Container volume affects drought experiments in grapevines: Insights on xylem anatomy and time of dehydration

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Abstract
Plant stress experiments are commonly performed with plants grown in containers to better control environmental conditions. Nevertheless, the container can constrain plant growth and development, and this confounding effect is generally ignored, particularly in studies on woody species. Here, we evaluate the effect of the container volume in drought experiments using grapevine as a model plant. Grapevines grown in small (7 L, S) or large (20 L, L) containers were subjected to drought stress and rewetting treatments. We monitored plant stomatal conductance ($g_s$), midday stem water potential ($Ψ_s$), and photosynthetic rate ($A_N$) throughout the experiment. The effect of the container volume on the stem and petiole xylem anatomy, as well as on the total leaf area (LA), was assessed before drought imposition. The results showed that LA did not differ between plants in L or S containers, but S vines exhibited a higher theoretical hydraulic conductance at the petiole level. Under drought L and S similarly reduced $g_s$ and $A_N$, but plants in S containers reached lower $Ψ_s$ than those in L. Nevertheless, upon rewetting droughted plants in S containers exhibited a faster stomata re-opening than those in L, probably as a consequence of the differences in the stress degree experienced and the biochemical adjustment at the leaf level. Therefore, a suitable experimental design should consider the container volume used in relation to the desired traits to be studied for unbiased results.

1 | INTRODUCTION

Physiological research in plants are often carried out with plants grown in containers of different sizes and shapes since this allows for a more precise control of experimental conditions. Such is the case in perennial crops like grapevines, for which the volume of the containers used reported in the literature mostly ranges from 3 to 60 L (Corso et al., 2015; Griesser et al., 2015; Hochberg, Bonel, et al., 2017; Lovisolo & Schubert, 1998; Tomsesi et al., 2015). With limited spaces, for instance, in growth chambers or greenhouses, reducing the size of growing containers is the only way to maximize the number of replicates (Poorter et al., 2012). Similarly, high-throughput phenotyping in breeding systems often uses automated equipment, and the use of small containers minimizes the space requirement and the effort of the transport robots (Granier et al., 2006; Tisné et al., 2013). Although this may improve the efficiency of the experimental setup, constraining the root biomass due to small container volumes can lead to physiological and phenotypic changes in plants (Sinclair et al., 2017). Such confounding effects of container volumes are generally ignored (Passioura, 2006; Poorter et al., 2012). The most commonly described
effect of small containers is a restraint of above-ground biomass. Poorter et al. (2012) estimated that about 65% of experiments utilize containers of an inadequate volume for raising the plants. Using inadequate container volume can result in alterations in root physiology (Kharkina et al., 1999), changes in the root:shoot ratio (Mokany et al., 2006), root temperature (Passioura, 2006), and restriction of plant-available water or nutrients (NeSmith & Duval, 1998; Ray & Sinclair, 1998). Container volume restriction was also shown to reduce root hydraulic conductance (Nardini & Pitt, 1999).

Particularly when performing drought experiments, it is pivotal to understand if and how the container volume can affect the results. For example, when investigating drought effects on the grapevine cultivar Merlot—using the same clone, rootstock material and instrumentation—leaf stomata closed at a water potential (Ψ) of about −0.8 MPa in 7 L pots (Hochberg, Bonel, et al., 2017) but around −1.1 MPa in 40 L pots (Herrera et al., 2017). Also, two different meta-analyses on grapevines showed that stomatal conductance (gs) of drought stressed plants obtained from field studies were more than two times higher than data obtained from potted plants (Lavoie-Lamoureux et al., 2017; Medrano et al., 2002a); however, that study did not analyse the effect of variation in pot volumes.

Therefore, in the present study, we aimed to characterize the differences in performing drought stress experiments using two different container volumes (7 and 20 L). We used grapevines since this species is considered a model plant for drought stress studies, along with being of great economic interest worldwide (Gambetta et al., 2020). We hypothesized that differences in the substrate volume would result in differences in plant development and water relations among groups when subjected to drought. Furthermore, we explored the rewatering phase (re-irrigation after drought) as the recovery rate is closely related to drought stress resilience.

2 | MATERIALS AND METHODS

2.1 | Experimental design and plant material

The experiment was conducted in 2018 on 40 two-years-old *Vitis vinifera* cv. Zweigelt plants grafted on Kober 5BB rootstock. The plants were grown during the 2017 season in 7 L containers filled with a commercial substrate supplemented with 20% perlite and without limitations of water and nutrients. During the winter dormancy (December 2017), half of the plants were transplanted to 20 L containers (large; L) while the second half remained in the 7 L (small; S) ones. Both container types were 29 cm high, while the upper diameter was 21 and 36.5 cm in S and L, respectively. On January 9, all plants were placed inside a greenhouse chamber located at BOKU UFT (Tulln, Austria). Chamber conditions were set as: temperature 21/16 °C day/night, artificial light source (400 μmol m−2 s−1) 12 h per day and constant air relative humidity of ~45% (Figure S1). Plants were arranged in four rows with container volumes distributed under a fully randomized design. Bud break occurred on January 19, and only two shoots per vine were maintained. The two shoots were trained vertically with the aid of bamboo sticks, and all inflorescences were removed. Vines were then allowed to grow without water limitations for 46 days (establishment period) by irrigating all pots daily to saturation using pressure compensated drippers (Netafim PCJ 2 L h−1). The 20 L pots had two drippers each to ensure even distribution of water throughout the pot.

At the end of the establishment period (March 6), petiole and stem tissues were sampled from three different plants per pot category for anatomical analyses. Thereafter, all plants were standardized to 10 leaves per shoot by trimming the apex, attempting to maintain the leaf area and whole plant transpiration constant across treatments during the drought experiment. At that time, every shoot had on average 13.7 ± 2.5 and 15.7 ± 2.0 leaves in L and S containers, respectively, and leaf area did not differ between container volumes (*P* > 0.1, Table 1).

### Table 1

| Parameter                        | Small (7 L)       | Large (20 L)      | *P*-value |
|----------------------------------|-------------------|-------------------|-----------|
| Number of leaves per shoot       | 15.7 ± 2.0        | 13.7 ± 2.5        | 0.670     |
| Leaf length (cm)                 | 11.2 ± 0.51       | 11.9 ± 0.61       | 0.414     |
| Leaf area (cm² leaf⁻¹)           | 152.1 ± 10.8      | 163.2 ± 13.4      | 0.554     |
| Total LA-1 (cm² vine⁻¹)          | 3650 ± 412        | 3656 ± 94         | 0.989     |
| Total LA-2 (cm² vine⁻¹)          | 3074 ± 346        | 3320 ± 367        | 0.293     |

2.2 | Drought and rewatering experiment

After the establishment period and sampling for anatomical analyses, the remaining 17 plants per container category were randomly assigned to two different irrigation treatments: nine plants were under deficit irrigation conditions, and eight plants served as well-watered controls. Well-watered (W) control plants in 20 L containers (L-W) and 7 L (S-W) were irrigated daily by adding water equivalent to 120% of the average weight loss during the previous 24 h, measured with a digital scale (Acculab SV150C, Capacity/Resolution 50 kg/0.5 g, Sartorius group). Drought stressed (D) plants of both 20 L (L-D) and 7 L (S-D) were deficit irrigated with water weighing 25% of the...
average weight loss in control pots. After 10 days of deficit irrigation, a more severe drought was imposed by completely withholding irrigation to reduce the vines’ stem water potential \( \Psi_s \) to values \(< -1.2 \text{ MPa} \), which is considered as moderate to severe drought stress in grapevines (Gambetta et al., 2020). Given the differences in water content between containers, this took 5 days in S-D and 10 days in L-D. In the following rewatering period, D vines were then manually watered to saturation and thereafter daily drip-irrigated with the same volumes of their respective W controls. Stem water potential and leaf gas exchange were regularly monitored during the drought and rewatering periods of the experiment, as described below.

### 2.3 | Stem water potential and gas exchange measurements

Midday stem water potential (\( \Psi_s; \text{MPa} \)) was determined in mature basal leaves that were enclosed in plastic bags and aluminium foil for 30 min before cutting the petioles using a sharp blade. While still bagged, the leaves were immediately placed in a pressure chamber (Soil Moisture Co.) with the petiole protruding from the chamber lid. The chamber was then pressurized using nitrogen, and \( \Psi_s \) recorded. At each sampling date, one leaf for each of three vines per treatment was measured. In total, 9 and 10 sampling dates were performed in W and D vines, respectively. On average, we removed a total of three leaves per plant during the whole drought period, which accounted for approximately 15% of the canopy.

Drought stress integral (\( S_p \)) was calculated for the entire experiment using the following equation (Myers, 1988)

\[
S_p = \sum_{i=0}^{n} (\Psi_{i+1} - c)n
\]

where \( \Psi_{i+1} \) is the \( \Psi_s \) of each interval \( i, i + 1 \); \( c \) is the \( \Psi_s \) of reference measured at the beginning of the experiment (\(-0.28 \) and \(-0.25 \text{ MPa} \) in L-D and S-D, respectively); and \( n \) is the interval in number of days between the two measurements.

Gas exchange was measured on the youngest fully expanded, healthy, and light-exposed leaves using an infrared gas analyser (LC pro-SD, ADC BioScientific Ltd) to record leaf CO2 assimilation rate (\( g_{\text{\(
\end{equation}

...the theoretical hydraulic conductance of the section \((kh)\).
665, and 710 nm in the spectrophotometer (Genesys 10S, Thermo-Scientific). Chlorophyll A, B, and total carotenoid concentrations were calculated according to Wellburn (1994). Three technical replicates were performed per sample.

2.7 | Statistical analysis

Data were analysed using SPSS version 26.0 (IBM Corp.). One-way ANOVA was used to identify differences in traits between container volumes before the drought imposition (i.e. leaf area, xylem anatomy traits). For the same traits, cross-sections were used as replicates (i.e. n = 6 for stem, and n = 5 for petioles as one cross-section image was discarded). Two-way ANOVA was used to assess the effects of container volumes, irrigation, and their interaction as fixed factors (2 × 2 full factorial model) on gas exchange and water potential parameters at each sampling date; when needed, post hoc Tukey’s honestly significant difference test was used to separate means. To test if photosynthesis was affected by container volumes and irrigation other than through the effect of stomatal conductance, we used a mixed-effects model with g_s, container volume, and irrigation treatment as fixed factors and plant individuals as random variable to account for repeated measures of individual plants. The effect of container volume on vessel-size distribution was tested with Chi-square tests. Pigments concentration in the leaves were tested by comparing W and D treatments within each container volume category using a one-way ANOVA (P < 0.05).

3 | RESULTS

3.1 | Leaf area and xylem anatomy after the establishment period

Grapevines grew for 46 days with sufficient water supply, where the only difference was the container volume. After that period, the leaf development of the plants was very similar, and there was no significant effect of container volume at the start of the drought imposition on the number of leaves developed, the average leaf length and area, as well as for the leaf area per plant (Table 1). The average leaf area per plant was 3650 and 3656 cm² in small and large containers, respectively. At that stage, all plants were trimmed to 10 leaves, after which the leaf area was 3074 and 3320 cm² per plant (P > 0.20) in small and large containers, respectively.

Container volume significantly affected xylem anatomy in petioles (Figure 1) but not in stems (Table 2). The petiole of plants in small containers had significantly higher vessel lumen fraction (0.186 ± 0.006) than of plants in large containers (0.099 ± 0.023) and marginally but significantly (P < 0.10) larger average vessel areas (805 and 654 μm² in S and L, respectively). Furthermore, in small containers, the petioles had a higher kh (P < 0.01) than the ones in large containers (8.65 and 3.63 kg m⁻¹ MPa⁻¹ s⁻¹, respectively; Table 1). Vessel size distribution (Figure 1A) showed that petioles of vines from small containers had a higher proportion of large vessels (>1400 μm²) and a lower proportion of small vessels (<400 μm²). The contribution of each vessel category to the total kh (Figure 1B) shows how the presence of larger vessels in S plants largely contributes to the higher kh.

3.2 | Drought effect

Fully irrigated vines maintained high g_s (average 0.21 mol m⁻² s⁻¹), A_N (average 13.5 μmol m⁻² s⁻¹), and Ψ_s (average −0.27 MPa) throughout the experiment (Figure 2), and little differences between large or small containers were evident, except for Days 8, 14, 16, 17, and 18 of the experiment (DOE) where control vines in large containers (L-W) showed higher g_s values than in small containers (S-W; Table S1). In plants under drought, g_s and water potential declined fast. Throughout the deficit irrigation period, vines in large (L-D) and small (S-D) containers gradually reduced g_s at a very similar rate. After 3 days (DOE 3) drought-stressed plants had significantly lower g_s than the W controls, and at DOE 5, they reached a minimum of 0.07 mol m⁻² s⁻¹ (−70% of the fully irrigated controls) irrespective of container volume. Photosynthetic rate (A_N) decreased following the stomatal closure over the period of deficit irrigation, although A_N

| Anatomy trait     | Stem                               | Petiole                              | P-value |
|-------------------|------------------------------------|--------------------------------------|---------|
|                  | Small (mm)                         | Large (mm)                           |         |
| D_v (μm²)        | 0.0721 ± 0.0028                    | 0.0736 ± 0.0032                      | 0.369   |
| VD (# mm⁻²)      | 58.5 ± 3.7                         | 59.2 ± 1.6                           | 0.431   |
| VA_mean (μm²)    | 3055 ± 261                         | 3186 ± 261                           | 0.366   |
| Vessel area fraction | 0.196 ± 0.029                     | 0.192 ± 0.016                       | 0.451   |
| kh (kg m⁻¹ MPa⁻¹ s⁻¹) | 42.9 ± 6.1                      | 46.0 ± 8.6                           | 0.388   |
|                  | Small (g)                          | Large (g)                            |         |
|                  | 0.0351 ± 0.0013                    | 0.0318 ± 0.0016                      | 0.145   |
|                  | 235.0 ± 19.4                       | 160.7 ± 41.2                         | 0.141   |
|                  | 805.6 ± 51.4                       | 654.5 ± 59.2                         | 0.090   |
|                  | 0.186 ± 0.006                      | 0.099 ± 0.023                        | 0.007   |
|                  | 8.65 ± 0.76                        | 3.63 ± 0.81                          | 0.002   |

Note: Values are average ± st (n = 5 petioles and n = 6 for stems from three different plants per treatment). For each trait, differences between the averages of small and large containers were assessed by one-way ANOVA; P-values <0.1 are presented in bold.
declined less than \( g_s \) (Figure 2). At DOE 5, L-D and S-D recorded \( A_N \) values of 8.4 and 7.1 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), respectively, when L-D was 40% lower than L-W and S-D was 50% lower than S-W.

After the 10 days of deficit irrigation, irrigation was completely stopped to reach severe stress. Up to that point, the \( \Psi_s \) values of both L-D and S-D had decreased to an average of \(-0.8 \) MPa without significant differences between them. Without irrigation, the \( \Psi_s \) values of S-D decreased very fast, reaching a minimum of \(-1.55 \) MPa, whereas in L-D, the dehydration happened at a much lower rate and \( \Psi_s \) values never went below \(-1.2 \) MPa, even though the S-D plants were without irrigation for five more days (Figure 2). Without irrigation, \( g_s \) continued to decline in both drought groups reaching a minimum of \( \sim 0.026 \) mol m\(^{-2}\) s\(^{-1}\) in both L-D and S-D vines. In drought plants, \( g_s \) remained \( <0.05 \) mol m\(^{-2}\) s\(^{-1}\) until rewatering initiation. Similarly, \( A_N \) continued to decrease gradually and reached a minimum value of 3.44 and 2.22 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in S-D and L-D, respectively, right before the

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**FIGURE 1**  Vessel size (\( \mu \text{m}^2 \)) frequency distribution in petioles cross sections (A) and their respective relative contribution (in \%) to the total \( k_h \) (B) of grapevines grown in large (20 L) or small (7 L) containers. Data represent average \pm SE of five cross sections (sampled from three different plants). Significant differences between container volumes in (A) are indicated as * or ***, for \( P < 0.05 \) or 0.001, respectively, after a Chi-squared test.

**FIGURE 2**  Midday stem water potential (\( \Psi_s \)), stomatal conductance (\( g_s \)), and assimilation rate (\( A_N \)) of grapevines in small (7 L) and large (20 L) containers over the period of drought and rewatering. S-W, small well-watered; S-D, small drought-stressed; L-W, large well-watered; L-D, large drought-stressed. Data are presented as the average \pm SE (\( n = 4 \) in W; \( n = 7-9 \) in D). Vertical dashed lines indicate the start of the complete irrigation withholding (orange) and of the rewatering period for the small (purple) and large (grey) containers. Statistics for each date of measurements of \( A_N \) and \( g_s \) are presented in Tables S1 and S2, respectively.
rewatering initiation. In general, during the entire drought period, S-D showed a trend towards lower values than L-D (Figure S4), but no statistical differences were observed at any date of measurement.  

3.3 | Rewatering period

Since irrigation was re-started at different times for L-D and S-D containers, we present the gs and AN data in parallel as days of rewatering (DOR) to permit a direct comparison between L and S (Figure 3).

At 0 DOR, Ψs was significantly lower in S-D vines (−1.55 MPa) compared to L-D (−1.27 MPa). These values reflect that the drought in S-D was more severe than in L-D. However, in both groups, Ψs increased fast and reached values >−0.5 MPa after 48 h with no significant difference compared to the irrigated controls (Figure 2). Throughout the rest of the rewatering, Ψs remained between −0.3 and −0.4 MPa in all plants.

While Ψs recovered quickly, AN and gs restoration was much slower and did not reach values comparable to the irrigated controls in both container volume categories (Figure 3). Within each container category, the differences between W and D plants during the rewatering were recorded always significant (P<0.05) except for AN in S vines at DOR 5, 6, and 13 (Figure S5), a result arising from a higher variability in D plants rather than from a real increase in the AN values to the W control levels. In plants in small containers, AN and gs increased faster than in large containers upon rewatering (Figure 3). The effects of water stress and subsequent rewatering on AN was explained by the response of gs (P < 0.001), while container volume or watering had no additional effect on AN (P > 0.1; Figure 4).

Pigments concentration in leaves were affected by drought, albeit only on the plants in large containers. In large containers, the concentration of chlorophyll A and B was significantly lower (−28% for total chlorophyll) and the concentration of carotenoids higher (−227%) in previously stressed compared to control plants (Table 3). In small containers, pigment concentrations were not affected by drought or, at least, after the rewatering period.

4 | DISCUSSION

The first hypothesis we tested was whether differences in the substrate volume would result in differences in plant development. The results from this study highlight the impact of the container volume used on the xylem architecture at the petiole level, albeit not of the stems (Table 1). The xylem anatomy results revealed that the petiole vessels of vines grown in small containers were larger than those in large containers, resulting in a highly significant increase of the theoretical hydraulic conductance (kh). The regulation of vessel diameter in plants is in general poorly understood as it involves different endogenous (hormone crosstalk and transport, turgor-driven cell expansion) and environmental (temperature, water, nutrient status) factors (Hacke et al., 2017). The plant hormone Auxin (indole-3-acetic acid) is involved in the regulation of plant vascular development, and its interplay with other plant hormones (cytokinins and brassinosteroids) is likely responsible for xylem development (De Rybel et al., 2016; Hacke et al., 2017). However, the mechanistics of this process and how it is modified by the environment remains unclear. While previous studies reported xylem architecture modifications in grapevines as response to drought acclimation during the same season (Hochberg, Bonel, et al., 2017) or after consecutive drought seasons...
Concentration (mg g⁻¹ of leaf fresh weight) of chlorophyll A and B, total chlorophyll, and total carotenoids in leaves of grapevines grown in small or large containers after drought and rewatering period. S-W, small well-watered; S-D, small drought-stressed; L-W, large well-watered; L-D, large drought-stressed

|               | Chlorophyll A (mg g⁻¹ FW) | Chlorophyll B (mg g⁻¹ FW) | Total chlorophyll (mg g⁻¹ FW) | Carotenoids (mg g⁻¹ FW) |
|---------------|---------------------------|---------------------------|-------------------------------|-------------------------|
| S-W           | 2.28 ± 0.11               | 0.65 ± 0.03               | 2.93 ± 0.14                   | 0.30 ± 0.01             |
| S-D           | 2.33 ± 0.10               | 0.68 ± 0.03               | 3.01 ± 0.13                   | 0.28 ± 0.02             |
| L-W           | 2.43 ± 0.11               | 0.70 ± 0.03               | 3.12 ± 0.14                   | 0.30 ± 0.02             |
| L-D           | 2.00 ± 0.07               | 0.26 ± 0.03               | 2.26 ± 0.08                   | 0.98 ± 0.07             |

Note: Values are average ± st (n = 6). For each column and within each container volume category, significant differences between irrigation treatments are indicated as: *, ***, or ns, for P < 0.05, 0.001, or not significant, respectively, after a one-way ANOVA.

(Tombesi et al., 2018), we show that xylem architecture modification may also be a response to different substrate volumes. Drought can lead to the formation of narrower vessels (Hacke et al., 2017; Lovisolo & Schubert, 1998), but in our case, there was no drought stress situations during the establishment period. Our results rather suggest that the greater availability of resources in the large containers resulted in the acclimation of a less efficient hydraulic system at the petiole level (Mencuccini, 2003). Nevertheless, we should bear in mind that vessel size alone cannot explain a complex hydraulic system; recent research in grapevines showed that the contribution of narrower vessels to the sap flow is underestimated when considering only the lumen size and not the overall xylem pathway heterogeneity (Bouda et al., 2019).

The second hypothesis tested in the study was whether morphological changes induced by the different container volumes used during the establishment period would impact the water relations of the plants. Despite the differences in petiole's xylem architecture between container volumes, stomatal regulation under drought did not differ. In all drought exposed vines, gs rapidly decreased following the reduction of Ψw, as stomatal regulation is among the first responses to drought and precedes xylem dysfunction through embolism formation (Buckley, 2019; Charrier et al., 2018; Hochberg, Herrera, et al., 2016; Martin-StPaul et al., 2017). The effect of deficit irrigation over 10 days on water relations dynamics was similar for plants in small and large containers. However, when irrigation was withheld completely, Ψw declined faster in S-D than in L-D plants (Figure 2), probably because of the similar leaf areas but different soil volumes and thus available water. Nevertheless, both S-D and L-D reached levels of severe water stress (gs < 0.05 mol m⁻² s⁻¹; Flexas et al., 2002) at a similar time (11 DOE; Table S1), suggesting that the stronger Ψw decline observed in S-D could have been mainly driven by differences in daytime and/or night-time transpiration rates (Coupeledru et al., 2016; Dayer et al., 2020a, 2020b) that were not recorded here. The minimum xylem tension was lower in S-D (~1.55 MPa) than in L-D (~1.27 MPa) plants; bearing in mind that S-D petioles had larger vessels, which are associated with higher embolism vulnerability (Lovisolo & Schubert, 1998), we would expect a higher degree of embolism in the petioles of S-D plants (Hochberg, Albuquerque, et al., 2016; Hochberg, Herrera et al., 2016; Hochberg, Windt, et al., 2017). Put another way, the xylem size modifications recorded in S-D petioles would suggest a trade-off between a higher water transport efficiency at the cost of a more vulnerable system (Venturas et al., 2017). However, leaf gas exchange in S-D vines increased faster than in L-D ones after re-irrigation, indicating on one hand that the lower water potential recorded in S-D did not lead to substantial embolism formation (therefore allowing for a fast stomata re-opening), and on the other that other factors such as metabolic responses (discussed below) determined the slower gas exchange restoration in L-D, reminding us that the stomatal action is not solely controlled by soil water availability (Medrano et al., 2002b).

Despite the faster stomatal re-opening after drought observed in S plants, both S-D and L-D plants did not recover gs and AN completely to the levels of their respective controls. Previous research reported a fast recovery of water potential upon re-irrigation of drought-stressed grapevines coupled with a slower recovery of gas exchange mainly due to stomatal limitations (Flexas et al., 2009; Lovisolo et al., 2008; Pou et al., 2008). Drought promotes the accumulation of abscisic acid (ABA) in the leaves, and ABA maintains the stoma closed for some days (Degu et al., 2019; Gambetta et al., 2020; Tombesi et al., 2015). In our experiment, stomatal conductance and consequently AN after 9 (L-D) or 13 (S-D) days of re-irrigation remained lower than in controls (Figure 3), and no modifications in the AN ~ gs correlation were observed (Figure 4). Interestingly, the chlorophyll concentration in the leaf was found to be significantly reduced only in the drought stressed vines grown in large containers (Table 3). Degradation of chlorophyll in combination with an increased concentration of total carotenoids suggests that L-D vines suffered strong stress that led to damage of the photosynthetic apparatus, triggering photoprotection via carotenoids (Munne-Bosch & Alegre, 2000). This was not observed in S-D vines and partially explains the faster increase of photosynthesis upon rewatering as compared to L-D. Why leaves of plants grown in large containers suffered greater metabolic stress than leaves of small containers deserves further research. The most likely explanation is that the time of exposure more than drought intensity drives metabolic adjustment to drought in leaves. Under the conditions of this experiment, S-D plants experienced more severe water stress in terms of intensity (more negative water potential) but for a shorter time (16 days before re-irrigation), while L-D experienced a less intense drought (less negative water potential) but...
for a longer time (21 days before re-irrigation). Indeed, by calculating the stress integral (Myers, 1988) during the experiments, L-D reached 69 MPa day while S-D 40 MPa day (Figure 5). The imposed conditions resulted in L-D having stomata closed (i.e. \( g_s < 0.05 \text{ mol m}^{-2} \text{ s}^{-1} \)) for a longer time than S-D (+5 days) with the consequent increase in excess of excitation energy and the production of reactive oxygen species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993). Previous studies on grapevines showed that stomatal closure to values below species (Munné-Bosch & Alegre, 2000; Smirnoff, 1993).

Some similarities to our results can be found in Romero et al. (2017), who compared the drought and rewatering of potted against field-grown grapevines. In that experiment, potted vines suffered a faster and more severe water stress as compared to field plants as well as a faster functioning recovery upon rewatering; authors attributed such behaviour to differences in plant size—as field-grown plants were much larger in canopy than potted vines—and hypothesized that a larger canopy might require a more prolonged and coordinated embolism repair process (Romero et al., 2017). Nevertheless, it is plausible that similar to our experiment, field-grown plants experienced a longer drought exposure (i.e. a greater drought stress integral) with negative biochemical consequences (attributed to longer stomatal closure) such as the ones observed here for chlorophyll and carotenoids at the leaf level.

**5 | CONCLUSION**

Using different container volumes led to differences in xylem differentiation, whereby the vessel diameters in the petioles of plants grown in 7 L containers were significantly affected. If we consider bigger planting containers (20 L in our study) as closer to the natural growing conditions, we conclude that using pot volumes of 0.5 or 3 L—as is common in experimental setups—should have an effect at least as strong. In our dry-down experiment where drought was imposed fast (few days) the predominant response was a similar stomatal closure. However, under different drought conditions (long term or slow decrease in water availability) differences in xylem architecture might additionally affect results. In our case, studying the post-drought rewatering produced different results that were mainly environmentally driven (soil volume). Therefore, a suitable experimental design should consider the container volume to be used in relation to the desired traits to be studied for unbiased results. Timing and intensity of drought are strongly affected by container volumes, as shown in our study, but there might be some experimental setups in which the container volume does not represent a limiting factor, such as the study of specific hydraulic traits (e.g. Dayer et al., 2020a) or the comparison of short-term drought responses among genotypes. However, the study of long-term adaptation strategies (such as growth modulation or metabolism adjustment, among others), might suffer from some artefacts due to the wrong choice of the container. We conclude that much care is to be taken when upscaling pot-experiment results to field conditions.

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**AUTHOR CONTRIBUTIONS**

Jose Carlos Herrera and Joseph Mattocks: Planned and executed the experiment with the help of Tadeja Savi and under the supervision of Astrid Forneck. Joseph Mattocks and Susanne Scheffknecht: Performed the microscopy analyses under the supervision of Sabine Rosner. Federica De Berardinis: Performed the sampling and analysis of pigments. Jose Carlos Herrera and Tadeja Savi: Drafted the original manuscript. Peter Hietz: Critically reviewed and edited the manuscript. All authors read and approved the manuscript.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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