

Contrasting Responses of Two Grapevine Cultivars to Drought: The Role of Non-structural Carbohydrates in Xylem Hydraulic Recovery

Marco Vuerich¹, Elisa Petrusa¹, Francesco Boscutti¹, Enrico Braidot¹, Antonio Filippi^{1,2}, Francesco Petruzzellis³, Martina Tomasella³, Giuliana Tromba⁴, Mauro Pizzuto⁵, Andrea Nardini³, Francesca Secchi⁶ and Valentino Casolo^{1,*}

¹Dipartimento di Scienze Agroalimentari, Ambientali e Animali, Via delle Scienze 91, Udine 33100, Italy

²Dipartimento di Area Medica, Università di Udine, Piazzale Kolbe 4, Udine 33100, Italy

³Dipartimento di Scienze della Vita, Università di Trieste, Via L. Giorgieri 10, Trieste 34127, Italy

⁴Elettra-Sincrotrone Trieste, Area Science Park, Basovizza, Trieste 34149, Italy

⁵Vivai Cooperativi Rauscedo, Via Udine, 39, Rauscedo (PN) 33095, Italy

⁶Dipartimento di Scienze Agrarie, Forestali, Alimentari (DISAFA), Università di Torino, Largo Paolo Braccini 2, Grugliasco (TO) 10095, Italy

*Corresponding author: E-mail, valentino.casolo@uniud.it

Xylem embolism is one of the possible outcomes of decreasing xylem pressure when plants face drought. Recent studies have proposed a role for non-structural carbohydrates (NSCs) in osmotic pressure generation, required for refilling embolized conduits. Potted cuttings of grapevine Grenache and Barbera, selected for their adaptation to different climatic conditions, were subjected to a drought stress followed by re-irrigation. Stem embolism rate and its recovery were monitored in vivo by X-ray micro-computed tomography (micro-CT). The same plants were further analyzed for xylem conduit dimension and NSC content. Both cultivars significantly decreased Ψ_{pd} in response to drought and recovered from xylem embolism after re-irrigation. However, although the mean vessel diameter was similar between the cultivars, Barbera was more prone to embolism. Surprisingly, vessel diameter was apparently reduced during recovery in this cultivar. Hydraulic recovery was linked to sugar content in both cultivars, showing a positive relationship between soluble NSCs and the degree of xylem embolism. However, when starch and sucrose concentrations were considered separately, the relationships showed cultivar-specific and contrasting trends. We showed that the two cultivars adopted different NSC-use strategies in response to drought, suggesting two possible scenarios driving conduit refilling. In Grenache, sucrose accumulation seems to be directly linked to embolism formation and possibly sustains refilling. In Barbera, maltose/maltodextrins could be involved in a conduit recovery strategy via the formation of cell-wall hydrogels, likely responsible for the reduction of conduit lumen detected by micro-CT.

Keywords: Embolism • Micro-CT • Refilling • Starch • Sucrose • Vessel diameter

Introduction

Over recent decades, terrestrial plants have faced increasing frequency and intensity of water stress events (Dai 2011). Intense or prolonged drought can threaten plant growth and survival, with negative effects on crops (Lesk et al. 2016) and natural vegetation (Adams et al. 2017). Vascular plants can sustain photosynthesis and growth by maintaining the balance between the water absorbed by roots from the soil and the water lost by transpiration (Venturas et al. 2017, Nardini et al. 2018). However, severe drought events can lead to a drop of xylem pressure below species- and organ-specific critical thresholds, leading to embolism-induced disruption of the continuity of water columns in xylem conduits (Tyree and Sperry 1989, Mayr et al. 2014) and ultimately causing plant dehydration and dieback (Tyree 1988, Tyree and Zimmermann 2002, Nardini et al. 2013, Choat et al. 2018). In woody angiosperms, such detrimental consequences are thought to occur when xylem hydraulic conductance drops by >60–80% compared to the well-hydrated status (Nardini et al. 2013, Urli et al. 2013, Adams et al. 2017).

The drought-induced decline of water potential depends on the hydraulic strategy and stomatal behavior that is species/variety dependent and shifts between two theoretical extremes: conservative and dissipative (Klein et al. 2014, Charrier et al. 2018). However, the interaction with environmental conditions and drought intensity can shape this response (strategy), leading to a modulation of stomatal response, even in the same species/variety (Martínez-Vilalta and Garcia-Forner

2017, Hochberg et al. 2018). Different sensitivities in stomatal regulation and photosynthetic behaviors of plants have been claimed to lead to different trajectories of non-structural carbohydrate (NSC) content and plant hydraulic functioning under drought. These would range from (i) a progressive decline of energy sources (carbon starvation) (McDowell et al. 2008) if carbon demand strongly exceeds carbon supply to (ii) a complete failure of hydraulic conductance depending on the species-specific vulnerability to embolism. The first scenario is more likely for plants displaying a conservative water-use strategy, whereas the second is expected in species maintaining high levels of gas exchanges, even under water shortage. Nonetheless, also in the first case, direct links between NSC decline, water potential dynamics, and stomatal regulation need to be cautiously considered (Garcia-Forner et al. 2017). However, most drought events do not suddenly reach critical thresholds leading to hydraulic failure, and woody plants can respond to water deprivation with medium-, long-term acclimation responses including morpho-anatomical changes such as the production of new xylem conduits (Zhang et al. 2019), increased conduit wall thickness and wood density, as well as leaf hydraulics changes (Sorek et al. 2021), restrictions of vessel at petiole level (Falchi et al. 2020) and pit membrane modifications (Hacke et al. 2001, Sperry et al. 2006, 2007). When drought ends and water supply is restored, plants can also recover their hydraulic functioning by means of rapid physiological responses. Indeed, it has been suggested that, under certain conditions, plants can refill embolized conduits (Triflò et al. 2015, Savi et al. 2016, Yoshimura et al. 2016, Beikircher and Mayr 2017, Gleason et al. 2017, Love and Sperry 2018).

The actual occurrence of embolism repair has been intensively investigated and debated (Martorell et al. 2014, Ooeda et al. 2016, Secchi and Zwieniecki 2016, Triflò et al. 2017, Brodersen et al. 2018, Nardini et al. 2018). This phenomenon is frequently accompanied by the increase of the sugar pool at the expense of starch (Yoshimura et al. 2016, Beikircher and Mayr 2017, Pagliarani et al. 2019, Wang et al. 2019, Tomasella et al. 2021), leading to the hypothesis of an active osmotic role of NSCs during the refilling process (Nardini et al. 2011a, Brodersen and McElrone 2013, Knipfer et al. 2016), and in particular to generate a local positive pressure driving water flow into gas-filled conduits (Secchi and Zwieniecki 2011, 2012).

Despite experimental evidence supporting hydraulic recovery in tracheophytes based on refilling of embolized conduits, the validity of these findings is still under debate, in particular for reports suggesting refilling under residual moderate xylem tension (Cochard and Delzon 2013). Synchrotron X-ray micro-computed tomography (micro-CT) has been used to visualize in vivo embolism formation and subsequent refilling (Brodersen et al. 2013). In particular, several micro-CT experiments on embolism repair were performed on *Vitis vinifera*, with some studies confirming the occurrence of this phenomenon (Holbrook et al. 2001, Brodersen et al. 2013, Knipfer et al. 2015), but others reported a lack of conduits refilling under residual

negative pressure after rehydration, at least in the apical part of the plant (Charrier et al. 2016).

These dissimilar results do not surprise, considering the critical role of plant physiological status at the end of drought in determining whether embolism repair is energetically possible (Savi et al. 2016, Tomasella et al. 2019, 2021, Triflò et al. 2021). Moreover, the actual mechanisms of water stress response in *V. vinifera* are complicated by several anatomical and physiological peculiarities due to its vine habitus and ecology, features that are also variable among cultivars (Lovisolo et al. 2010). In particular, a critical point is the range of regulation of stomatal closure in different cultivars (Hochberg et al. 2018, Villalobos-González et al. 2019), which might reflect also in NSC metabolism under drought and thus possibly on the occurrence of xylem refilling process upon rehydration. In drought-stressed grapevines, starch depletion has been observed (Rodrigues et al. 1993). However, NSCs were shown to be involved in active osmotic adjustment in immature leaves, while a major role is played by inorganic ions (Patakas and Noitsakis 2001, Patakas et al. 2002). Recent studies on canes and leaf petioles of grapes suggested that the variation in NSCs mainly depends on the type of drought stress (i.e. short and severe vs mild and prolonged) and on the hydraulic behavior displayed under stress (Falchi et al. 2019, 2020, Morabito et al. 2021).

Considering that high resistance to embolism is generally associated with genotypes distributed in dry environments (Maherali et al. 2004), we tested the relationship between hydraulics and carbon metabolism under drought and recovery in two red grape varieties, i.e. Grenache, the most widely planted red grape variety, considered as drought resistant and well adapted to Mediterranean regions, and Barbera, native from Northern Italian region and more associated to continental climate conditions. Based on micro-CT analysis, we analyzed xylem embolism in grapevine cuttings exposed to a severe drought and their xylem functionality recover upon rehydration by refilling vessels. Concurrently, we investigated the role of NSCs during the drought stress and subsequent recovery, focusing on different compounds potentially important for osmoregulation and for energetic metabolism (i.e. glucose, sucrose, maltose/maltodextrins and starch). We expected sugar levels to increase under drought, followed by a reduction near to pre-stress values during recovery. We specifically aimed at describing potential cultivar-based differences in xylem embolism formation/recovery, as coupled to NSC metabolism.

Results

Relationship between stomatal conductance and stem water potential

Under drought stress, both cultivars underwent a reduction of gas exchange rates directly related to the water potential decrease. However, the interpolation curves obtained from punctual measurements showed that Grenache reached a leaf conductance to water vapor $<50 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ at Ψ_{pd} of

approximately -0.7 MPa, while in Barbera the same level of gas exchange was reached at lower values (about -1.0 MPa) (Supplementary Fig. S1). Consistently, the absolute lower values of Ψ_{stem} were reached in Barbera.

Water status

Experimental treatments significantly affected the predawn water potential (Ψ_{pd}) (Table 1), while neither the cultivar nor the interaction between factors was significant. When grapevine plants were exposed to drought, in both cultivars a similar and significant decrease of Ψ_{pd} was observed (Fig. 1A). After rewatering, Ψ_{pd} returned to pre-drought values. The degree of xylem embolism was statistically influenced by the treatment and cultivar, but not by their interaction (Table 1). Both cultivars showed a two-fold increase in embolism percentage during drought, followed by a recovery after rewatering (Fig. 1B), as documented by micro-CT images in terms of the number of gas- vs water-filled conduits

Table 1 ANOVA (two-way) applied to Ψ_{pd} and xylem embolism in relation to cultivar, treatment and their interactions

Response variable	Factor	df	F-value	P
Ψ_{pd}	Cultivar	1.39	0.20	0.655
	Treatment	2.39	36.38	<0.001
	Cultivar * treatment	2.39	0.25	0.786
Xylem embolism	Cultivar	1.32	4.72	0.037
	Treatment	2.32	4.84	0.015
	Cultivar * treatment	2.32	0.02	0.981

Significant relationships are in bold.
df = degrees of freedom; F = Fisher value; P = level of significance.

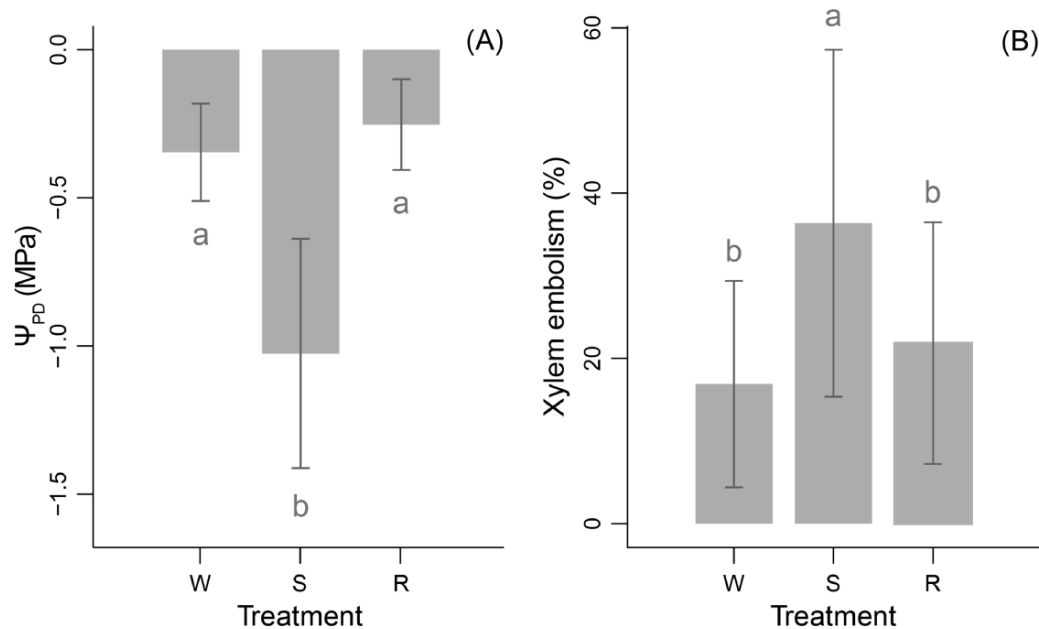


Fig. 1 Predawn leaf water potential (Ψ_{pd}) and xylem embolism percentage in cv. Barbera and Grenache under different water status. Ψ_{pd} (A) and xylem embolism (B) in well-watered (W), water-stressed (S) and recovered (R) plants of cv. Barbera and Grenache. Means \pm SD ($n = 14$) are shown. Different letters correspond to statistically significant differences ($P < 0.05$).

(Fig. 2). Well-watered Grenache plants displayed a significantly lower level of embolism rate (Table 1) ($20.0 \pm 16.3\%$) than Barbera plants ($31.4 \pm 18.4\%$).

Non-structural carbohydrates

The stem content of the total soluble NSC was significantly affected by the treatment, following the same trend of embolism rate (Table 2). In both cultivars, total soluble NSC content was higher in water-stressed (S) plants than in well-watered (W) and re-irrigated (R) plants (Fig. 3A). Starch level in stems did not show significant changes with respect to treatment, but it was influenced by cultivar (Table 2), as its content was more than the double in Barbera (12.5 ± 10.2 mg g^{-1} DW) compared to Grenache (5.0 ± 4.5 mg g^{-1} DW).

Among the analyzed sugar components (i.e. glucose, sucrose and maltose/maltodextrins), sucrose was the most abundant soluble carbohydrate in both cultivars, representing about two-thirds of the total NSC. Although its content showed a similar pattern observed for total sugars, it was not significantly affected either by the experimental treatments or by their interaction (Table 2).

Glucose represented a minor component of NSCs (approximately 1 mg g^{-1} DW) and did not display significant changes between treatments, cultivar or their interaction (Table 2).

Maltose and maltodextrins content was significantly different between cultivars (Table 2), reaching approximately 20% and $<10\%$ of the total NSC in Barbera and Grenache, respectively (Fig. 3B). The two cultivars exhibited contrasting trends when subjected to treatments (Table 2). Maltose and maltodextrins increased during recovery in Barbera but decreased

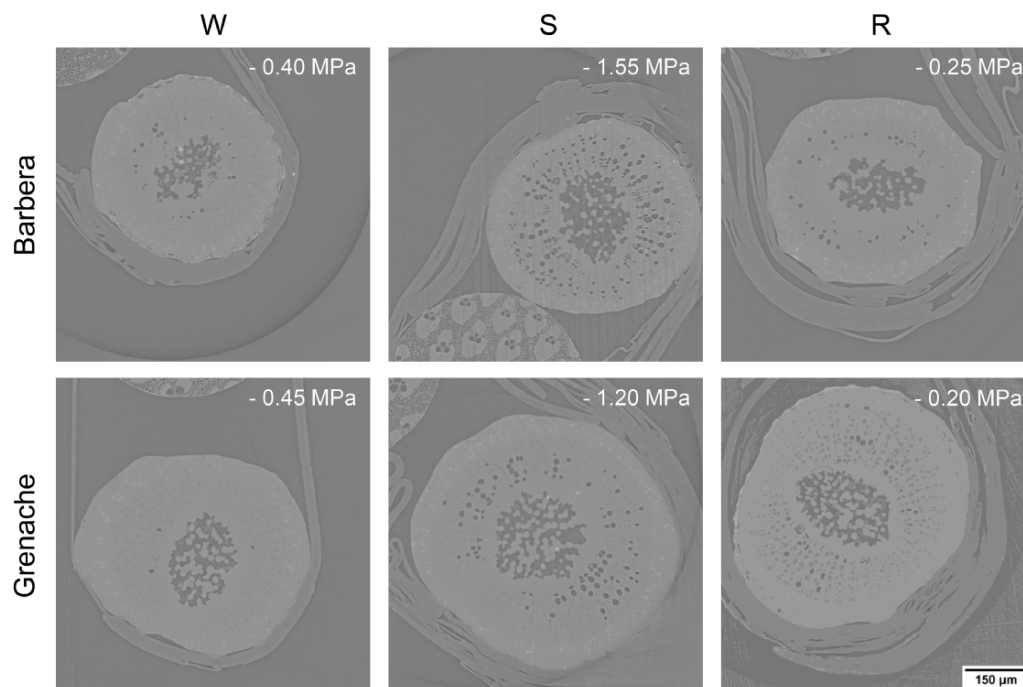


Fig. 2 Xylem embolism visualization by the micro-CT image analysis in plant stems of cv. Barbera and Grenache under different water status. Imaging of transverse sections of micro-CT scans showing stem xylem functionality as the occurrence of water-filled vessels (light gray) or embolized conduits (appearing as black gray) in well-watered (W), water-stressed (S) and re-irrigated (R) plants of cv. Barbera and Grenache. Ψ_{pd} values of these samples at the time of scanning were shown in the images. Bars = 150 μm .

Table 2 ANOVA (two-way) applied to the total soluble NSC, sucrose, glucose, maltose/maltodextrins and starch content in relation to cultivar, treatment and their interactions

Response variable	Factor	df	F-value	P
Total soluble NSC	Cultivar	1.38	1.35	0.252
	Treatment	2.38	5.89	<0.006
	Cultivar * treatment	2.38	1.35	0.272
Sucrose	Cultivar	1.39	4.72	0.858
	Treatment	2.39	2.11	0.134
	Cultivar * treatment	2.39	0.46	0.632
Glucose	Cultivar	1.33	0.005	0.942
	Treatment	2.33	0.026	0.974
	Cultivar * treatment	2.33	0.107	0.342
Maltose/maltodextrins	Cultivar	1.35	36.80	<0.001
	Treatment	2.35	0.026	0.209
	Cultivar * treatment	2.35	0.107	0.004
Starch	Cultivar	1.33	13.77	<0.001
	Treatment	2.33	1.15	0.330
	Cultivar * treatment	2.33	1.13	0.337

Significant relationships are in bold.
df = degrees of freedom; F = Fisher value; P = level of significance.

in Grenache. The stem concentration of each NSC investigated for each treatment and for each cultivar is reported in [Supplementary Table S1](#).

Relationship between Ψ_{pd} , NSC content and xylem embolism

A significant negative relationship between the xylem embolism rate and Ψ_{pd} was observed for the two cultivars ([Table 3](#),

[Fig. 4](#)). In both cultivars, the relationship between the concentration of the total soluble NSC and the xylem embolism rate showed consistent trends, with an increasing total soluble NSC along with the increasing embolism rate ([Table 3; Fig. 5](#)).

Significant relationships emerged between the concentration of both starch and sucrose and the xylem embolism level, with cultivar-specific trends ([Table 3](#)). In Barbera, a negative significant relationship emerged between starch and embolism rate, while in Grenache the relationship was positive, with a lower slope ([Fig. 6A](#)). Among the single sugar compounds analyzed, only sucrose content was positively influenced by the embolism level in the Grenache cultivar, whereas no significant relationship was observed in Barbera ([Fig. 6B](#)).

Vessel area

Barbera and Grenache differed in the distribution of vessel size classes in their stems ($P < 0.001$). In fact, Grenache showed a significantly higher number of wide vessels ([Supplementary Fig. S2A](#)). The very short recovery time (2 d) likely did not allow the production of new vessels ([Supplementary Fig. S2B](#)). Nonetheless, when comparing the diameter of vessels in stressed vs recovered samples, a significant difference was observed in Barbera, which showed an apparent increase in the percentage of narrower vessels in recovered ones ($P < 0.001$) ([Supplementary Fig. S3](#)).

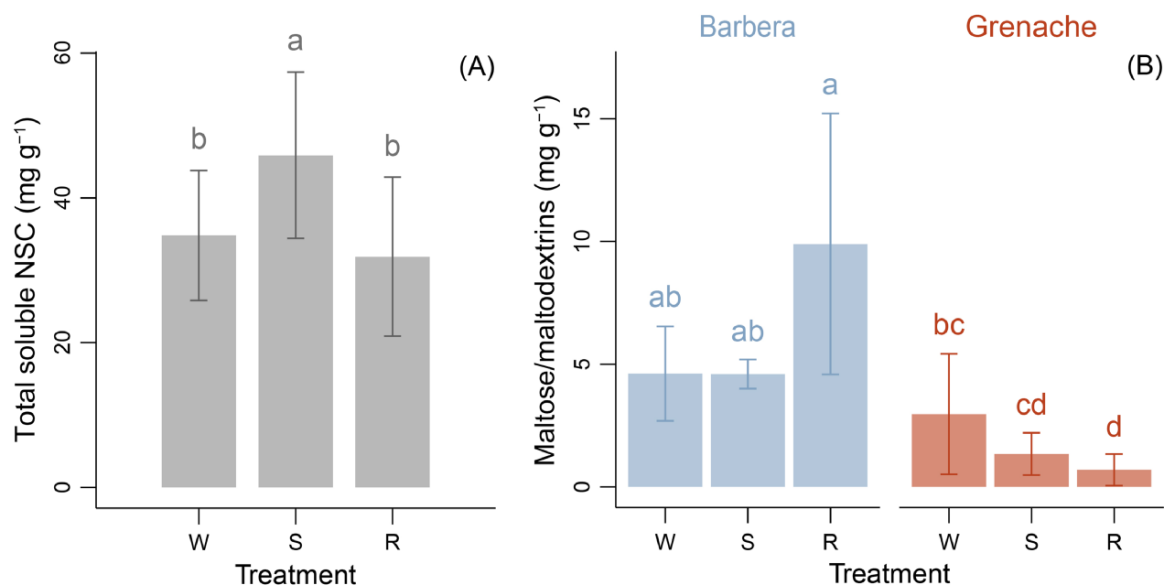


Fig. 3 Variation of NSCs under different water status. The total soluble NSC (A) in stems of well-watered (W), water-stressed (S) and recovered (R) plants ($n = 14$). Maltose and maltodextrins content (B) in stems of well-watered (W), water-stressed (S) and recovered (R) plants of cv. Barbera (left) and Grenache (right) ($n = 7$). Means \pm SD are shown. Different letters correspond to statistically significant differences ($P < 0.05$).

Table 3 Results of ANOVA applied to the linear models describing the relationship between xylem embolism and Ψ_{pd} and NSCs and xylem embolism in stem of Barbera and Grenache grapevines; cultivar (Barbera and Grenache), Ψ_{pd} (in the case of xylem embolism) or xylem embolism (in the case of the total soluble NSC, sucrose, glucose, log(Maltose/maltodextrins) and log(Starch)) and their interactions were tested

Response variable	Explanatory variable	df	F-value	P
Xylem embolism	Cultivar	1.34	0.68	0.425
	Ψ_{pd}	1.34	27.50	<0.001
	Cultivar * Ψ_{pd}	1.34	0.92	0.345
Total soluble NSC	Cultivar	1.34	0.24	0.627
	Xylem embolism	1.34	7.10	0.011
	Cultivar * xylem embolism	1.34	1.66	0.207
Sucrose	Cultivar	1.32	0.05	0.831
	Xylem embolism	1.32	2.50	0.124
	Cultivar * xylem embolism	1.32	6.25	0.018
Glucose	Cultivar	1.34	0.59	0.449
	Xylem embolism	1.34	0.32	0.580
	Cultivar * xylem embolism	1.34	0.22	0.646
log(Maltose/maltodextrins)	Cultivar	1.32	17.81	<0.001
	Xylem embolism	1.32	0.58	0.598
	Cultivar * xylem embolism	1.32	0.01	0.913
log(Starch)	Cultivar	1.32	16.58	<0.001
	Xylem embolism	1.32	0.16	0.367
	Cultivar * xylem embolism	1.32	4.48	0.042

Significant relationships are in bold.

df = degrees of freedom; F = Fisher value; P = level of significance.

Discussion

Relationship between stomatal conductance and stem water potential

Young cuttings of *V. vinifera* subjected to the experimental drought underwent a decrease of Ψ_{stem} to values consistent with those recorded in field-growing grapevine plants experiencing severe summer water shortage (Lovisolo et al.

2010). Barbera and Grenache are originally adapted to different climate conditions and were expected to exhibit different hydraulic behaviors in response to drought (Schultz 2003, Shelden et al. 2017). Consistently, young cuttings from these cultivars revealed different levels of gas exchanges when Ψ_{stem} decreases, confirming the near-isohydric behavior of Grenache (Gerzon et al. 2015) and suggesting the near-anisohydric behavior of Barbera.

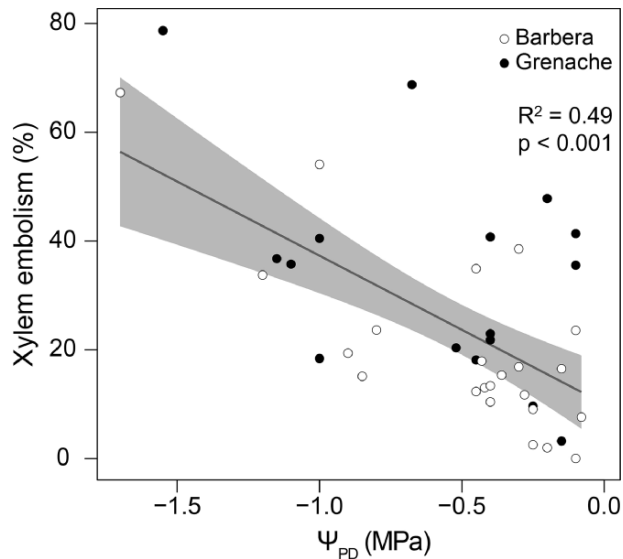


Fig. 4 Overall relationships between predawn leaf water potential and xylem embolism. Relationships between the predawn leaf water potential and xylem embolism according to the outcomes of applied linear models. Shaded areas represent the confidence intervals (0.95).

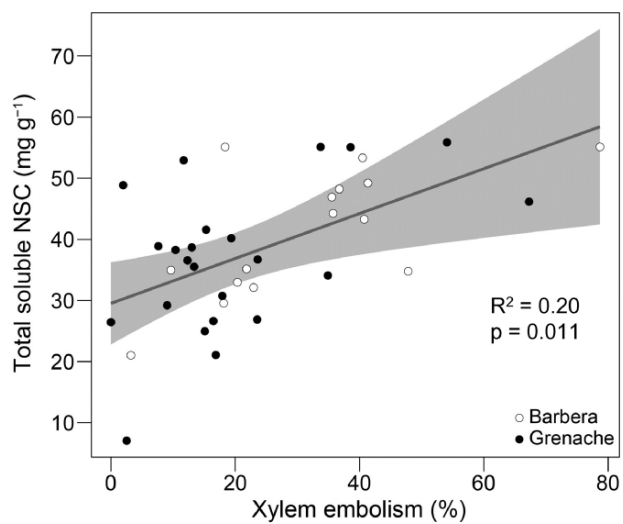


Fig. 5 Overall relationships between xylem embolism and the total soluble NSC. Relationships between xylem embolism and the total soluble NSC, according to the outcomes of applied linear models. Shaded areas represent the confidence intervals (0.95).

Water status

Despite Grenache showed a higher number of wide xylem vessels compared to Barbera and a constitutive varietal difference in the degree of xylem embolism was detectable, its pattern under drought was similar in both cultivars. In both cultivars, the observed decrease in water potential under water shortage was coupled to the increase of xylem embolism rates, and this behavior and relative susceptibility to xylem embolism under dehydration was likely associated with cultivar-specific vessel

network characteristics (Jacobsen et al. 2015, Venturas et al. 2017).

The recovery phase allowed plants to return to the same Ψ_{pd} values measured before the water stress treatment. A complete recovery of xylem functional status to pre-drought levels was also observed, indicating that grapevine can recover from embolism through the refilling of empty vessels, as already shown in previous studies in the genus *Vitis* (Brodersen et al. 2018, 2013, Knipfer et al. 2016) and in other woody species (Wheeler et al. 2013, Secchi and Zwieniecki 2014, Trifilò et al. 2014). However, it should be stressed that even if a recovery of water-filled vessels was demonstrated through micro-CT imaging in both cultivars after rewatering, only actual measurements of the vessel conductivity by means of tracers could demonstrate that refilled vessels regained full functionality of water transport (Pratt and Jacobsen 2018, Pratt et al. 2020).

Active role of NSCs in the xylem refilling mechanism

Previous observations of grapevine responses to drought showed a reduction of NSC content in leaves (Rodrigues et al. 1993, Patakas et al. 2002). Instead, our results showed that grapevine responds to water stress with an accumulation of NSCs at the stem level, consistently with what observed also in leaf petioles of cultivar Merlot (Vuerich et al. 2021) and in stems of Grenache when exposed to slow-developing drought (Morabito et al. 2021). Consistently with these apparently contrasting observations, Savi et al. (2019a) reported for the cultivar Riesling an even more complex scenario, where the NSC increase or decrease during drought is depending on organ and carbohydrate type.

The observed NSC accumulation in the stem is compatible with the role that soluble carbohydrates play in osmoregulation when woody plants are exposed to drought (Peltier et al. 2021, Tomasella et al. 2021), low temperature (Baffoin et al., 2021, Lintunen et al. 2016, Casolo et al. 2020) or salinity (Gil et al. 2013, Pellegrini et al. 2020). This has also been reported for *V. vinifera* exposed to water stress (Patakas and Noitsakis 2001, Degu et al. 2019), although inorganic ions like K^+ (Monder et al. 2021) are considered the major drivers of this process in grapevine (Patakas et al. 2002). In short severe drought, the observed increase in stem soluble NSCs in both cultivars was consistent with that reported when a slow-developing drought was imposed in Grenache (Morabito et al. 2021). This result suggests that the type of stress may not be the only driver modulating the response of grapevines to drought, but other traits such as rootstock genotype and scion size might be involved.

When the total soluble NSC was plotted vs embolism rate, a positive relationship emerged for both cultivars. This finding is consistent with the relationships between soluble NSCs and xylem embolism levels reported for other woody plants (Yoshimura et al. 2016, Liu et al. 2019, Trifilò et al. 2019, Nakamura et al. 2021), as well as with observations of NSC consumption coupled to embolism recovery at the end of the water stress (Tomasella et al. 2019).

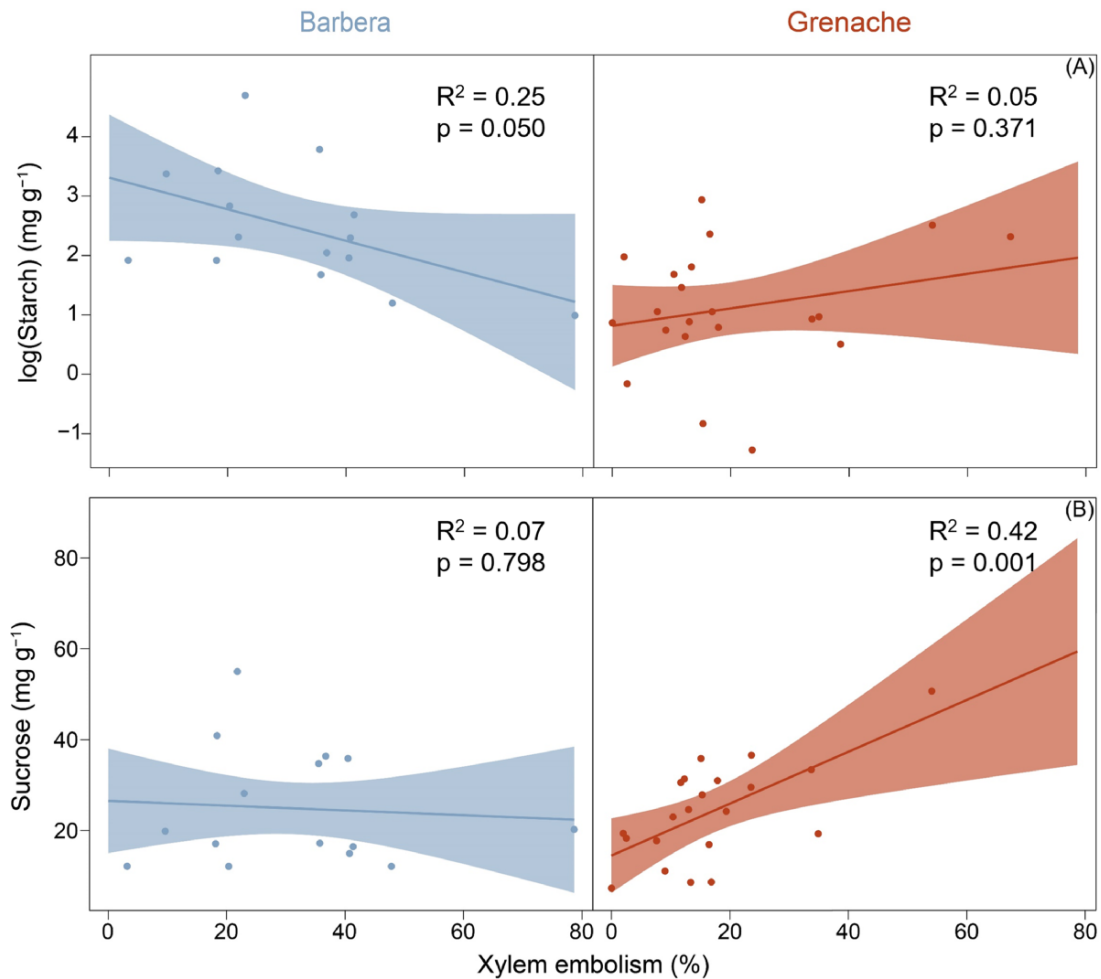


Fig. 6 Overall relationships between xylem embolism, starch and sucrose. Relationships between xylem embolism and the content of starch in cv. Barbera (blue) and Grenache (red) (A); relationships between xylem embolism and the content of sucrose in cv. Barbera (left) and Grenache (right) (B), according to the outcomes of applied linear models. Shaded areas represent the confidence intervals (0.95).

Previous studies have reported that the contents of different sugar types in plants subjected to drought were variable (Pagliarani et al. 2019, Savi et al. 2019b) as observed also in grapevine leaf, stem, root (Savi et al. 2019a) and petioles (Falchi et al. 2020). In our experiment, when considering separately sucrose, glucose and maltose (including maltodextrins), it appeared that only in Grenache the sucrose sustained soluble NSC response to drought (positive relationship with xylem embolism). On the contrary, when the relationship between starch and xylem embolism was considered, the scenario was the opposite, with Barbera displaying a significant negative relationship between starch and xylem embolism. Our data suggest that only in Grenache the refilling model proposed by Secchi and Zwieniecki (2016) was likely operating. In fact, in Grenache embolism recovery was apparently coupled to sucrose levels, which were directly correlated with the total NSC. In the above-cited model, soluble NSCs were proposed to derive from starch by the acid invertase pathway producing glucose and fructose (Pagliarani et al. 2019). Our data in Grenache confirm a direct involvement of sucrose that probably derives from

the maltose/maltodextrin pool (representing soluble, ready-to-use starch), rather than starch. The low content of maltose in stressed and recovered Grenache plants also indicates that sucrose production guaranteed from photosynthesis is limited under such conditions. On the contrary, in Barbera it may be hypothesized that embolism repair could occur with a different mechanism, with starch hydrolyzed from stress to recovery through the amylase pathway, producing maltose and then increasing glucose which is consumed during recovery.

Indirect role of NSCs in xylem refilling by vessel size reduction

The progressive reduction of vessel dimensions is a long-term acclimation response to drought observed in many woody plants, including grapevine (Lovisolo and Schubert 1998). This response has been suggested to play a role in the mitigation of embolism-induced loss of hydraulic conductance risk (Blackman et al. 2010, Jupa et al. 2021). Nevertheless, the time-lapse of the present experiment was not long enough to allow

for the production of new xylem during or after drought stress, as indeed observed in leaf petioles of grapevines subjected to different water treatments by [Falchi et al. \(2020\)](#).

However, the recorded reduction in vessel diameter observed in Barbera raises some interesting questions. This finding, coupled to the lack of a relationship of sucrose with xylem embolism and to the increase in maltose/maltodextrins during the recovery phase, may suggest the presence in this cultivar of a refilling mechanism different from the one proposed by [Secchi and Zwieniecki \(2016\)](#). Maltodextrins are water-soluble short-chain carbohydrate polymers produced by the breakdown of starch ([Beck and Ziegler 1989](#)), which are known to form highly hygroscopic aqueous gels ([Kennedy et al. 1995](#), [Chronakis 1998](#), [Loret 2004](#)), also in combination with pectin ([Evageliou 2000](#)). We speculate that, during recovery, maltodextrins could be transferred into the apoplast, soaking xylem vessels. This could lead to a change in the density of cell walls, detected by micro-CT as a cell-wall swelling and leading to apparently reduced diameter of conduit lumen. Consistently with such a speculative hypothesis, gel formation has been already observed in embolized vessels of grapevine ([Jacobsen and Pratt 2012](#), [Pratt and Jacobsen 2018](#)). Interestingly, the authors argued that vessel occlusion by gels, differently from tyloses, could be reversible and likely involved in the process of xylem refilling, as also hypothesized by [Sun et al. \(2008\)](#), where the matrices of wound-induced vascular occlusions were demonstrated by cytohistological analyses to be rich in pectin.

The interaction of pectin-hydrogels at the pit membrane level with xylem sap cations has been suggested to be involved in the modulation of xylem hydraulic resistance ([Nardini et al. 2011b](#); [Plavcová et al. 2011](#), [Zwieniecki et al. 2001](#)). This hypothesis has been recently challenged ([Kaack et al. 2019](#)), although the same study reported the presence of pectins in the annulus of pit membranes, which could interact with maltodextrins. An even more fascinating hypothesis involving

carbohydrate polymers in the control of vessel refilling, named ‘pit membrane osmosis’, was proposed by [Hacke and Sperry \(2003\)](#). Maltodextrins might be released into embolized vessels, lowering Ψ and producing a hydrogel held in the cell wall which, due to its polymerization with pectins, cannot move across pits that would thus act as an osmotic ‘membrane’. The hygroscopic capacity of maltodextrins accumulated in the cell wall of embolized conduits might drive water inflow from both neighboring parenchyma cells and water-filled conduits, thus allowing reconnection to the transpiration stream. Alternatively, modification of surface tension induced by maltodextrins might favor hydraulic isolation of refilling conduits, via the interaction with pit membrane features, making a possible local generation of positive pressure via osmotic mechanisms, while the bulk of still water-filled conduits are under residual negative pressure ([Zwieniecki and Holbrook 2000](#)).

In conclusion, our results showed that 1-year-old cuttings of *V. vinifera* can recover from xylem embolism under moderate residual tension, through an active refilling process likely mediated by soluble NSCs in a direct (Grenache) or indirect (Barbera) way ([Fig. 7](#)). In the model proposed for Grenache, starch does not undergo any significant decrease (as observed for maltose), but the total soluble NSC content (in particular sucrose) is directly correlated with the degree of embolism, consistent with the hypothesis proposed by [Secchi and Zwieniecki \(2016\)](#), in which sugar accumulation in the xylem sap is necessary to generate a local positive pressure forcing water in the embolized vessels. On the other hand, in Barbera the increase of maltose and maltodextrins parallels a decrease in starch. We suggest the possible formation of cell-wall pectin-hydrogel, due to maltose/maltodextrins accumulation deriving from starch degradation, that could act as a hygroscopic sponge unable to move through pits but drawing water from neighboring parenchyma cells.

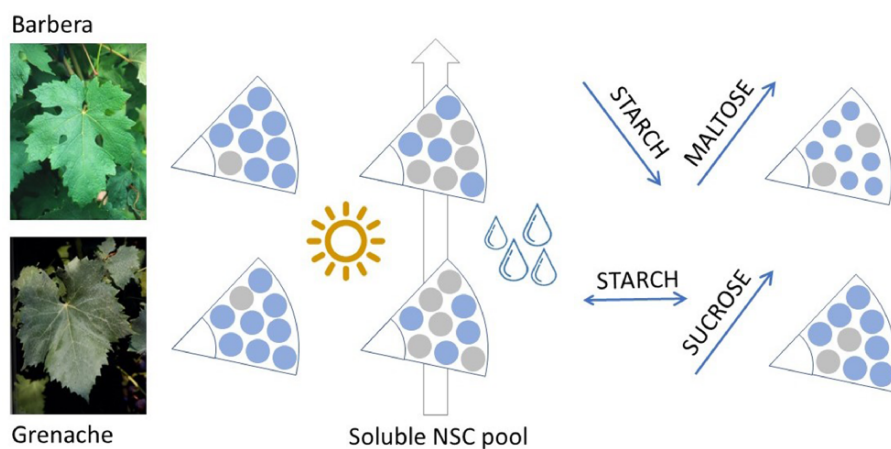


Fig. 7 The proposed model of the alternative pathways involved in embolism recovery for Barbera and Grenache. Barbera responded to drought by converting starch to maltose, producing a hydrogel effect leading to an apparent decrease in vessel diameter; Grenache increased sucrose concentration without a parallel reduction in starch and promoting recovery by refilling likely osmotic mechanism.

Material and Methods

Plant material and growth conditions

One-year-old cuttings of grapevine (*Vitis vinifera* L.) cv. Barbera and Grenache (VCR 207 and VCR3 clones, respectively), both grafted on 15-cm-long SO4 rootstock, were provided by Vivai Cooperativi Rauscedo (VCR) (Rauscedo, Italy). In late May 2019, cuttings were planted in 1-l pots filled with field-sieved soil supplemented with 10% perlite and randomly allocated in a growth chamber at the University of Udine (Italy) (mean temperature 22°C, light:dark photoperiod 12:12 h, artificial photosynthetic photon flux density of 92 $\mu\text{mol s}^{-1} \text{m}^{-2}$, relative humidity ca. 40%). Plants were irrigated to field capacity every 1–3 d, until early September, when the experiment started. When used at the micro-CT beamline, plants of both cultivars showed similar sizes, compatible with the sample holder.

Experimental design

The experimental treatments were applied in early September 2019. At the beginning of the experiment, seven plants per cultivar were kept as controls and daily irrigated for the whole duration of experiment (W, well-watered), while 14 plants per cultivar were subjected to drought stress by suspending irrigation for 6 d (S, water stress). At the end of the drought period, half of the stressed plants were rewatered to field capacity (R, water stress treatment followed by recovery) and allowed to recover >48 h (Supplementary Fig. S4).

Measurements of stomatal conductance and stem water potential

Stomatal conductance (g_s) was measured to assess the hydraulic behavior of the two cultivars under drought on fully expanded leaves using a portable infrared gas analyzer (ADC-LCPro+ system, The Analytical Development Company Ltd., Hoddesdon, UK). Measurements were performed using a 6.25-cm² leaf chamber equipped with artificial irradiation (1,200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$), set with a chamber temperature of 25°C to avoid overheating. The g_s was monitored in the morning (between 10:00 am and 12:00 pm) during the whole duration of drought experiment, in parallel with stem water potential measurements.

Stem water potential (Ψ_{stem}) was measured on fully expanded non-transpiring leaves. Prior to measurements, leaves were placed in humidified aluminum foil-wrapped plastic bags for 20 min before excision. After excision, leaves were allowed to equilibrate for an additional 15 min, and water potential was measured using a Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

To evaluate the water status of cuttings during experiments performed at the synchrotron, predawn leaf water potential (Ψ_{pd}) was measured on the second fully expanded leaf of each plant. Leaves were bagged immediately before measurement, and they were excised from the shoot using a sharp blade. Leaves were placed into a pump-up pressure chamber (PMS Instrument Company, Albany, OR, USA), and Ψ_{pd} was recorded when the initial xylem sap emerged from the cut end of the petiole.

X-ray micro-CT observations

All control, stressed and recovered plants of each cultivar were analyzed via micro-CT at the Elettra synchrotron radiation source (Trieste, Italy), using the phase contrast facility available at the SYRMEP beamline (www.elettra.trieste.it). Plants were transported from the growth chamber to the micro-CT station one night before the start of analysis and kept in the dark.

To reduce sample movement during the scan rotation, each plant was removed from the pot, root and soil were secured in a plastic bag and the whole plant was wrapped in plastic film and secured to a wooden axis fixed to the beamline sample holder such that stem distance was 12 cm from the detector. Grapevine stem was observed at the second to fourth node from the rootstock using a pixel size of 2.9 μm at a mean energy of 22 keV, with 1,800 projections

acquired during the sample rotation >180°. Scans were performed in free propagation phase contrast modality with an exposure time of 100 ms. In total, seven plants per treatment and cultivar were scanned, and each plant was scanned only once to avoid possible damage to living cells (Petruzzellis et al. 2018). After scanning, each stem of recovered samples was excised under the rootstock junction point by a razor blade, and the occurrence of water droplets on the surface was carefully observed under a 20 \times magnification lens in order to exclude xylem sap exudation under root positive pressure (Wegner 2014). The micro-CT slices were reconstructed by applying a phase retrieval pre-processing filter (Paganin et al. 2002), before the use of the conventional filtered back-projection algorithm implemented in the SYRMEP TOMOPROJECT software (Brun et al. 2015). One central slice per sample was selected and analyzed by means of the Fiji software (Schindelin et al. 2012). For each sample, the transverse areas of all gas-filled (dark gray) and water-filled (light gray) xylem conduits were measured. The embolism rate (as a proxy of percentage loss in conductivity, PLC) was calculated as the percentage of the transversal area of embolized xylem conduits with respect to the total xylem conduit area. It must be noted that these values estimated on the basis of 2D images could differ from the actual sample hydraulic conductivity, especially when comparing different varieties and species (Pratt et al. 2020). Hence, PLC values reported in this study should be considered only as proxies of the impact of embolism on plant hydraulic properties.

NSC analysis

NSC content was measured on the same samples used for X-ray micro-CT observations. After the scan, a 4-cm stem segment was collected just above the scanned portion and microwaved at 700 W for 3 min in a water-saturated atmosphere to stop enzymatic activities. Samples were then ground into fine powder in a mortar under liquid nitrogen and oven dried at 56°C for 24 h. Then, 15 \pm 1 mg of powder was suspended in 0.3 ml ethanol 80% (v/v) twice and incubated at 80°C for 30 min, and then the resulting supernatant dried at 60°C overnight. A third suspension was made with 0.5 ml of 50 mM Tris-HCl pH 7.5 and used to resuspend the crystallized carbohydrates resulting from the previous steps. The supernatants obtained after incubation at 80°C for 30 min and a 14,000 \times g centrifugation for 5 min were used for soluble NSC measurement, while the pellet was resuspended in 1 ml of sodium acetate buffer (0.2 M NaCH₃COO, pH 4.6) and used for starch quantification. The total soluble NSC fraction was quantified by a VICTOR3 Multilabel Counter Plate Reader (Perkin Elmer, Boston, MA, USA) by means of the anthrone-sulfuric acid assay (Yemm and Willis 1954). A glucose standard curve was used to compare the colorimetric response of the samples, whose absorbance was read at 620 nm, and the NSC content was expressed as mg (glucose) g⁻¹ (DW).

Glucose was analyzed by enzymatic hexokinase/glucose-6-P dehydrogenase assay (Landhäusser et al. 2018), sucrose and maltose/maltodextrins, followed the same process, after conversion into glucose by 25 U invertase or 25 U amyloglucosidase for the latter sugars, respectively. Starch digestion was performed overnight at 55°C, using 100 U of α -amylase and 25 U of amyloglucosidase per sample. To prevent further degradation, the samples were boiled for 3 min. The starch fraction was quantified by means of enzymatic assay, as previously mentioned. Known amounts of amylose were also processed and analyzed to obtain a calibration curve. The final concentration of starch in the sample was then expressed as mg (starch) g⁻¹ (DW). All the chemicals were purchased from Sigma-Aldrich, Milan, Italy.

The portion of water-soluble starch (Landhäusser et al. 2018), derived from starch degradation, represents maltose and short-chain carbohydrate polymers identified as maltodextrins.

Statistical analyses

All the statistical analyses were performed in R statistical software (R Core Team 2021). The effect of the different treatments, cultivars and their interaction was tested separately for each parameter using two-way analysis of variance

(ANOVA) analysis through the 'anova' function in the 'stats' R package. The relationships between NSC content (i.e. glucose, sucrose, maltose/maltodextrins and starch), Ψ_{pd} and embolism rate in the two cultivars were tested using linear models. It should be noted that the relationship between Ψ_{pd} and embolism percentage is not a classical vulnerability curve, which should be based on the relationship between PLC and minimum daily water potential experienced by the plants and is generally approximated by a sigmoidal function. Model assumptions (i.e. normality and homoscedasticity) were checked by the diagnostic plot of residuals. Logarithmic transformation was used where the assumptions of normality and homoscedasticity were not satisfied. Outlier records were detected by the function 'outlier Test' of the 'car' package (Fox and Weisberg 2019) and removed from analysis (where $P < 0.01$). For significant tests ($\alpha = 0.05$), Tukey's honestly significant differences post hoc analysis was carried out through the 'TukeyHSD' function in the 'stats' R package. Xylem conduit size distribution was compared within each cultivar, between treatments. The xylem conduit distribution was represented by density plots. Paired differences in conduit size distribution were tested by the two-sample Kolmogorov–Smirnov test ($P < 0.05$).

Data Availability

The relevant data is available in the public Mendeley Data repository with doi:10.17632/cjdzvnp9px.1 (Vuerich et al., 2023).

Funding

Elettra Sincrotrone Trieste (20180165).

Acknowledgments

This study was performed thanks to VCR that provided grapevine cuttings prepared for the micro-CT experiment. Thanks are also due to Francesca di Lillo e Nicola Sodini (SYRMEP beamline staff) for their support during micro-CT experiments. Finally, we would like to thank Alberto Loschi for his help during grapevine care before the start of the experiment.

Author Contributions

V.C., A.N. and E.P. conceived and designed the research. M.P. projected and prepared specific grape cuttings; M.V., V.C., F.B., F.P., E.P., A.F., M.T., A.N. and G.T. were involved in micro-CT observations; M.V. and F.B. performed the image reconstruction; F.S., E.B. and F.P. evaluated hydraulics and anatomy; M.V., E.P. and V.C. performed the NSC measurements. M.V. and F.B. performed the statistical analysis. V.C., E.P., M.V. and A.N. wrote the manuscript. All the authors contribute to the data discussion, revised the manuscript and approved the manuscript.

Disclosures

The authors have no conflicts of interest to declare.

References

- Adams, H.D., Zeppel, M.J.B., Anderegg, W.R.L., Hartmann, H., Landhäusser, S.M., Tissue, D.T., et al. (2017) A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nat. Ecol. Evol.* 1: 1285–1291.
- Baffoin, R., Charrier, G., Bouchardon, A.-E., Bonhomme, M., Améglio, T. and Lacoite, A. (2021) Seasonal changes in carbohydrates and water content predict dynamics of frost hardiness in various temperate tree species. *Tree Physiol.* 41: 1583–1600.
- Beck, E. and Ziegler, P. (1989) Biosynthesis and degradation of starch in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40: 95–117.
- Beikircher, B. and Mayr, S. (2017) Annual patterns of xylem embolism in high-yield apple cultivars. *Funct. Plant Biol.* 44: 587–596.
- Blackman, C.J., Brodribb, T.J. and Jordan, G.J. (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol.* 188: 1113–1123.
- Brodersen, C.R., Knipfer, T. and McElrone, A.J. (2018) *In vivo* visualization of the final stages of xylem vessel refilling in grapevine (*Vitis vinifera*) stems. *New Phytol.* 217: 117–126.
- Brodersen, C.R. and McElrone, A.J. (2013) Maintenance of xylem network transport capacity: a review of embolism repair in vascular plants. *Front. Plant Sci.* 4: 1–11.
- Brodersen, C.R., McElrone, A.J., Choat, B., Lee, E.F., Shackel, K.A. and Matthews, M.A. (2013) *In vivo* visualizations of drought-induced embolism spread in *Vitis vinifera*. *Plant Physiol.* 161: 1820–1829.
- Brun, F., Pacile, S., Accardo, A., Kourousias, G., Dreossi, D., Mancini, L., et al. (2015) Enhanced and flexible software tools for X-ray computed tomography at the Italian synchrotron radiation facility Elettra. *Fundam. Inform.* 141: 233–243.
- Casolo, V., Braidot, E., Petrusa, E., Zancani, M., Vianello, A. and Boscutti, F. (2020) Relationships between population traits, nonstructural carbohydrates, and elevation in alpine stands of *Vaccinium myrtillus*. *Am. J. Bot.* 107: 639–649.
- Charrier, G., Delzon, S., Domec, J.-C., Zhang, L., Delmas, C.E.L., Merlin, I., et al. (2018) Drought will not leave your glass empty: low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. *Sci. Adv.* 4: 1–9.
- Charrier, G., Torres-Ruiz, J.M., Badel, E., Burlett, R., Choat, B., Cochard, H., et al. (2016) Evidence for hydraulic vulnerability segmentation and lack of xylem refilling under tension. *Plant Physiol.* 172: 1657–1668.
- Choat, B., Brodribb, T.J., Brodersen, C.R., Duursma, R.A., López, R. and Medlyn, B.E. (2018) Triggers of tree mortality under drought. *Nature* 558: 531–539.
- Chronakis, I.S. (1998) On the molecular characteristics, compositional properties, and structural-functional mechanisms of maltodextrins: a review. *Crit. Rev. Food Sci. Nutr.* 38: 599–637.
- Cochard, H. and Delzon, S. (2013) Hydraulic failure and repair are not routine in trees. *Ann. For. Sci.* 70: 659–661.
- Dai, A. (2011) Drought under global warming: a review. *WIREs Clim. Change* 2: 45–65.
- Degu, A., Hochberg, U., Wong, D.C.J., Alberti, G., Lazarovitch, N., Peterlunger, E., et al. (2019) Swift metabolite changes and leaf shedding are milestones in the acclimation process of grapevine under prolonged water stress. *BMC Plant Biol.* 19: 19:69.
- Evageliou, V. (2000) Effect of sucrose, glucose and fructose on gelation of oxidised starch. *Carbohydr. Polym.* 42: 261–272.
- Falchi, R., Petrusa, E., Braidot, E., Sivilotti, P., Boscutti, F., Vuerich, M., et al. (2020) Analysis of non-structural carbohydrates and xylem anatomy of leaf petioles offers new insights in the drought response of two grapevine cultivars. *Int. J. Mol. Sci.* 21: 1457.
- Falchi, R., Petrusa, E., Zancani, M., Casolo, V., Beraldo, P., Nardini, A., et al. (2019) Summer drought stress: differential effects on cane anatomy

- and non-structural carbohydrate content in overwintering Cabernet Sauvignon and Syrah vines. *BIO Web Conf.* 13: 03007.
- Fox, J. and Weisberg, S. (2019) An R companion to applied regression, Third edition. Sage, Thousand Oaks CA.
- García-Fórner, N., Biel, C., Savé, R. and Martínez-Vilalta, J. (2017) Isohydic species are not necessarily more carbon limited than anisohydic species during drought. *Tree Physiol.* 37: 441–455.
- Gerzon, E., Biton, I., Yaniv, Y., Zemach, H., Netzer, Y., Schwartz, A., et al. (2015) Grapevine anatomy as a possible determinant of isohydic or anisohydic behavior. *Am. J. Enol. Vitic.* 66: 340–347.
- Gil, R., Boscaiu, M., Lull, C., Bautista, I., Lidón, A. and Vicente, O. (2013) Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? *Funct. Plant Biol.* 40: 805–818.
- Gleason, S.M., Wiggins, D.R., Bliss, C.A., Young, J.S., Cooper, M., Willi, K.R., et al. (2017) Embolized stems recover overnight in *Zea mays*: the role of soil water, root pressure, and nighttime transpiration. *Front. Plant Sci.* 8: 662.
- Hacke, U.G. and Sperry, J.S. (2003) Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*: refilling under negative pressure. *Plant Cell Environ.* 26: 303–311.
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D. and McCulloh, K.A. (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.
- Hochberg, U., Rockwell, F.E., Holbrook, N.M. and Cochard, H. (2018) Iso/anisohydry: a plant–environment interaction rather than a simple hydraulic trait. *Trends Plant Sci.* 23: 112–120.
- Holbrook, N.M., Ahrens, E.T., Burns, M.J. and Zwieniecki, M.A. (2001) In vivo observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiol.* 126: 27–31.
- Jacobsen, A.L. and Pratt, R.B. (2012) No evidence for an open vessel effect in centrifuge-based vulnerability curves of a long-vesselled liana (*Vitis vinifera*). *New Phytol.* 194: 982–990.
- Jacobsen, A.L., Rodriguez-Zaccaro, F.D., Lee, T.F., Valdovinos, J., Toschi, H.S., Martinez, J.A., et al. (2015) Grapevine xylem development, architecture, and function. In *Functional and Ecological Xylem Anatomy*. Edited by Hacke, U. pp. 133–162. Springer International Publishing, Cham.
- Jupa, R., Krabičková, D., Plichta, R., Mayr, S. and Gloser, V. (2021) Do angiosperm tree species adjust intervessel lateral contact in response to soil drought? *Physiol. Plant* 172: 2048–2058.
- Kaack, L., Altaner, C.M., Carmesin, C., Diaz, A., Holler, M., Kranz, C., et al. (2019) Function and three-dimensional structure of intervessel pit membranes in angiosperms: a review. *IAWA J.* 40: 673–702.
- Kennedy, J.F., Knill, C.J. and Taylor, D.W. (1995) Maltodextrins. In *Handbook of Starch Hydrolysis Products and Their Derivatives*. Edited by Kearsley, M.W. and Dziedzic, S.Z. Springer US, Boston, MA.
- Klein, T., Hoch, G., Yakir, D. and Korner, C. (2014) Drought stress, growth and nonstructural carbohydrate dynamics of pine trees in a semi-arid forest. *Tree Physiol.* 34: 981–992.
- Knipfer, T., Cuneo, I., Brodersen, C. and McElrone, A.J. (2016) In-situ visualization of the dynamics in xylem embolism formation and removal in the absence of root pressure: A study on excised grapevine stems. *Plant Physiol.* 171: 1024–1036.
- Knipfer, T., Eustis, A., Brodersen, C., Walker, A.M. and McElrone, A.J. (2015) Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure: contrasting response of wild grapevines to drought stress. *Plant Cell Environ.* 38: 1503–1513.
- Landhäusser, S.M., Chow, P.S., Dickman, L.T., Furze, M.E., Kuhlman, I., Schmid, S., et al. (2018) Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. *Tree Physiol.* 38: 1764–1778.
- Lesk, C., Rowhani, P. and Ramankutty, N. (2016) Influence of extreme weather disasters on global crop production. *Nature* 529: 84–87.
- Lintunen, A., Paljakka, T., Jyske, T., Peltoniemi, M., Sterck, F., von Arx, G., et al. (2016) Osmolality and non-structural carbohydrate composition in the secondary phloem of trees across a latitudinal gradient in Europe. *Front. Plant Sci.* 7.
- Liu, J., Gu, L., Yu, Y., Huang, P., Wu, Z., Zhang, Q., et al. (2019) Cortical photosynthesis drives bark water uptake to refill embolized vessels in dehydrated branches of *Salix matsudana*. *Plant Cell Environ.* 42: 2584–2596.
- Loret, C. (2004) Rheological characterisation of the gelation behaviour of maltodextrin aqueous solutions. *Carbohydr. Polym.* 57: 153–163.
- Love, D.M. and Sperry, J.S. (2018) In situ embolism induction reveals vessel refilling in a natural aspen stand. *Tree Physiol.* 38: 1006–1015.
- Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., et al. (2010) Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Funct. Plant Biol.* 37: 98–116.
- Lovisolo, C. and Schubert, A. (1998) Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *J. Exp. Bot.* 49: 693–700.
- Maherali, H., Pockman, W.T. and Jackson, R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85: 2184–2199.
- Martínez-Vilalta, J. and García-Fórner, N. (2017) Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydic concept: deconstructing the iso/anisohydic concept. *Plant Cell Environ.* 40: 962–976.
- Martorell, S., Diaz-Espejo, A., Medrano, H., Ball, M.C. and Choat, B. (2014) Rapid hydraulic recovery in *Eucalyptus pauciflora* after drought: linkages between stem hydraulics and leaf gas exchange. *Plant Cell Environ.* 37: 617–626.
- Mayr, S., Schmid, P., Laur, J., Rosner, S., Charra-Vaskou, K., Dämon, B., et al. (2014) Uptake of water via branches helps timberline conifers refill embolized xylem in late winter. *Plant Physiol.* 164: 1731–1740.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178: 719–739.
- Monder, H., Maillard, M., Chérel, I., Zimmermann, S.D., Paris, N., Cuéllar, T., et al. (2021) Adjustment of K⁺ fluxes and grapevine defense in the face of climate change. *Int. J. Mol. Sci.* 22.
- Morabito, C., Orozco, J., Tonel, G., Cavalletto, S., Meloni, G.R., Schubert, A., et al. (2021) Do the ends justify the means? Impact of drought progression rate on stress response and recovery in *Vitis vinifera*. *Physiol. Plant* 174: 1–13.
- Nakamura, T., Ishida, A., Kawai, K., Minagi, K., Saiki, S.-T., Yazaki, K., et al. (2021) Tree hazards compounded by successive climate extremes after masting in a small endemic tree, *Distylium leptotum*, on subtropical islands in Japan. *Glob. Change Biol.* 27: 5094–5108.
- Nardini, A., Battistuzzo, M. and Savi, T. (2013) Shoot desiccation and hydraulic failure in temperate woody angiosperms during an extreme summer drought. *New Phytol.* 200: 322–329.
- Nardini, A., Lo Gullo, M.A. and Salleo, S. (2011a) Refilling embolized xylem conduits: is it a matter of phloem unloading? *Plant Sci.* 180: 604–611.
- Nardini, A., Salleo, S. and Jansen, S. (2011b) More than just a vulnerable pipeline: xylem physiology in the light of ion-mediated regulation of plant water transport. *J. Exp. Bot.* 62: 4701–4718.
- Nardini, A., Savi, T., Trifilo, P. and Lo Gullo, M.A. (2018) Drought stress and the recovery from xylem embolism in woody plants. In *Progress in Botany*. Edited by Cánovas, F.M., Lüttge, U. and Matyssek, R. pp. 197–231. Springer-Verlag Berlin Heidelberg.

- Ooeda, H., Terashima, I. and Taneda, H. (2016) Structures of bordered pits potentially contributing to isolation of a refilled vessel from negative xylem pressure in stems of *Morus australis* Poir: testing of the pit membrane osmosis and pit valve hypotheses. *Plant Cell Physiol.* 58: 354–364.
- Paganin, D., Mayo, S.C., Gureyev, T.E., Miller, P.R. and Wilkins, S.W. (2002) Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *J. Microsc.* 206: 33–40.
- Pagliarani, C., Casolo, V., Ashofteh Beiragi, M., Cavalletto, S., Siciliano, I., Schubert, A., et al. (2019) Priming xylem for stress recovery depends on coordinated activity of sugar metabolic pathways and changes in xylem sap pH: how does the biology of xylem apoplast impact the recovery from water stress in poplar? *Plant Cell Environ.* 42: 1775–1787.
- Patakas, A., Nikolaou, N., Zioziou, E., Radoglou, K. and Noitsakis, B. (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. *Plant Sci.* 163: 361–367.
- Patakas, A. and Noitsakis, B. (2001) Leaf age effects on solute accumulation in water-stressed grapevines. *J. Plant Physiol.* 158: 63–69.
- Pellegrini, E., Forlani, G., Boscutti, F. and Casolo, V. (2020) Evidence of non-structural carbohydrates-mediated response to flooding and salinity in *Limonium narbonense* and *Salicornia fruticosa*. *Aquat. Bot.* 166: 103265.
- Peltier, D.M.P., Guo, J., Nguyen, P., Bangs, M., Gear, L., Wilson, M., et al. (2021) Temporal controls on crown nonstructural carbohydrates in southwestern US tree species. *Tree Physiol.* 41: 388–402.
- Petrizzellis, F., Pagliarani, C., Savi, T., Losso, A., Cavalletto, S., Tromba, G., et al. (2018) The pitfalls of in vivo imaging techniques: evidence for cellular damage caused by synchrotron X-ray computed micro-tomography. *New Phytol.* 220: 104–110.
- Plavcová, L., Hacke, U.G. and Sperry, J.S. (2011) Linking irradiance-induced changes in pit membrane ultrastructure with xylem vulnerability to cavitation. *Plant Cell Environ.* 34: 501–513.
- Pratt, R.B., Castro, V., Fickle, J.C., Madsen, A. and Jacobsen, A.L. (2020) Factors controlling drought resistance in grapevine (*Vitis vinifera*, chardonnay): application of a new micro CT method to assess functional embolism resistance. *Am. J. Bot.* 107: 618–627.
- Pratt, R.B. and Jacobsen, A.L. (2018) Identifying which conduits are moving water in woody plants: a new HRCT-based method. *Tree Physiol.* 38: 1200–1212.
- R Core Team (2021) R: a language and environment for statistical computing.
- Rodrigues, M., Chaves, M., Wendler, R., David, M., Quick, W., Leegood, R., et al. (1993) Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation. *Aust. J. Plant Physiol.* 20: 309–321.
- Savi, T., Casolo, V., Luglio, J., Bertuzzi, S., Trifilò, P., Lo Gullo, M.A., et al. (2016) Species-specific reversal of stem xylem embolism after a prolonged drought correlates to endpoint concentration of soluble sugars. *Plant Physiol. Biochem.* 106: 198–207.
- Savi, T., García González, A., Herrera, J.C. and Forneck, A. (2019a) Gas exchange, biomass and non-structural carbohydrates dynamics in vines under combined drought and biotic stress. *BMC Plant Biol.* 19.
- Savi, T., Petruzzellis, F., Moretti, E., Stenni, B., Zini, L., Martellos, S., et al. (2019b) Grapevine water relations and rooting depth in karstic soils. *Sci. Total Environ.* 692: 669–675.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9: 676–682.
- Schultz, H.R. (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought: hydraulic conductance and stomatal behaviour. *Plant Cell Environ.* 26: 1393–1405.
- Secchi, F. and Zwieniecki, M.A. (2011) Sensing embolism in xylem vessels: the role of sucrose as a trigger for refilling. *Plant Cell Environ.* 34: 514–524.
- Secchi, F. and Zwieniecki, M.A. (2012) Analysis of xylem sap from functional (nonembolized) and nonfunctional (embolized) vessels of *Populus nigra*: chemistry of refilling. *Plant Physiol.* 160: 955–964.
- Secchi, F. and Zwieniecki, M.A. (2014) Down-regulation of plasma intrinsic protein1 aquaporin in poplar trees is detrimental to recovery from embolism. *Plant Physiol.* 164: 1789–1799.
- Secchi, F. and Zwieniecki, M.A. (2016) Accumulation of sugars in the xylem apoplast observed under water stress conditions is controlled by xylem pH: xylem pH regulates sugar accumulation in apoplast. *Plant Cell Environ.* 39: 2350–2360.
- Shelden, M.C., Vandeleur, R., Kaiser, B.N. and Tyerman, S.D. (2017) A comparison of petiole hydraulics and aquaporin expression in an anisohydric and isohydric cultivar of grapevine in response to water-stress induced cavitation. *Front. Plant Sci.* 8.
- Sorek, Y., Greenstein, S., Netzer, Y., Shtein, I., Jansen, S. and Hochberg, U. (2021) An increase in xylem embolism resistance of grapevine leaves during the growing season is coordinated with stomatal regulation, turgor loss point and intervessel pit membranes. *New Phytol.* 229: 1955–1969.
- Sperry, J.S., Hacke, U.G., Feild, T.S., Sano, Y. and Sikkema, E.H. (2007) Hydraulic consequences of vessel evolution in angiosperms. *Int. J. Plant Sci.* 168: 1127–1139.
- Sperry, J.S., Hacke, U.G. and Pittermann, J. (2006) Size and function in conifer tracheids and angiosperm vessels. *Am. J. Bot.* 93: 1490–1500.
- Sun, Q., Rost, T.L. and Matthews, M.A. (2008) Wound-induced vascular occlusions in *Vitis vinifera* (Vitaceae): tyloses in summer and gels in winter. *Am. J. Bot.* 95: 1498–1505.
- Tomasella, M., Casolo, V., Aichner, N., Petruzzellis, F., Savi, T., Trifilò, P., et al. (2019) Non-structural carbohydrate and hydraulic dynamics during drought and recovery in *Fraxinus ornus* and *Ostrya carpinifolia* saplings. *Plant Physiol. Biochem.* 145: 1–9.
- Tomasella, M., Casolo, V., Natale, S., Petruzzellis, F., Kofler, W., Beikircher, B., et al. (2021) Shade-induced reduction of stem nonstructural carbohydrates increases xylem vulnerability to embolism and impedes hydraulic recovery in *Populus nigra*. *New Phytol.* 231: 108–121.
- Trifilò, P., Casolo, V., Raimondo, F., Petrusa, E., Boscutti, F., Lo Gullo, M.A., et al. (2017) Effects of prolonged drought on stem non-structural carbohydrates content and post-drought hydraulic recovery in *Laurus nobilis* L.: the possible link between carbon starvation and hydraulic failure. *Plant Physiol. Biochem.* 120: 232–241.
- Trifilò, P., Kiorapostolou, N., Petruzzellis, F., Vitti, S., Petit, G., Lo Gullo, M.A., et al. (2019) Hydraulic recovery from xylem embolism in excised branches of twelve woody species: relationships with parenchyma cells and non-structural carbohydrates. *Plant Physiol. Biochem.* 139: 513–520.
- Trifilò, P., Nardini, A., Gullo, M.A.L., Barbera, P.M., Savi, T. and Raimondo, F. (2015) Diurnal changes in embolism rate in nine dry forest trees: relationships with species-specific xylem vulnerability, hydraulic strategy and wood traits. *Tree Physiol.* 35: 694–705.
- Trifilò, P., Natale, S., Gargiulo, S., Abate, E., Casolo, V. and Nardini, A. (2021) Stem photosynthesis affects hydraulic resilience in the deciduous *Populus alba* but not in the evergreen *Laurus nobilis*. *Water* 13.
- Trifilò, P., Raimondo, F., Lo Gullo, M.A., Barbera, P.M., Salleo, S. and Nardini, A. (2014) Relax and refill: xylem rehydration prior to hydraulic measurements favours embolism repair in stems and generates artificially low PLC values. *Plant Cell Environ.* 37: 2491–2499.
- Tyree, M.T. (1988) A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. *Tree Physiol.* 4: 195–217.
- Tyree, M.T. and Sperry, J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40: 19–36.
- Tyree, M.T. and Zimmermann, M.H. (2002) Xylem Structure and the Ascent of Sap. Springer, Berlin, London.

- Urli, M., Porte, A.J., Cochard, H., Guengant, Y., Burlett, R. and Delzon, S. (2013) Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiol.* 33: 672–683.
- Venturas, M.D., Sperry, J.S. and Hacke, U.G. (2017) Plant xylem hydraulics: what we understand, current research, and future challenges. *J. Integr. Plant Biol.* 59: 356–389.
- Villalobos-González, L., Muñoz-Araya, M., Franck, N. and Pastenes, C. (2019) Controversies in midday water potential regulation and stomatal behavior might result from the environment, genotype, and/or rootstock: evidence from Carménère and Syrah grapevine varieties. *Front. Plant Sci.* 10.
- Vuerich, M., Braidotti, R., Sivilotti, P., Alberti, G., Casolo, V., Braidot, E., et al. (2021) Response of merlot grapevine to drought is associated to adjustments of growth and nonstructural carbohydrates allocation in above and underground organs. *Water* 13.
- Vuerich, M., Petrusa, E., Boscutti, F., Braidot, E., Filippi, A., Petruzzellis, F., et al. (2023) Data from: contrasting responses of two grapevine cultivars to drought: the role of non-structural carbohydrates in xylem hydraulic recovery. *Mendeley Data* 1.
- Wang, S., Alseekh, S., Fernie, A.R. and Luo, J. (2019) The structure and function of major plant metabolite modifications. *Mol. Plant* 12: 899–919.
- Wegner, L.H. (2014) Root pressure and beyond: energetically uphill water transport into xylem vessels? *J. Exp. Bot.* 65: 381–393.
- Wheeler, J.K., Huggett, B.A., Tofte, A.N., Rockwell, F.E. and Holbrook, N.M. (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant Cell Environ.* 36: 1938–1949.
- Yemm, E.W. and Willis, A.J. (1954) The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* 57: 508–514.
- Yoshimura, K., Saiki, S.-T., Yazaki, K., Ogasa, M.Y., Shirai, M., Nakano, T., et al. (2016) The dynamics of carbon stored in xylem sapwood to drought-induced hydraulic stress in mature trees. *Sci. Rep.* 6.
- Zhang, Y., Xu, J., Su, W., Zhao, X. and Xu, X. (2019) Spring precipitation effects on formation of first row of earlywood vessels in *Quercus variabilis* at Qinling Mountain (China). *Trees Struct. Funct.* 33: 457–468.
- Zwieniecki, M.A. and Holbrook, N.M. (2000) Bordered pit structure and vessel wall surface properties. Implications for embolism repair. *Plant Physiol.* 123: 1015–1020.
- Zwieniecki, M.A., Melcher, P.J. and Michele Holbrook, N. (2001) Hydrogel control of xylem hydraulic resistance in plants. *Science* 291: 1059–1062.