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STUDIES

Functional hydraulic sectoring in grapevines as evidenced by sap flow, dye infusion, leaf removal and micro-computed tomography

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Abstract

The supply of water to a plant canopy is dependent on the xylem pathway connecting roots to leaves. In some plants, sectored xylem pathways can restrict resource distribution, resulting in variable quality of organs in the shoots, yet little is known about the effects of sectoring in crop cultivars. In this study, we combined sap flow measurements and infusion of xylem-specific dyes to document functional conductive area and flow pathways from roots to shoots of 20-year-old Thompson Seedless and 8-year-old Chardonnay grapevines. Sap flow measurements and dye infusion demonstrated that water flowed predominantly in discrete xylem (visually identifiable from the trunk surface) sectors along the trunk axis, each supplying limited portions of the canopy. Functional conductive area in the trunk was proportional to that in the shoots even though sector size varied considerably between vines. Leaf area removal experiments further demonstrated sectoring in grapevines; sap flow decreased by >90 % in trunk sectors connected to excised shoots while it remained constant in trunk sectors supplying intact portions of the canopy. Despite the functional sectoring in grapevines, a high degree of interconnectivity of trunk xylem in the tangential direction was confirmed with synchrotron-based micro-computed tomography (microCT) and dye crossover infusion studies. Fruit attached to dyed canes was also similarly sectored; no clusters exhibited dye on non-dyed canes, while 97 % of clusters attached to dyed canes exhibited dye infusion. The dye travelled down the cluster rachis and appeared to accumulate at the pedicel/berry junction, but only on dyed canes. These findings suggest that xylem in grapevine trunks is integrated anatomically, but functions in a sectored manner due to high axial hydraulic conductivity. The functional sectoring of grapevine xylem documented here has important implications for management practices in vineyards and for fruit cluster uniformity within single grapevine.

Keywords: Active sapwood; plant hydraulics; synchrotron; *Vitis*; xylem network.

Introduction

Xylem is responsible for delivering water and nutrients from roots to plant canopies, and the degree of connectedness of a xylem network can affect its hydraulic efficiency and safety (Tyree and Zimmermann 2002). A high degree of connectedness provides redundancy in case of injury or hydraulic dysfunction and promotes sharing of water and nutrients between various

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plant organs with different water status (Dietrich *et al.* 2018). However, it can also allow pathogens and embolism to spread more easily throughout the hydraulic network (Loepfe *et al.* 2007; Lee *et al.* 2013). Anatomical differences distinguish the hydraulic pathways of well-connected (i.e. integrated) versus sectored xylem networks at both the trunk (Schenk *et al.* 2008) and cellular scale (Carlquist 2001, 2009; Sano *et al.* 2011), and those connections have important implications for water transport to the canopy (Bouda *et al.* 2019). Sectored plants have larger springwood and conduit diameters, lower conduit density (Zanne *et al.* 2006), increased conduit isolation (Ellmore *et al.* 2006; Zanne *et al.* 2006), decreased lateral flow and conduit to conduit pitting (Orians *et al.* 2004; Ellmore *et al.* 2006), and straighter and more parallel conduits (Espino and Schenk 2009) than integrated plants. Xylem sectoring can therefore impact within-plant resource allocation and tissue variability in different parts of the canopy (Orians and Jones 2001; Orians *et al.* 2002), and thus contribute to non-uniformity of fruit quality and production in cropping systems.

Ring-porous tree species, which have large diameter earlywood vessels, are significantly more sectored than diffuse-porous species (Orians *et al.* 2005; Zanne *et al.* 2006), where the large diameter earlywood vessels can be physically disconnected from both the smaller diameter latewood vessels in the same annual ring, but also disconnected from subsequent growth rings (Wason *et al.* 2019). While grapevines (*Vitis vinifera*) are technically considered a semi-diffuse porous species, their xylem contains long, wide vessels and has axial conductivity similar to ring-porous trees. The anatomical similarities that grapevine vessels share with hydraulically sectored species suggest that grapevine xylem may also be functionally sectored. Studies examining grapevine response to freezing (Wample *et al.* 2001), pathogen movement (Stevenson *et al.* 2004) and movement of pathogenic toxins (F. Peduta, UC Davis, pers. comm.) only into some portions of the canopy support the idea that grapevine xylem is sectored. While our understanding of the functional implications of xylem network organization in current year growth has been recently explored (Brodersen *et al.* 2010, 2011, 2013a,b; Lee *et al.* 2013; Knipfer *et al.* 2015; Bouda *et al.* 2019), links to the trunk xylem in grapevines are not well-understood.

Dye infusion has been used to assess hydraulic sectoriality in a variety of plant species (Orians *et al.* 2004; Taneda and Tateno 2007; Umebayashi *et al.* 2007; Espino and Schenk 2009), where xylem-mobile dyes are infused upstream of the foliage to assess the movement of liquid through the network. Ellmore *et al.* (2006) observed that patterns of dye transport infused into single roots were closely linked to xylem sectoriality in two tree species; dye spread to 38 % of the branches in sectored *Quercus rubra* versus 94 % of the branches of the integrated *Betula papyrifera*. Dye infusion is also commonly used to estimate sapwood tissue area for scaling sap flow velocities to volumetric water use (e.g. Braun and Schmid 1999; Sano *et al.* 2005; Pearsall *et al.* 2014). Sap flow velocities can vary dramatically along the trunk radius (e.g. Jiménez *et al.* 2000 for several species) and around the circumference for a given plant. Pearsall *et al.* (2014) found that sap flow velocities can vary by 3-fold consistently throughout the entire day around the circumference of a grapevine trunk, which is likely related to the amount of leaf area supplied by a given portion of the xylem. Knowledge of how much of the secondary xylem is functional, and how it is connected to the canopy are important for understanding water use throughout the canopy and for a whole plant. Highlighting active tissue area with dye infusion can validate flow measurements, determine total conductive tissue area and define radial flow patterns.

This study combined sap flow measurements, canopy manipulation, dye infusion and synchrotron-based micro-computed tomography (microCT) to better understand the 3D structure of xylem in mature grapevines and its implications for water transport. We hypothesized that (i) grapevines exhibit functional sectoriality, wherein certain sectors of the root system and trunk cross-section primarily supply water to certain portions of the canopy and (ii) the low axial resistance of grapevine xylem promotes sectoring even though tangential pathways around the trunk circumference do exist.

Materials and Methods

Site descriptions

Field-grown *V. vinifera* (own-rooted cv. Thompson Seedless clone 2A and cv. Chardonnay) vines were used in this study. The Thompson Seedless vines were planted in a 1.4-ha vineyard in 1987 at the University of California Kearney Agricultural Center (Parlier, CA, USA). Vine and row spacing were 2.15 and 3.51 m, respectively (7.55 m^2 per vine). Vines were irrigated with 4 L h^{-1} in-line drip emitters spaced every 0.30 m in the vine row. Vines were head-trained and cane-pruned during the dormant portion of the growing season to ~12–15 nodes in length. Cultural practices to control diseases and insect pests were performed by field station personnel as described previously (Daane and Williams 2003; Williams *et al.* 2003). A follow-up experiment was conducted between 31 August and 4 September 2020 on 8-year-old Chardonnay vines growing in an experimental vineyard at the University of California in Davis, CA, USA. This experiment was conducted to evaluate whether fruit on vines was similarly impacted by the hydraulic sectoriality of the xylem. Chardonnay vines were head-trained and cane-pruned during each dormant season. Fruit was fully mature at the time of the experiment and ready for harvest.

Sap flow measurements

Sap flow was measured using methods described previously in Pearsall *et al.* (2014). Sensors consisted of three, 20-mm-long, 1.2-mm-diameter needles, each with one Type E point thermocouple and a fourth needle containing a coiled 18-Ω nichrome wire heater. Sensor needles were formed by cutting off the tip of a 35-mm-long hypodermic needle (18 G 1.5 short bevel, Becton Dickinson and Co., Franklin Lakes, NJ, USA) forming a 20-mm tube. The cut end of the tube was then sealed with solder. Either a thermocouple or a nichrome heater was then inserted into the tube, and sealed in place with an epoxy resin. Heaters were formed by coiling a length of nichrome wire around itself. The exposed ends of the heater wire were connected via extension wire, through a control switch, to a 12-V battery.

A Dremel rotary tool (Robert Bosch Tool Corporation, Dremel, Racine, WI, USA) with a 1.25-mm-diameter drill bit was used at low speed for needle installation, and a drill guide was used to ensure the desired needle spacing. Sensor needles were coated with grafting wax as they were inserted into drill holes in order to improve thermal contact and to prevent fungal growth. Sensor needles were installed in vines such that thermocouple needles were placed 6 mm upstream (–) and 6 and 18 mm downstream (+) from the heater. The thermocouples located –6 mm and +6 mm from the heater were used for HRM measurements, whereas thermocouples –6 mm upstream and +18 mm downstream were used for the

compensation heat-pulse method measurements. The heat-pulse sensors were wired with extension cable to AM25T multiplexers controlled by CR10X dataloggers (Campbell Scientific Inc., Logan, UT, USA), and measurements were taken every 30 min. Heaters were activated from a 12-V power supply for 2 s.

Dye infusion

Dye infusions were performed as a single dye trunk infusion on vines with full canopies (single infusion), a single dye trunk infusion on vines with part of the canopy previously removed (leaf area removal infusion), a dual trunk infusion on vines with full canopies (dual trunk infusion) and a single dye root infusion on vines with full canopies (root infusion). For leaf area removal infusion method, ~70 % of the vine's leaf area was removed a few days prior to the infusion and impacts on sap flow were recorded at the time of removal. The single infusion method was also used on the Chardonnay vines used in the follow-up fruit evaluation experiment conducted in the summer of 2020. In all cases, the dye perfusion into the xylem vascular system was driven by the transpiration-induced flow of the intact leaves, and vines were dissected after the infusion to evaluate connections and allow for documentation (i.e. dissection of vines, like those shown in Fig. 3, was completed only after the infusion to verify dye penetration into various parts of the vines). Infusions were conducted post-veraison for all vines and after the canopy had fully formed.

Acid fuchsin solution (0.1 % in degassed distilled water) was the primary dye used for all infusions methods; crystal violet solution (0.1 % in degassed distilled water) was used as a second dye for the dual infusion method. A 0.1 % solution of acid fuchsin was found by Umebayashi *et al.* (2007) to be an ideal xylem-mobile dye and aqueous concentration for visualizing water movement in xylem. Comparatively, the positive charge associated to crystal violet causes it to be taken up more slowly than other dyes (Larson *et al.* 1993; Umebayashi *et al.* 2007) although it has been used successfully for staining of active xylem tissue (Larson *et al.* 1993; Stevenson *et al.* 2004). Consequently, crystal violet usage was limited to infusions when two dyes were used.

For trunk infusions, flexible plastic sheets were cut to form a cone around the trunk below the sap flow sensors. Cones were attached to the trunk with a gap-filling clay and duct tape to prevent leaks, and then filled with dye solution. A 6.35-mm chisel was then driven into the trunk with a mallet below the surface of the dye to create an infusion point in the trunk to a depth of ~2.5 cm at an angle of ~45° from the trunk (Fig. 1C). The infusion point was created with the chisel immersed in the dye solution to prevent vessel embolism. Injection points for the single and dual infusions were located on ridges visible on the bark surface that could be followed to the canopy as they spiral vertically around the trunk. Sap flow sensors were installed on these same outgrowths. Infusion points were similarly made on the trunk in sectors for the leaf area removal experiments, which had been visually identified as associated with the removed canopy. Dye solution in the cones was maintained at a constant level above the infusion sites for the duration of the infusion. For root infusions, one lateral root per vine of ~10-mm diameter was excavated by hand. Loose dirt was cleaned from the root surface, and the root was surrounded by a plastic container and immersed in a dye solution. The root was then cut with pruning shears while submerged in the dye solution.

Harvested vine data collection and analysis

Dye infusions for all treatments were performed for 70–80 min during the midday hours in full sunlight. The relatively short infusion duration compared with other studies (Braun and Schmid 1999; Ellmore *et al.* 2006) was a consequence of acid fuchsin's relatively fast diffusion rate and its propensity to diffuse into non-water-conducting vessels in infusions lasting longer than 2 h (Umebayashi *et al.* 2007). Dye was visually detected in the leaves within the duration of the infusion (Fig. 1A and B).

Upon completion of the infusion, canes and clusters were removed with pruning shears from vines and inspected for the presence of dye with a hand lens or stereomicroscope depending on the size of the organ. Individual canes and clusters were labelled for either presence or absence of dye in the shoot and its affiliated fruit, leaves and petioles. The trunk was then cut below the infusion point for trunk infusions, or the vine and root system were excavated for root infusions. Trunks of Thompson

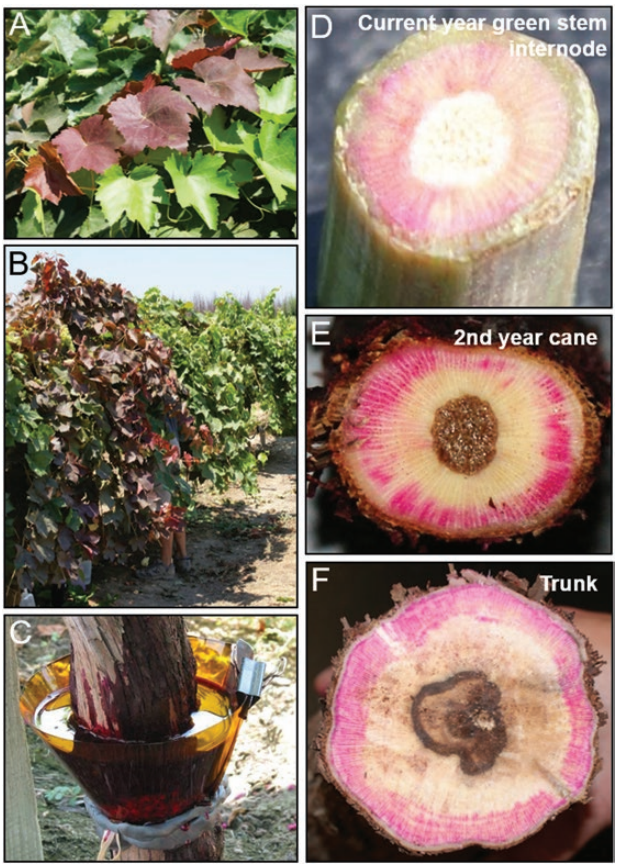

Figure 1. Dye infiltration experiments conducted on mature Thompson Seedless (A–C). Leaves (A) and the whole canopy (B) of a dyed vine showing complete staining at the end of the experiment. All infusions were performed on intact vines with shoots still attached during the summer after the canopy was fully formed to track transpiration-driven flow. Dye was delivered to the canopy using collars attached to the trunk (C) or single roots as described in the methods. Right column—Uptake patterns of dye in three different grapevine stems of varying ages. (D) Current year green grapevine stem internode showing complete dye uptake in all active xylem tissue. (E) Dye pattern in a second-year-old cane highlights active tissue area in outer xylem (i.e. current year xylem), with minimal uptake further from the cambium. (F) Active tissue area of mature grapevine trunk. Section diameters of the current year shoot, second year cane and mature trunk in D–F were ~1, 2–3, and 10 cm, respectively.

Seedless vines were immediately cut transversely with a battery-powered reciprocating saw in 10-cm increments (see numbered segments in Figs 4 and 5) to observe the dye path, at which point the perimeter of the xylem stained by dye was traced directly on each segment using a coloured pencil to mark its location prior to dye diffusion. Vines were then placed in large plastic bags and stored at 4 °C for preservation. For the Chardonnay dye infusion experiment completed in 2020, the vines were harvested similarly to above, but also included an assessment of dye infusion into each cluster on the four experimental vines.

Physiological attributes of each vine and the dye flow path were assessed. Vines were re-assembled and photographed with a Nikon D40 digital camera (Nikon Corporation, Tokyo, Japan) to document trunk morphology with respect to dyed shoots. Cross-sections were photographed and images were combined with the re-assembled vine to observe dye movement throughout the trunk. APS Assess (v2002, American Phytopathological Society) was used to assess cross-sectional photographs and determine the trunk's active tissue area and the total dyed area. Digital editing of the images was performed using Adobe Photoshop v9.0.2 (San Jose, CA, USA) to remove image backgrounds and improve dye visibility for interpretation purposes. Images from the Chardonnay infusions during the summer of 2020 were captured with an iPhone X (Apple, Inc.) and a Leica M165C stereomicroscope.

Synchrotron-based X-ray microCT

Sections of grapevine xylem were harvested from trunks of plants used in the dye infusion studies in the field. Harvested sections extended 4–5 cm radially into the xylem and included several annual growth increments. Sections were dried at 40 °C for 48 h in a drying oven prior to scanning at the x-ray microtomography beamline (8.3.2) at the Advanced Light Source at Lawrence Berkeley National Laboratory (Berkeley, CA, USA) using the methods described in Brodersen *et al.* (2011). Samples were rotated in the field of view in 0.125° increments around 180° in a 17-keV x-ray beam, yielding 1440 2D projection images per sample, with a voxel (volumetric pixel element) size of 4.5 μm^3. The sample was then advanced vertically and repeatedly scanned to create a seamless data set along the trunk axis. The images were then normalized using a filtered back-projection algorithm. Normalized 2D images were reconstructed using Octopus 8.3 software (Institute for Nuclear Sciences, University of Ghent, Belgium) to create a 3D, 16-bit series or stack of tagged image file (TIF) format files. Each TIF image was composed of 3D voxels, where voxel intensity was based on x-ray attenuation. Each voxel was then assigned an *x*, *y* and *z* coordinate in 3D space. Images were processed with an edge-preserving filter in Avizo 6.2 software (VSG, Burlington, MA, USA) to increase contrast between plant tissue and vessel lumen. Connections between all vessels within the xylem section were identified by scrolling through the stacks of transverse image slices in 3D data sets, and using the intervessel threshold criteria established in Brodersen *et al.* (2010). Avizo visualization software allows for virtual slices in any orientation to be displayed such that longitudinal planes could be visualized that enable determination of vessel connectedness throughout the segment. Examples of tangential connections are illustrated in the 3D renderings below and false colouring was used to illustrate connectedness of vessels within the segment.

Results

Dye infusion experiments demonstrated that active xylem is limited to the outer trunk tissue of the mature grapevines studied here. Vines subjected to dye infusion had an average stained depth of 6.7 mm from the cambium (Figs 1 and 2B). Measurements of dyed tissue extended to a maximum depth of 1.0 cm into the sapwood, with no dyed or active tissue visible at a further radial depth in any of the vines sampled. Micro-computed tomography and low magnification observations of the transverse sections showed that no tyloses or heartwood formation was present within this region of tissue. The transverse sections also showed an unstained gap between the heartwood and the active, dye-stained sapwood. While relatively consistent, the stained xylem depth did vary around the vine circumference, and was often deeper in discrete sectors of tissue (i.e. 3–4 cm wide around the circumference of the trunk—see pink vs. blue dyed portions on opposite sides of the trunk in Fig. 2B). These sectors were formed by uneven growth of the trunk xylem, and created a noticeable outgrowths visible on the bark, and could often be traced to individual

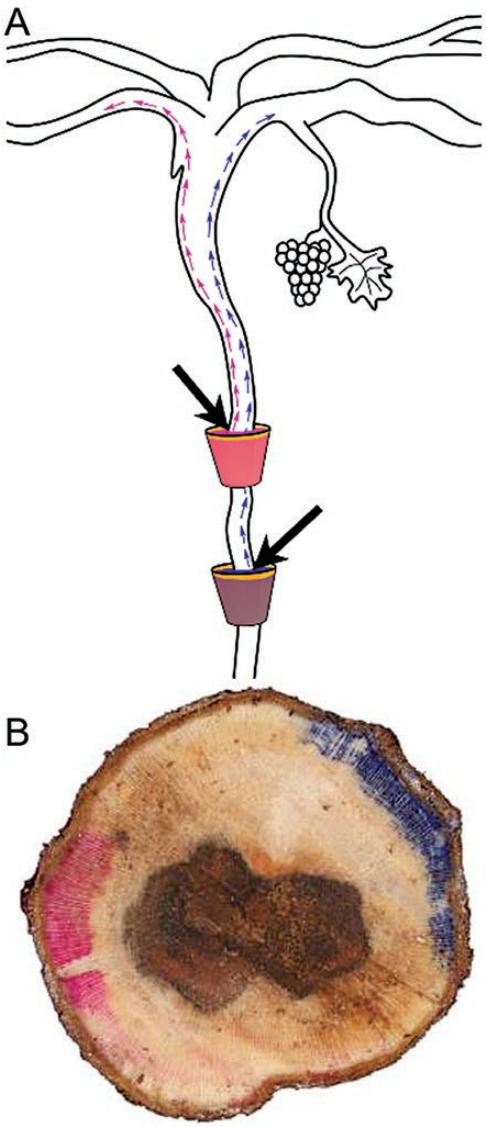

Figure 2. (A) Schematic of the dual infusion method. Coloured arrows show sap flow pathways; black arrows denote infusion points. (B) Trunk cross-section following acid fuchsin (pink) and crystal violet (blue). Dye infiltrated ~1.0 cm radially for all dye infusions performed. Sap flow remained in discrete xylem sectors as highlighted by the dyed flow paths (A) and dyed trunk cross-sectional area (B).

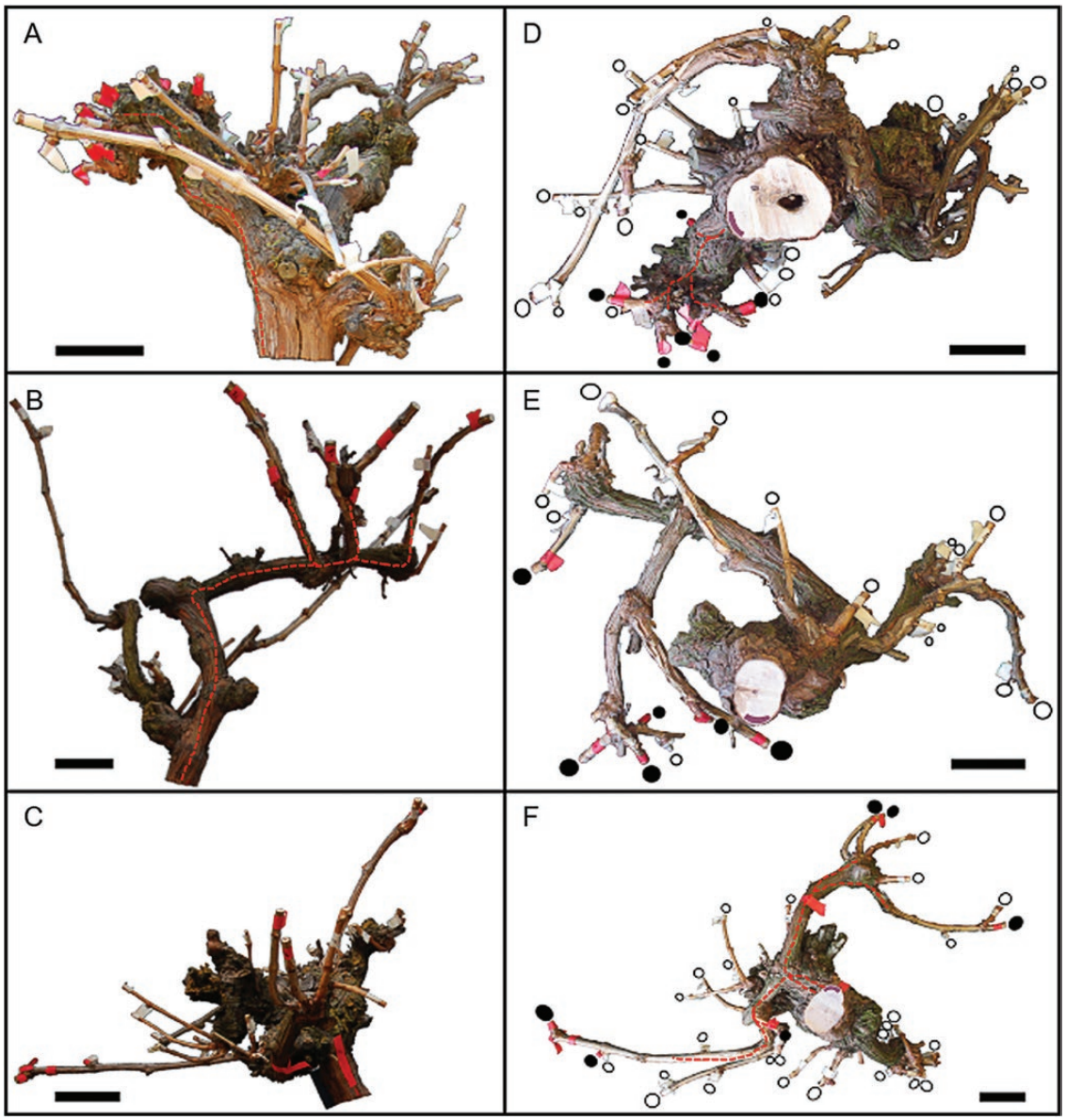

Figure 3. Resulting patterns of dye uptake and distribution following trunk infusion with 0.1 % acid fuchsin in three representative Thompson Seedless grapevines for side view images (A–C) and skyward view images (i.e. if you were laying on the ground looking up the trunk towards the sky) (D–F) looking up at the dyed trunk cross-section. Dye infusions were performed on intact vines in the summer after the canopy was fully formed (i.e. intact leaves drove the transpiration-driven uptake of the dye). Images shown here represent the vine head after shoots excision upon completion of dye infusion to verify its translocation patterns. Side images of grapevine heads show the external pattern of dye uptake by vine leaves. Dye was visible in canes identified with red tape, whereas those wrapped in white tape did not uptake dye. Dyed shoots and leaves were found to remain clustered in sectors associated with the xylem bundle into which dye had been infused. In panel (F), dyed shoots appeared to be spread far apart, but traces showed their spurs originated from the same part of the head. Dyed xylem was enhanced as pink on cross-section to improve visibility. Closed circles indicate dyed canes; open circles indicate canes are undyed. Circle size is proportional to each cane's dimensions. Bars = 10 cm. $N > 10$ vines were used for these dye infiltrations—images show three representative vines.

canes by following the outgrowth as it spiraled around the trunk into the canopy towards the leaves. Single and dual dye infusions provided further support that dye followed discrete pathways through xylem from the infusion point to the canopy (Figs 2 and 3), and these discrete bundles also provided the primary route of dye transport with root infusions (Fig. 4). As seen in the trunk cross-sectional images, there was limited lateral or radial dye movement observed outside of the xylem sector associated with the infiltrated root (Fig. 4). Interestingly, the xylem sector associated with the infused root would often spiral around the trunk as observed in 10-cm-diameter serial transverse sections moving closer to the canopy, and yet the dye remained in those discrete xylem sectors. It was evident that the majority of the canes containing dye were directly supplied by the dyed xylem sector in a given vine (Fig. 3). These experiments also confirmed extremely high sap flow rates in these vines, suggestive of low axial resistance. Dye introduced into the base of vine trunks was detected in the leaves at a distance of 4.11 m away from the insertion point within 35 min. The first leaves to show obvious dye transport were always leaves positioned at the top of the canopy and in full sun exposure.

Radial sap flow values further emphasized the significance of lateral, circumferential flow through active sapwood (see

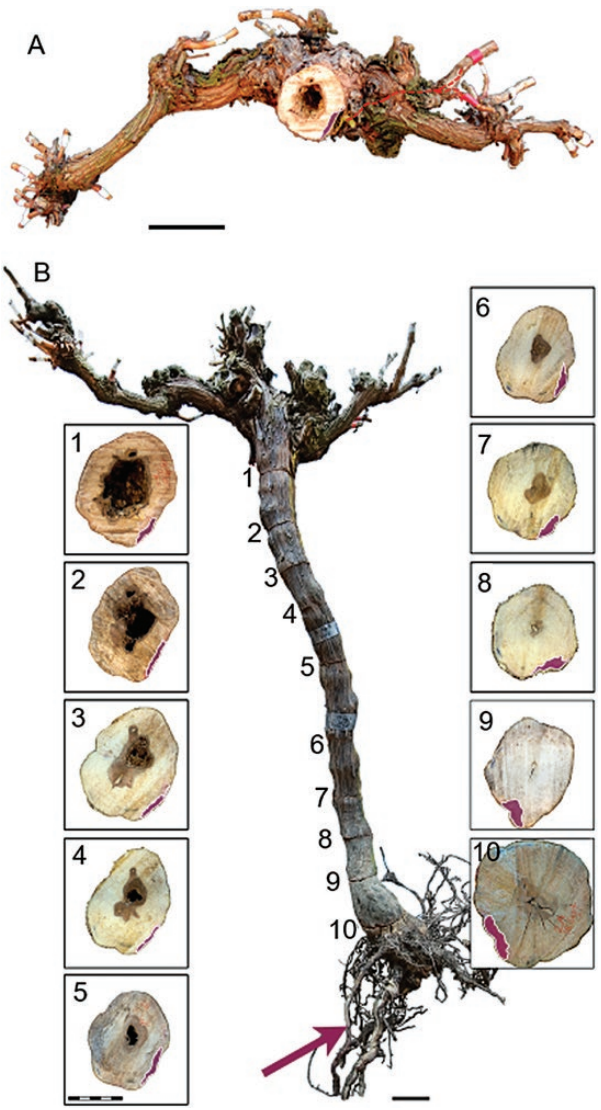

Figure 4. Dye pattern of a grapevine stained with 0.1 % acid fuchsin via root infusion. Dye infusions were performed on vines with intact canopies, and shoots were excised from the head after the infusion. (A) Skyward view of the grapevine head following dye infusion and shoot excision, showing cross-sectional dyed area (dye visible on lower right portion of cross-section) and distribution of canes with dyed leaves. Dye was visible in canes identified with red tape, whereas those wrapped in white tape did not uptake dye. Bar = 5 cm. (B) Cross-sectional correlation between dye pattern and location within the trunk. Arrow indicates the root used for the infusion. Numbered images correlate with numbered location on grapevine trunk. All cross-section images are oriented relative to the front of the vine as seen in this image. Perceivable dye movement through the cross-sections is due to twisting of the trunk (i.e. torsion like a twisted rope). Dye remained in the xylem bundle it was introduced into in the root. Bar = 10 cm; subset image 5 bar = 5 cm and corresponds to all cross-sectional images.

previously published data in Pearsall 2011). Sap flow velocity was inversely proportional to radial distance, a finding which supports measurements of active tissue extending radially up to ~1.0 cm from the cambium, a gap of non-conductive sapwood, and extensive heartwood formation. In many vines, heartwood was observed to have decomposed into a physical hole in the middle of the trunk, prohibiting radial fluid movement (Figs 2–5). The physical barrier produced by heartwood, combined with visual evidence of active tissue and sap flow data showing a radial decrease in flow velocity, confirm the potential for lateral, circumferential flow pathways within active sapwood.

Partial shoot removal performed for crossover infusions markedly decreased measured sap flow in the xylem sector directly associated with the excised canopy (Fig. 5). The sap flow velocity measured by Sensor 1 (Fig. 5B), which was not directly associated with the removed canopy region, had a minor reduction in sap flow over pre-removal flow rates. The marked decrease in flow measured by Sensor 2 indicated which xylem sector directly supplied the removed canopy region (Fig. 5C). The minimal decrease in flow velocity measured by Sensor 1 indicated a minor degree of lateral circumferential flow from xylem sectors on the opposing side of the vine to the removed canopy area (Fig. 5B).

These findings of lateral circumferential flow through active sapwood were supported by dye infusion observations. Crossover infusions after canopy removal revealed dye movement from the infusion point on the xylem bundle associated with Sensor 2 to the remaining canopy primarily fed by xylem where Sensor 1 was installed (Fig. 5A). In contrast with the uniform, discrete dye sectors observed in the cross-sections of the single, dual and root infusions (Figs 2–4), the dye pattern produced during crossover infusions showed nearly lateral sap movement in response to a lack of immediate canopy demand (Fig. 5A). The cross-sectional image at the fifth position of the crossover infusion experiment (Fig. 5A) demonstrates a significant amount of lateral dye movement at a distance of only 4.5 cm downstream from the infusion point (i.e. the infusion hole in the trunk was 6.35 mm in width, but the dye spread laterally beyond the hole width as it ascended the trunk).

The 7-mm deep × 5-mm tall × 4.5-mm wide section of wood imaged with microCT spanned ~6 years of growth. In the full sample, there were 44 tangential connections that crossed ray parenchyma. The average cell diameter of those connecting cells was 94.6 μm, the average tangential distance crossed by connecting cells was 201.7 μm and the average angle of ascent of the connecting cells was 36°. The length of the connection along the 36° angle was 436 μm and the average radial distance between connecting cells was 348.2 μm. In this sample, there was potential connectivity between all xylem conduits. There were some isolated tangential connections, yet the majority of the tangential connections occurred as entire files of vessels, spanning multiple growth years, moving as a radial group across ray parenchyma to merge with neighbouring vessel files (Fig. 6). Micro-computed tomography imaging showed xylem vessels closer to the heartwood show increased connectivity than the section near the cambium. The entire sample is eventually entirely integrated within its 7-mm axial length spanning moving from cambium towards the older rings. Connectivity between xylem in the trunk and that in the arms and active current year shoots is illustrated for a vine with vertical shoot positioning in Fig. 9. The most recent annual ring (seventh year) in this theoretical vine shows the pathway for dye transport in the active sapwood that likely involves low axial resistance along the trunk and arm followed by tangential flow in a small band of active xylem around the outside of the arm. Large heartwood and rotted cores in the mature vines used in our experiments would further assure that any crossover of dye from one side of the trunk to the canopy on the opposite side of the vine would need to move tangentially around the trunk and pass through lateral xylem vessel wall connections, similar to patterns seen in the crossover experiment (Fig. 5A).

A follow-up study was conducted in the summer of 2020 to assess the impacts of hydraulic sectoriality on fruit of

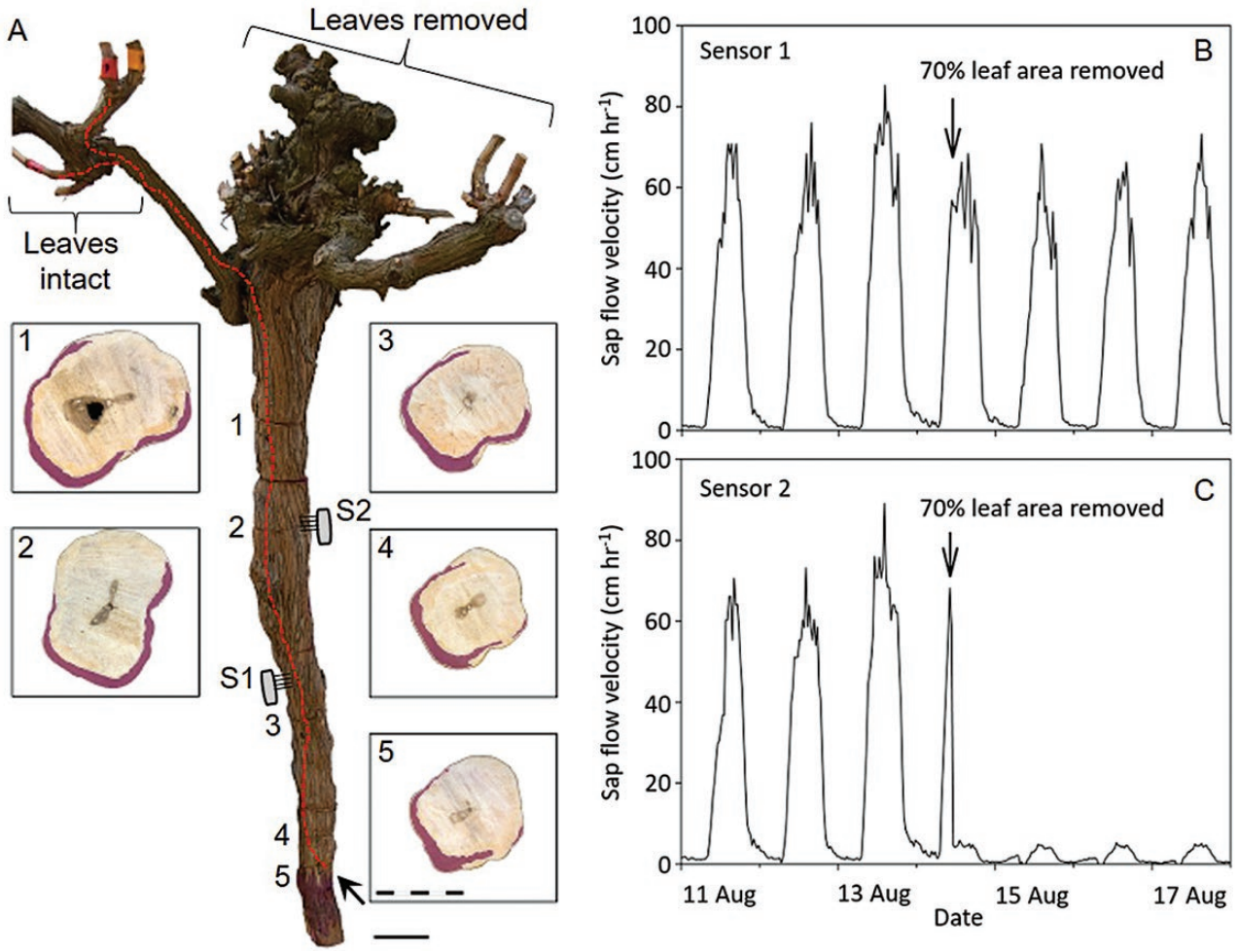

Figure 5. Dye movement pattern and sap flow data for grapevine following crossover trunk infusion. (A) Image of dye grapevine showing positions of sap flow sensors, point of infusion (black arrow at base of trunk), cross-sectional images at five locations along the trunk and dye path highlighted in dashed red line. All cross-sectional images are oriented towards the front view of the vine as seen in this image. Approximately 70 % of the vine's leaf area was removed from the right side of the vine 4 days prior to the infusion. Sap flow sensors were positioned in the xylem sectors believed to be associated with delivery to the left (Sensor 1; S1) and right (Sensor 2; S2) sides of the vine's canopy. The numbered images place the location along the trunk from where the cross-section originated. Image 5 is directly above the infusion point. The increasing dyed cross-sectional area higher in the trunk (images 1–3) indicates the spread of dye from the xylem sector that was initially infused (images 4, 5) into a larger section of the xylem. All shoots on the left side of the vine were dyed, further demonstrating dye movement through xylem connections. Bar = 10 cm; for subset image #5 the bar is 5 cm. (B and C) Sap flow velocity results for S1 and. The observed decrease in sap flow by Sensor 2 following the 70 % reduction in leaf area and the unchanged sap flow measured by Sensor 1 indicates that the left and right xylem bundles primarily serve the left and right sections of the canopy, respectively.

Chardonnay vines growing in an experimental orchard at the UC Davis. These vines were well past veraison and the fruit would have been harvested around the time of this experiment. The single infusion method revealed hydraulic sectoriality in these vines similar to that described for Thompson Seedless reported above with only a portion of the canopy exhibiting dye infusion into shoots (Fig. 7; $n = 4$ vines were used for this experiment). All shoots and clusters were excised from the vines and assessed for the presence of dye in the xylem tissue (Fig. 8). On shoots that were dyed in the leaves and xylem cross-section (Fig. 8F), 32 out of 33 clusters exhibited clear signs of dye in the rachis cross-section (Fig. 8A–C). All berry pedicels that were inspected from dyed clusters also exhibited dye infusion all the way to the berry attachment point ($n = 15$; Fig. 8D and E). The dye appeared to accumulate at the beginning of the brush (i.e. the central vascular bundle) where the berry connects to the pedicel (Fig. 8D and E); this pattern is consistent with limited xylem flow in post-veraison berries. Conversely, in shoots that did not exhibit dye infusion on all the vines, there was no evidence of dye penetrating into the cluster rachis; there were no dyed clusters (0 out of 76) on dye free shoots.

Discussion

Using sap flow measurements, dye infusion and canopy manipulation, we demonstrated that the active xylem of mature grapevines is young relative to the vine age and functionally sectored with water flowing predominantly in discrete sectors (visually identifiable from the outside surface) of the trunk axis that supply limited portions of the canopy, including the fruit. Despite the functional sectoring, a high degree of interconnectivity of grapevine trunk xylem in the tangential direction was confirmed with microCT and dye crossover infusion studies. These findings suggest that xylem in grapevine trunks is integrated anatomically, but functions in a sectored manner due to low axial resistance and higher lateral resistance. Based solely on their very wide vessel

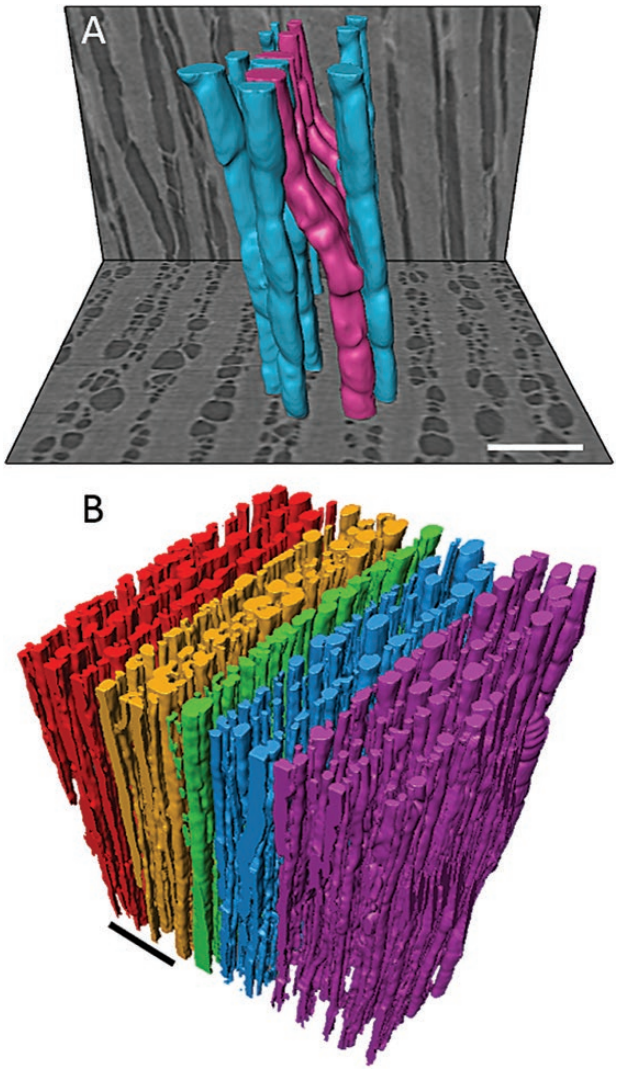

Figure 6. Micro-computed tomography images of angled connections (pink) between vessel files (blue) (A) offer an explanation for lateral xylem flow across ray parenchyma, and of a section of trunk equivalent to 3.5-mm spanning from cambium to heartwood (B), equivalent to the most c. 3 years of growth. Within this 3.5-mm image section, connected xylem share the same colour. However, by 7-mm depth, all vessels were interconnected. Bar = 1000 μm in both panels.

Figure 7. Images of Chardonnay vines growing in a vineyard in Davis, CA, USA, that were used in a dye infusion experiment evaluating whether fruit is similarly impacted by hydraulic sectoring. Dye was infiltrated into the trucks of using the wood chisel method described for experiments above, and remained in only a portion of the trunk cross-section (red outlined in the cross-section image in the bottom right of each panel) and shoots (red outlined in the whole canopy image). Nearly all clusters (32 out of 33) on dyed canes exhibited dye, while no clusters in the rest of each vine became dyed. N = 4 vines were used for this experiment.

diameters (>100 μm), it might not be surprising that grapevines have sectored xylem like other large-vesseled species (Ellmore *et al.* 2006; Zanne *et al.* 2006). However, hydraulically integrated plants often exhibit increased lateral flow and reduced axial and lateral resistance (Ellmore *et al.* 2006) compared to sectored counterparts. Grapevines present an interesting twist to these reported patterns because they appear to be anatomically integrated with extensive lateral connections, but functionally sectored primarily due to very low axial resistance associated with long vessels often found in lianas. It is notable that the high integration of the trunk xylem is in stark contrast to the xylem of young shoots and canes (Brodersen *et al.* 2010; Bouda *et al.* 2019), which exhibit a much more sectored xylem network. There is high connectivity within some groups of vessels within 1-year-old stems near the pith, but those metaxylem vessels have few connections to the older vessel groups closer to the cambium (Brodersen *et al.* 2010; Lee *et al.* 2013). Furthermore, tangential connections as seen frequently in the trunk xylem here are rare in the younger xylem. This developmental shift in the degree of xylem integration (i.e. from highly sectored to highly integrated in the young vs. old wood) may serve multiple roles. While high integration would allow for greater distribution of water through multiple pathways, both drought- or freeze-thaw-induced embolism and pathogens can migrate through the same pathway (Lee *et al.* 2013). Thus, having a less integrated xylem network in the tissue most susceptible to both embolism and pathogens would potentially protect the trunk and root system from potential damage. The degree of integration may also be the result of the changing biomechanical roles of current year stems compared to trunks, where the advantage of flexibility transitions to structural support and load-bearing in more mature trunk xylem. As seen in the crossover experiment, the loss of a significant part of the canopy still allows xylem not directly connected to the remaining canopy to contribute significantly to flow.

Hydraulic sectoring was thought to result from physical splitting of the xylem and isolation of portions of the network, but Espino and Schenk (2009) recently demonstrated that functional sectoring preceded physical splitting in the

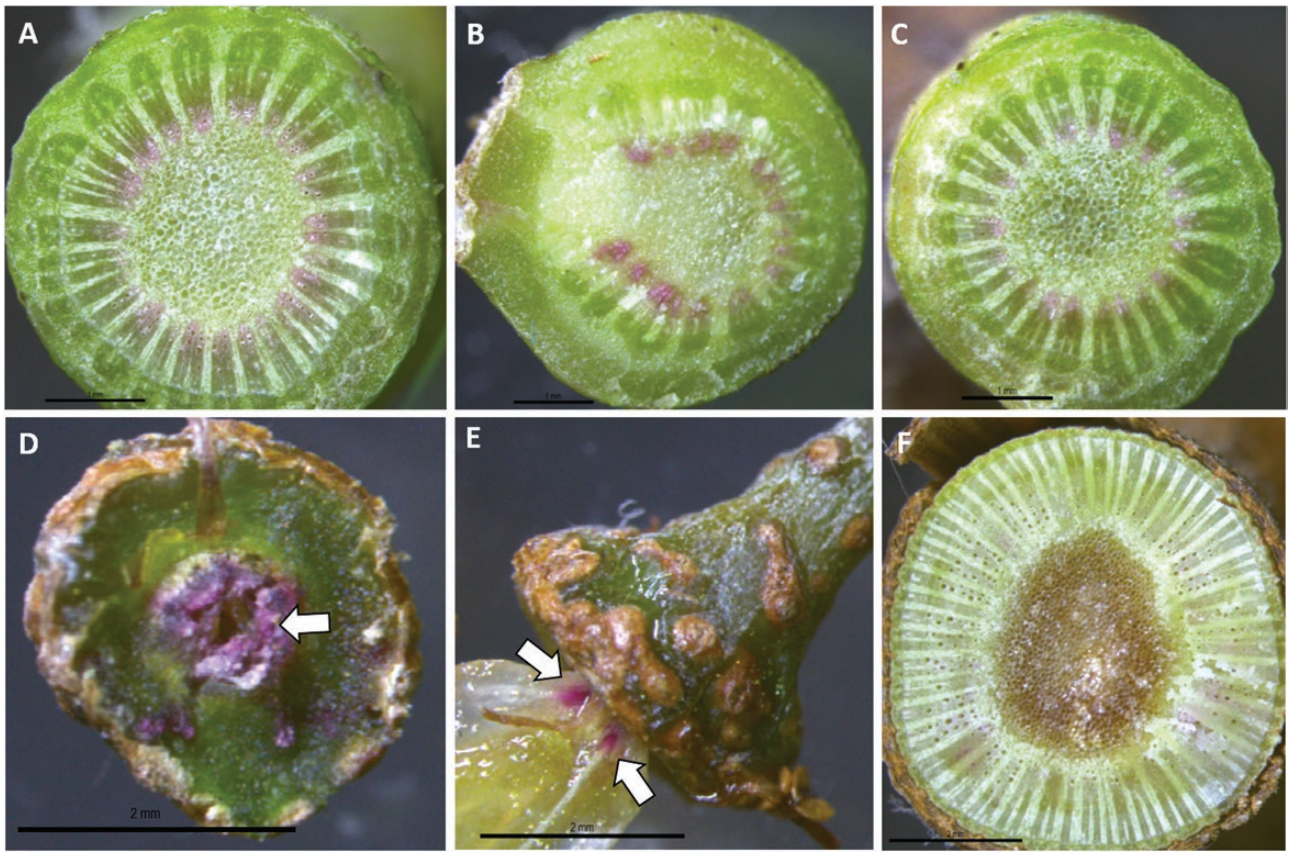

Figure 8. Images from dyed shoots and clusters from Chardonnay vines shown in Fig. 7. Nearly all clusters (32 out of 33) on dyed canes exhibited dye, while no clusters in the rest of each vine became dyed. Dye infiltrated into clusters on dyed canes (F) as seen in cluster rachises (examples seen in panels A–C) and berry pedicels (D and E). Dye travelled only into clusters on dyed canes, and progressed through the pedicel to the brush at the point of berry attachment (arrows). On canes without dye, no clusters were dyed (i.e. 0 out of 76 clusters inspected) demonstrating that fruit is similarly impacted by xylem hydraulic sectoring. Similar patterns were found in all vines (n = 4).

desert shrub *Ambrosia dumosa*. Proposed to be an adaptive response to environment, hydraulic sectoring increases under heterogeneous field conditions such as patchy water or nutrient availability (Larson *et al.* 1993; Espino and Schenk 2009), similar to conditions in a drip-irrigated vineyard. In an evaluation of 18 woody tree and shrub species, sectored species had an increased tolerance to light, wind and drought conditions and thrived in environments with heterogeneous moisture availability (Zanne *et al.* 2006). Drier environments promoted sectoring, whereas plants in flood-prone environments were more integrated (Zanne *et al.* 2006). Conversely, integrated plants may be better able to exploit nutrient-rich patches and prioritize nutrient transport to the fastest-growing regions of the plant (Orians *et al.* 2004). It is currently unknown if irrigation can alter xylem anatomy in a way to alter the functional status of xylem. Sectoriality has also been proposed as a potential safety mechanism for bypassing embolized conduits (Taneda and Tateno 2007). Lateral water flow increases via interconduit pits (Burgess and Bleby 2006; Nadezhdina 2010), explaining an inverse relationship between conduit pitting and sectoriality (Orians *et al.* 2004; Ellmore *et al.* 2006). Pit membrane structure allows for the safe transport of water through the xylem network by creating a physical barrier to embolisms and pathogens (Choat *et al.* 2008), further identifying survival characteristics inherent in integrated plants yet less prevalent in anatomically sectored plants.

Previous studies combining sap flow measurements with dye infusion have shown that 21-year-old grapevines did not form heartwood (Braun and Schmidt 1999), and that even the oldest xylem rings were active in water transport. Our current and previous (Pearsall *et al.* 2014) results suggest that sap flow is limited to a small band of active sapwood located close to the cambium. Numerous studies have found similar findings across several species where the majority of water transport occurs in the current year xylem of woody plants (Ellmore and Ewers 1986; Melcher *et al.* 2003; Fukuda *et al.* 2015; Wason *et al.* 2019). Braun and Schmid (1999) performed their dye uptake by placing the entire severed trunk in a bucket of dye solution, which potentially opens up parts of the xylem upon excision that were not active in the intact vine. This effect would be exacerbated in grapevines, which have a very long mean vessel length. Therefore, these authors may have counted any xylem vessels that could potentially transport water as vessels that are conducting the transpiration stream, even if sap flow through these vessels was minimal. Poyatas *et al.* (2007) argued that minimally invasive sap flow techniques are needed to best assess dynamic changes in radial sap flow profiles. Insertion probes are needed to detect these radial profiles, while the heat balance technique used by Braun and Schmidt (1999) does not accurately assess these patterns. Additionally, observations of a 38-year-old grapevine (*V. vinifera* cv. 'Mission') revealed extensive heartwood formation and obvious physical sectoring of the

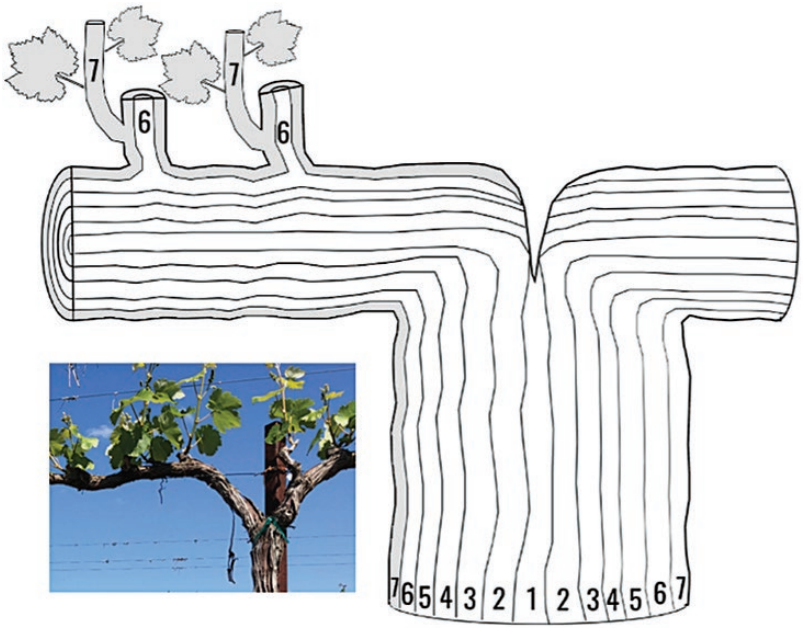

Figure 9. Cartoon depiction of a longitudinal slice through a 7-year-old bilateral cordon-trained and spur-pruned grapevine with vertical shoot positioning illustrating connectivity of the xylem in the current annual ring (seventh year of growth) in the trunk to that in the cordon, two bud spur (in its second year of growth) and current year shoots. Numbers represent each successive annual growth ring. Annual dormant season pruning of shoots eliminates connectivity of the current year to older xylem and creates a situation where bulk supply of water to active shoots comes from the side of the trunk in closest proximity given the high axial conductance of grapevine xylem. Water travelling up the trunk directly below the cordon with active shoots would travel rapidly along the axis and then have to traverse its circumference in a thin band of active sap wood through lateral connections between adjacent vessels as illustrated in Figs 5 and 6.

grapevine trunk (C. R. Brodersen *et al.*, unpubl. data). Absence of heartwood in grapevines observed by Braun and Schmidt (1999) suggests that integrated, radial flow is possible in grapevines regardless of vine age. Heartwood formation in older vines may depend upon water stress-induced damage or pathogen infection, effectively preventing spread of disease by production of a physical barrier. Vineyards in California struggle with extensive trunk pathogen diseases that contribute trunk wood damage (e.g. Bruez *et al.* 2016) similar to that shown in Figs 2–5 in this study. The mature Thompson Seedless vineyard exhibited trunk disease symptoms and may contribute to differences seen between our current findings and previous study (Pearsall *et al.* 2014) and previous work by other groups (i.e. Braun and Schmid 1999).

To obtain volumetric water use, sap flow velocities measured with heat dissipation techniques are multiplied using active cross-sectional area of the xylem (e.g. Bush *et al.* 2010). Our previous work on grapevines in this and other vineyards demonstrated high variability of flow rates around a vine trunk circumference. Sectors of xylem bulging out from the trunk's surface with higher sap flow velocities were connected directly to more leaf area on the arms above (Pearsall *et al.* 2014). This within plant variability can make it difficult to accurately scale sap flow velocities to volumetric water use, and can be compounded by the presence of trunk diseases. Sectored sectors of xylem have been shown to supply water to individual segments of plant canopies (Dye *et al.* 1991), and sap flow in these segments can be different from one another depending on the evaporative demand and the portion of the canopy they supply. The high sap flow rates (i.e. 0.12 m min^{-1}) found in our study vines suggests low axial resistance that would functional sectoring in the vines with preferential flow paths between xylem sectors in the trunk and portions of the canopy that were fully sun-exposed. Pearsall *et al.* (2014) also showed that targeting of trunk sectors closely connected to leaf area above can contribute to interannual variability for the same plant when sensors are repositioned to limit wounding effects.

We conclude that grapevine exhibits functional hydraulic sectoring, as observed through dye infusions, despite findings of anatomical pathways needed to function as an integrated plant, evidenced through microCT imaging and sap flow data. Alternate pathways via tangential connections are utilized based upon changes in canopy demand. Tangential connections that cross rays were observed with microCT imaging between vessel files and provide a pathway through which tangential flow can occur. Furthermore, pruning techniques commonly used in commercial viticulture may induce further sectoring in an otherwise integrated plant. Annual shoot pruning ensures that grapevine canopies are smaller than they would be under natural conditions and may negate the need for (i.e. less cross-sectional area of the trunk is needed to support a canopy that is kept smaller by pruning) and connectivity to older portions of the xylem located in the permanent wood structures (i.e. trunks) (Fig. 9). Our follow-up experiment verified that hydraulic sectoriality also carries over to fruit attached to dyed shoots. It was long thought that grape berries become hydraulically isolated as they mature, but work by various groups is consistent with our current results that the xylem remains conductive into the berry through ripening (Keller *et al.* 2006; Choat *et al.* 2009; Knipfer *et al.* 2015). Only the clusters attached to shoots that were dyed exhibited these direct connections; thus, delivery of water from one portion of the truck cross-section supplied only certain clusters on the vine. Such sectoring could contribute to varied resource distribution and lack of fruit uniformity within a vine.

Contributions by the Authors

A.J.M. and L.E.W. secured funding to support the work; A.J.M., L.E.W., and K.R.P. designed the experiments; all authors contributed to the data collection, summary and representation; A.J.M. produced the first draft of the manuscript; all authors contributed edits for the final draft.

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Conflict of Interest

None declared.

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Literature Cited

Bouda M, Windt CW, McElrone AJ, Brodersen CR. 2019. In vivo pressure gradient heterogeneity increases flow contribution of small diameter vessels in grapevine. *Nature Communications* **10**:5645.

Braun P, Schmid J. 1999. Sap flow measurements in grapevines (*Vitis vinifera* L.) 1. Stem morphology and use of the heat balance method. *Plant and Soil* **215**:39–45.

Brodersen CR, Choat B, Chatelet DS, Shackel KA, Matthews MA, McElrone AJ. 2013a. Xylem vessel relays contribute to radial connectivity in grapevine stems (*Vitis vinifera* and *V. arizonica*; *Vitaceae*). *American Journal of Botany* **100**:314–321.

Brodersen CR, Lee EF, Choat B, Jansen S, Phillips RJ, Shackel KA, McElrone AJ, Matthews MA. 2011. Automated analysis of three-dimensional xylem networks using high-resolution computed tomography. *The New Phytologist* **191**:1168–1179.

Brodersen CR, McElrone AJ, Choat B, Lee E, Shackel KA, Matthews MA. 2013b. In vivo visualizations of drought-induced embolism spread in *Vitis vinifera*. *Plant Physiology* **161**:1820–1829.

Brodersen CR, McElrone AJ, Choat B, Matthews MA, Shackel KA. 2010. The dynamics of embolism repair in xylem: in vivo visualizations using high-resolution computed tomography. *Plant Physiology* **154**:1088–1095.

Bruez E, Baumgartner K, Bastien S, Travadon R, Guérin-Dubrana L, Rey P. 2016. Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. *Australian Journal of Grape and Wine Research* **22**:288–295.

Burgess SS, Bleby TM. 2006. Redistribution of soil water by lateral roots mediated by stem tissues. *Journal of Experimental Botany* **57**:3283–3291.

Bush SE, Hultine KR, Sperry JS, Ehleringer JR. 2010. Calibration of thermal dissipation sap flow probes for ring- and diffuse-porous trees. *Tree Physiology* **30**:1545–1554.

Carlquist S. 2001. *Comparative wood anatomy: systematic, ecological, and evolutionary aspects of dicotyledon wood*, 2nd edn. Berlin: Springer-Verlag.

Carlquist S. 2009. Non-random vessel distribution in woods: patterns, modes, diversity, correlations. *Aliso* **27**:39–58.

Choat B, Cobb AR, Jansen S. 2008. Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *The New Phytologist* **177**:608–625.

Choat B, Gambetta GA, Shackel KA, Matthews MA. 2009. Vascular function in grape berries across development and its relevance to apparent hydraulic isolation. *Plant Physiology* **151**:1677–1687.

Daane KM, Williams LE. 2003. Manipulating vineyard irrigation amounts to reduce insect pest damage. *Ecological Applications* **13**:1650–1666.

Dietrich L, Hoch G, Kahmen A, Körner C. 2018. Losing half the conductive area hardly impacts the water status of mature trees. *Scientific Reports* **8**:15006.

Dye PJ, Olbrich BW, Poulter AG. 1991. The influence of growth rings in *Pinus patula* on heat pulse velocity and sap flow measurement. *Journal of Experimental Botany* **42**:867–870.

Ellmore GS, Ewers FW. 1986. Fluid flow in the outermost xylem increment of a ring-porous tree, *Ulmus americana*. *American Journal of Botany* **73**:1771–1774.

Ellmore GS, Zanne AE, Orians CM. 2006. Comparative sectoriality in temperate hardwoods: hydraulics and xylem anatomy. *Botanical Journal of the Linnean Society* **150**:61–71.

Espino S, Schenk HJ. 2009. Hydraulically integrated or modular? Comparing whole-plant-level hydraulic systems between two desert shrub species with different growth forms. *The New Phytologist* **183**:142–152.

Fukuda K, Kawaguchi D, Aihara T, Ogasa MY, Miki NH, Haishi T, Umebayashi T. 2015. Vulnerability to cavitation differs between current-year and older xylem: non-destructive observation with a compact magnetic resonance imaging system of two deciduous diffuse-porous species. *Plant, Cell & Environment* **38**:2508–2518.

Jiménez MS, Nadezhdina N, Cermák J, Morales D. 2000. Radial variation in sap flow in five laurel forest tree species in Tenerife, Canary Islands. *Tree Physiology* **20**:1149–1156.

Keller M, Smith JP, Bondada BR. 2006. Ripening grape berries remain hydraulically connected to the shoot. *Journal of Experimental Botany* **57**:2577–2587.

Knipfer T, Eustis A, Brodersen C, Walker AM, McElrone AJ. 2015. Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure. *Plant, Cell & Environment* **38**:1503–1513.

Knipfer T, Fei J, Gambetta GA, McElrone AJ, Shackel KA, Matthews MA. 2015. Water transport and xylem anatomy of the grape (*V. vinifera*) pedicel during fruit development: Insights into xylem function and structure using x-ray computed microtomography. *Plant Physiology* **168**:1590–1602.

Larson DW, Mattes-Sears U, Kelly PE. 1993. Cambial dieback and partial shoot mortality in cliff-face *Thuja occidentalis*: evidence for sectored radial architecture. *International Journal of Plant Science* **154**:496–505.

Lee EF, Matthews MA, McElrone AJ, Phillips RJ, Shackel KA, Brodersen CR. 2013. Analysis of HRCT-derived xylem network reveals reverse flow in some vessels. *Journal of Theoretical Biology* **333**:146–155.

Loepfe L, Martinez-Vilalta J, Piñol J, Mencuccini M. 2007. The relevance of xylem network structure for plant hydraulic efficiency and safety. *Journal of Theoretical Biology* **247**:788–803.

Lovisolo C, Schubert A, Sorce C. 2002. Are xylem radial development and hydraulic conductivity in downwardly-growing grapevine shoots influenced by perturbed auxin metabolism? *The New Phytologist* **156**:65–74.

Melcher PJ, Zwieniecki MA, Holbrook NM. 2003. Vulnerability of xylem vessels to cavitation in sugar maple. Scaling from individual vessels to whole branches. *Plant Physiology* **131**:1775–1780.

Nadezhdina N. 2010. Integration of water transport pathways in a maple tree: responses of sap flow to branch severing. *Annals of Forest Science* **67**:107–116.

Orians CM, Ardón M, Mohammad BA. 2002. Vascular architecture and patchy nutrient availability generate within-plant heterogeneity in plant traits important to herbivores. *American Journal of Botany* **89**:270–278.

Orians CM, Jones CG. 2001. Plants as resource mosaics: a functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. *Oikos* **94**:493–504.

Orians CM, Smith SD, Sack L. 2005. How are leaves plumbed inside a branch? Differences in leaf-to-leaf hydraulic sectoriality among six temperate tree species. *Journal of Experimental Botany* **56**:2267–2273.

Orians CM, van Vuuren MMI, Harris NL, Babst BA, Ellmore GS. 2004. Differential sectoriality in long-distance transport in temperate tree species: evidence from dye flow, ^{15}N transport, and vessel element pitting. *Trees* **18**:501–509.

Pearsall KR. 2011. *Dual heat pulse sensors for determining volumetric water use in grapevines*. M.S. Thesis, University of California, Davis.

Pearsall KR, Williams LE, Castorani S, Bleby TM, McElrone AJ. 2014. Evaluating the potential of a novel dual heat-pulse sensor to measure volumetric water use in grapevines under a range of flow conditions. *Functional Plant Biology* **41**:874–883.

Sano Y, Morris H, Shimada H, Ronse De Craene LP, Jansen S. 2011. Anatomical features associated with water transport in imperforate tracheary elements of vessel-bearing angiosperms. *Annals of Botany* **107**:953–964.

Sano Y, Okamura Y, Utsumi Y. 2005. Visualizing water-conduction pathways of living trees: selection of dyes and tissue preparation methods. *Tree Physiology* **25**:269–275.

Schenk HJ, Espino S, Goedhart CM, Nordenstahl M, Martinez Cabrera HI, Jones CS. 2008. Hydraulic integration and shrub growth form linked across continental aridity gradients. *PNAS* **105**(32):11248–11253.

Stevenson JF, Matthews MA, Rost TL. 2004. Grapevine susceptibility to Pierce's disease I: relevance of hydraulic architecture. *American Journal of Enology and Viticulture* **55**:228–237.

Taneda H, Tateno M. 2007. Effects of transverse movement of water in xylem on patterns of water transport within current-year shoots of kudzu vine, *Pueraria lobata*. *Functional Ecology* **21**:226–234.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap*. Springer-Verlag Berlin Heidelberg, Germany.

Umebayashi T, Utsumi Y, Koga S, Inoue S, Shiiba Y, Arakawa K, Matsumura J, Oda K. 2007. Optimal conditions for visualizing water-conducting pathways in a living tree by the dye injection method. *Tree Physiology* **27**:993–999.

Wample RL, Hartley S, Mills L. 2001. Dynamics of grapevine cold hardiness. In: Rantz JM, ed. *Proceedings of the American Society of Enology and Viticulture 50th Anniversary Meeting*, 81–93. American Society of Enology and Viticulture, Davis, CA USA.

Wason JW, Brodersen CR, Huggett BA. 2019. The functional implications of tracheary connections across growth rings in four northern hardwood trees. *Annals of Botany* **124**:297–306.

Williams LE, Phene CJ, Grimes DW, Trout TJ. 2003. Water use of Thompson Seedless grapevines in California. *Irrigation Science* **22**:1–18.

Zanne AE, Sweeney K, Sharma M, Orians CM. 2006. Patterns and consequences of differential vascular sectoriality in 18 temperate tree and shrub species. *Ecology* **20**:200–206.