while leaf gas exchange is at a minimum. It is unknown whether the strategy utilized by *V. riparia* would continue to be effective over multiple cycles of water stress given the possible demands on carbohydrate reserves.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Mid-day measurements of stomatal conductance of three grapevine species, which correspond to transpiration measurements of Fig. 4b. Bars are means ± SE of the same five plants. Different letters 'a' and 'b' indicate significant differences between treatments within each species and letters 'x' and 'y' indicate significant differences between species within each treatment ($P < 0.05$).