

Water availability dynamics have long-term effects on mature stem structure in *Vitis vinifera*

Sarel Munitz^{1,2}, Yishai Netzer^{1,2,3,4}, Ilana Shtein^{1,2}, and Amnon Schwartz¹

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¹ R.H. Smith Institute of Plant Science and Genetics in Agriculture, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

² The Eastern Regional Research and Development Center, Ariel 40700, Israel

³ Biotech engineering department, Ariel University, Ariel 40700, Israel

⁴ Author for correspondence (e-mail: ynetzer@gmail.com)

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PREMISE OF THE STUDY: The stem of *Vitis vinifera*, a climbing vine of global economic importance, is characterized by both wide and narrow vessels and high specific hydraulic conductivity. While the effect of drought stress has been studied in 1- and 2-yr-old stems, there are few data documenting effects of drought stress on the anatomical structure of the mature, woody stem near the base of the vine. Here we describe mature wood anatomical responses to two irrigation regimes on wood anatomy and specific hydraulic conductivity in *Vitis vinifera* Merlot vines.

METHODS: For 4 years, irrigation was applied constantly at low, medium, or high levels, or at alternating levels at two different periods during the growing season, either early spring or late summer, resulting in late season or early spring deficits, respectively. The following variables were measured: trunk diameter, annual ring width and area, vessel diameter, specific hydraulic conductivity and stem water potential.

KEY RESULTS: High water availability early in the season (late deficit) resulted in vigorous vegetative growth (greater trunk diameter, ring width and area), wider vessels and increased specific hydraulic conductivity. High water availability early in the season caused a shift of the vessel population towards the wider frequency classes. These late deficit vines showed more negative water potential values late in the season than vines that received low but relatively constant irrigation.

CONCLUSIONS: We concluded that high water availability during vegetative growth period of *Vitis* increases vessels diameter and hydraulic conductivity and causes the vines to be more vulnerable to drought stress late in the season.

KEY WORDS drought stress; long-term effect; mature *Vitis* stem; specific hydraulic conductivity; vessel diameter; vessel distribution; *Vitis vinifera* L. cv. Merlot; water availability; xylem structure.

Most cultivated vineyards worldwide are located in semi-arid and arid regions where drought stress is prevalent (Chaves et al., 2007), and yet, compared to other woody plants, grapevines (*Vitis vinifera*) are often described as relatively vulnerable to drought stress (Choat et al., 2010; Zufferey et al., 2011; Jacobsen and Pratt, 2012; Jacobsen et al., 2015). Drought stress induces embolism and a loss of hydraulic function (Schultz and Matthews, 1988; Hargrave et al., 1994; Lovisolo et al., 1998; Choat et al., 2010; Brodersen et al., 2014) and negatively affects vegetative growth and pruning mass of vines (Matthews, 1987; Intrigliolo and Castel, 2010; Shellie and Bowen, 2014; Munitz et al., 2016).

Vulnerability to drought in *Vitis* is due in part to its bimodal distribution of vessel diameters in two distinct vessel size groups—wide and narrow (Carlquist, 1985; Ewers et al., 1990; Wheeler and LaPasha, 1994;

Shtein et al., 2016). Wide diameter vessels are considered to be more hydraulically efficient, but tend to be more vulnerable to embolism within the same species (Sperry and Tyree, 1988; Lo Gullo and Salleo, 1991; Hargrave et al., 1994; Cai and Tyree, 2010; Christman et al., 2012; Scoffoni et al., 2016). The accepted air-seeding theory suggests that the increased vulnerability to embolism of wide vessels is linked to their enlarged total area of intervessel pits. A wide pit area raises the average size of the “rare” largest pore, consequently increasing the risk of air seeding (Choat et al., 2003; Wheeler et al., 2005; Jansen et al., 2009; Cai and Tyree, 2010).

In 1-yr-old stems of *Vitis*, primary xylem may be still functional and is much more vulnerable to cavitation than secondary xylem vessels active in mature stems (Pratt and Jacobsen, 2018). This is due to the partial secondary wall thickenings in primary xylem

elements compared to scalariform arrangement of intervessel bordered pits of secondary xylem vessel elements (Pratt, 1974; Choat et al., 2005; Chatelet et al., 2006; Sun et al., 2006; Brodersen et al., 2011, 2013a; Rolland et al., 2015; Hochberg et al., 2016b). Indeed, visualization techniques (microCT/NMR, Brodersen and Roddy, 2016) have shown that in 1-yr-old stems of *Vitis*, the spread of embolism proceeds from the pith toward the cambium through the primary xylem (Choat et al., 2010; Brodersen et al., 2013a, b; Knipfer et al., 2015; Vergenst et al., 2015; Hochberg et al., 2016a).

Recent reported anatomical information on vines is based mainly on analysis of 1-yr-old stems (Schultz and Matthews, 1993; Lovisollo et al., 1998; Schubert et al., 1999; Sun et al., 2006; Brodersen et al., 2011; Chatelet et al., 2011; Santarosa et al., 2016). In 1-yr-old stems, hydraulic structure is reported to vary among *Vitis* cultivars and rootstocks (Chouzouri and Schultz, 2005; Chatelet et al., 2011; Gerzon et al., 2015; Jacobsen et al., 2015; Hochberg et al., 2015; Santarosa et al., 2016; Shtein et al., 2016) and to be affected by environmental parameters (Schubert et al., 1999).

Evidence suggests that xylem architecture and hydraulic properties of the mature trunk differ from those of the young shoot. Since vessel length and diameter are correlated with stem diameter, mature vine trunks tend to have wider and longer vessels compared to young stems (Ewers and Fisher, 1989; Jacobsen et al., 2012, 2015). In addition, Sun et al. (2006) showed that the ratio between the area of the pith and the xylem decreases with stem maturation (Sun et al., 2006). Although several studies analyzing anatomical features of xylem in mature *Vitis* trunks have been published (Zimmermann and Jeje, 1981; Fahn et al., 1986; Ewers et al., 1990; Schweingruber, 1990; Wheeler and LaPasha, 1994; Tibbetts and Ewers, 2000; Shtein et al., 2016), only two have described anatomical responses of 1-yr-old stems to varied environments (Lovisollo et al., 1998; Hochberg et al., 2015).

In the present study, the effect of water availability during growing season was examined. Three levels of constant water treatments were chosen in addition to either early water restriction (during vegetative growth) or late restriction (during fruit maturation). Our goal was to determine how both the duration and amount of water availability influenced hydraulic conductivity potential of new xylem in older stems. The hypothesis was that water availability may alter the xylem structure and hydraulic conductivity, thus affecting the adaptability of the vines to later drought stress.

MATERIALS AND METHODS

Plant material and experimental design

This study was carried out in a 100-ha commercial vineyard located in the Judean Plain, Israel (31°49'N, 34°53'E, 124 m a.s.l.). This region has a semi-arid climate with predominantly winter rainfall (average 463 mm yr⁻¹) and high evapotranspiration (average 1512 mm yr⁻¹). The vineyard was planted in 1998 with *Vitis vinifera* L. cv. Merlot grafted to 140 Ruggeri, and trained onto a two-wire vertical trellis. Row direction was north-south with a slight tendency to the west, and vine and row spacing were 1.5 m and 3 m respectively (2222 vines ha⁻¹). The soil was loam (48% sand, 29% silt and 23% clay, field capacity 28% volume, wilting point 14% volume). Pest management and fertilization in the vineyard were applied according to standard local agricultural practice. No rainfall events were recorded during the growing seasons, and average winter rainfall (463 mm) measured during trial years was similar to the average rainfall measured in the past 20 years.

The experimental design was a complete randomized block design with five irrigation treatments each replicated four times. Each block comprised three rows (one data and two border rows). Each plot comprised 16 vines per line, with the outer two vines at each end being buffer vines and the inner 12 vines being measurement vines (a total of 240 measurement vines, i.e., 12 vines × 5 treatments × 4 replicates).

Irrigation treatments

During 2009–2012, five irrigation treatments representing different levels of deficit irrigation were applied as percentages of crop evapotranspiration (ET_c). Crop evapotranspiration was calculated by multiplying reference evapotranspiration (ET_o) by the crop coefficient (K_c), i.e., ET_c = ET_o × K_c. ET_o was calculated using data obtained from the adjacent meteorological station, and K_c was calculated according to Netzer et al. (2009) following nondestructive measurements of leaf area index. The irrigation method was detailed by Munitz et al. (2016). Irrigation treatments followed two strategies: static irrigation and dynamic (seasonally changing) irrigation. Dynamic treatments involved alternation of the percentage of ET_c along the growing season according to phenological stages (stage I, stage II, stage III) as defined by Kennedy (2002): stage I, from bloom to bunch closure; stage II, from bunch closure to veraison (color change to red); and stage III, from veraison to harvest. Dynamic irrigation treatments were early deficit (0, 20, 50% of ET_c) and late deficit (50, 20, 20% of ET_c). Static irrigation treatments were low irrigation (20% of ET_c), medium irrigation (35% of ET_c), and high irrigation (50% of ET_c).

Stem water potential (Ψ_s)

Stem water potential (Ψ_s) was measured using a pressure chamber (Arimad 2, Kfar Charuv, Israel). Three sunlit, mature, fully expanded leaves from each plot (12 leaves per treatment) were bagged 2 h before measurement in plastic bags covered with aluminum foil. The time elapsing between leaf excision and chamber pressurization was less than 15 s. The measurements were conducted 1 day before irrigation was applied.

Trunk diameter

Diameters of 48 vines per treatment (12 vines per plot × 4 replicates) were measured monthly with a digital caliper (075430, Signet, Taiwan). So that data would be consistent, all measured vines (240) were marked 30 cm above ground with colored tape, and all measurements were taken at this point.

Anatomical sampling

At the end of the experiment (December 2012), xylem cores from representative vines were sampled 50 cm above ground with an increment borer (5.15 mm core, 3-threaded 8", Haglöf, Långsele, Sweden). Twelve cores were sampled from each treatment (3 cores per plot × 4 replicates, 60 cores total). Trunk diameter (D; mm) at the drilling location was recorded. Cores were placed in sterilized water and stored at 4°C until cross-sectioned with a sliding microtome (NR17800, Reichert, Vienna, Austria) at a thickness of 90 μm. Visual contrast was increased by staining cross sections for 60 s in Reactif Genevois solution (Fahn, 1954), then flushed with

distilled water. Photographs of stained cross sections were obtained using a stereomicroscope (Olympus SZ2-ILST) coupled with a digital camera (Olympus LC20) equipped with image acquisition software (LCmicro 5.1, Olympus, Tokyo, Japan) at 20× magnification.

Image analysis

Cross sections were analyzed by separately quantifying variables in the visible field for each of four recent growth rings (2009–2012) using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). The abbreviations for structural variables were taken from Scholz et al. (2013). The following anatomical variables were measured (Fig. 1): annual ring width (W_r ; μm), bark width (W_b ; μm), xylem radius (r_x ; μm), and inner xylem radius (r_i ; μm). Vessel lumen area (A_v ; μm^2) was measured, and vessels (n) were counted using the “analyze particles” tool (see Fig. 2 for explanation); analyzed area (A ; mm^2) was also measured. A total of 12,177 vessels were measured and used for subsequent hydraulic conductivity calculations. Because of image resolution limitations, the lower limit of vessels analysis was 12 μm . The detailed calculations for trunk and vessel parameters are presented in Table 1.

Theoretical specific hydraulic conductivity (K_s) calculations

The theoretical specific hydraulic conductivity (K_s ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was calculated using the modified Hagen–Poiseuille’s equation (Tyree and Ewers, 1991):

$$K_s = \left(\frac{\pi \rho}{128 \eta A_w} \right) \sum_{i=0}^n (d_i^4),$$

where K_s is the specific hydraulic conductivity, ρ is the density of the fluid in kg m^{-3} (assumed to be 1000 kg m^{-3}), η is the dynamic viscosity of the fluid in MPa s^{-1} (assumed to be $1 \times 10^{-9} \text{ MPa s}^{-1}$), A_w is the area (m^2) of the xylem cross section analyzed, d is the diameter (m) of the i^{th} vessel and n is the total number of vessels in the measured area.

Hydraulic conductivity per annual ring (K_{ar} , $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was calculated by multiplying the theoretical xylem-specific hydraulic conductivity (K_s) by annual growth ring area (A_r , m^2).

Frequency classes for calculation of total vessel number and total conductivity were established at intervals of 20 μm (Fig. 3). For calculation of vessel density and vessel average diameter, vessels were separated into two size categories ($>100 \mu\text{m}$, $\leq 100 \mu\text{m}$), since *Vitis* has a bimodal distribution of xylem vessels (Fig. 3A).

Statistical analysis

The software program JMP7 (SAS Institute, Cary, NC, USA) was used for all statistical procedures. Data were analyzed via analysis of variance (ANOVA), and means were separated according to the least significant difference (LSD) at $p \leq 0.05$ using the Tukey–Kramer test.

RESULTS

Stem vessel distribution

The distribution of stem xylem vessels in all irrigation treatments showed a classic bimodal pattern. The small vessels ($<100 \mu\text{m}$) constituted the majority (61.3%) of total vessel number, while the large

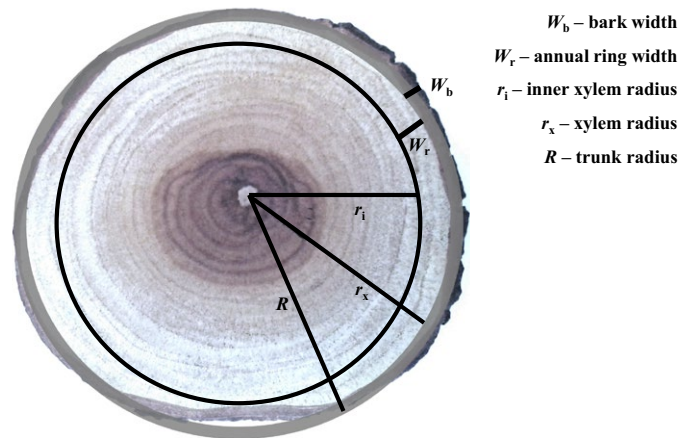


FIGURE 1. Schematic cross section with abbreviations used for trunk variables and calculations.

vessels comprised only 38.7% of total vessels (Fig. 3A). In contrast, the theoretical hydraulic conductivity showed a reverse distribution, where the large vessels contributed 97.2% of total conductivity (Fig. 3B), whereas the contribution of the more abundant small vessels was negligible.

Seasonal changes in trunk diameter

As an integrative indicator of vegetative growth, seasonal changes in trunk diameter were monitored monthly during 2011–2012 (following 2 years of differential irrigation application after 11 years of identical irrigation). Seasonal trends of trunk diameter development were similar in both years in all irrigation treatments (Fig. 4); an increase in trunk diameter began 2 weeks after bud break and continued until stage II (mid-June), then remained stable until the next season. In 2011, a decrease in trunk diameter was apparent during stage III, in all irrigation treatments. Vines in different irrigation treatments showed differences in trunk diameter throughout the entire experimental period (Fig. 4). Among static irrigation vines, trunk diameter increased with applied water amounts, even though the trunk of the medium irrigation vines was slightly narrower than expected (Fig. 4A). In the dynamic irrigation treatments, the early-deficit vines exhibited the narrowest trunk diameter of all vines in all irrigation treatments during the entire measuring period. The late-deficit vines had an intermediate trunk diameter throughout the measuring period (Fig. 4B).

Structural variables

Vines subjected to the static irrigation treatments differed in their annual ring width, with a positive effect of applied water amounts on ring width (Table 2). Within the dynamic irrigation treatments, the late-deficit vines had the widest annual ring width (901.5 μm), very similar to the width of the high-irrigation vines (Table 2). Surprisingly, the early-deficit vines had the narrowest annual ring width (686 μm) compared to the high and medium irrigation and late-deficit vines. The general trend in the annual ring area resembled the trend in annual ring width. Ring area was positively affected by increasing water amounts in static treatment vines. In dynamic irrigation treatments, early-deficit vines had the smallest annual ring area of the five treatments, while the late-deficit vines

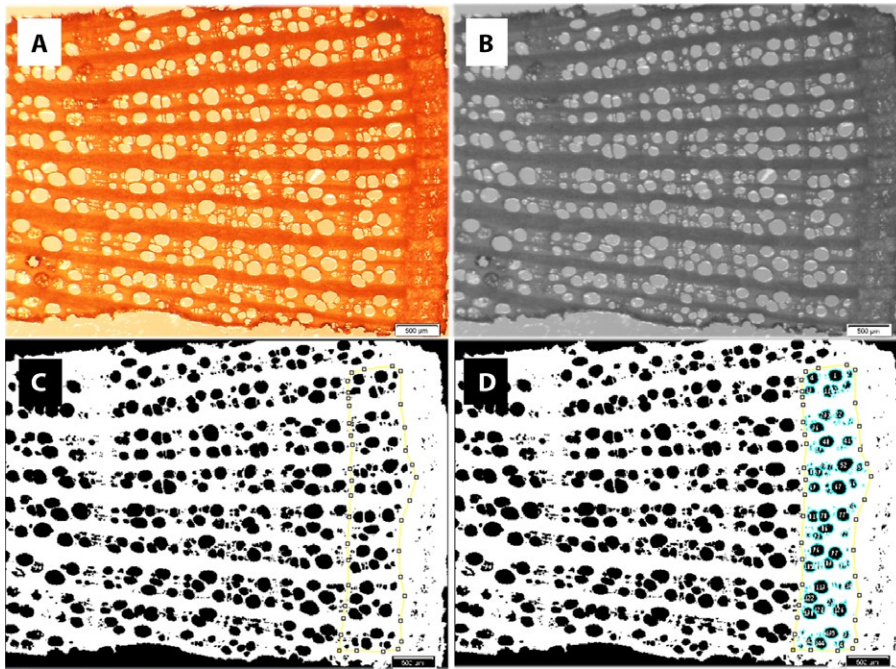


FIGURE 2. Stem cross section of *Vitis vinifera* cv. Merlot. (A) Stereomicrograph. (B) Image processing to 8-bit. (C) Image conversion to black and white (binarization) and selection of annual ring to be analyzed (yellow line). (D) Measurement of vessel area.

had a relatively large ring area, intermediate between that of the high- and medium-irrigation vines (Table 2).

The density of small ($\leq 100 \mu\text{m}$) and large ($> 100 \mu\text{m}$) vessels was not affected by irrigation treatments, although a nonsignificant reduction in the density of small vessels in high-irrigation vines was observed. The overall vessel diameter of the high-irrigation vines was wider than that of all the other vines (Table 2). The large ($> 100 \mu\text{m}$) vessel diameter of the high-irrigation vines was wider than that of the low- and medium-irrigation vines. In the dynamic irrigation treatments, early-deficit vines had narrower large vessels than in any of the vines in all other irrigation treatments, while the late-deficit vines had the widest (Table 1). The trend for small ($\leq 100 \mu\text{m}$) vessel diameter was less clear, with early-deficit and medium irrigation vines exhibiting wider vessels than all other vines. The reason for the unclear trend in the small vessels diameter may be derived from the fact that our method of measuring small vessels was limited to $12 \mu\text{m}$ and did not include all vessels. The trend in specific hydraulic conductivity was similar to that of large ($> 100 \mu\text{m}$) vessel diameter, where the high-irrigation and late-deficit vines had significantly higher hydraulic conductivity (Table 1) than vines in other treatments. Hydraulic conductivity per annual ring increased with increasing water amounts in the static irrigation treatments, whereas in dynamic treatments the early-deficit vines had

the lowest conductivity, while the late-deficit vines had high conductivity (slightly lower than the high-irrigation vines).

The relationship between hydraulic conductivity per annual ring and seasonal water amount (Fig. 5A) was weak ($R^2 = 0.21$), while the relationship between hydraulic conductivity per annual ring and water amounts applied during stage I (bloom to bunch closure, Fig. 5B) was stronger ($R^2 = 0.60$, $p < 0.001$).

Stem hydraulic conductivity distribution among vessel size classes

Specific stem hydraulic conductivity was calculated separately for each vessel size class (Fig. 6). Among static irrigation treatments, the conductivity distribution was similar, but several differences among treatments were observed. In low-irrigation vines, a higher percentage of the calculated hydraulic conductivity was derived from narrow vessel classes, in the medium irrigation vines—from wide vessel classes, and in the high-irrigation vines—from the widest vessel classes (Fig. 6A). In the dynamic irrigation

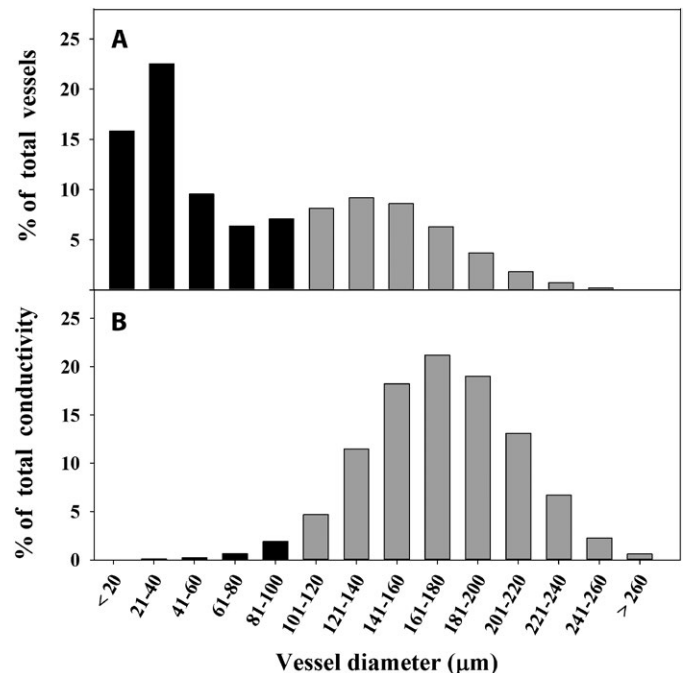


FIGURE 3. (A) Distribution of stem xylem vessels according to diameter classes (μm) in Merlot vines of all irrigation treatments. (B) Distribution of hydraulic conductivity according to diameter classes (μm) in Merlot vines of all irrigation treatments. Vessel classes were divided into two size categories: $\leq 100 \mu\text{m}$ (black bars) and $> 100 \mu\text{m}$ (gray bars). Data represent vessels from all irrigation treatments during 2009–2012, $n = 12177$ vessels.

TABLE 1. Calculations used for trunk and vessel characteristics.

Variable	Abbreviation	Unit	Formula
Vessel diameter	d	μm	$d = (4A/\pi)^{0.5}$
Vessel density	V_d	mm^{-2}	$V_d = n/A$
Trunk radius	R	μm	$R = D/2$
Xylem radius	r_x	μm	$r_x = R - W_b$
Annual ring area	A_r	mm^2	$A_r = \pi(r_x)^2 - \pi(r_p)^2$

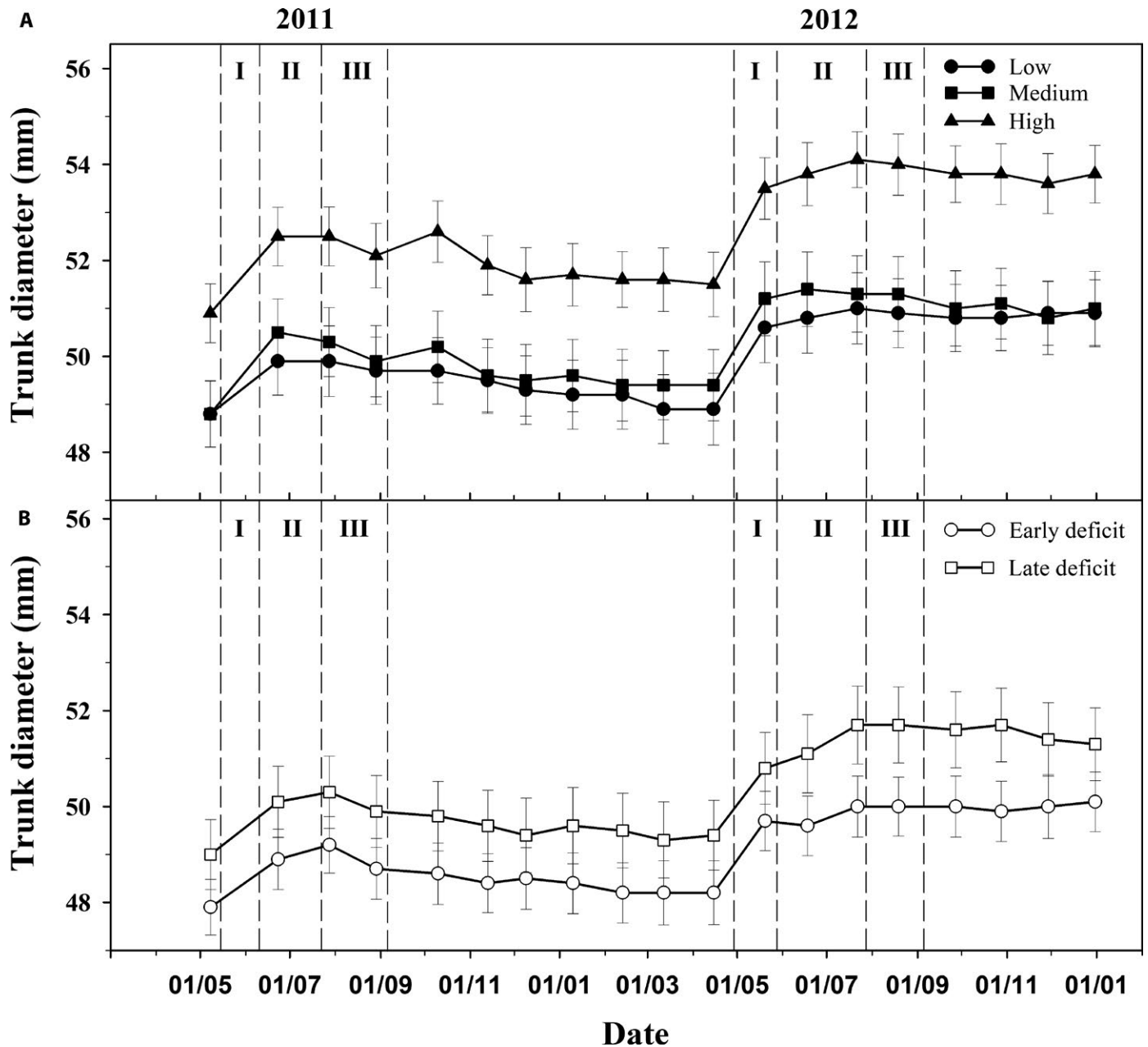


FIGURE 4. (A) Biennial pattern of trunk diameter development of Merlot vines exposed to low irrigation (closed circles), medium irrigation (closed squares), and high irrigation (closed triangles), in 2011 and 2012. (B) Biennial pattern of trunk diameter development of Merlot vines exposed to early (open circles) and late water deficit (open squares), in 2011 and 2012. Each point is the mean of 48 vines (12 vines per replicate). The vertical bars denote one standard error.

treatments, the early-deficit vines had a higher amount of hydraulic conductivity derived from narrower frequency classes, while the hydraulic conductivity in late-deficit vines shifted toward the wide-frequency classes (Fig. 6B).

Stem water potential

Differences in water potential (Ψ_s) between vines in different irrigation treatments were observed during stages II and III (Fig. 7). The daily trend in Ψ_s was similar among treatments, with a steep decrease in Ψ_s values being recorded throughout

the morning, followed by stabilization and improvement in Ψ_s during the afternoon (Fig. 7). In stage II, an improvement in Ψ_s values during the afternoon was apparent only in the high- and medium-irrigation vines (Fig. 7B). On both measuring days, the high-irrigation vines had the highest Ψ_s values during the day, the medium-irrigation vines were at an intermediate level, and the low-irrigation vines had the significantly lowest Ψ_s . In the dynamic irrigation treatments, the early-deficit vines had low Ψ_s values during stage II (lower than the low-irrigation vines) and intermediate values during stage III (resembling those of the medium-irrigation vines). The late-deficit vines had significantly

TABLE 2. Irrigation treatments and anatomic and hydraulic variables. 'Hulda' Merlot vineyard, 2009–2012.

Irrigation treatment	Water amount (mm season ⁻¹)	Annual ring width (μm)	Annual ring area (mm ²)	Large vessel density (mm ⁻²)	Small vessel density (mm ⁻²)	Overall vessel average diameter (μm)	Large vessel average diameter (μm)	Small vessel average diameter (μm)	Specific hydraulic conductivity (kg m ⁻¹ MPa ⁻¹ s ⁻¹)	Hydraulic conductivity per annual ring (kg m ⁻¹ MPa ⁻¹ s ⁻¹)
Low	105	719.9 ^{bc}	104.4 ^{bc}	9.9	16.9	80.3 ^b	147.9 ^b	37.5 ^b	142.5 ^b	0.0152b
Medium	184	835.6 ^{ab}	124.2 ^{ab}	9.6	14.7	84.5 ^b	147.1 ^b	43.3 ^a	144.0 ^b	0.0179b
High	256	891.4 ^a	141.5 ^a	10.3	12.6	90.5 ^a	154.9 ^a	40.3 ^b	188.6 ^a	0.0273a
Early deficit	143	686.0 ^c	100.9 ^c	9.7	15.5	83.9 ^b	142.1 ^c	45.5 ^a	127.2 ^b	0.0128b
Late deficit	141	901.5 ^a	134.3 ^a	9.3	15.8	83.9 ^b	158.5 ^a	38.3 ^b	188.3 ^a	0.0257a
<i>P</i>		<0.0001	<0.0001	N.S.	N.S.	N.S.	0.0004	0.0018	<0.0001	<0.0001
<i>F</i> _{4,75}		10.09	12.67	1.19	1.39	1.66	5.68	4.73	6.7	14.56

Notes: Values represent means ($n = 12$). Within each column, means followed by different letters are significantly different ($P < 0.05$) according to Tukey–Kramer test.

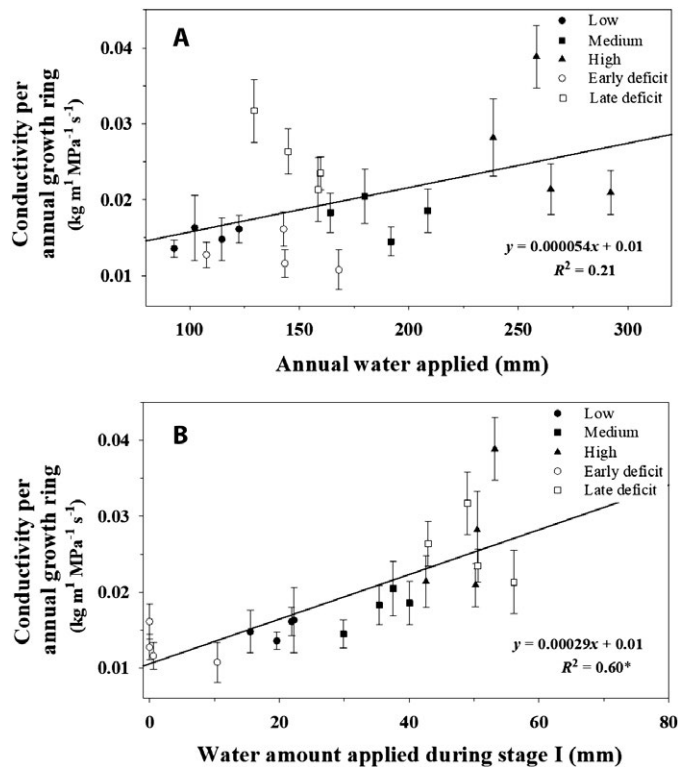


FIGURE 5. (A) Relationship between annual water applied and hydraulic conductivity per annual growth ring in Merlot vines of all irrigation treatments. (B) Relationship between water amount applied during stage I (bloom to bunch closure) and hydraulic conductivity per annual growth ring in Merlot vines of all irrigation treatments. Each point is the mean of 12 annual rings from the same year (3 vines per replicate \times 4 replicates) with 4 points per treatment. The vertical bars denote one standard error. *Significant at $P < 0.001$.

low Ψ_s values on both dates, even compared to the values of the low-irrigation vines (Fig. 7).

DISCUSSION

Vegetative growth

Vegetative growth can serve as a good indicator of water availability (Tyree and Ewers, 1991; Munitz et al., 2016). In the present

study, we continuously monitored trunk growth. In all irrigation treatments, an increase in trunk diameter occurred mainly early in the season (from 2 weeks before blooming until bunch closure) (Fig. 4). Early-season trunk growth has been previously reported in Merlot (Ton and Kopyt, 2004) and other *Vitis vinifera* cultivars (Myburgh, 1996; Ton and Kopyt, 2004; Intrigliolo and Castel, 2007; Montoro et al., 2011; Papi and Storchi, 2012; Edwards and Clingeleffer, 2013).

The high-irrigation and late-deficit vines, which received higher water amounts early in the season, had the widest trunk diameter of all vines. Similarly, annual ring width and area, which represent annual vegetative growth, were also positively correlated with high irrigation early in the season (Stage I). The high-irrigation and late-deficit vines had significantly wider ring width and area in comparison to the low-irrigation and early-deficit vines (Table 2). The dominance of early season vegetative growth in *Vitis vinifera* can be explained by the fact that cambial activity to produce new vascular elements takes place mainly during the early stage of the growing season (until 20 d after bunch closure; Bernstein and Fahn, 1960).

Hydraulic structure

A significant increase (9–11%) in the large vessel diameter was recorded in the high-irrigation and late-deficit vines (Table 2), even though no significant difference in the density of the large vessels was found. Reductions in average vessel diameter in response to drought stress has been previously reported in *Vitis vinifera* 1-year-old shoots (Lovisolo et al., 1998) and petioles (Hochberg et al., 2015). Interestingly, we found the opposite in small vessels: the vessels of the early-deficit vines that received minimal water early in the season had the widest diameter (Table 2). Although the majority of the total vessels were small vessels (61%), they contributed only a negligible 3% of total theoretical hydraulic conductivity (Fig. 3). A similar phenomenon of dominance of wide vessels with respect to total hydraulic conductivity has also been recorded in other species (Hargrave et al., 1994; Tibbetts and Ewers, 2000).

From inspection of the hydraulic conductivity distribution, it can be deduced that high water amounts early in the season result in a higher percentage of hydraulic conductivity derived from wider frequency classes (Fig. 6). It is well known that in *Vitis*, as in many other lianas, wide vessels are formed at the beginning of the growing period and narrow vessels are formed at the end (extreme diffuse-porous), causing a bimodal distribution (Pratt, 1974; Kozlowski, 1983; Ewers et al., 1990; Wheeler and LaPasha, 1994). Applying large amounts of water during the formation of wide vessels and reducing

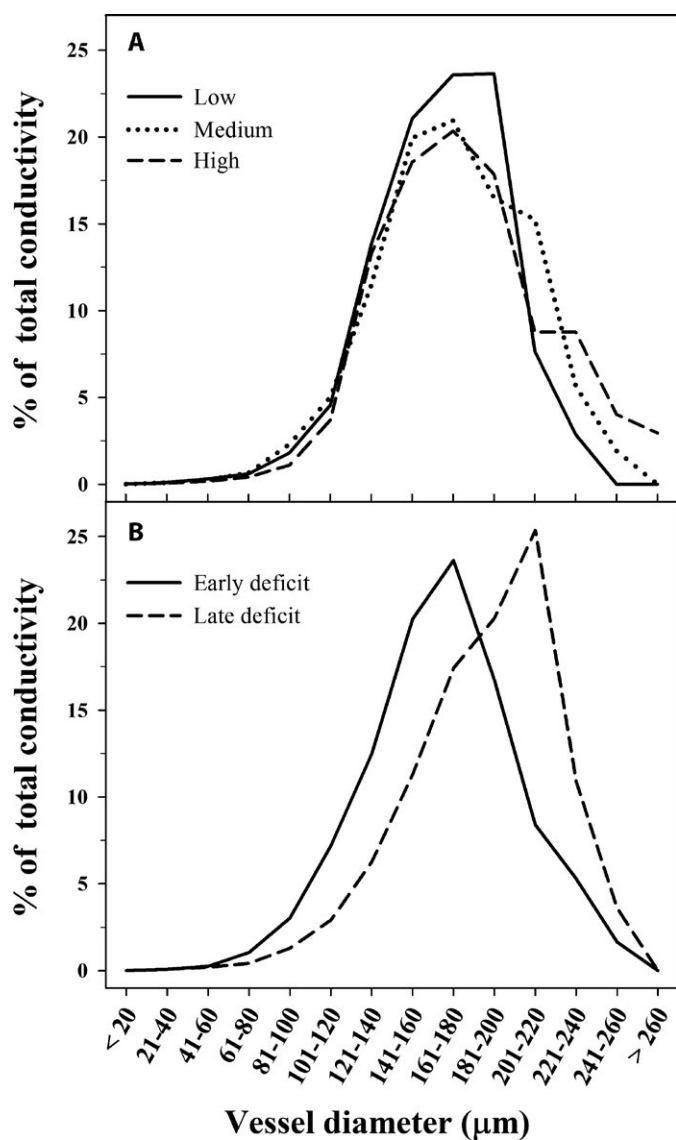


FIGURE 6. (A) Distribution of hydraulic conductivity according to diameter classes (μm) in Merlot vines exposed to low irrigation (solid line), medium irrigation (dotted line), and high irrigation (dashed line). (B) Distribution of hydraulic conductivity according to diameter classes (μm) in Merlot vines exposed to early (solid line) and late water deficit (dashed line). Data represent vessels from all analyzed years (2009–2012), $n = 12,177$ vessels.

water allocation during the formation of narrow vessels (late deficit) should result in larger wide vessels and smaller narrow vessels, leading to an increased proportion of hydraulic conductivity being derived from wide vessels. As a result, early-irrigation (late-deficit) vines are expected to be more susceptible to embolism formation in comparison to low-irrigation vines.

Specific hydraulic conductivity (K_s) represents the conducting efficiency of the xylem tissue (Tyree and Ewers, 1991). The high-irrigation and late-deficit vines had significantly increased K_s (24–33%) compared to other vines (Table 2). It can be concluded that water shortage early in the growth season (bloom to bunch closure) results in decreased K_s . Since there is a good correlation

between measured and calculated hydraulic conductivity (Salleo et al., 1985; Hargrave et al., 1994; Lovisolo et al., 1998; Nolf et al., 2017), our theoretical K_s results indicate a decline in total hydraulic conductivity as a response to early-season drought stress. Hydraulic conductivity per annual growth ring (K_{ar}) is an integrated parameter combining both hydraulic conductivity (K_s) and vegetative growth (trunk diameter and ring width). High-irrigation and late-deficit vines had significantly increased hydraulic conductivity per annual ring (K_{ar} , Table 2). Interestingly, the high-irrigation vines had 9% higher K_{ar} (not significant) than the late-deficit vines. This difference can be attributed to the reduction of water amounts in late-deficit vines during bunch closure, while cambial activity in the stem persisted for a further 20 d (Bernstein and Fahn, 1960).

Reinforcement of the notion that most of the vegetative growth and xylem development occur early in the season can be found in the correlation between the amounts of applied water and hydraulic conductivity per annual ring, K_{ar} (Fig. 5). While annual water applied had a weak and nonsignificant correlation with K_{ar} ($R^2 = 0.21$), water amount applied during stage I was strongly and significantly correlated to K_{ar} ($R^2 = 0.6$, $P < 0.001$).

Water potential

Stem water potential is known to be a sensitive indicator of vine water status (Choné et al., 2001; Chaves et al., 2010; Munitz et al., 2016). Indeed, on both measuring days, static-irrigation vines differed significantly in their stem water potentials, according to the water amounts applied, throughout the entire day. Those differences in stem water potential were present from bunch closure until harvest (Munitz et al., 2016). Interestingly, late-deficit vines were more drought-stressed (more negative stem water potential) than low-irrigation vines on both measuring days, even though they received on average 31% more water before the measuring days. Those differences in stem water potential cannot be attributed to a broader canopy, since the late-deficit and low-irrigation vines had similar leaf area on measuring days (Munitz et al., 2016). Water potentials of -1.4 MPa, recorded on both measuring days, have been reported to cause a 30–80% decrease in hydraulic conductivity of *Vitis vinifera* shoots (Alsina et al., 2007; Choat et al., 2010; Jacobsen and Pratt, 2012).

The increased drought stress in late-deficit vines can be explained by a greater hydraulic conductivity loss. As stated before, wide vessels are more susceptible to embolism formation. Late-deficit vines, with a higher percentage contribution of wide vessels to total hydraulic conductivity, are expected to undergo greater hydraulic loss at a similar water potential. Increased hydraulic loss will, in turn, lead to increased drought stress, e.g., more negative water potential (Tyree et al., 1991).

Structural variables as compared to values from literature

Generally, our results are in agreement with values presented in the literature. The annual increase in trunk diameter of mature vines measured in this study (1.5–2.5 mm, Fig. 4), is similar to that reported for a number of *Vitis vinifera* cultivars (0.5–3.5 mm) (Bernstein and Fahn, 1960; Myburgh, 1996; Ton and Kopyt, 2004). Similarly, the range of annual ring width measured in this study (720–901 μm) is consistent with values reported for mature *Vitis vinifera* cultivars (100–1300 μm) (Perold, 1927; Bernstein and Fahn, 1960), and the range of annual ring area found in this study

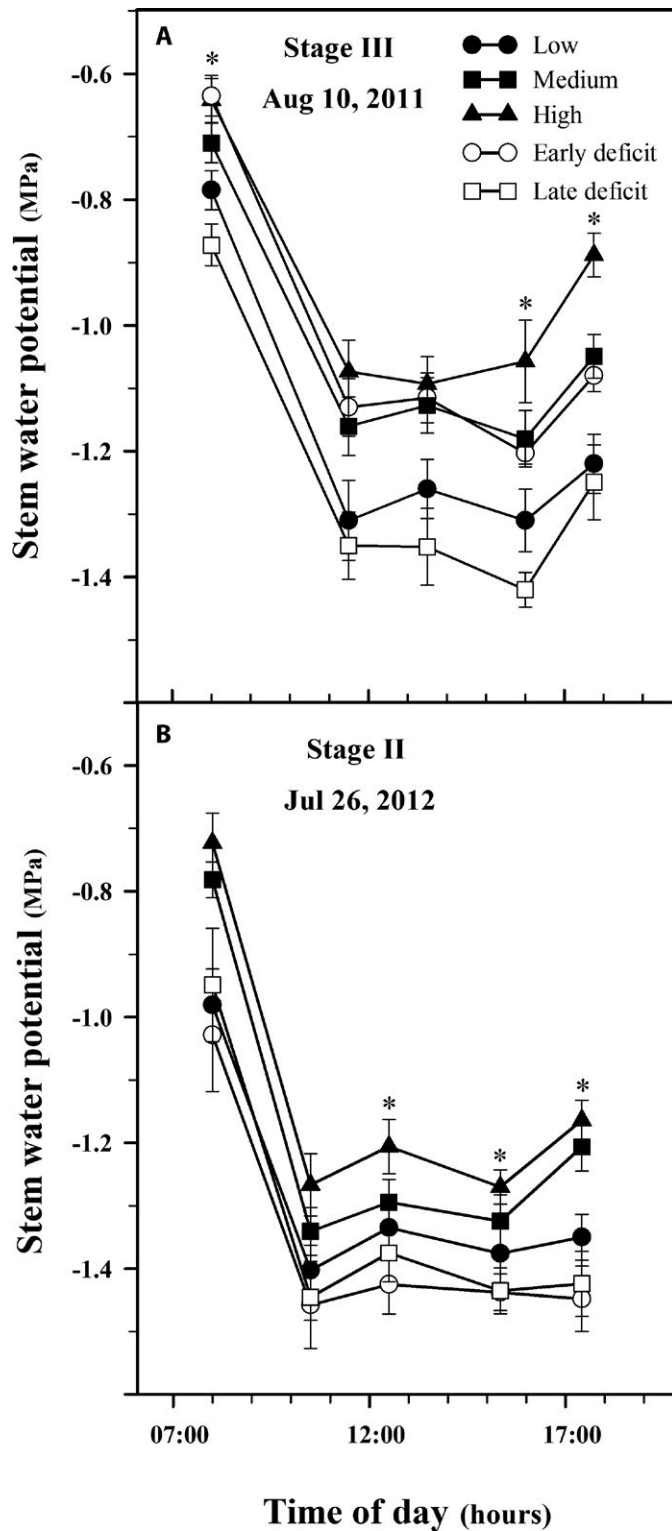


FIGURE 7. (A) Daily pattern of stem water potential of Merlot vines exposed to low irrigation (closed circles), medium irrigation (closed squares), high irrigation (closed triangles), early deficit (open circles) and late deficit (open squares) measured on 10 August 2011. (B) Daily pattern of stem water potential of Merlot vines exposed to low irrigation (closed circles), medium irrigation (closed squares), high irrigation (closed triangles), early deficit (open circles) and late deficit (open squares) measured on 26 July 2012. Each point is the mean of 12 leaves (3 vines per replicate). The bars denote one standard error. Asterisks indicate significant difference ($P < 0.01$) between irrigation treatments according to Tukey–Kramer test. Detailed values and statistics presented in Appendix S1.

difference of 11 μm in vessel diameter (Table 2), the difference between the two cultivars stands at more than 50 μm . This implies an inherent genetic distinction in trunk xylem anatomy between *Vitis vinifera* cultivars, as reported previously for petioles and shoots (Chouzouri and Schultz, 2005; Chatelet et al., 2011; Tombesi et al., 2014; Gerzon et al., 2015; Hochberg et al., 2015; Santarosa et al., 2016). Typical values of specific hydraulic conductivity of liana stems (Milburn, 1979) are 65–349 $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$; our K_s results (142–188 $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) are in the middle of this range. Values of K_{ar} reported for *Vitis vinifera* cv. Cabernet Sauvignon (0.07–0.11, $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) are considerably higher than those calculated in this study, due to the wider vessels and ring area measured in Cabernet Sauvignon vines.

CONCLUSIONS

The anatomical structure of woody stems constitutes the long-term “memory” of the vine, yet very little information about drought stress effects on anatomical features of mature *Vitis* stems is available. One example of this “memory” is the fact that the current year’s vine canopy develops while consuming water conducted through vessels differentiated in previous years. Most of the vine’s canopy develops in the early stages of the growing season, about 60 days from budbreak (Ben-Asher et al., 2006; Intrigliolo et al., 2009; Romero et al., 2010; Munitz et al., 2016). Cambial tissue begins its activity about 2 weeks after bud break (Bernstein and Fahh, 1960). Vessels become hydraulically active about 4 weeks after their initial differentiation is initiated (Halis et al., 2012; Jacobsen et al., 2015, 2018): 2 weeks for differentiation, expansion, and formation of secondary walls and at least two additional weeks for the creation of perforation plates by autolysis of axial cell walls. In practice, current year vessels are functional no less than 54 days after bud break, when canopy development has almost ceased.

There is a lack of information about the effects of drought stress on *Vitis* xylem structure (Lovisololo et al., 1998), especially in mature stems. Our research can contribute to the understanding of mature stem xylem structure and how it is affected by drought stress in the long term. This long-term memory of xylem structure can be relevant for other woody plants, particularly in those in which xylem annual rings are hydraulically active for several years. High water availability during xylem formation period, results in wider vessels and increased hydraulic conductivity. This improved hydraulic system, even though favorable during periods of high water availability, is more prone to cavitation during drought periods.

(104–134 mm^2) are lower than that of Cabernet Sauvignon vines (120–240 mm^2 , Shtein et al., 2016).

The average diameter of large vessels measured in this study (147–158 μm) is considerably smaller than that reported for *Vitis vinifera* cv. Cabernet Sauvignon. While in the current study, water availability of Merlot vines in different irrigation regimes triggered a maximal

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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