Chapter 5
Grapevine Xylem Development, Architecture, and Function

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1 *Vitis* as a Model Xylem System

The genus *Vitis* (Vitaceae) has been thoroughly studied, in part because of commercial and agricultural interests in grapevine. This has led to myriad studies on many aspects of grapevine biology, including efforts to understand grapevine molecular, physiological, structural, and development biology.

Early efforts to sequence entire genomes of model plant species such as *Arabidopsis*, poplar, and rice also included efforts to sequence *Vitis*. This resulted in the early sequencing of the entire genome of *V. vinifera* "Pinot Noir" (Jaillon et al. 2007; Velasco et al. 2007). Additional molecular research has also been conducted on the *Vitis* chloroplast genome (Jansen et al. 2006) and on the use of other molecular techniques to compare across the genus *Vitis* (Myles et al. 2010).

In addition to ongoing molecular and genetic studies, many physiological studies have examined grapevine functional biology, including water relations and hydraulics (Zufferey et al. 2000; Schultz 2003; Domec and Johnson 2012). These studies have included xylem responses to pathogens and infection (Chatelet et al. 2006; Thorne et al. 2006; Sun et al. 2013) and to drought (Schultz and Matthews 1988; Lovisolo and Schubert 1998; Lovisolo and Schubert 1998; Lovisolo et al. 2010). Additionally, active processes that occur within the xylem have been examined, including the formation of tyloses and gels following infection, wounding, and embolism (Sun et al. 2007, 2008; Pérez-Donoso et al. 2007; Jacobsen and Pratt 2012).

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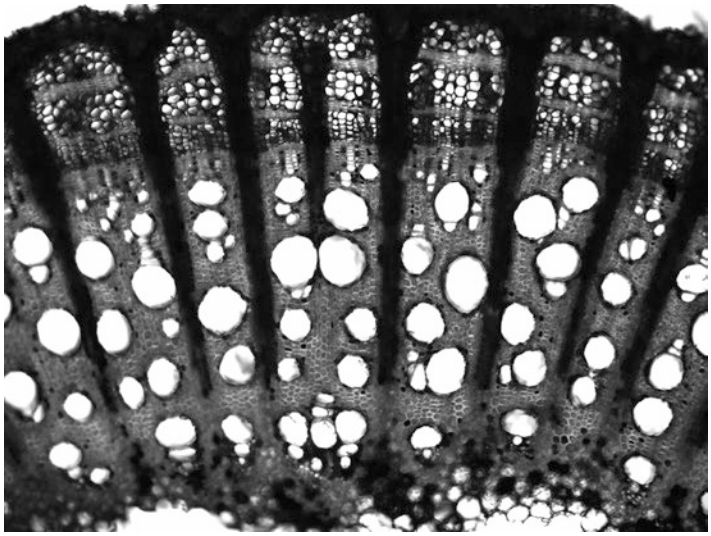

Fig. 5.1 Representative xylem micrograph from a 1-year-old stem of *Vitis vinifera*. The starch within the section has been stained dark to highlight the location of living cells with the xylem, particularly as they occur in wide xylem rays and in paratracheal axial parenchyma cells. This section is from a stem harvested toward the end of the growing season as is evidenced by the presence of many very narrow vessels near the cambium containing secondary cell walls and the absence of large expanding vessel elements lacking secondary cell walls. The cells of the phloem are also clearly visible

The hydraulics of grapevine stems has also been examined (e.g., Sperry et al. 1987; Tibbetts and Ewers 2000; Choat et al. 2010; Jacobsen and Pratt 2012).

Finally, grapevine hydraulic structure has been the subject of many recent studies. Grapevine has several unique features of its xylem and phloem structure, many of which are associated with its liana habit (Fig. 5.1; Chap. 6). Grapevine stems have wide diameter vessels, which are relatively isolated from other large vessels within the secondary xylem tissue (Fig. 5.1). This makes these vessels relatively easy to resolve using newly developing images technologies (Holbrook et al. 2001; Brodersen et al. 2010, 2011).

2 Secondary Xylem Vessel Development

2.1 General Patterns of Woody Plant Vessel Development

Within flowering plants, water is transported throughout the plant body primarily through xylem vessel elements. Many individual vessel elements connect at axially located perforation plates to form long continuous tubes through xylem tissue that are termed vessels. Vessels begin and end at terminal vessel elements, which contain one end wall that is not open (i.e., a perforation plate is only present on one side

of the vessel element) (Handley 1936). Individual vessels may extend several meters in length, but most vessels are quite short (Zimmermann and Jeje 1981; Zimmermann and Potter 1982; Ewers et al. 1990; Jacobsen et al. 2012).

Xylem vessel elements are formed from meristematic cells in either the procambium or the vascular cambium and each vessel element matures individually (Esau 1953). Following differentiation, vessel elements expand to what will become their mature volume before starting to form secondary wall layers along their sides (Esau and Charvat 1978). At this stage of vessel element development, the protoplasm is highly vacuolated (Esau 1936; Esau and Charvat 1978). During early vessel element development, the end walls, located basally and apically in the cell where perforation plates will later occur, become thickened (Esau and Hewitt 1940; Esau and Charvat 1978; Benayoun et al. 1981). The term perforation partition has also been used to refer to the areas of the cell wall that will later open to form perforation plates (Meylan and Butterfield 1981).

Maturation of vessel elements into hydraulically functional tubes is a multistage process that may occur over several weeks in the secondary xylem. Vessel element differentiation and expansion are followed by the deposition of lateral secondary walls. Deposition does not occur in the areas that will become perforation plates (usually at the end walls, but they may occur on the lateral walls in some species; Esau 1953) or in the areas along lateral walls that will become pits (Barnett 1982; Chaffey et al. 1997; see Chap. 3 for information on pit formation in conifers). Following the formation of lateral secondary walls within vessel elements, the end walls begin to thin (Esau 1936). Other cellular changes also occur, including the rupture of the tonoplast, the degradation of other organelles within the cell, and the deposition of lignin (reviewed in Fukuda 1997 and Bollhöner et al. 2012). At this stage, each individual vessel element maintains end walls and intact cell membranes filled with dilute protoplasm and may remain in this stage for up to several weeks (Esau and Hewitt 1940; Halis et al. 2012; Bollhöner et al. 2012).

Death of vessel elements, the opening of end walls, and the lysing of the remaining cellular contents occur during the final stages of vessel element development and signal their transition to becoming hydraulically active. Vessel element end walls open at the same time that the cell membrane disintegrates (Esau and Hewitt 1940; Murmanis 1978; Benayoun et al. 1981). Within a single vessel, individual vessel elements die at different times, although usually starting first basally and progressing in the apical direction. Both open basal and closed apical vessel elements may be found simultaneously within a single vessel (Esau 1936; Eames and MacDaniels 1947; Halis et al. 2012). Thus, individual vessel elements may become hydraulically active before the entire vessel activates (Halis et al. 2012) and the end walls of still living vessels form the barrier between the apoplast and symplast during this time. Once all of the vessel elements within a vessel undergo autolysis, the vessel becomes fully hydraulically functional, with water able to move from element to element through open perforation plates along the entire length of a vessel or through pits into lateral vessels.

The differentiation, development, and maturation of vessels may extend over several weeks in woody species. It has been reported that vessel element development

took about five weeks in the earlywood vessel elements of the bole and branches of mature oak trees (Zasada and Zahner 1969). Others have reported that the final stages of vessel element maturation, including the disintegration of the end wall was quite gradual (Esau 1953; Murmanis 1978). Cell death of vessel elements and the onset of hydraulic function may also be delayed an additional several weeks after the full expansion of cells and formation of mature secondary walls. This has been described previously in grapevine (Halis et al. 2012). Indeed, vessel elements as well as fibers may remain alive (i.e., retain an intact cell membrane) at locations distant from the vascular cambium in some species (Zasada and Zahner 1969; Almeida-Rodriguez and Hacke 2012; Bollhöner et al. 2012).

The timing of these processes in woody plants differs from cell culture-, primary xylem-, or *Arabidopsis*-based studies which have reported that vessel elements differentiate, mature, and undergo cell death rather rapidly (Benayoun et al. 1981; Fukuda 1997; Groover and Jones 1999; Turner et al. 2007; Bollhöner et al. 2012), although metaxylem vessel elements have been described as remaining alive for longer periods and for up to 30 cm from growing tips in some nonwoody species as well (St. Aubin et al. 1986). Rapid vessel element maturation and subsequent cell death suggest that nonwoody systems may have limitations as models for understanding development of vessels within the secondary xylem of woody plants.

2.2 *Vessel Differentiation, Expansion, and Maturation in Grapevine*

Secondary xylem vessel element development in grapevine follows a similar pattern as described for other woody angiosperms. Vessel elements within the secondary xylem tissue are initiated early in the growing season, following initial shoot expansion. Throughout most of the growing season, new vessels are continuously formed. Thus, when xylem is examined in cross section, secondary vessels that are still differentiating and expanding, that are still living and have complete secondary walls, and hydraulically functional vessels are all visible simultaneously (Fig. 5.2a–d). This pattern has been described in *Vitis vinifera* "Cabernet" (Halis et al. 2012) and is also apparent in samples from both *V. vinifera* "Glenora" and *V. vinifera* "Chardonnay" (Fig. 5.2a–d), and thus may be general to *V. vinifera*. Interestingly, these vessels are not easily apparent unless active xylem staining is employed (compare Fig. 5.1 to Fig. 5.2a–d; see below for additional method details).

We examined vessel lifespan in 1-year-old shoots of field-grown *V. vinifera* L. "Glenora," a table grape that grows well in southern California and produces pale-red medium-sized seedless berries (see Jacobsen and Pratt 2012 for additional information on this variety) and from which samples were readily available and in close proximity to the laboratory. Additionally, a limited number of observations were also conducted on 1-year-old shoots of field-grown *V. vinifera* L. "Chardonnay." All samples were collected from well-watered plants on or near the campus of California State University, Bakersfield during the 2012 and 2013 growing seasons.

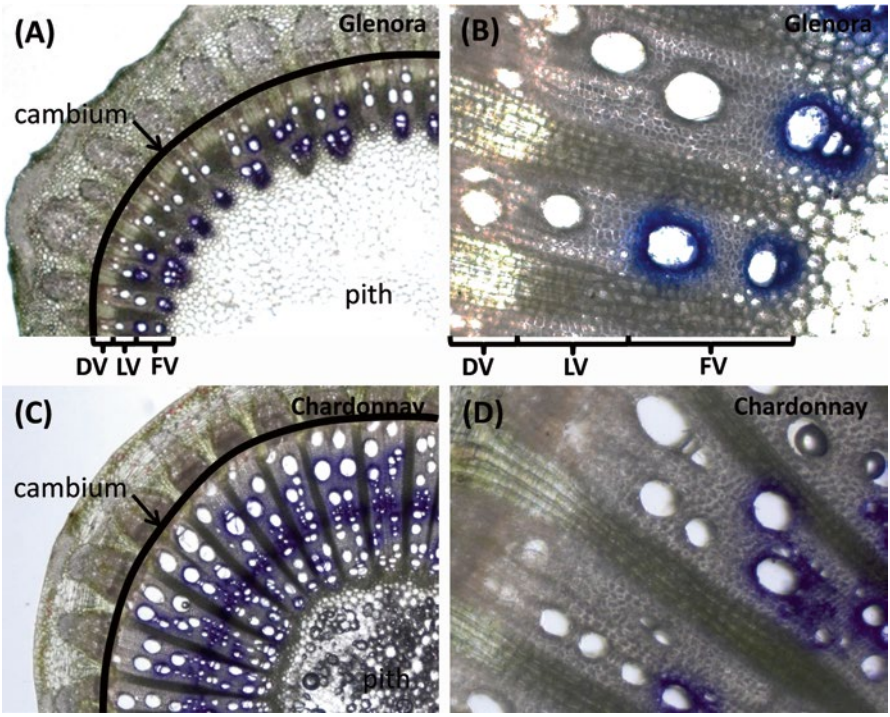

Fig. 5.2 Xylem cross sections collected during the middle of the growing season from 1-year-old stems of *Vitis vinifera* "Glenora" (**a**, **b**) and *V. vinifera* "Chardonnay" (**c**, **d**) that have been stained for active xylem vessels using crystal violet and are shown at both low (**a**, **c**; 25×) and higher (**b**, **d**; 100×) magnification. Only vessels which have already undergone autolysis and are hydraulically functional stain purple (i.e., they are functional vessels, FV). The remaining, nonstained vessels, are shown in two different stages of vessel development, those that do not yet have a complete secondary cell wall and may still be expanding (differentiating/developing vessels, DV) and those that have a complete secondary cell wall but with vessel elements that are still alive and filled with dilute protoplasm (living vessels, LV). Both examined varieties have many nonhydraulically functional vessels and contain a relatively narrow band of vessels that would be contributing significantly to long distance water transport within the stem. The vascular cambium is identified in panels A and C to highlight that vessels only become hydraulically functional once they are relatively distant from the cambium and that the large vessels nearest the cambium are not hydraulically active. This pattern is apparent throughout most of the growing season as long as new vessels are continuing to form (see Figs. 5.3 and 5.4)

Samples collected in 2012 were used to hone the staining technique described below to ensure that flushing, staining, and sample preparation did not disrupt living vessel elements (see St. Aubin et al. 1986 for discussion of the challenges of preparing samples without disruption of end walls, particularly when fixation was used).

In 2013, 1-year-old shoots were removed from plants at the site of initial bud emergence so that the entire portion of 1-year-old growth was removed from the point of its connection to older tissue. *Vitis vinifera* "Glenora," samples were collected 15 April, 23 April, 3 May, 7 June, 27 June, 17 July, and 26 October.

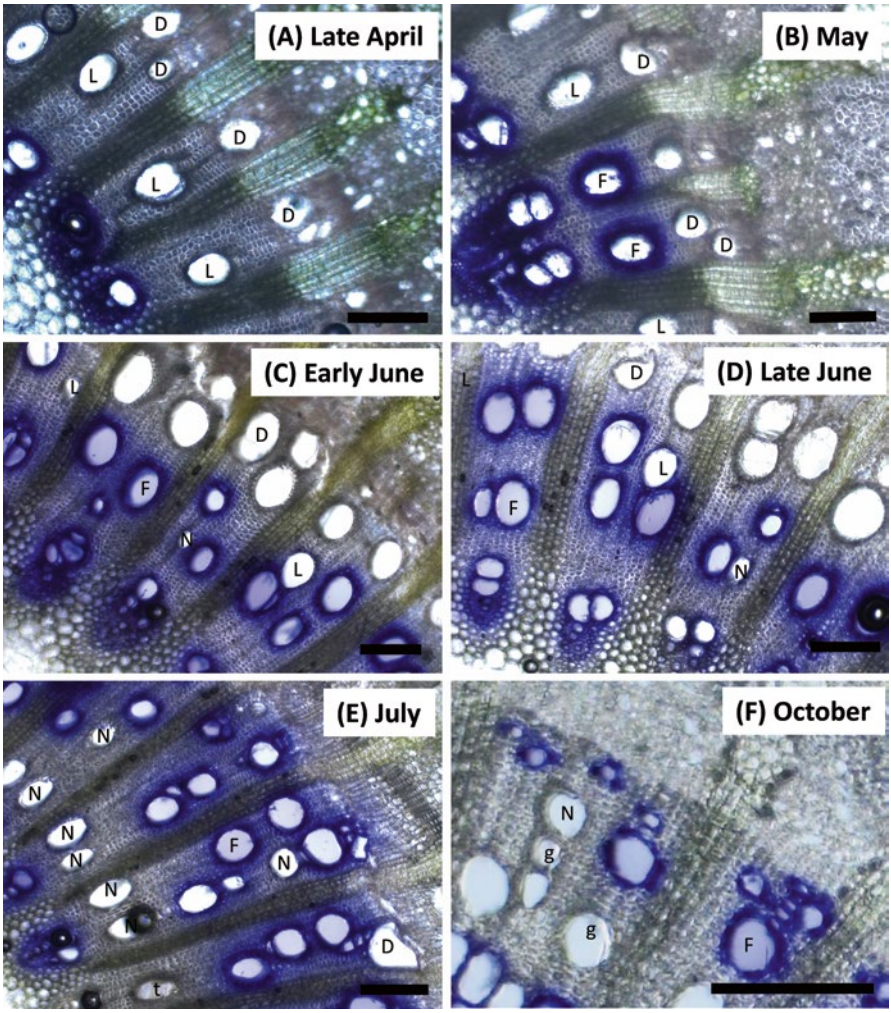

Fig. 5.3 Micrographs showing representative staining patterns from sampling times (scale bars indicate 200 μm) selected to illustrate patterns in vessel development throughout the growing season. Vessels were identified as falling within one of the following vessel lifespan classes: developing (D), living (L), potentially hydraulically functional (F), or nonhydraulically functional (N). Several vessels in each of these classes are identified in the panels above to illustrate their appearance. Potentially hydraulically active vessels were stained with crystal violet and appear purple in micrographs. New vessel formation only ceased at the end of the growing season, at the sampling period coinciding with the onset of leaf abscission (October). Vessel occlusions, including tyloses (t) and gels (g) are visible in some sections

These collection times corresponded to early shoot growth (April), blooming (May), fruit development (June), fruit ripening (July), and the initiation of leaf abscission (October). Samples were collected from *V. vinifera* "Chardonnay" on 18 June and 17 July.

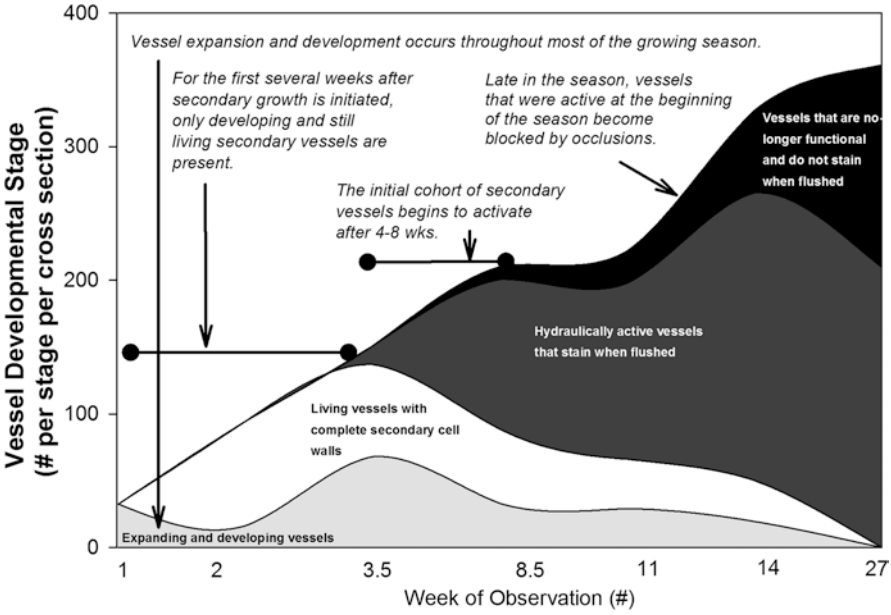

Fig. 5.4 The number of vessels occurring in different vessel lifespan classes as determined throughout the 2013 growing season, beginning in April and ending in October, and based on observations from 25 stem sections of current year growth from well-watered *V. vinifera*. Gray-scale fills indicate different vessels classes and are based on mean vessel numbers. Important transitions in vessel development are indicated in italics within the figure. When analyzed across the growing season, potentially hydraulically active vessels accounted for approximately 35 % of vessels in cross sections. Vessels that are not able to transport sap represented the majority of vessels within the secondary xylem tissue, including developing and living vessels (54 %) and vessels that were no longer hydraulically functional (11 %)

At each sampling date four large shoots were collected at predawn, double bagged in large plastic bags with a moist paper towel, and transported to the laboratory (<10 min transport time). In October, only a single large shoot was sampled due to limited ability to find shoots that still maintained green nonabscising leaves from among the shoots that had been tagged and followed since bud growth initiation earlier in the season. Immediately upon arrival in the laboratory, a 0.20 m stem segment was trimmed underwater from each large shoot at 1 m distance from the proximal end. All samples were collected at this same distance so that samples were of progressively older stems throughout the growing season from shoots initiated at a similar time. This meant that samples became progressively wider in diameter at each sampling time due to continuing secondary growth.

The ends of shoot segments were shaved with fresh razor blades and samples were inserted into a tubing apparatus and flushed for 1 h at 100 kPa using a degassed 20 mM KCl solution filtered to 0.1 μm (inline filter, GE Water and Process Technologies, Trevose, PA, USA). A 0.10 m segment was then cut underwater 0.06 m from the proximal end of the longer flushed segments. This shorter segment was stained using a 0.1 % (mass/volume) crystal violet dye solution, adjusted to a pH

of 2 using HCl, and ultra-filtered (0.1 μm, MAGNA, nylon supported filter, Osmonics, Inc., Minnetonka, MN, USA). Stain was pulled up through stem samples at approximately 1–2 kPa for 20–30 min following the methods of Jacobsen et al. (2007). At the end of this time period, dye could be seen emerging from the distal ends of stem samples. This stain was selected because it easily passes through pit membranes, readily stains secondary cell walls (also termed gentian violet, Chamberlain 1901; Ruzin 1999) with a preference for lignified walls, and has been used in many studies as an indicator of active xylem vessels (Kolb and Davis 1994; Tibbetts and Ewers 2000; Jaquish and Ewers 2001; Jacobsen et al. 2007; Jacobsen and Pratt 2012).

After staining, segments were briefly (1–3 min) flushed at 1–2 kPa with the same solution used to flush samples prior to staining. This removes dye from many of the hydraulically active vessel lumens, but does not remove dye from already stained cell walls. This reduced the spread of dye into adjacent tissue prior to imaging and also reduced the amount of dye transferred by sectioning. Dyed stems were sectioned in the central portion of segments, in a region of the stem that was not submerged beneath dye during the staining process. This ensured that dye was limited to the region around conductive vessels into which dye had been pulled. Because dye slowly spreads following staining, each stem was sectioned and immediately photographed following staining.

All secondary xylem vessels were quantified into one of the four following classes: (1) Developing (not stained purple, near the cambium, without a thick secondary wall), (2) living (not stained purple, close to the cambium, thick secondary wall), (3) potentially hydraulically functional (stained purple), and (4) nonhydraulically functional (not stained purple, in a region of the stem where other vessels are or have been hydraulically functional; may contain gels or tyloses). Vessels located within the primary xylem tissue were also observed, but they were classified only as being either conductive or nonconductive. To confirm the presence of gels in older nonconductive secondary xylem vessels, some fresh sections were analyzed under a dissecting scope with no coverslip or mounting material. This allowed for easy identification of gel-filled vessels because gels do not recede from cut vessels and were often visible actively extruding from cut vessels (see Jacobsen and Pratt 2012, their Fig. 4; see also Fig. 5.11a–d in the present chapter).

Vessels located within primary xylem tissue, located in vascular bundles, were already hydraulically active during the first sampling period in early April (not shown). Interestingly, the vast majority of these primary xylem vessels remained potentially hydraulically active throughout the growing season. More than 93 % of the primary xylem vessels in all of the examined sections were hydraulically active from early April through late June. The percentage of potentially hydraulically active primary xylem vessels began to decline in July (mean of 76 % potentially hydraulically active) and had further declined by the onset of leaf abscission in October (mean of 27 % potentially hydraulically active vessels).

Secondary xylem production was initiated in a few of the observed samples prior to early April, but at the time of the first observations of samples there were no secondary xylem vessel elements yet displaying fully formed secondary cell walls. The first secondary xylem vessel elements to fully expand and start to form secondary cell walls were observed in late April (Fig. 5.3a). These vessel elements were still

living and not yet potentially hydraulically active. The first potentially hydraulically active secondary xylem vessels were observed in May (Fig. 5.3b), more than 3 weeks after initial observations of vessel differentiation. The entire initial cohort of secondary xylem vessels were not observed to be potentially hydraulically active until early June, nearly 8 weeks after initial observations of vessel differentiation. Thus, the observed amount of time from the initial differentiation of vessel elements to the appearance of hydraulically active vessels was approximately 4–8 weeks (Figs. 5.3a–f and 5.4). This included at least 2 weeks to differentiate, expand, and form secondary walls and an additional 2–4 weeks before autolysis and opening of perforation plates. The same patterns of vessel element development were also apparent in samples from Chardonnay (data not shown; see Fig. 5.2d).

The production of secondary xylem and secondary xylem vessel development continued throughout most of the growing season (Figs. 5.3a–f and 5.4). A band of differentiating and developing vessels could be found near the vascular cambium at every sampling period, except for October (Figs. 5.3a–f and 5.4). Beginning at the time of fruit harvest (July) and continuing to the period of leaf abscission (October), vessel development declined and a cohort of narrow diameter vessels became hydraulically active immediately adjacent to the vascular cambium (Fig. 5.3f). Similarly, living vessels were present throughout most of the growing season and were visible as fully expanded vessel elements, usually removed from the vascular cambium by at least one or two developing vessels, that were not yet potentially hydraulically active (Figs. 5.2a–d and 5.3a–f).

Individual vessels did not remain potentially hydraulically active throughout the growing season. Rather, there was a steady change in the suite of vessels that were potentially hydraulically active and vessels that were active early in the season were mostly nonhydraulically functional later in the season (Fig. 5.3e, f). In general, none of the secondary xylem vessels that would have been hydraulically active in May and June were still active late in the season. This was indicated by the failure of early season vessels to stain with active xylem staining later in the season.

When analyzed across the growing season, hydraulically active vessels represented only a small proportion of total vessels (Fig. 5.4). The region of xylem containing vessels that were hydraulically functional was small and vessels are only active for a limited portion of time (Fig. 5.3a–f). Staining of samples that have been flushed to remove emboli indicated that potentially hydraulically active vessels account for only 35 % of vessels visible within cross sections of 1-year-old stems in *V. vinifera* and, due to embolism formation, the in situ number of hydraulically active vessels would likely have been even lower. Vessels not able to transport sap represented the majority of vessels. The majority of these vessels, particularly in the early half of the season, were vessels that were still developing or living (~54 % of visible secondary xylem vessels). This included a large band of vessels located near the cambium.

Later in the season, the proportion of vessels that were not able to transport sap also included an expanding band of inactive vessels occurring near the pith. This band slowly expanded out toward the vascular cambium throughout the late period of the growing season. These inactive vessels did not become conductive following flushing of stems under positive pressure, indicating that this loss was semipermanent to permanent. This was most likely due to the formation of gels or tyloses (Sun

et al. 2007, 2008; Jacobsen and Pratt 2012; Sun et al. 2013). The high proportion of nonhydraulically functional vessels in grapevine may pose a particularly challenging problem in ongoing research examining grapevine structure and function (see Sect. 5 of this chapter).

3 Grapevine Stem Hydraulic Architecture

Grapevine stem xylem vessels are relatively unexceptional in their dimensions when standardized by sample diameter, as is required for cross-species comparisons (Jacobsen et al. 2012). Secondary vessels in 1-year-old grapevine stems, the type commonly used in hydraulic studies, contain vessels that are much smaller in diameter, on average, than the reported global mean (compare standardized values in Table 5.1 to a global mean

Table 5.1 Mean and maximum vessel diameters from stems of several *Vitis* species and varieties compiled from several different sources

Species	Variety (if reported)	Mean vessel diameter (μm)	Maximum vessel diameter (μm)	Source
Not standardized by sample diameter (*ranges indicate that the reported mean varied with stem diameter, node, or treatment*):				
V. labrusca	–	Approx. 300	–	Zimmermann and Jeje (1981)
V. rotundifolia	–	36–245.5	–	Ewers and Fisher (1989), Ewers et al. (1990)
V. vinifera	Freisa	74–101	>140	Lovisolo and Schubert (1998)
V. vinifera	Grenache	–	108.7	Lovisolo et al. (2008)
V. vinifera	Chardonnay	60.5–67.6	–	Sun et al. (2006)
V. vinifera	Glenora	28.6–57.9	68.6–158.1	Current chapter, Fig. 5.10
V. vinifera	–	69.18	–	Sperry et al. (2005)
V. vinifera	Nebbiolo	61–107	>140	Schubert et al. (1999)
Standardized samples (*n* = 6–23 *per variety*; 4–8 *mm sampled stem diameters from 1-year-old segments*):				
V. labrusca	Catawba	29.1 ± 2.7	64.7 ± 3.1 (75.5)	Jacobsen, unpublished data
V. labrusca	Concord	24.2 ± 1.17	49.8 ± 4.7 (99.3)	Jacobsen, unpublished data
V. vinifera	Cabernet Savignon	18.4 ± 0.8	42.5 ± 2.6 (55.6)	Jacobsen, unpublished data
V. vinifera	Chardonnay	25.0 ± 1.0	57.3 ± 1.6 (74.7)	Jacobsen, unpublished data
V. vinifera	Flame	29.6 ± 2.0	55.3 ± 2.3 (66.2)	Jacobsen, unpublished data
V. vinifera	Glenora	25.6 ± 1.1	59.8 ± 1.4 (73.4)	Jacobsen, unpublished data

Data are divided by those reported in the literature from a wide range of stem diameters, ages, growing locations, and treatments and those that have been standardized by stem diameter and age specifically so that values are comparable between varieties and comparable to the global values reported in Jacobsen et al. (2012). Samples that have been standardized by diameter are all from 1-year-old stem samples collected in summer 2013 from varieties growing on or near campus at California State University, Bakersfield, USA and mean ± 1 SE are reported. Parenthetical values reported after maximum vessel diameters represent the largest diameter vessel recorded within samples from that variety

Table 5.2 Mean and maximum vessel length from stems of several *Vitis* species and varieties compiled from several different sources

Species	Variety (if reported)	Mean vessel length (m)	Maximum vessel length (m)	Source
*V. labrusca/riparia**	–	0.416*	1.4*	Sperry et al. (1987)
*V. labrusca**	–	1.435*	7.5*	Zimmermann and Jeje (1981)
V. labrusca	Catawba	–	0.78 ± 0.13	Jacobsen, unpublished data
V. labrusca	Concord	–	0.63 ± 0.10	Jacobsen, unpublished data
*V. rotundifolia**	–	0.322*	1.73*	Ewers and Fisher (1989), Ewers et al. (1990)
V. vinifera	Cabernet Savignon	–	0.54 ± 0.09	Jacobsen, unpublished data
V. vinifera	Chardonnay	–	0.67 ± 0.09	Jacobsen, unpublished data
V. vinifera	Chardonnay	–	0.5 to 0.9	Choat et al. (2010)
V. vinifera	Flame	–	0.73 to 0.6	Jacobsen, unpublished data
V. vinifera	Glenora	0.116	0.85	Jacobsen and Pratt (2012)
V. vinifera	–	0.207	–	Sperry et al. (2005)
V. vinifera	–	0.128	–	Wheeler et al. (2005)

Samples that are followed by an asterisk (*) are from wider diameter samples and may be from stems that are multiple years of age, whereas all vessel length values that are not followed by an asterisk are from 1-year-old stem segments. All samples reported from Jacobsen (unpublished data) were sampled in July 2014 from plants growing on or near campus at California State University, Bakersfield, USA and mean ± 1 SE are reported (n = 4–6 per variety). Mean vessel lengths were determined using different methods that have been shown to produce similar results (reviewed in Jacobsen et al. (2012)) and maximum vessel lengths were determined using air injection at 100 kPa (Greenidge 1952)

diameter of 67.89 μm for 3,005 woody angiosperms; Zanne et al. 2010). Similarly, mean and maximum vessel lengths are relatively short for lianas and are similar to the global mean across woody tree, shrub, and liana species (compare values in Table 5.2 to a global mean vessel length of 0.13 m and a global mean maximum vessel length of 0.93 m for 148 species; Jacobsen et al. 2012). However, when both vessel length and width are considered, grapevine has a combination of vessel length and width that results in the average size of grapevine conduits being larger than average when compared against the data available globally to date (Jacobsen and Pratt 2012).

Most species show an increase in both vessel length and width with increasing stem diameter (reviewed in Jacobsen et al. 2012) and, consistent with this general trend, grapevine vessel dimensions vary considerably with stem diameter and sample age (discussed below and shown in Fig. 5.10; Lovisolo and Schubert 1998; Schubert et al. 1999). Thus, especially in older samples or in wide diameter stems, grapevine xylem may contain very wide (approximately 300 μm diameter) and long (~8 m) vessels (Zimmermann and Jeje 1981).

The vessel network within grapevine secondary xylem has some unique features (Figs. 5.5a, b, 5.6a, b, and 5.7a, b). Large diameter vessels in grapevine are generally not directly connected laterally to other large vessels. Examination of serial sections of grapevine xylem suggests that large diameter vessels tend to terminate

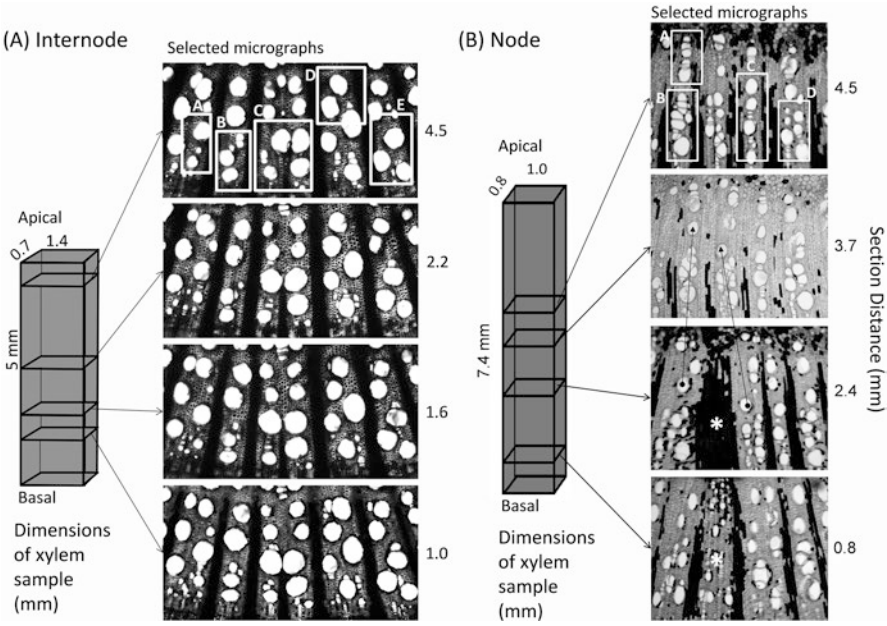

Fig. 5.5 Serial sections (at 100 μm intervals) were made through the xylem of internode (**a**) and node (**b**) segments of 1-year-old grapevine stems and were examined and used to construct three-dimensional models of the vessel network. Representative micrographs from the examined xylem samples are shown above along with a model illustrating the location of the sections. Inset boxes within the uppermost micrograph identify specific vessel groups that were selected for more detailed analysis of vessel network structure and vessel connectivity (see Figs. 5.6 and 5.7). For the node segment (**b**) the *asterisks* indicate a xylem section that exits the stem xylem to enter a leaf and the two-sided arrows that extend across panels identify the same vessel in each panel in their new location following the rearrangement of vessels around the exit of the leaf trace

abruptly, with one large vessel connecting to the next predominately at the vessel termini. The region of overlap between these large vessels occurs over just a few hundred microns and with only one or two vessel elements overlap (Jacobsen, unpublished data). Rather, the vast majority of connections between large diameter vessels occurs through the connection of large diameter vessels by relays of many shorter and narrower vessels (Fig. 5.6a; Brodersen et al. 2013). This is particularly evident within internode regions (Fig. 5.6a), but also occurred within nodes (Fig. 5.6b) and may be an important, but thus far unexplored, feature of grapevine hydraulic function.

Vessel network architecture differs between node and internode regions (Figs. 5.5a, b, 5.6a, b, and 5.7a, b). Xylem node regions contain a vessel network that is more disordered than the network found within internodes (Fig. 5.7a, b). Numerous vessels terminate within nodes, which represents a higher proportion of

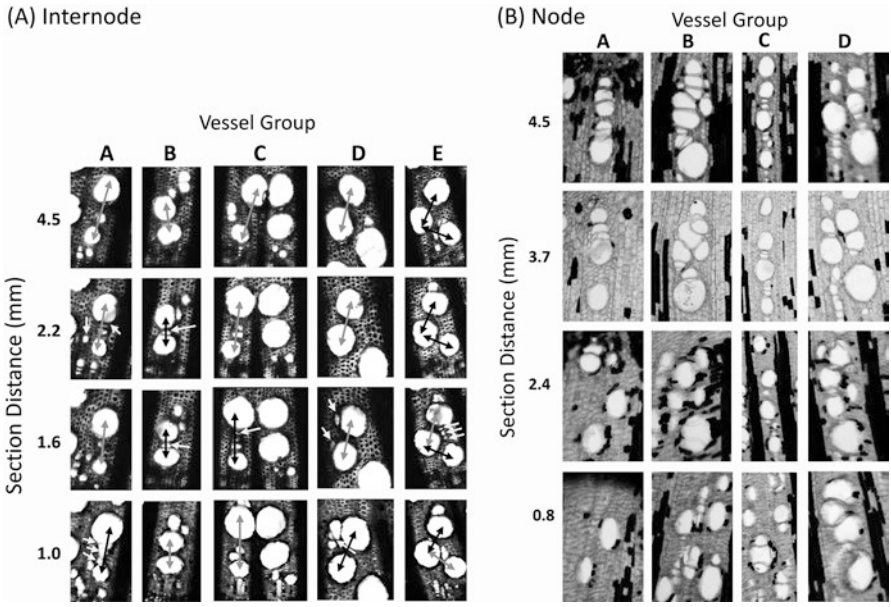

Fig. 5.6 Selected micrographs from serial sections of grapevine stem xylem located in an internode (**a**) and node (**b**) are shown, with specific vessel groups highlighted to show patterns of vessel connectivity through the xylem (see Fig. 5.5 for the location of vessel groups relative to one another). In Panel A, large vessels that are connected along part of their axial pathway are indicated with gray arrows, but they are not connected within the panels containing the gray arrows. Black arrows indicate the specific image(s) in which large vessels are visible as connected through vessel relays within that particular image. Large vessels rarely come into direct contact with one another and instead connect through the development of relays of narrower and shorter vessels (indicated with small white arrows). The connection of large vessels by shorter and narrower vessel relays is particularly evident in internode section (**a**), but is also visible within the node (**b**). Vessel connections are not indicated in panel B because the more disorganized vessel connections of the node make these connections much more complex; however, the connectivity of the node section shown above (**b**) can been seen in three-dimensional modeling for the entire sampled segment in Fig. 5.7b

vessels terminating per unit length than in internode sections (Figs. 5.6a, b and 5.7a, b; Jacobsen, unpublished data). This is similar to previous reports for *V. vinifera* as well as for other species, which also report that vessels are more likely to terminate within nodes (Salleo et al. 1984). Within the node, rays of parenchyma tissue were also more heavily rearranged, in part because of the loss of xylem segments into leaves, tendrils, and side branches at nodes, but also due to the termination and creation of additional xylem segments (Fig. 5.5a, b). This represents a significant nodal rearrangement in the connectivity of xylem conduits within the stem. This rearrangement is also visible in the much more tortured pathways of vessels through the xylem within nodes (Fig. 5.7a, b).

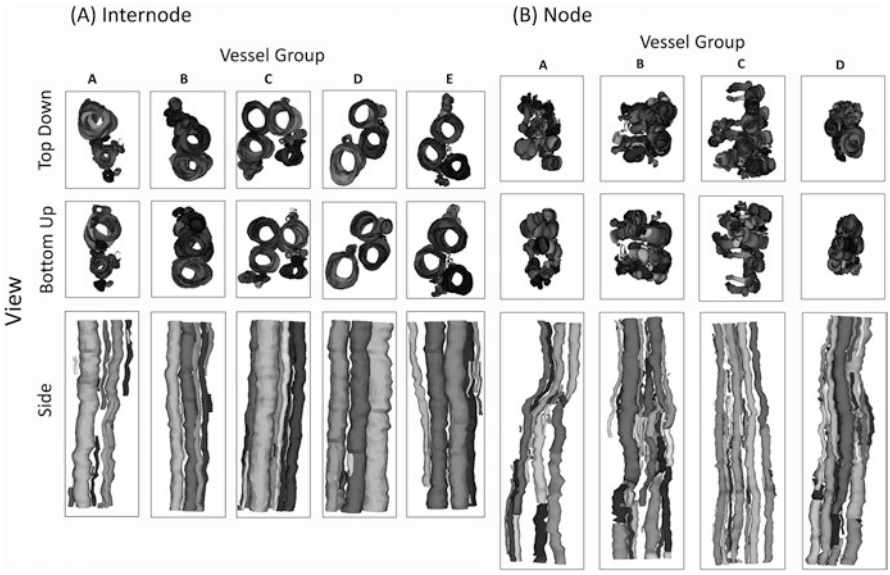

Fig. 5.7 Three-dimensional models of the vessels within a grapevine stem xylem internode (**a**) and node (**b**) xylem section based on combining serial sections (at 100 μm intervals) that were made through the same xylem segments presented in Figs. 5.5 and 5.6. Serial sections were aligned using Reconstruct (v. 1.1.0.1) and three-dimensional models were constructed using Image J (v. 1.44p) analysis of the stacked images as described in Yahya et al. (2011). Vessel terminations are modeled as being "flat" although they would likely be pyramidal within the examined specimens; this is largely due to the limitations of having images created from the stacking of 100 μm thick sections. The vessel network is relatively ordered within the internode (**a**) and open vessel lumens can be seen in both the bottom and top views of the xylem, illustrating that vessel pathways are relatively linear. The vessel network becomes much more tortured in the node (**b**) where numerous vessel groupings are rearranged following the exit of a leaf trace from the stem (Fig. 5.5) and the twisting, rearrangement, and tortured pathway of vessels means that lumens are not visible as being "open" through the "bottom up" and "top down" views through the node. Along the length of a single vessel, the diameter of the vessel changes considerably, as described previously in Akachuku (1987) and Ewers and Fisher (1989)

4 Hydraulic Function

4.1 Resistance to Water Stress-Induced Cavitation

Grapevine varieties are generally highly susceptible to water-stress induced cavitation as indicated by rapid declines in hydraulic conductivity with small declines in water potential (Fig. 5.8). Indeed, across many different varieties and several different studies, grapevine species and varieties have consistently been shown to be highly susceptible to cavitation and water stress (Table 5.3). Dehydrated grapevine shoots from two different grapevine varieties have also been reported to display a majority of acoustic emissions (AEs) between −0.2 and −1.5 MPa with early peaks

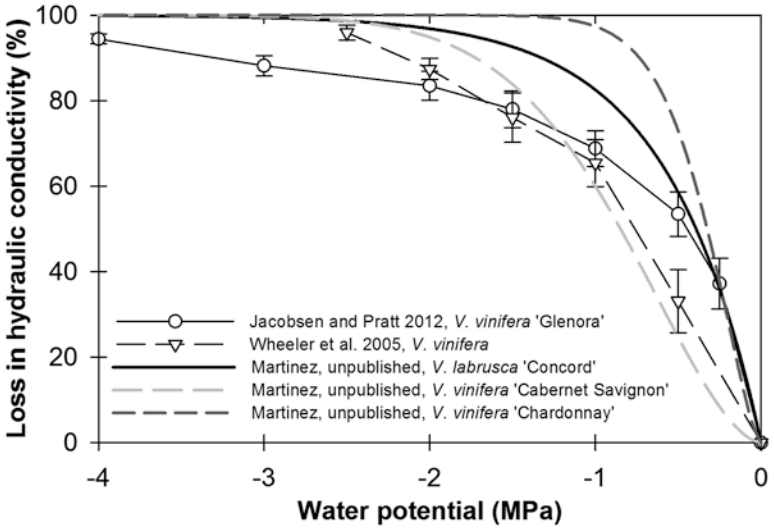

Fig. 5.8 Vulnerability to cavitation curves for five selected varieties of grapevine, showing the percentage loss in hydraulic conductivity with declining water potential. All curves were measured on 1-year-old samples using the centrifuge method (Alder et al. 1997)

in AEs at approximately −0.5 MPa (Schultz 2003). Acoustic emissions are indicative of cavitation events within the xylem. The production of many acoustic emissions (AEs) at relatively high water potentials as grapevine shoots are dehydrated represents an independent (of hydraulic methods) and in vivo confirmation that grapevine xylem is highly susceptible to water stress. Most recently, single vessel injection experiments conducted on 1-year-old stems of *V. vinifera* "Chardonnay" have confirmed the presence of a large proportion of highly susceptible vessels within the xylem (M. Venturas unpublished data).

High vulnerability to cavitation in the xylem of 1-year-old shoots of grapevine is consistent with grapevine physiology. Many grapevine varieties have been identified as being near isohydric and even varieties that display slightly anisohydric behavior do not typically experience water potentials below −2 MPa even when severely stressed. Indeed, the water potentials of "stressed" dehydrated plants have been reported to range from −0.6 to −2.0 MPa in many studies (Loveys and Kriedemann 1973; Matthews and Anderson 1988; Schultz and Matthews 1988; Lovisolo and Schubert 1998; Flexas et al. 1999; Choné et al. 2001; Schultz 2003; Lovisolo et al. 2008; Lovisolo et al. 2010). Whole plant leaf specific hydraulic conductance (k_l) has been shown to rapidly decline with minor declines in water potential (see whole shoot dehydration P_{50} reported in Table 5.3; Schultz 2003; Zufferey et al. 2011) and whole plant hydraulic resistance increased rapidly when water potentials declined from −0.6 to −1.2 MPa (Schultz and Matthews 1988). This decline in whole plant conductance is due to a combination of stomatal closure, cavitation, and other modifications of xylary and extraxylary pathways that lead to a decrease in conductance in roots, stems, branches, and leaves.

Table 5.3 Water potential at 50 % loss in hydraulic conductivity (P_{50}) calculated from centrifuge-based vulnerability curves (centrifuge), dehydration-based curves where the percentage loss in hydraulic conductivity was calculated relative to hydrated K_s (dehydration decline relative to hydrated plant K_s), and water potential at 50 % loss in whole plant leaf specific hydraulic conductance (k_l) as calculated for whole shoot dehydration experiments (dehydration decline in k_l) for two *Vitis* species and several different varieties

Vulnerability curve method[a]	Plant material	P_{50} (MPa)[b]	n	Sample length (m)	Source
Centrifuge	*V. labrusca* L. "Catawba"	−0.20 ± 0.07	6	0.14	Martinez, unpublished data
Centrifuge	*V. labrusca* L. "Concord"	−0.37 ± 0.07	6	0.14	Martinez, unpublished data
Centrifuge	*V. vinifera* L. "Cabernet Savignon"	−0.76 ± 0.10	6	0.14	Martinez, unpublished data
Dehydration decline in k_l	*V. vinifera* L. "Chasselas"	−0.41	12	whole shoot	Zufferey et al. (2011)
Centrifuge	*V. vinifera* L. "Chardonnay"	−0.7	6	0.145	Drayton (2009), Choat et al. (2010)
Centrifuge	*V. vinifera* L. "Chardonnay"	−0.28 ± 0.05	11	0.14	Martinez, unpublished data
Centrifuge	*V. vinifera* L. "Glenora"	−0.16 ± 0.03	4	0.14	Jacobsen and Pratt (2012)
Centrifuge	*V. vinifera* L. "Glenora"	−0.31 ± 0.11	4	0.27	Jacobsen and Pratt (2012)
Centrifuge	*V. vinifera* L. "Glenora"	−0.34 ± 0.06	12	0.14	Jacobsen and Pratt (2012)
Centrifuge	*V. vinifera* L. "Glenora"	−0.55 ± 0.12	12	0.27	Jacobsen and Pratt (2012)
Dehydration decline relative to hydrated plant K_s	*V. vinifera* L. "Glenora"	−0.51	31	0.14; K_s	Jacobsen and Pratt (2012)
Dehydration decline in k_l	*V. vinifera* L. "Grenache"	−0.32	19	whole shoot	Schultz (2003)
Dehydration decline in k_l	*V. vinifera* L. "Syrah"	−0.51	15	whole shoot	Schultz (2003)
Centrifuge	*V. vinifera* L. (variety not reported)	−0.76	≥6	0.142	Wheeler et al. (2005)

[a]Dehydration-based curves where percentage loss in hydraulic conductivity is calculated relative to a flushed maximum value have not been included due to methodological problems with this technique (see text for details)

[b]All P_{50} were calculated using a Weibull fit

For each reported P_{50} value, the sample size (n) and the length of measured samples are reported. Data were compiled from several different sources as indicated

4.2 Seasonal Changes in Hydraulics

Changes in xylem hydraulic function seasonally have been described previously for *Vitis* (Sperry et al. 1987; Tibbetts and Ewers 2000; Choat et al. 2010; Jacobsen and Pratt 2012) and have also been found in the *V. vinifera* and *V. labrusca* varieties reported on in the present chapter (Fig. 5.9; Jacobsen et al., unpublished data). In general, grapevine xylem-specific hydraulic conductivity (K_s) has been shown to increase throughout the growing season. This change occurs concurrent with changes in xylem cavitation resistance, with stems grown later in the growing season showing increased resistance to cavitation and reduced levels of conductivity loss at more negative water potentials (Choat et al. 2010; their Fig. 2).

The studies included above have examined changes in hydraulics at different times during the season, but seasonal changes in hydraulics have also been examined through the examination of changes occurring along a single 1-year-old shoot. Grapevines exhibit rapid growth and an individual stem may grow several meters throughout the growing season. Along this shoot, basal segments are initiated early in the season and the earlywood of these segments represents xylem formed during this period. These segments produce more secondary growth compared to those initiated later in the season and they are wider in diameter by the end of the growing season. Distal segments of 1-year-old stems represent xylem that was initiated later in the growing season and was produced during drier and hotter conditions. These stems have less time for secondary growth to occur and are narrower at the end of the growing season.

Examination of xylem structural and functional traits as they vary along 1-year-old shoots provides the opportunity to evaluate some of the changes that have occurred throughout the growing season. Within 1-year-old shoots, stem segments that are narrower in diameter (i.e., produced later in the season) are up to 10 times more resistant to cavitation (i.e., they have more negative P_{50}) than segments that are

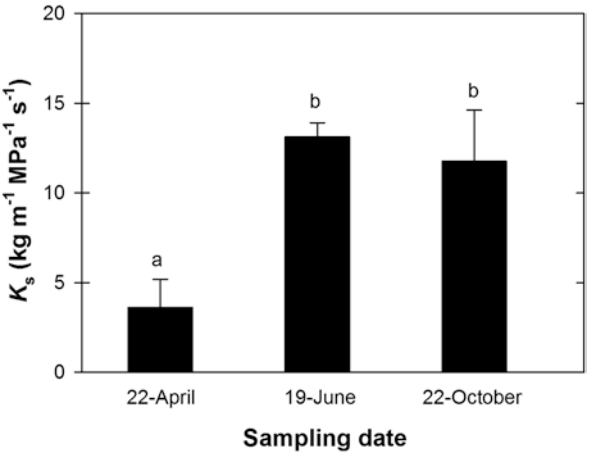

Fig. 5.9 Xylem-specific hydraulic conductivity (K_s) measured at three sample times in 2013 ($n=6$ per sample time; mean ± 1 SE) on *V. vinifera* "Glenora". Different letters indicate significant differences in K_s. Early season stem segments exhibited decreased xylem-specific conductivity (K_s) compared to samples measured later in the growing season ($P=0.017$, $F=5.71$, ANOVA)

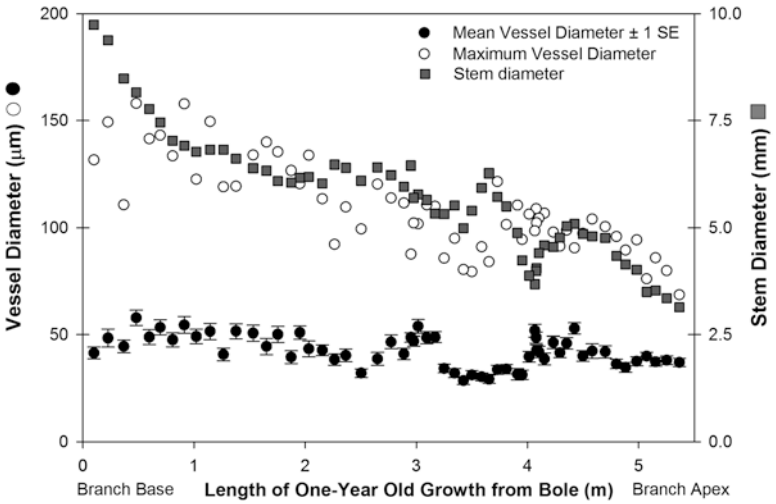

Fig. 5.10 Mean (±1 SE) and maximum vessel diameter as measured along the length of stem grown within a single growing season in grapevine. Each point represents a value from a single internode section beginning at the point of shoot initiation (internode 1) and continuing to the branch tip (internode 60). Stem diameter decreased from the branch base to the branch tip (*gray squares*) and vessel traits varied with both stem diameter and internode number. Maximum vessel diameter (*open circles*), in particular, was particularly sensitive to changes in internode number and stem diameter

wider in diameter (i.e., produced earlier in the season with a longer time period for secondary growth to occur) (Jacobsen and Pratt 2012; their Fig. 2). They also are higher in their stem hydraulic conductivity (Lovisolo and Schubert 1998; Schubert et al. 1999). These changes are consistent with the patterns in grapevine vessel development described above, whereby vessels take several weeks to become functional and different cohorts of vessels are active during different times of the season. These changes are also consistent with changes in vessel anatomy.

Vessel structure changes throughout the growing season (Fig. 5.10). Stems grown earlier in the growing season contain vessels that are wider in diameter compared to those produced later in the growing season (Fig. 5.10; Lovisolo and Schubert 1998; Schubert et al. 1999). This pattern is particularly evident when only the widest vessels within a cross section are examined (Fig. 5.10). This may be due, at least in part, to a longer period of secondary growth in stem segments that are initiated early, as evidenced by their wider diameter, but may also relate to other factors.

Thus, plant hydraulic structural and functional traits, particularly in grapevine, appear to be quite variable intra-annually and this may be due to different vessels becoming hydraulically active throughout the season and/or the formation of structurally different vessels within stems that are initiated during different times during the season. This is a functionally interesting pattern and suggests that xylem may be quite dynamic in a way that has not been widely appreciated (for instance, see Fig. 4.11 in Uwe's Poplar Chapter). Plants may have considerable control over the types

of vessels that are grown or activated and further research on the development of vessels and their onset of functionality (as well as loss in functionality) throughout the growing season may be particularly interesting.

5 Methodological Considerations in Study of Grapevine Hydraulic and Function

Although much of the data on grapevine hydraulics has been consistent and suggests high susceptibility to water stress, a few recent studies have called some of these findings into question (e.g., Choat et al. 2010; McElrone et al. 2012). Some of this confusion is likely linked to issues related to imaging of grapevine stems containing living or gel-filled vessels, but may also be linked to other complications. It is, therefore, worthwhile to consider how some of the unique aspects of grapevine xylem may impact the methodologies used to examine it and may impact the interpretation of some studies and results.

5.1 Challenges Related to In Vivo Imaging

The high proportion of nonhydraulically functional vessels in grapevine may be a particularly challenging problem in ongoing research examining grapevine structure and function using current in vivo imaging technologies. These nonhydraulically active vessels, including cells with completed secondary walls and degraded organelles that are still alive and also inactivated vessels that are gel filled (Fig. 5.11a–d), appear to be difficult to identify when the latest imaging technologies such as magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), and high resolution computed tomography (HRCT) are utilized. The problem arises because living or developing vessels and gel-filled vessels that are not conductive appear similar in images to cells that are mature and hydraulically active, as demonstrated by the inability of MRI images to differentiate glass tubes filled with water, saline solution, or pectin gel (Pérez-Donoso et al. 2007).

This limitation of imaging techniques is, in some cases, at the heart of a debate regarding the accuracy of methods to measure resistance to cavitation of xylem. Some authors have taken the data generated from imaging as a "gold standard" by which to judge other methods and interpreted disagreements with imaging as an indication that the nonimaging methods are flawed (Choat et al. 2010; Cochard et al. 2014). However, it appears that an alternative explanation is likely. To date, imaging data have yet to be shown to accurately estimate hydraulic conductivity, percentage loss in hydraulic conductivity, or cavitation resistance when compared against standard techniques. The difficulty in properly analyzing and interpreting grapevine images generated using these techniques is perhaps best illustrated through the repeated inability of these imaging techniques (e.g., Choat et al. 2010; McElrone et al. 2012) to generate data

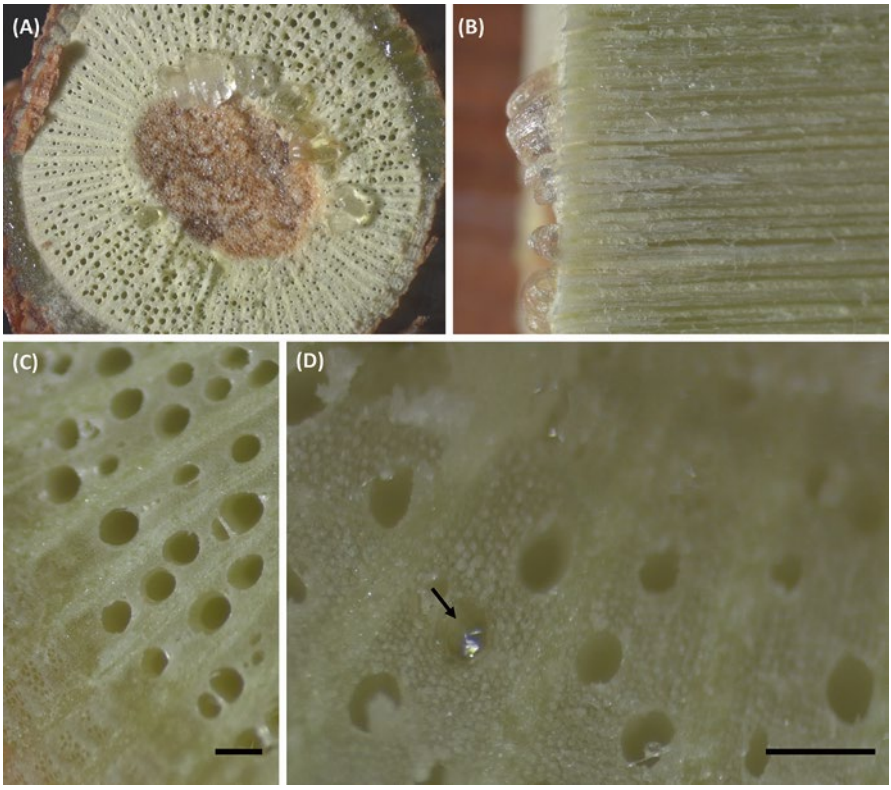

Fig. 5.11 Grapevine stems produce gels in response to both drought and wounding as seen in both cross (**a**) and longitudinal (**b**) sections of 1-year-old stems, where gels are visible exuding out of vessels. Gels appear to be produced in vessels during dehydration, but are not usually apparent when hydrated samples are sectioned ((**c**); note the absence of gels). When gels are present in vessels, they are easily seen when samples are freshly sectioned because gels do not recede from vessel ends ((**d**); gel-filled vessel is indicated by the *black arrow*). Scale bars in panels (**c**) and (**d**) indicate 200 μm

that agree with other standard and thoroughly verified hydraulic methods (i.e., the centrifuge method and dehydration methods as presented in Jacobsen and Pratt 2012 using the methods verified by Sperry et al. 2012; Tobin et al. 2013, and Hacke et al. 2015). This suggests that image analysis is not yet a verified tool in the examination of hydraulic function, at least in the case of grapevine xylem. Difficulties with in vivo imaging may also extend to imaging analyses conducted in other species where imaging data appear inconsistent with other physiological and standard hydraulic methods (e.g., Cochard et al. 2014), particularly for species such as oak and poplar where living vessel elements have been identified occurring well away from the vascular cambium (see Sect. 2.1 of the current chapter).

5.2 Challenges Posed by Dynamic Xylem Processes

Not all techniques or analyses for examination of resistance to water stress-induced cavitation may be appropriate for grapevine. The use of dehydration vulnerability curves, particularly those conducted over several weeks or longer (Alsina et al. 2007; Choat et al. 2010), may be heavily impacted by the formation of gels within vessels (Jacobsen and Pratt 2012) or the activation of new and functionally different xylem vessels. Of particular concern with dehydration measures is the formation of occlusions within the xylem during dehydration which results in error in measurements of the maximum xylem-specific hydraulic conductivity of samples (K_{smax}). This issue arises due to the analysis of these curves using percentage loss in hydraulic conductivity (PLC) as calculated relative to a flushed maximum value for each sample.

The K_{smax} error produced by the formation of gels within vessels and when flushed or vacuumed samples are affected by vessel clogging is not consistent across samples or sampling periods and it is difficult to identify and may be impossible to correct for. This variability and its potential impact on PLC estimates was illustrated in the data reported by Drayton (2009), the original source for much of the data published in Choat et al. (2010). Drayton (2009) reported that some, but not all samples, exhibited declines in conductivity following flushing and the declines were not consistent across seasonal measures. Declines were most notable in *V. vinifera* "Cabernet Sauvignon" samples collected in May, which exhibited a mean of approximately−20 % loss in conductivity following flushing (Drayton 2009). A decline in conductivity following flushing indicates the blockage of xylem conduits and is most likely linked to the exudation of gels in grapevine.

Although K_{smax} error may be dealt with by analyzing K_s of unflushed stems and possibly the calculation of PLC relative to hydrated and nonflushed samples, K_s is rarely reported for vulnerability curves. For this reason, dehydration vulnerability curves that are based on PLC calculated using a flushed maximum have not been included in the data reported within the present chapter. Additionally, centrifuge-based curves, which may be generated over a short time period, may be preferable when examining grapevine.

Changes in xylem seasonally may also be linked to some ongoing methods issues. For instance, this may explain, in part, the difficulty of interpreting hydraulic data collected over the growing season (Choat et al. 2010) and lends support to the suggestion that study design should carefully control for season (Jacobsen et al. 2007; Jacobsen and Pratt 2012; Hacke et al. 2015). These issues may be further complicated by seasonal variation in the formation of gels within the xylem (Sun et al. 2008). Additionally, few studies have examined plant hydraulic traits seasonally (however see Kolb and Sperry 1999; Jacobsen et al. 2007, and Jacobsen et al. 2014) and it is likely that measurements of plant hydraulics conducted at a single sampling time may not, in some cases, represent the function of a plant throughout the growing season. This may complicate the experimental design of hydraulic studies, but may be necessary to understand dynamic changes in the cohort of active vessels and the ability for xylem function to change.

5.3 *Active Xylem Staining Confirms Xylem Vulnerability to Water Stress*

Traditional staining protocols may provide the means to study xylem structure and function in a manner that avoids or corrects for most of the methodological issues described above. Staining allows for the examination of the distribution of hydraulically functional vessels and the identification of vessels that remain conductive during exposure to water stress treatments.

We examined the use of staining in grapevine as a means to examine which vessels remain conductive as plants were dehydrated. We collected 12 large branches (>3 m) from a single hydrated field-grown plant to reduce experimental variability and we divided these branches between two different water stress treatments: centrifugation or benchtop dehydration (six large shoots per treatment with three treated and three control). We included both benchtop dehydrated and centrifuged samples as an additional comparison of dehydration versus centrifuge methods for studying cavitation resistance in grapevine. All treatment samples were exposed to approximately −0.5 MPa, which is near the pressure at 50 % loss in hydraulic conductivity for the grapevine variety used for this experiment (Jacobsen and Pratt 2012). Control samples were collected from matched large branches that were kept hydrated, but were allowed to sit for the same amount of time as treated branches between collection and staining.

Following a water stress treatment through centrifugation of a 0.14 m stem segment or benchtop dehydration of a >3 m large shoot, one 0.10 m sample was excised and stained for active xylem vessels from each treated large branch and from a matched control branch. The 0.10 m sample was sampled from the center of centrifuged segments and from >1 m from the base of dehydrated shoots. Samples were taken from >1 m away from the cut end to avoid measurement of introduced emboli as well as potential wounding effects from sampling near the cut end (Sun et al. 2006). Sections were stained using crystal violet dye (1 % w/v) that was pulled up through segments using a mild tension (−2 kPa). Only vessels that were not embolized or otherwise blocked (i.e., by gels, tyloses, or closed end walls) were able to transport this dye, so that hydraulically active vessels could be easily identified as having purple stained secondary walls.

With this comparison, using traditional staining, we found that approximately 33 % of the vessels in hydrated control samples were not hydraulically active and were presumably developing and living or filled with gels or tyloses (Fig. 5.12a, b; 32.6 ± 1.8 % of vessels did not stain purple, $n = 6$). This is consistent with the pattern of vessel element development described above and the proportion of living vessels that we have previously found.

The percentage of active vessels was determined for each treated sample, using the control sections as a baseline for determining which vessels would have been hydraulically active prior to exposure to water stress so as not to overestimate the loss in conductivity. The estimated percentage loss in active vessels was not different between dehydrated (Fig. 5.12c, d) and centrifuged (Fig. 5.12e, f) stem samples

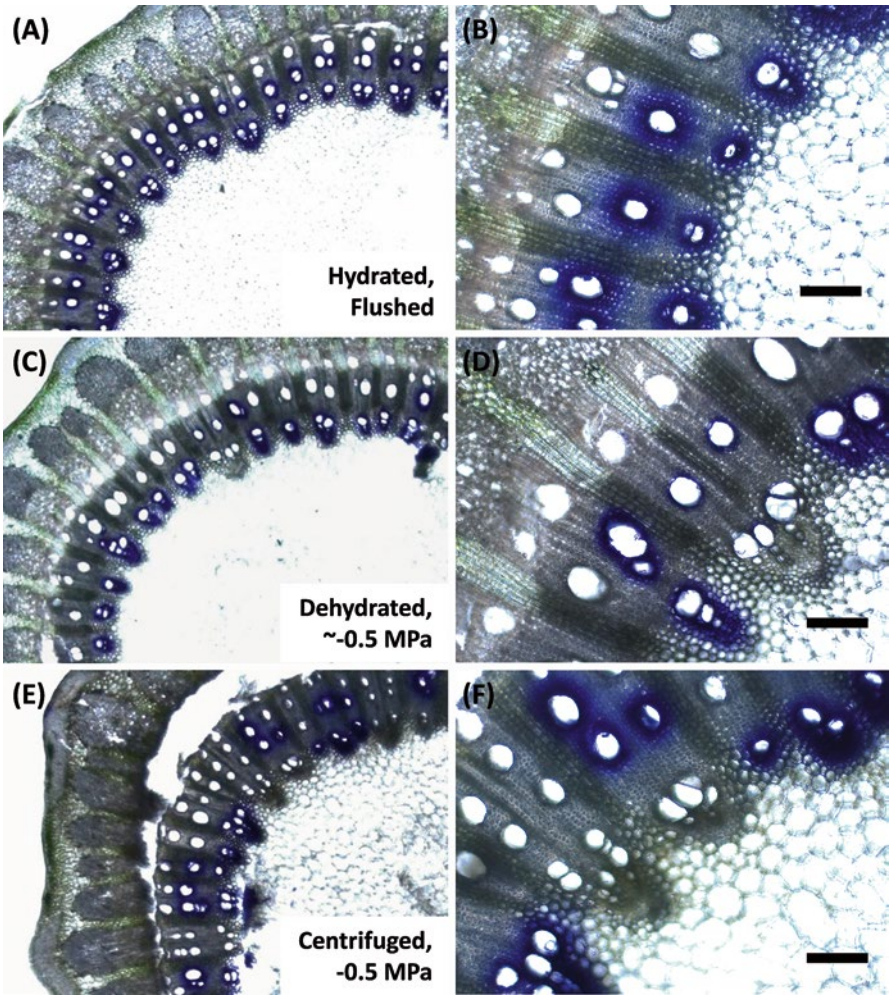

Fig. 5.12 Representative segments from approximately 8 mm diameter stems of grapevine that have been stained for active xylem vessels using crystal violet (purple) shown at both low ((**a**, **c**, **e**); 25×) and higher ((**b**, **d**, **f**); 100×; scale bars indicate 200 μm) magnification. Large branches (>3 m) were collected from a hydrated field-grown plant. Control segments were excised from hydrated branches, flushed, and then stained to determine which vessels were potentially active (**a**, **b**). Approximately 33 % of the vessels in hydrated control samples were not hydraulically active and were presumably developing or living ((**a**, **b**); 32.6 ± 1.8 % of vessels did not stain purple, $n = 6$). These vessels are visible predominantly near the cambium and are not stained purple (**a**, **b**). Additional segments were exposed to two treatments prior to staining, dehydration (**c**, **d**) of large branches or centrifugation (**e**, **f**), to a water potential of approximately −0.5 MPa ($n = 3$ per treatment). Segments from treated branches were then stained to determine which vessels remained functional (**c**, **d**, **e**, **f**). The percentage of active vessels was determined for each treated sample, assuming that if any vessels within a ring of vessels were active in control segments, then all of the vessels within that ring had previously been active. The estimated percentage loss of active vessels, when living vessels were excluded, was similar between dehydrated and centrifuged stem samples and this pattern was consistent among the three samples of each that were examined (54.2 ± 2.2 % and 60.9 ± 3.2 % of previously hydraulically active vessels were no longer active for dehydrated and centrifuged samples, respectively, $n = 3$ per treatment). Stem segments were sampled from a field grown plant of *V. vinifera* "Glenora" in April–May 2012 and sampling time was carefully matched across treatments and controls

(54.2 ± 2.2 % and 60.9 ± 3.2 % of previously hydraulically active vessels were no longer active for dehydrated and centrifuged samples, respectively, mean ± 1 SE; $P = 0.164$, $t_4 = -1.704$, $n = 3$ per treatment).

The agreement of centrifuged and dehydrated samples when stained using this standard technique suggests that: (1) grapevine is indeed highly susceptible to water stress, (2) centrifuge and dehydration treatments result in the same impact on hydraulically conductive vessels when measures are conducted in a way that avoids the errors that may result from PLC-based estimates (Choat et al. 2010) or from artifacts associated with the interpretation of images (McElrone et al. 2012), and (3) many vessels within grapevine xylem are not potentially hydraulically active at any given time. This also agrees with the findings of Sperry et al. (2012), Tobin et al. (2013), and Hacke et al. (2015) that centrifuge and dehydration measures generally agree.

5.4 *Open Vessels Do Not Impact the Reliability of Standard Hydraulic Measures*

The presence of open vessels (i.e., vessels that do not contain a terminal vessel element along their length within a measured sample) and how they may impact hydraulic measures has been an additional issue of concern to some (McElrone et al. 2004; Choat et al. 2010; Cochard et al. 2010; McElrone et al. 2012). While some vulnerability curve methods, such as the cavitron, appear to be prone to vessel-length associated measurement artifacts (Cochard et al. 2010; Wang et al. 2014), the standard centrifuge method has been rigorously tested for short- and long-vesselled samples and these studies have found no evidence for an open-vessel artifact when the standard, Alder et al. (1997), rotor design was used (Jacobsen and Pratt 2012; Sperry et al. 2012; Tobin et al. 2013; Jacobsen et al. 2014; Hacke et al. 2015). This includes careful methods comparisons conducted for grapevine, including data presented in the current chapter as well as in Jacobsen and Pratt (2012) and Tobin et al. (2013).

Measurements of hydraulic conductivity in grapevine are unaffected by the presence of open vessels when even a large number of vessels (40 %) are open. We measured the maximum vessel length on 1-year-old *V. vinifera* shoots using the air injection method (Greenidge 1952) ($n = 7$). We then collected 4 large 1-year-old branches, transported them to the laboratory, and excised stem segments greater than the longest measured maximum vessel length (Fig. 5.13a). Stem segments 1.05 m were vacuum flushed for 1 h using custom tubes filled with the same solution described above as being used for flushing samples and then both ends were trimmed to an initial measurement length of 1.00 m. These large segments were mounted into a tubing apparatus and both hydraulic resistance and conductivity determined. The segments were then shortened from the proximal end, with these same hydraulic parameters being measured at different lengths, until a final length of 0.05 m was obtained. This represented a length at which approximately 40 % of vessels were open through measured samples (Fig. 5.13a).

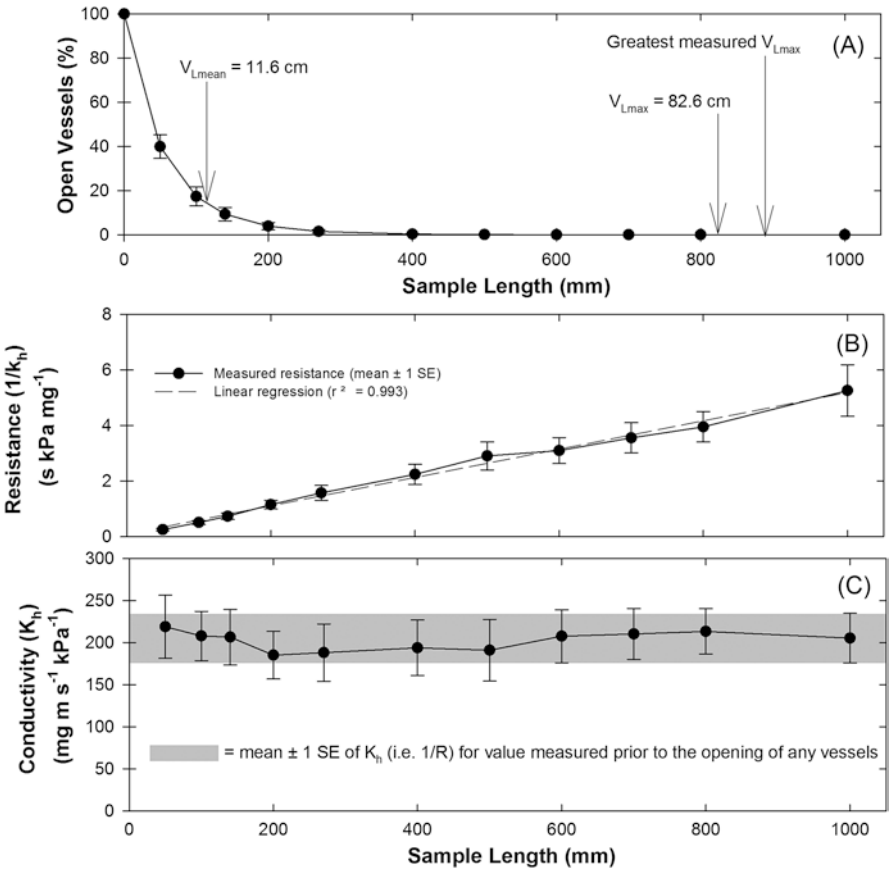

Fig. 5.13 One-year-old shoots of grapevine collected from the same individual and used to determine the proportion of open vessels using silicon injection ($n=6$) for the vessel length distribution (V_{Lmean}) and air injection ($n=7$) for the maximum vessel length (V_{Lmax}) (**a**) and to determine the hydraulic resistance (**b**) and conductivity (**c**) with varying sample length ($n=4$). The mean V_{Lmax} and the single greatest V_{Lmax} from among the measured samples and the mean V_{Lmean} are indicated. Resistance declined linearly as samples were shortened from 1 m to 0.05 m (**b**), while conductivity remained constant (**c**)

Hydraulic conductivity was not different between samples that had no open vessels and those that contained approximately 40 % of their vessel open (Fig. 5.13c). As samples were shortened, the conductivity remained constant (Fig. 5.13c). The resistance, which does not include path length as part of the calculation of this parameter, declined linearly with sample length (Fig. 5.13b) as expected and this also indicates that the opening of increasing numbers of vessels was not affecting resistance.

This result is similar to previously published studies, including a study that examined the effect of segment length on a different liana species and also found no effect of open vessels when segments were shortened from no vessels open to

approximately 40 % of vessels open (Chiu and Ewers 1993). When samples are very short (less than 1–2 cm in length) and have many vessels open, conductivity has been shown to be impacted (Sperry et al. 2005), but samples this short are generally not utilized in hydraulic experiments and perhaps should be avoided because of potential impacts on conductivity estimates. Thus, for samples within the range of those typically measured (>5 cm), hydraulic conductivity estimates are reliable and are not impacted by the presence of open vessels.

6 Conclusion

Study of the xylem structure and function of grapevines presents unique challenges, but has the potential to increase our understanding of plant hydraulics, particularly through increased appreciation for the dynamic nature of processes occurring within xylem vessels. Vessels are not static and go through a lifespan that includes a period of prefunctional development, a limited period of hydraulic function, and a period of postfunctionality that may include the formation of either gels or tyloses within the vessel lumen. Large seasonal changes in the cohort of active vessels and plant hydraulic traits suggest that the xylem may be able to dynamically respond throughout the season as conditions and hydraulic requirements change.

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