OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2023.57.1.7236

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Micro-CT constitutes a valuable tool in assessing the impact of cordon constriction on the vascular morphology of grapevines

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Associate editor:
François Delmotte

Received:
28 October 2022
Accepted:
21 February 2023
Published:
23 March 2023

ABSTRACT

The impact of permanent cordon training systems on the vasculature of grapevines has to date, not been investigated in depth. This study used optical microscopy (stereo microscope) and X-ray microtomography (micro-CT) to quantify the morphological properties of the xylem conduits of cane samples collected from the distal region of cordons, which had been established using four different training techniques. These treatments included one system where the cordon was wrapped very tightly around the cordon wire, a practice that is common in Australia and some other countries. The study also used micro-CT to observe the cordons directly, providing clear insight into the effects of the training methods on the localised structure of the cordons themselves. While the cordons in this study were only four years old at the time of their scanning and 3D reconstruction, significant differences were found between the different training methods. At one of the two trial sites, cordons which were wrapped tightly around the cordon wire had a significantly lower xylem conduit volume in relation to total cordon volume than those which had been woven through a plastic clip system centred between parallel cordon wires. The xylem conduits of woven cordons, in turn, had a lower volume than those which had been trained on top of the cordon wire and secured in place with plastic ties. Cordons which had been wrapped tightly around the cordon wire also had significantly thinner vessels and fewer connections per unit volume between vessels than other treatments at this site, as well as a lower theoretical specific hydraulic conductivity (Ks). No definitive patterns of differences between treatments were observed in the morphological properties of cane samples, either by stereo microscope or micro-CT. The results of this study suggest that the choice of cordon training method may have a notable impact on the capacity of the xylem for normal hydraulic function. Training methods which constrict the vasculature of the cordon, in particular tightly wrapping the cordon around the cordon wire, may have long-term negative outcomes on cordon health and productivity.

KEYWORDS: grapevine, constriction, water status, xylem, hydraulic conductivity

INTRODUCTION

Grapevines, like all vascular plants, are dependent on a functional vascular system for the movement of water and essential mineral nutrients. Sap is transported through the xylem network via a negative pressure gradient controlled by changes in leaf transpiration rate and soil moisture content (Tyree and Sperry, 1988). Individual xylem vessels are comprised of a series of elongated cells (vessel elements) with dead cell walls, stacked end-to-end and interconnected by perforated end walls (Esau, 1977; Tyree and Ewers, 1991). The xylem conduit is composed of many individual vessels interconnected by lateral pits (small openings in the lignified secondary cell walls) (Venturas *et al.*, 2017). In the centre of each pit is a pit membrane, allowing for the movement of water between xylem vessels while limiting the spread of embolisms (air-filled conduits that are not available for water conduction) and vascular pathogens (Choat *et al.*, 2008). The hydraulic properties of the xylem change and develop with wood age, with older stems typically having vessels of greater diameter (Ewers and Fisher, 1989; Jacobsen *et al.*, 2012; Jacobsen *et al.*, 2015) and a greater ratio between xylem width and stem diameter (Sun *et al.*, 2006). Physiological factors, including water availability, may also impact elements of xylem morphology, including vessel diameter (Lovisolo and Schubert, 1998; Munitz *et al.*, 2018), and hydraulic conductivity (Schultz and Matthews, 1993; Tyree and Ewers, 1991).

The training method is a factor that has not been studied in depth in regard to its impact on the vascular morphology of grapevine perennial wood structures (O'Brien *et al.*, 2021). In Australia and many other winegrowing regions around the world, it is a common practice for canes to be wrapped tightly around the cordon wire during the establishment of permanent cordon arms. As these arms grow, they have the unfortunate tendency to become tightly constricted over time, with the cordon wire often becoming visibly embedded within the wood of the cordon. This situation is often accompanied by severe decline and dieback, leading some to believe that the normal functionality of the vasculature of the cordon may be compromised by the strangulation effect induced by tight wrapping (Caravia *et al.*, 2015). This decline may be observed both in the presence or absence of vascular pathogens, which themselves are contributors to the formation of vascular occlusions and necrosis (Chatelet *et al.*, 2006; Rudelle *et al.*, 2005). There is likely a complicated relationship between vascular constriction observed after tight wrapping and the incidence and symptomology of vascular pathogen infection (O'Brien *et al.*, 2023), as strangled cordon arms could be more susceptible to initial infection and/or more likely to express symptoms once infected. Another unknown is whether the physical stress of tightly wrapping developing cordon arms around the cordon wire could induce a wound response resulting in the restriction of the normal flow of water and nutrients along the cordon. Such interruptions to the vine's ability to transport water to its most distal regions could have a severe outcome on cordon health and productivity.

The vascular morphology of grapevines may be examined by different forms of microscopy, allowing for the quantification of differences in the xylem characteristics of seasonal and permanent wood structures. Micro-computed tomography (micro-CT) has previously been used as a tool to model grapevine graft unions (Milien *et al.*, 2012) and the 3D spatial distribution of xylem network connections in grapevine cane internode segments (Wason *et al.*, 2021). It has also been used to successfully distinguish between asymptomatic and defective wood suffering from symptoms of black rot, necrosis, and decay, affecting individual xylem vessels in both canes and perennial wood samples (Vaz *et al.*, 2020). The aim of our current study was to investigate the impact of different cordon training techniques on the xylem morphology of canes originating from the distal portion of permanent cordons as well as cordons themselves using X-ray microtomography (micro-CT) and direct observation by optical microscopy (stereo microscope). We hypothesise that tightly wrapping the cordon around the cordon wire during establishment may constrict the vasculature of the perennial wood of the cordon, having a negative impact on the capacity of the xylem for normal hydraulic functionality.

MATERIALS AND METHODS

1. Experimental design

Treatments were applied in the spring of 2018 at two sites. Site A was a newly planted (3-year-old) vineyard site in Williamstown, South Australia (34°40'21.4"S 138°53'27.0"E) of Cabernet-Sauvignon (WA Cape Selection), grafted onto 1103 Paulsen, and planted at 3 m × 2 m inter-row and intra-row spacing. Site B was a newly reworked 20-year-old planting of Shiraz (clone BVRC12) in Eden Valley, South Australia (34°38'32.1"S 139°05'53.0"E) on its own roots, planted at 2.8 m × 1.8 m inter-row and intra-row. Both sites had a north–south row orientation and rows featuring a single foliage wire for the use of a sprawl canopy system. Cordon wire height at both sites was 1 m above the ground. The total amount of irrigation discharged at both sites was ~1.0 ML/ha during each growing season of the trial (2018-2022).

Treatments were randomly allocated to different panels. At Site A, six sets of three panels of three vines each (54 vines per treatment) were assessed for ongoing analysis. Three permanent cordon training (securing) techniques were applied and included (i) tightly wrapped (T), where canes selected as permanent cordon arms were wrapped tightly around the cordon wire, (ii) cordon placed on top of wire (P), where canes selected as permanent cordon arms were placed on top of the wire and secured in place at three or four positions with plastic ties; and (iii) cordon woven through clips (W), where canes selected as permanent cordon arms were woven through a plastic clip system centred between parallel cordon wires (Figure 1). At site B, an additional treatment, (iv) s-bend (S), was applied where canes selected as permanent cordon arms were wrapped around two parallel cordon wires in a loose, s-shaped bend. Three sets of

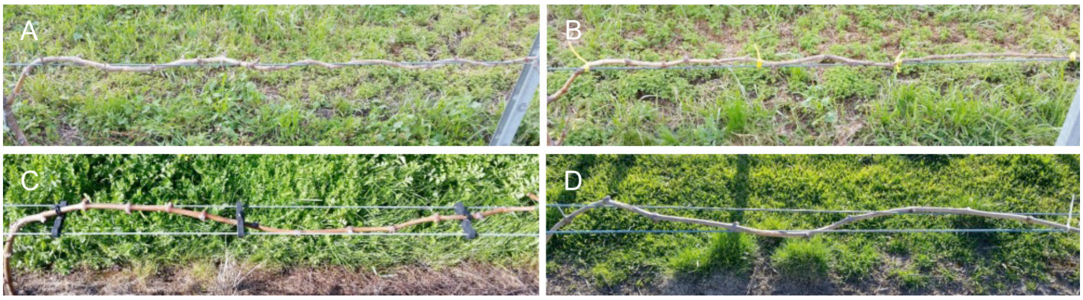

FIGURE 1. Applied cordon training methods. (A) Cordon wrapped tightly wrapped around cordon wire, T (B) Cordon placed on top of cordon wire, P (C) Cordon woven through plastic clip system, W (D) Cordon trained around parallel cordon wires in s-shaped bend, S.

three panels of three vines each (27 vines per treatment) were assessed at this site.

2. Measurements

2.1. Stereo microscopy of 1-year-old canes

One-year-old cane samples were collected from the distal portions of cordon arms during pruning in the winter of 2019, 2020, and 2021 for microscopic examination. Samples consisting of nodes three and four and their interjoining internode were taken from canes originating from the basal node of the previous year's 2-node spur. All samples were stored at 4°C until further processing. Three canes were randomly selected from each set of panels for a total of 18 samples assessed per treatment for site A and nine samples assessed per treatment for site B. Major and minor diameters were measured at the mid-point between nodes with digital callipers and averaged to determine cane diameter. Secateurs were used to cut cane sections approximately 1 cm long from the centre of each internode. A razor blade was then used to quarter cross-sections and to give the transverse face being observed a clean and straight surface. Samples were then observed directly with a stereo microscope (Model SMZ25, Nikon, Tokyo, Japan). The length and width of xylem vessels within the region of interest of captured images were measured using ImageJ version 1.53e (NIH, USA) software (Figure 2). Length and width were then averaged to obtain vessel diameter. The area of the xylem (including both the primary and secondary xylem) within the region of interest was measured to determine vessel density.

2.2. X-Ray microtomography (micro-CT) of 1-year-old canes

One cane sample was randomly selected from each set of panels for a total of six samples assessed per treatment at site A and three samples assessed per treatment at site B. Secateurs were used to remove the nodes from each cane sample, leaving the internode as long as possible. Internodes were then examined using a SkyScan 1276 Micro-CT system (Bruker, Belgium). Images were reconstructed and analysed using associated SkyScan software (NRecon version 2.1.0.1, CT Analyzer version 1.20.5.0) (Figure 3). Xylem vessels were isolated from other low-density regions so that the volume of the xylem conduit could be measured relative to total cane volume and its other morphological features could be investigated. Using 3D analysis in CT Analyzer, Euler analysis (Odgaard and Gundersen, 1993) was used to provide a measure of connectivity density, indicating the number of redundant connections between vessel structures per unit volume. Utilising the same software, a model-independent 3D thickness was measured for the xylem vessels of each cane sample.

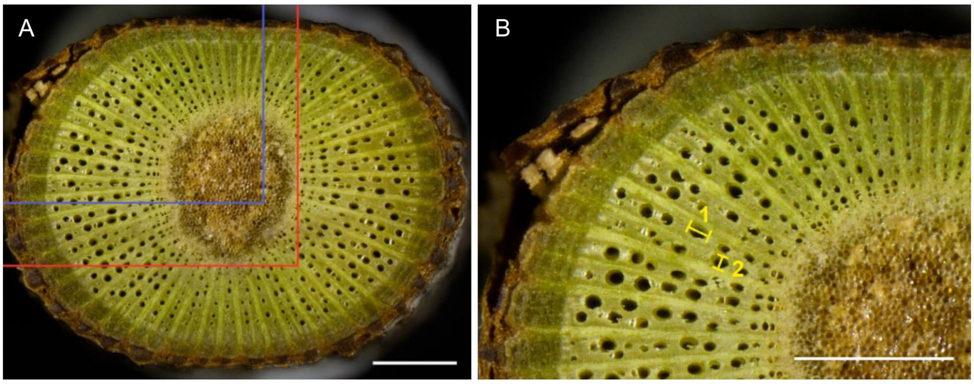

FIGURE 2. Cane samples as imaged by optical microscopy. (A) Full cross-section. Red lines indicate where quartering cuts were made. Blue lines indicate the edge of the region of interest. (B) Region of interest showing example measures of vessel length (1) and width (2). Scale bars = 1000 µm.

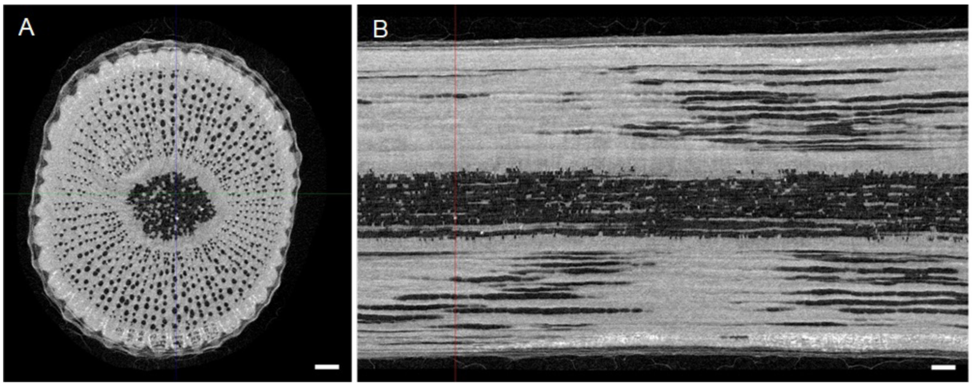

FIGURE 3. X-ray microtomography (micro-CT) of cane sample sourced from the distal portion of a cordon arm. (A) Cross-section (B) Longitudinal section. Resolution = 5.5 μm. Scale bars = 600 μm.

2.3. X-Ray microtomography (micro-CT) of cordons

In the winter of 2022, cordon samples were collected from one vine in each set of panels for a total of six samples per treatment at site A and three samples per treatment at site B. Samples of approximately 30 cm in length were taken directly from the centre of each cordon arm. All bark was removed from the samples, which were then surface disinfested in bleach and placed in a 40°C oven for 24 hours. Cordon samples were then examined using a large volume Nikon XT H 225ST (Nikon Metrology, Tring, UK) micro-CT system, using the helical scanning modality-special feature for long objects to capture ~20 cm of the length of each cordon sample. Images were analysed using CT Analyzer (version 1.20.5.0) (Figure 4). Nodes were removed from the analysis, and the investigation focused only on the internode segments of the captured cordon. Xylem vessels were isolated from other low-density regions so that xylem conduit volume could be measured relative to total cordon volume and so that the other morphological features of the xylem could be investigated, including connectivity density and vessel thickness, as described above (2.2).

2.4. Determination of theoretical specific conductivity

Theoretical specific hydraulic conductivity (K_s) was calculated according to the Hagen–Poiseuille equation:

$$K_{\mathrm{s}} = \left(\frac{\pi\rho}{128\eta A_{\mathrm{w}}}\right)\sum_{i=1}^{n}(d_i^4)$$

where ρ is the density of water (998.2 kg m^{-3} at 20°C), η is the viscosity of water (1.002 10^{-9} MPa s at 20°C), A_w is the sapwood cross-sectional area (m^2), d is the diameter of the ith vessel, and n is the number of vessels.

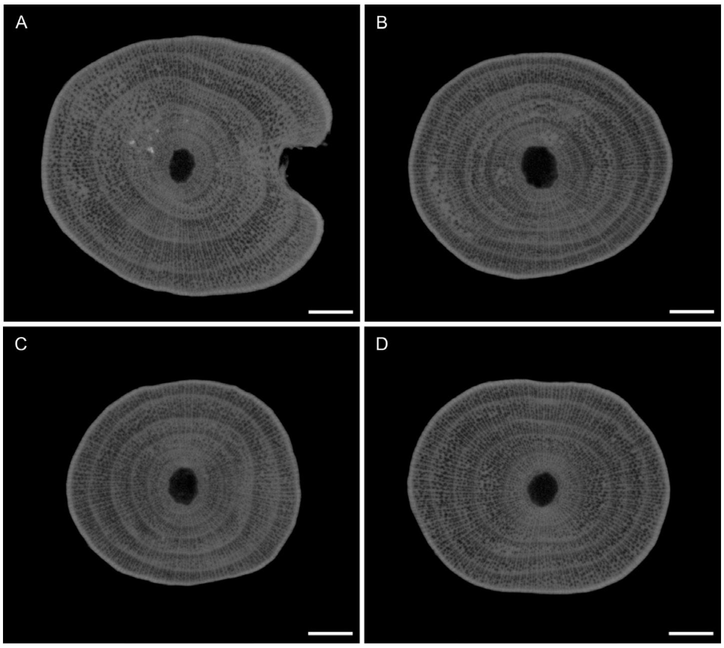

FIGURE 4. X-ray microtomography (micro-CT) of cordon samples in a cross-sectional view. (A) Cordon wrapped tightly around cordon wire, T (B) Cordon placed on top of cordon wire, P (C) Cordon woven through clip system, W (D) Cordon trained in s-bend, S. Resolution = 35 μm. Scale bars = 4 mm.

TABLE 1. Quantitative xylem characteristics of distal cane samples as observed by stereo microscopy and micro-CT (mean ± std).

Site A								
		Stereo Microscope		Micro-CT				
		Vessel diameter/cane diameter (%)	Vessel density (no./mm^2)	Xylem conduit volume/cane volume (%)	Vessel density (no./mm^2)	Vessel thickness (µm)	Connectivity density (no./mm^3)	K_s (kg m^{-1} MPa^{-1} s^{-1})
2019	T	0.47 ± 0.08	65.7 ± 18.1	6.8 ± 1.1	15.4 ± 1.1	74.3 ± 8.6	11.5 ± 3.0	17.6 ± 6.3
	P	0.49 ± 0.06	62.8 ± 26.1	7.0 ± 0.7	16.0 ± 2.2	73.4 ± 5.5	16.6 ± 7.2	16.7 ± 3.2
	W	0.42 ± 0.08	54.6 ± 15.5	7.3 ± 0.5	17.6 ± 2.7	76.2 ± 5.8	13.7 ± 2.9	22.0 ± 7.6
p-value		0.027	ns	ns	ns	ns	ns	ns
2020	T	0.44 ± 0.06	60.4 ± 13.9	7.4 ± 2.0	17.9 ± 4.9	74.1 ± 5.9	26.6 ± 14.2	20.8 ± 6.0
	P	0.44 ± 0.05	51.0 ± 7.0	7.8 ± 0.7	20.6 ± 2.6	68.5 ± 5.9	51.4 ± 15.9	16.9 ± 5.3
	W	0.42 ± 0.05	52.2 ± 11.6	7.5 ± 1.0	17.8 ± 0.8	72.2 ± 6.3	24.7 ± 3.0	19.0 ± 5.2
p-value		ns	0.032	ns	ns	ns	0.004	ns
2021	T	0.45 ± 0.05	45.9 ± 4.0	9.3 ± 1.9	28.3 ± 6.9	65.9 ± 7.8	97.5 ± 30.8	18.4 ± 6.5
	P	0.45 ± 0.08	42.3 ± 6.0	7.2 ± 0.7	21.0 ± 2.1	69.3 ± 4.6	56.3 ± 5.2	18.2 ± 3.7
	W	0.47 ± 0.07	47.0 ± 5.6	9.2 ± 0.7	25.8 ± 5.2	68.7 ± 6.8	86.5 ± 25.4	19.6 ± 5.5
p-value		ns	0.027	0.021	ns	ns	0.021	ns
Site B								
		Stereo Microscope		Micro-CT				
		Vessel diameter/cane diameter (%)	Vessel density (no./mm^2)	Xylem conduit volume/cane volume (%)	Vessel density (no./mm^2)	Vessel thickness (µm)	Connectivity density (no./mm^3)	K_s (kg m^{-1} MPa^{-1} s^{-1})
2019	T	0.60 ± 0.08	68.3 ± 10.7	11.6 ± 0.6	16.7 ± 2.5	92.2 ± 8.9	22.3 ± 6.4	43.1 ± 9.6
	P	0.63 ± 0.08	66.1 ± 20.3	12.0 ± 0.6	15.8 ± 3.3	96.5 ± 13.1	21.7 ± 6.2	49.8 ± 17.2
	W	0.66 ± 0.15	72.7 ± 36.6	12.4 ± 0.7	18.0 ± 2.4	94.7 ± 4.5	51.4 ± 27.2	51.9 ± 4.1
	S	0.60 ± 0.06	64.6 ± 16.2	14.8 ± 4.0	21.2 ± 1.7	94.0 ± 12.4	59.6 ± 30.2	53.8 ± 18.1
p-value		ns	ns	ns	ns	ns	ns	ns
2020	T	0.57 ± 0.10	63.1 ± 6.3	12.8 ± 1.4	20.6 ± 2.6	85.5 ± 7.7	45.3 ± 8.8	39.4 ± 10.4
	P	0.59 ± 0.08	59.7 ± 7.7	11.8 ± 0.5	21.0 ± 2.8	82.0 ± 4.9	42.6 ± 6.5	32.9 ± 3.3
	W	0.54 ± 0.05	61.4 ± 8.3	11.6 ± 0.9	19.3 ± 0.6	84.5 ± 5.3	36.4 ± 2.1	35.1 ± 7.6
	S	0.58 ± 0.08	71.3 ± 17.4	12.0 ± 0.9	17.7 ± 1.1	89.3 ± 3.5	33.0 ± 12.9	40.4 ± 4.7
p-value		ns	ns	ns	ns	ns	ns	ns
2021	T	0.54 ± 0.05	60.3 ± 9.9	12.3 ± 1.4	23.6 ± 3.2	87.0 ± 2.2	120.1 ± 33.3	46.3 ± 1.3
	P	0.54 ± 0.03	71.1 ± 10.1	12.4 ± 0.8	24.4 ± 2.7	84.7 ± 3.6	107.9 ± 18.8	44.7 ± 6.1
	W	0.55 ± 0.07	59.0 ± 8.6	12.0 ± 1.1	31.4 ± 4.9	90.3 ± 2.9	159.9 ± 58.6	74.2 ± 10.5
	S	0.57 ± 0.05	69.3 ± 7.0	12.5 ± 0.6	27.7 ± 2.5	83.2 ± 4.0	175.9 ± 42.5	48.4 ± 9.8
p-value		ns	0.012	ns	ns	ns	ns	0.005

Means were separated by ANOVA using Fisher's LSD test at a significance level of $p \leq 0.05$. ns = not significant.

2.5. Vegetative growth

Pruning weights were collected from 18 vines per treatment at site A and nine vines per treatment at site B each winter with a digital scale. The number of canes of each pruned vine were counted for the determination of average cane weight. Cordon length was measured with a flexible measuring tape so that growth components could be reported on a per-metre basis.

3. Statistical analysis

ANOVA was performed using XLSTAT Version 2022.3.2 (Addinsoft SARL, Paris, France). Means were separated using Fisher's LSD test at a significance level of $p \leq 0.05$ for all data.

RESULTS

1. Analysis of 1-year-old canes

At site A in 2019, based on observation by a stereo microscope, cane samples collected from cordons which were placed on top of the wire and secured in place had a significantly higher vessel diameter relative to cane diameter than cordons which were woven through the plastic clip system centred between parallel cordon wires (Table 1). In 2020 and 2021, no difference was observed between the vessel diameters of different treatments. No difference was observed in vessel density between treatments in 2019. In 2020, cane samples from cordons which had been wrapped tightly around the cordon wire had significantly more vessels per area than other treatments. In 2021, cane samples from cordons which had been placed on top of the cordon wire and secured in place had significantly fewer vessels per area than other treatments. At site B, based on observation by stereo microscope, no difference was observed in the diameter of vessels relative to cane diameter for any of the treatments in any year of the study. No difference was observed in vessel density in 2019 or 2020. In 2021, significantly more vessels per area were observed in samples collected from cordons which were placed on top of the cordon wire or wrapped in the s-shaped bend than those which were wrapped tightly around the cordon wire or woven through the plastic clip system centred between parallel wires.

Examining the characteristics of cane samples using X-ray microtomography (micro-CT), no difference was observed in the total volume of the xylem conduit relative to the total cane volume between treatments in 2019 or 2020 at site A (Table 1). In 2021, the average volume of the xylem conduit of samples collected from cordons placed on top of the wire was determined to be smaller than that of other treatments. No difference was observed in vessel density or vessel thickness in any year of the study. No difference was observed in connectivity density in 2019. Interestingly, in 2020 cane samples from cordons placed on top of the wire

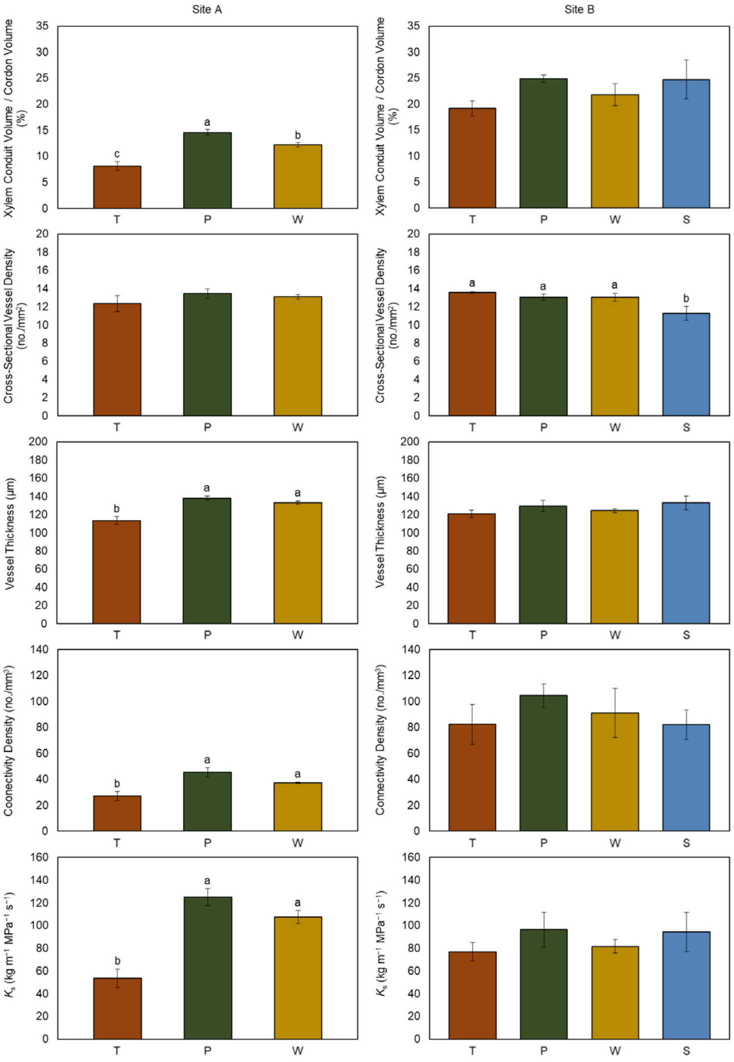

FIGURE 5. Anatomical measurements of cordon samples obtained by micro-CT. T = tightly wrapped, P = placed on top, W = woven through clips, S = s-bend. Means were separated by ANOVA using Fisher’s LSD test at a significance level of $p \leq 0.05$, and different letters indicate significant differences between treatments at each site.

had significantly more connections per volume than other treatments, while in 2021, they had fewer connections than other treatments. At site B, no significant difference was observed between treatments for any of the investigated morphological properties of cane samples collected in any year of the study, as observed by micro-CT. However, the theoretical specific hydraulic conductivity (K_s) of canes from cordons woven through the plastic clip system was determined to be higher than other treatments at this site in 2021. No difference in K_s between treatments was observed at site A in any year.

2. Analysis of cordons

At site A, the total volume of all xylem vessels in the xylem conduit relative to total cordon volume was found to be significantly lower in cordons which had been wrapped tightly around the cordon wire than in other treatments (Figure 5). Cordons which were woven through the plastic clip system centred between parallel wires had a greater xylem conduit volume than those which had been wrapped tightly around the wire but a lower volume than those which had been placed on top of the wire and secured in place, which had the greatest xylem conduit volume relative to cordon volume of all treatments. Site B followed a similar trend, although not significantly. At this site, cordons which had been wrapped around two parallel wires in the s-shaped bend appeared to have a similar xylem conduit volume to those which had been placed on top of the cordon wire.

When considering cross-sectional vessel density, there appeared to be no difference between cordons which had been wrapped tightly around the wire, placed on top of the cordon wire, or woven through the plastic clip system at either of the two experimental sites. At site B, however, significantly fewer average vessels per area were observed in cordons which were wrapped around two parallel wires in the s-bend than all three other treatments. At site A, cordons which had been wrapped tightly around the wire were determined to have significantly thinner xylem vessels than those which had been placed on top of the cordon wire or woven through the plastic clip system. At site B, no significant difference was observed between the average thickness of the xylem vessels of any treatment, including cordons which had been

TABLE 2. Vegetative growth components measured at winter pruning (mean ± std).

		Site A			Site B		
	Treatment	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)
2019	T	13.6 ± 2.8	24.4 ± 12.8	0.34 ± 0.20	17.9 ± 1.9	42.5 ± 16.1	0.75 ± 0.28
	P	12.5 ± 2.5	28.7 ± 12.2	0.35 ± 0.14	17.8 ± 4.0	42.4 ± 11.2	0.73 ± 0.15
	W	11.1 ± 0.8	18.8 ± 6.4	0.21 ± 0.07	18.3 ± 3.1	44.9 ± 22.5	0.81 ± 0.45
	S	-	-	-	18.4 ± 3.0	40.9 ± 14.1	0.74 ± 0.22
	p-value	0.047	ns	ns	ns	ns	ns
2020	T	13.4 ± 2.6	40.2 ± 13.9	0.56 ± 0.25	24.3 ± 4.3	31.2 ± 9.8	0.76 ± 0.30
	P	13.8 ± 2.7	41.7 ± 12.3	0.59 ± 0.23	22.8 ± 6.2	28.5 ± 9.8	0.65 ± 0.31
	W	14.1 ± 2.9	44.7 ± 13.0	0.64 ± 0.24	22.3 ± 2.1	26.2 ± 5.9	0.59 ± 0.18
	S	-	-	-	21.7 ± 1.9	28.5 ± 9.0	0.61 ± 0.16
	p-value	ns	ns	ns	ns	ns	ns
2021	T	19.3 ± 3.0	51.6 ± 18.9	1.01 ± 0.43	26.0 ± 2.8	25.7 ± 8.0	0.66 ± 0.21
	P	20.1 ± 1.9	49.7 ± 10.9	0.99 ± 0.20	26.5 ± 5.3	20.9 ± 3.3	0.56 ± 0.19
	W	20.8 ± 2.5	53.5 ± 14.6	1.10 ± 0.29	27.5 ± 2.2	21.8 ± 5.1	0.60 ± 0.17
	S	-	-	-	26.7 ± 2.9	24.2 ± 8.0	0.64 ± 0.20
	p-value	ns	ns	ns	ns	ns	ns
2022	T	25.5 ± 4.8	73.7 ± 36.9	1.86 ± 0.93	26.6 ± 5.2	28.0 ± 10.4	0.73 ± 0.25
	P	26.4 ± 3.2	68.3 ± 44.8	1.76 ± 1.04	24.9 ± 3.7	22.1 ± 3.5	0.55 ± 0.14
	W	26.9 ± 2.9	103.7 ± 33.5	2.81 ± 1.01	26.7 ± 5.1	20.6 ± 5.3	0.55 ± 0.17
	S	-	-	-	28.8 ± 4.1	23.5 ± 7.0	0.69 ± 0.27
	p-value	ns	0.022	0.006	ns	ns	ns

Means were separated by ANOVA using Fisher's LSD test at a significance level of $p \leq 0.05$. ns = not significant.

wrapped in the s-shaped bend. At site A, cordons which were wrapped tightly around the cordon wire had a significantly smaller number of redundant connections between vessel structures per unit volume than other treatments. At site B, no difference was observed in the connectivity density between any treatments. The theoretical specific hydraulic conductivity of cordons which had been wrapped tightly around the cordon wire was significantly lower than other treatments at site A but not at site B.

3. Analysis of vine vegetative growth

In 2019 at site A, cordons which were wrapped tightly around the cordon wire had significantly more canes per metre than cordons which were woven through the plastic clip system centred between parallel wires (Table 2). In 2022, cordons woven through the clip system had a much higher average cane weight and pruning weight than other treatments at this site. No difference in pruning weight or its components were observed between treatments at site B in any year.

DISCUSSION

Cane samples in this trial were collected from the distal portion of the cordon arms as it was decided that canes originating from this portion of the cordon had the greatest potential to show negative effects from the constriction of tightly wrapping the cordon around the cordon wire. The reasoning behind this is that sap movement to the canes of the distal section of the cordon involved the greatest distance of travel through the vasculature of the cordon arms of the various treatments (Torregrosa *et al.*, 2021). When using optical microscopy to evaluate differences in the diameter of the xylem vessels of cane samples, little difference was observed between treatments at either site in any season of the trial. Vessel diameter was considered as a proportion of cane diameter, as vessel size is known to increase with cane size (Ewers and Fisher, 1989; Olson and Rosell, 2013). In 2019, at site A, canes from the distal portion of arms, which had been placed on top of the cordon wire and secured in place, had vessels of greater diameter than canes from cordons woven through the plastic clip system centred between parallel wires. No difference was observed in vessel diameter in 2020 or 2021 at site A or in any year of the trial at site B. Similar results were obtained when examining distal cane samples collected in the same manner with X-ray microtomography (micro-CT). Considering xylem conduit volume as a proportion of total cane volume, no difference was observed between treatments in any year of the study at site B. At site A, while no difference was observed between treatments in the first two years of the study, in 2021, cane samples from the distal portion of arms which had been placed on top of the cordon wire had a lower xylem conduit volume relative to total cane volume than other treatments. Considering vessel density, as observed by stereo microscope, no difference was observed between treatments at either site in 2019. At site A, in 2020, cane samples from cordons which had been wrapped tightly around the cordon wire had significantly more vessels per area than other treatments. In 2021, cane samples from cordons which had been placed on top of the cordon wire had significantly fewer vessels than other treatments. In contrast, in 2021 at site B significantly more vessels were observed in samples collected from cordons which were placed on top of the cordon wire or wrapped in the s-shaped bend than those which were wrapped tightly around the cordon wire or woven through the plastic clip system centred between parallel cordon wires. Using micro-CT, no differences were observed in vessel density between treatments at sites A or B in any year of the trial. Vessel density values evaluated by micro-CT appear lower than those evaluated by stereo microscope in this study as they consider the number of vessels in relation to total cross-sectional cane area rather than in relation to xylem area.

Using CT Analyzer, a model-independent 3D thickness was measured for the xylem vessels of each cane sample. No significant difference was observed between the thickness of the vessels of canes collected from treatments at either site in any year of the study. Another metric that was evaluated using 3D analysis was connectivity density, indicating the number of redundant connections between vessel structures per unit volume. The number of connections between vessels is an important consideration for water flow as vessels are of finite length, and transiting sap eventually must move from one vessel to another through lateral pit pairs (Tyree and Ewers, 1991). While at site B no difference was observed in connectivity density between treatments in any year; somewhat contradictory results were obtained at site A. In 2020, cane samples from cordons placed on top of the cordon wire had significantly more connections per volume than either of the two other treatments investigated at the site, while in 2021, they had fewer connections than the other treatments. No difference in theoretical specific hydraulic conductivity (K_s) between treatments was observed at site A in any year, while at site B, canes from cordons woven through the plastic clip system had significantly higher K_s than other treatments in 2021. This was due to these canes having thicker vessels and a greater vessel density than other treatments, though these differences, when considered alone, were not significant.

Examining cordons directly with micro-CT allowed for a direct assessment of the impact of the training method on localised cordon structure. At site A, highly significant ($p < 0.0001$) differences were observed between xylem conduit volume in relation to total cordon volume for the different treatments. Cordons which had been placed on top of the cordon wire had a greater xylem conduit volume than those which had been woven through the plastic clip system, which in turn had a greater xylem conduit volume than those which had been wrapped tightly around the cordon wire. The volume occupied by the xylem conduit of the woven cordons was, on average, 49.8 % higher than those which were wrapped tightly around the cordon wire, while the xylem conduit volume of cordons trained on top of the wire was 78.7 % higher than those which were wrapped tightly around the cordon wire. This indicates that the tight wrapping treatment is likely to have had a serious detrimental impact on the vascular health of the cordon. Such a reduction in the volume of the xylem conduit suggests that the constrictive effects of the tight wrapping reduced the capacity of the xylem for normal vascular function, as conductivity is determined by vessel structure, size, and efficiency (Schultz and Matthews, 1993; Tyree and Ewers, 1991). Indeed, the theoretical specific hydraulic conductivity (K_s) of tightly wrapped cordons was determined to be much lower than both other applied treatments at site A ($p < 0.0001$). At site B, there was also a trend of greater xylem conduit volume and K_s in cordons which had not been tightly wrapped, although these trends were not significant. Considering the 3D thickness of vessels, at site A, the vessels of cordons which had been tightly wrapped were significantly thinner than those which had been placed on top of the cordon wire or woven through the plastic clip system. However, at site B no difference was observed in vessel thickness between treatments.

No difference was observed in cross-sectional vessel density between cordons which had been wrapped tightly around the cordon wire, placed on top of the cordon wire, or woven through the plastic clip system at either of the two trial sites. Cordons which were wrapped in the s-shaped bend had significantly fewer average vessels per area than the three other treatments at site B. There is no obvious reason for why this was the case, but the shape of the cordon imparted by this training method (s-bend) had the largest curve out of any treatment and had the greatest deviation from a straight structure. No difference was observed in the connectivity density of different treatments at Site B. At site A, cordons which were wrapped tightly around the cordon wire had significantly fewer connections between vessel structures per volume than those which were trained on top of the cordon wire or woven through clips. As with canes, the number of connections within vessel networks of perennial cordons is an important element of the sap movement pathway. Increased connectivity may make the xylem conduit more efficient and hydraulically integrated, especially in the case of long vessels (Espino and Schenk, 2009; Jacobsen *et al.*, 2012). While no reduction in connectivity density was observed at site B, the reduction observed at site A in cordons which were wrapped tightly around the cordon wire, suggests that they may be suffering from numerous deleterious effects. Not only was their xylem conduit seemingly impeded by a reduction in volume as a result of their constrictive training method, but also a reduction in the connectivity of their vessel network. Interestingly, in 2021 their cane weights and pruning weights were lower than those of cordons which were woven through the plastic clip system, but not those that were trained on top of the wire.

The results of several studies suggest that while the reduced xylem volume observed in the tightly wrapped cordons of this trial may make them likely to suffer from a reduction in water transport function, they may not necessarily be more susceptible to any or all forms of vascular disease (Oswald, 2017; Pouzoulet *et al.*, 2017; Solla and Gil, 2002). Whether the restricted vasculature of these cordons could result in water stress symptoms appearing in the distal regions of such arms is another matter and is not immediately clear. While larger-diameter vessels have the benefit of conducting sap more efficiently (Dimond, 1966), they may also be more prone to the occurrence of embolisms (Hacke *et al.*, 2006). In the absence of measurements made during the growing season to assess the impact of the different training systems on sap transport and hydraulic conductivity in a direct manner, the anatomical measurements presented in this trial cannot conclude on the presence or absence of water stress. As such, the results do not give a clear indication of water stress in the cane samples collected from the distal portion of tightly wrapped cordons as opposed to the other investigated treatments. Observed differences in vessel diameter, vessel density, and connectivity density were minimal between treatments for cane samples observed both by micro-CT and optical microscopy. Where there were differences between treatments, no trend is immediately clear, such as in the case of the canes from cordons trained on top of the wire displaying a greater connectivity density than other treatments in one season and a lesser connectivity density than other treatments in the following season. Some of the differences in results observed between canes collected from the two sites may be related to the fact that different cultivars were investigated at each site, with Cabernet-Sauvignon being the cultivar of interest at site A and Shiraz being the cultivar of interest at site B. Another important distinction between sites is that site A was a newly planted block while site B was an older block that had been newly reworked. Differences observed at the same site between seasons are harder to explain, however, and could be related to water availability and climatic conditions in addition to the training method.

The direct observation of the cordons provided a clearer insight into the localised impact of the different training methods on the morphology of the vascular system of the cordons themselves. While significant results were not produced at site B for measurements of xylem volume in relation to cordon volume, they followed the same trend as site A. That is, cordons which were wrapped tightly around the cordon wire had a smaller xylem conduit volume than those which were woven through the plastic clip system, which in turn had a smaller xylem conduit volume than cordons which were placed on top of the cordon wire. These results make sense when one considers the constrictive nature of the different training methods. The cordons, which were wrapped tightly around the wire, displayed by far the greatest degree of constriction, with the distorted shape of the cordon from the presence of the cordon wire being very apparent (Figure 4). The pressure exerted from the cordon wire onto the cordon, in this case, was great enough for the wire to become partially embedded within the cordon wood. The cordons woven through the plastic clip system centred between parallel wires meanwhile had some pressure exerted on the cordon at the points where the plastic clips made contact with the wood of the cordon. In contrast, the cordons which had been trained on top of the cordon wire and secured in place had minimal to no pressure exerted on the wood as the tying material that was used (plastic ties) were expandable to a certain extent and allowed for the cordon to grow without becoming constricted at its anchor points. These ties were also replaced after roughly the 2-year mark if they appeared to be becoming too tight, reducing their risk of constricting the cordon as it grew. It should be noted that the plastic clip system presented in this trial could be used in an alternative manner with the cordon placed on top of all clips rather than woven through them, which would presumably apply less constriction to the arms but afford them less stability as well.

A recently conducted survey of ten vineyard sites over two growing seasons which visually assessed vines displaying varying degrees of cordon strangulation, dieback, and characteristic foliar symptoms of *Eutypa* dieback, did not find evidence of cordon strangulation being a driving force behind cordon decline (O'Brien *et al.*, 2023). In spite of this, however, it is a very common occurrence to see cordons which have been wrapped very tightly around the cordon wire displaying severe dieback, especially those around 15–20 years of age or older. Although this current study focused on the observation that newly trained cordons were only four years old, differences in the vascular morphology of the cordons were already apparent. The reductions in xylem conduit volume, vessel thickness, and connectivity density observed in cordons which were wrapped tightly around the cordon wire suggest that this training method may have a serious negative impact on the capacity of the xylem for normal hydraulic function, which could likely progressively become worse over time. This argument is further supported by K_s values for tightly wrapped cordons which were 50.2 % lower than cordons woven through the clip system and 57.2 % lower than cordons trained on top of the wire at one site.

Further examination of these same cordons over a longer period could provide a more in-depth insight into the long-term effects of the different training techniques on cordon vitality. In the short span of the trial at site A, cordons which were trained in alternative manners benefited from vascular conduits, which were of 49.8–78.7 % greater volume than those which had been wrapped tightly around the cordon wire. This is a notable increase and one that could likely have implications on the regular functionality of the cordon, as well as and perhaps even more importantly, in circumstances of stress. If alternative training methods could provide permanent cordons with a healthier vascular system, more equipped to endure the changing conditions required to sustain a long lifespan, then understanding their impact on vascular health could allow for more informed management decision-making. While there are certain benefits associated with wrapping developing cordon arms tightly around the cordon wire, avoiding constriction of the vascular system may be a reason of great enough concern to reconsider the practice.

ACKNOWLEDGEMENTS

The authors would like to thank the University of Adelaide. This work was supported by Wine Australia, with levies from Australia's grape growers and winemakers and matching funds from the Australian Government. We would also like to express our gratitude to the growers who allowed us access to their vineyards to conduct this study. The work was supported by the Australian Research Council (LE180100136). The authors thank Flinders Microscopy and Micro Analysis (FMMA), Adelaide, Australia, for providing access to the large-volume micro-CT system. We also thank Adelaide Microscopy for providing access to their instrumentation, including the Nikon SMZ25 stereo microscope and Bruker SkyScan 1276 micro-CT. An additional thank you to Craig Johns and Simonit & Sirch for their guidance on trial establishment, as well as Stephen Tyerman for productive discussions that improved this research.

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